Factors affecting outcome after autologous chondrocyte implantation for the treatment of osteochondral defects of the knee

By

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ABSTRACT

Some studies on autologous chondrocyte implantation (ACI) have demonstrated little benefit over other techniques and few have demonstrated a lasting benefit. A number of factors can contribute to failure and a scientific approach to elucidate these variables has not been reported.

This thesis reports on the use of a statistical approach - the Generalised Linear Model (GLM) to quantify the effect each factor has whilst considering the interplay of other variables. Data from a randomised controlled trial and several case-controlled studies will assess the efficacy of 2 different types of ACI, the influence of smoking, BMI, and physical activity. Non-modifiable risk factors that were assessed include the aetiology, site and size of the lesion, the duration of symptoms and number of previous operations prior to the index procedure and the presence of early osteoarthritis.

Site had a significant effect on outcome but size did not. The GLM predicted a point increase in the Modified Cincinnati Score (MCS) before surgery (MCS 0) would lead to a further 0.5 point increase in MCS 2 years postoperatively (MCS 24) ($p=0.001$). Other significant non-modifiable risk factors include age and sex of the patient. When treating lesions in the patella, duration of symptoms was a significant factor, but age was not.

The GLM predicted that smokers' MCS 24 (the Modified Cincinnati Score 2 years after surgery) was likely to be 15 less than non-smokers (p=0.002). Patients playing no sports experienced an 11.4 point decrease. For each increase in BMI, the MCS 24 was 2.4 less (p=0.001).

Factors that optimise outcome following surgery are; avoidance of numerous procedures prior to ACI and delay of more than one year before undergoing ACI. Current NICE guidelines prohibit the use of ACI as the first-line surgical procedure and prevent addressing the above 2 issues.

Poorer results were observed in obese patients. Weight loss and active lifestyle are essential pre-operatively. Furthermore, we recommend that pre-operative counselling for smokers is essential and that all smokers be offered a cessation programme.

DECLARATION

I declare that this is a group of patients that have been referred to and treated by The Royal National Orthopaedic Hospital Trust. All these patients have been treated following a local research ethical committee application (reference No 981028), and a multi-centre research committee application for the study of autologous cartilage cell implantation (ACI-C) compared with membrane carried cartilage cell implantation (MACI) for treatment of damaged cartilage lining of the knee (reference No 02/01/73). Professor George Bentley has been the principal investigator for these studies.

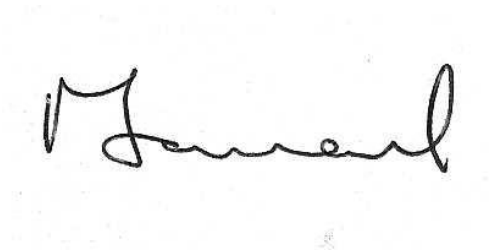
Specific Contribution To The Work

When I started in the research post, 691 procedures had been performed in the Joint Reconstruction Unit at The Royal National Orthopaedic Hospital as either part of a randomised controlled trial (RCT) or a prospective cohort study. When reporting on the RCT, there was missing data which I retrieved whilst also ensuring that new patients had adequate data collection. Specifically, out of 247 consecutive patients that had either an ACI or matrix-carried autologous chondrocytes implantation (MACI) procedure performed in a 3 year period, only 137 patients had functional scores recorded 2 years after surgery. I managed to get the latest functional scores in a further 69 patients.

This was also true for the patients in chapter 4 when assessing the efficacy of autologous chondrocytes implantation (ACI) in the patella-femoral joint. In chapter 5, I reviewed all 200 radiographs and also retrieved missing data. A colleague also reviewed 115 radiographs so that we could perform inter-observer error for two different types of radiographic scoring

systems. The work in chapter 6 was entirely my own. I created the new data capture form for smoking status, body mass index (BMI) and physical activity. I distributed the forms and collected data.

I certify that the work in this thesis is original, though the collection of data has been supported by others and will be acknowledged accordingly. The analysis of data and subsequent conclusions are entirely the work of the author. Further my research has been conducted ethically and the results are genuine.



Parag K. Jaiswal

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I would like to thank my preceding research fellow Derek Park for acting as an independent observer in one of the chapters, and also Simon Macmull whose help was invaluable in helping retrieve missing data.

I am also indebted to my co-supervisor, Caroline Dore and her colleague Suzie Cro for their statistical advice.

Finally, the journey to complete this thesis was long and arduous but made significantly easier with the love, support and patience of my wife, Roopa and my children Eva and Kara. I am sorry so much time was spent away from you! They say that behind every successful man there stands a woman; I am very fortunate to have one extraordinary woman and two beautiful girls who inspire me to greater heights....

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List of Abbreviations

ACI	Autologous chondrocytes implantation
ACI-C	Autologous chondrocytes implantation using synthetic collagen membrane
ACI-P	Autologous chondrocytes implantation using autologous periosteum
ACL	Anterior cruciate ligament
ANOVA	Analysis of variance
BMI	Body mass index
CCI	Characterised chondrocytes implantation
CONSORT	Consolidated Standards of Reporting Trials
CP	Chondromalacia Patellae
CRP	C-reactive protein
CTU	Clinical trials unit
ECM	Extracellular matrix
FGF	Fibroblast growth factor
GLM	Generalised Linear Model
ICRS	International Cartilage Repair Society
IKDC	International Knee Documentation Committe
IL-1	Interleukin-1
IL-6	Interleukin-6
JRU	Joint Reconstruction Unit (Royal National Orthopaedic Hospital, Stanmore)
K&L	Kellgren and Lawrence
KOOS	Knee osteoarthritis and outcome score
LFC	Lateral femoral condyle
MACI	Matrix-carried autologous chondrocytes implantation
MCS	Modified Cincinnati Score

MFC	Medial femoral condyle
MMPs	Matrix metalloproteases
MREC	Multi-centre ethics research committee
MRI	Magnetic Resonance Imaging
MSCs	Mesenchymal stem cells
NICE	National Institute of Health Clinical Excellence
OA	Osteoarthritis
OCDs	Osteochondral defects
OD	Osteochondritis dissecans
OR	Odds ratio
Pat.	Patella
PFJ	Patellofemoral joint
PFR	Patellofemoral replacement
PG	Peptidoglycans
RCT	Randomised controlled trial
RR	Relative risk
SF-36	Short-Form 36 Questionnaire
TGF-β	Transforming growth factor-Beta
TIMPs	Tissue inhibitors of metalloproteases
TKR	Total knee replacement
TNF	Tumour necrosis factor
UKR	Unicompartmental knee replacement
VAS	Visual Analogue Scale
WOMAC	Western Ontario and McMaster osteoarthritis index

Chapter 1:
The Biology of Cartilage

Introduction

Articular cartilage has a unique structure and function. It forms the articulating surface of diarthrodial joints and provides friction-free load-bearing on joint surfaces, hence allowing smooth movement without pain. This is accomplished by the production of a special matrix (by chondrocytes) which binds and contains water. In most synovial joints, articular cartilage provides these essential biomechanical functions for 8 decades or more. No synthetic material performs this well as a joint surface.

Composition

Articular cartilage consists of cells, matrix water, and matrix macromolecular framework. Its mechanical properties are derived primarily from its matrix. Cells contribute little to the volume of the tissue (approximately 1% in adult humans).

Chondrocytes

Chondrocytes are the only type of cell within normal articular cartilage and are responsible for synthesizing all the constituents of the extracellular matrix (ECM). They are derived from mesenchymal cells, which differentiate during skeletal development to form chondrocytes. The size, shape and metabolic activity differ according to zone they reside in (Aydelotte et al. 1996). All chondrocytes contain the organelles necessary for matrix synthesis (e.g. endoplasmic reticulum and Golgi membranes).

Chondrocytes are metabolically active and derive their nutrients from the synovial fluid. The nutrients must first pass through synovial tissue and fluid and then into cartilage matrix. This places a restriction on not only the size of materials but also the charge and molecular configuration (Fischer et al. 1995). The configuration of this system means that chondrocytes are exposed to low levels of oxygen relative to other tissues and hence depend primarily on anaerobic metabolism.

In growing individuals, chondrocytes produce new tissue to expand and remodel the articular surface. In the skeletally mature, they do not substantially change the volume of tissue, but replace degraded matrix macromolecules as well as remodelling the articular surface to a limited extent (Buckwalter 1995). During the formation and growth of articular cartilage, the cell density is high and the cells reach their highest level of metabolic activity as the chondrocytes proliferate rapidly and synthesize large volumes of matrix. After completion of growth, most chondrocytes probably never divide but rather continue to synthesize collagens, proteoglycans and non-collagenous proteins. The maintenance of articular cartilage requires on going remodelling of the matrix framework with an establishment of equilibrium between synthetic and degradative activity. Several mechanisms influence this balance. For example, immobilization of a joint has been shown to exceed the degradative process when compared to synthetic activity (Buckwalter 1995). Furthermore, with ageing, the capacity of the cells to synthesize some types of proteoglycans and their response to growth factors decrease (Guerne et al. 1995 and Martin et al. 1996). In the osteoarthritic patient, there is evidence to suggest that chondrocytes are able to synthesize new matrix but many of these molecules are degraded by proteolysis (Hollander et al. 1991).

As well as mechanical loads and hydrostatic pressures changes, chondrocytes also respond to soluble mediators, such as growth factors, interleukins and pharmaceutical agents. Although

chondrocytes maintain a stable matrix, their response to some factors (e.g. IL-1) may lead to degradation of ECM. The response to other types of messages used to commonly regulate body processes is limited. Cartilage has no nerve supply; hence neural impulses can not provide information. Immune responses (both cellular and humoral) are not likely to occur in cartilage (due to exclusion of monocytes and immunoglobulins from tissue by steric exclusion).

Extracellular matrix

The matrix of articular cartilage consists of two components: tissue fluid and the framework of structural macromolecules that give the tissue its form and stability. The interaction of the tissue fluid with the macromolecular framework gives the tissue its mechanical properties of stiffness and resilience (Arokoski et al.1999).

Tissue fluid

Water contributes 65% to 80% of the wet weight of articular cartilage and the interaction of water with macromolecules within the matrix influences the mechanical properties of the tissue significantly (Linn et al. 1965, Maroudas et al 1987). In order to balance the negatively-charged proteoglycans, the tissue fluid contains high concentrations of cations as well as gases, small proteins and metabolites. The interaction of water with the large aggregating proteoglycans help maintain the fluid within the matrix and the concentrations of electrolytes in the fluid. The concentration of positive ions (e.g. sodium) is increased due to the presence of large numbers of negatively-charged sulphate and carboxylate groups on macromolecules attracting positive charged ions and repelling negatively charged particles. Consequently the concentration of negative ions such as chloride decreases. The increase in the total concentration of inorganic ions causes an increase in osmolality of the tissue (i.e. it creates a Donnan effect) (Buckwalter and Mankin Part 1, 1997). The collagen network resists

the Donnan osmotic pressure caused by the inorganic ions associated with proteoteglycans thus maintaining the turgor of the tissue (Buckwalter and Mankin Part 2, 1997).

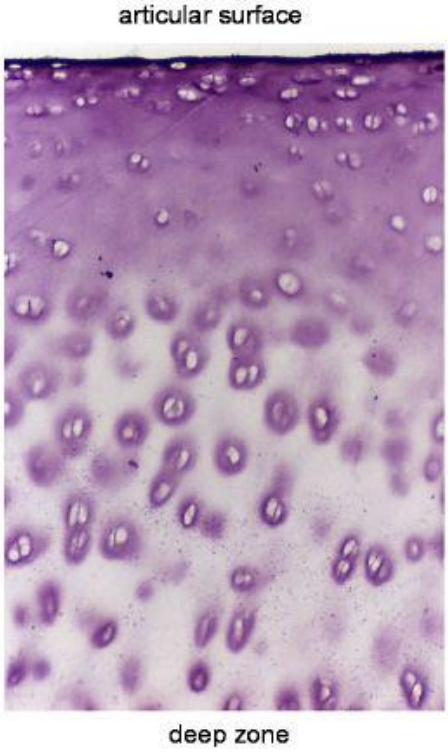
Structural macromolecules

Macromolecules contribute 20 to 40% of the wet weight of the tissue (Buckwalter and Rosenberg 1990). The three classes of macromolecules in articular cartilage are collagens, proteoglycans, and non-collagenous proteins. Collagens contribute 60% of the dry weight of cartilage; proteoglycans 25-35%; and non-collagenous proteins and glycoproteins 15-20%.

Collagens

Collagens are distributed fairly evenly throughout the depth of the cartilage except for the collagen-rich superficial zone (figure 1.1). The collagen fibrillar meshwork gives cartilage its form and tensile strength (Buckwater and Mow 1992).

Figure 1.1: Immunochemical localization of type II collagen in an intact articular cartilage region from the knee joint.



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Articular cartilage consists of multiple distinct collagen types of which type II collagen accounts for 90-95%. Others include Type VI, IX, X and XI. Types II, IX and XI form the cross-branded fibrils seen with electron microscopy. These fibrils are organized into a tight meshwork that extends throughout the tissue and provides the tensile stiffness and strength of articular cartilage. Type II collagen is the primary component of the cross-banded fibrils.

Type IX collagen molecules bind covalently to the superficial layers of the cross-banded fibrils and project into the matrix, where they can also bind covalently to other type IX collagen molecules (Eyre 1995). Type XI collagen molecules bind covalently to type II molecules and form part of the interior structure of the cross-banded fibrils. The functions of type IX and XI collagens remain unclear but it is presumed that they help form and stabilise the collagen fibrils assembled primarily from type II collagen. The projecting portions of type IX collagen may also help to bind together the collagen-fibril meshwork (Bruckner 1988).

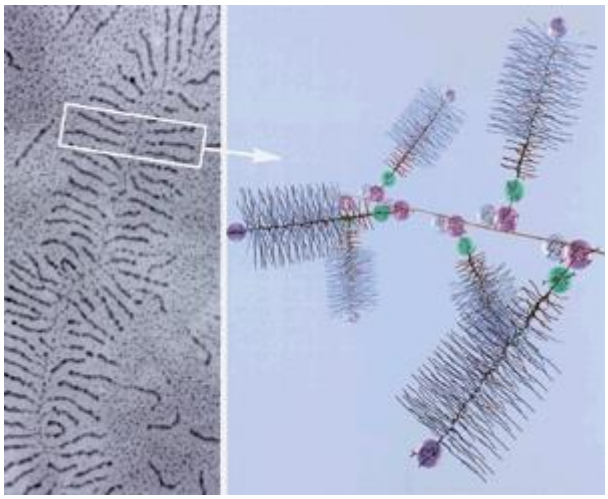
Type VI collagen appears to form an important part of the matrix surrounding the chondrocytes and helps chondrocytes attach to the matrix (Hagiwara et al.1993). Type-X collagen is only present near the cells of the calcified cartilage zone of the articular cartilage and the hypertrophic zone of the growth plate (where the longitudinal cartilage septa begin to mineralize). This suggests that it has a role in mineralization of the cartilage (Eyre et al. 1992).

Proteoglycans

Proteoglycans consist of a protein core and one or more glycosaminoglycan chains (long unbranched polysaccharide chains consisting of repeating disaccharides that contain an amino sugar (Hardingham 1992). Each unit of disaccharide has at least one negatively charged carboxylate or sulphate group. Hence these glycosaminoglycans form long strings of negative charges, attracting cations. Glycosaminoglycans found in cartilage include hyaluronic acid,

chondroitin sulphate, keratin sulphate and dermatan sulphate. The concentration of these molecules varies amongst sites within articular cartilage and also with age, injury to the cartilage and disease.

Figure 1. 2: The micrograph on the cover shows a portion of an aggregate isolated from cartilage that consists of a central filament of hyaluronan and a large number of attached monomer (aggrecan) proteoglycans



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The major classes of proteoglycans are aggrecans (large aggregating monomers) and small proteoglycans such as decorin, biglycan and fibromodulin (Poole et al. 1996). Aggrecans have large numbers of chondroitin sulphate and keratin sulphate chains attached to a protein core filament. Cartilage also contains large non-aggregating proteoglycans that resemble aggrecans in structure and composition and may represent degraded aggrecans (Buckwalter and Roughly 1994). Aggrecan molecules fill most of the interfibrillar space of the cartilage matrix, contributing 90% of the total matrix proteoglycan mass; large non-aggregating proteoglycans contribute 10% and small non-aggregating proteoglycans contribute 3%. The latter, due to their small size, may be present in equal or higher molar amounts even though they add relatively little to the total mass.

Most aggrecans non-covalently associate with hyaluronic acid and link proteins (small non-collagenous proteins) to form proteoglycan aggregates (see figure 1.2).

These large molecules have a central backbone of hyaluronan that can range in length from several hundred to more than 300 associated aggrecan molecules (Buckwalter and Kuettner 1985). Link proteins stabilise the association between monomers and hyaluronic acid and appear to have a role in directing the assembly of aggregates (Tang et al. 1996).

Biochemical and electron microscopy studies show two populations of proteoglycan aggregates within articular cartilage (Pita et al. 1990):

1) slow sedimenting population of aggregates with low chondroitin sulphate-to-hyaluronate ratio and few monomers per aggregate

2) faster sedimenting population of aggregates with higher chondroitin sulphate-to-hyaluronate ratio and more monomers per aggregate.

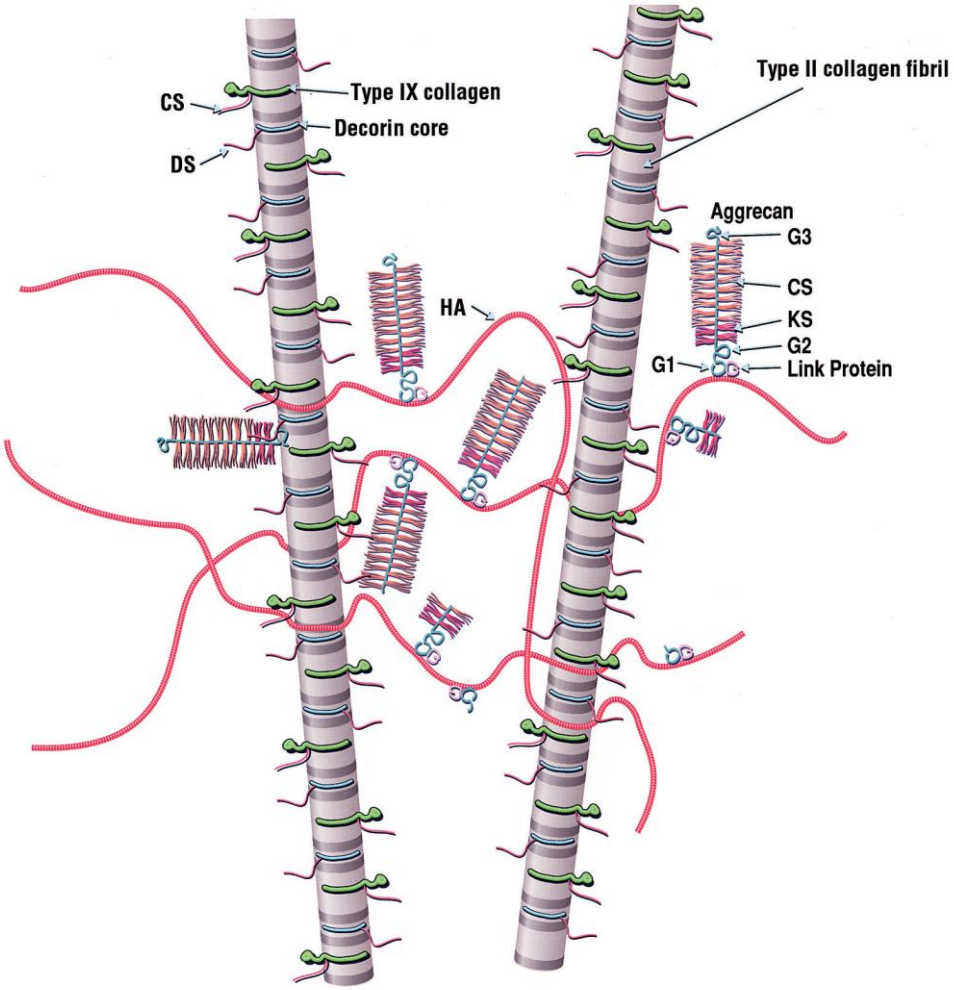
The superficial regions of cartilage contain primarily smaller aggregates whereas the deeper regions contain both types. Loss of larger aggregates appears to be one of the earliest changes associated with osteoarthritis and immobilization of the joint (Buckwalter and Roughly et al 1994). The smaller aggregates are thought to influence cell function by binding to other macromolecules.

Non-collagenous proteins and glycoproteins

There are a wide of variety of these molecules in articular cartilage but thus far only a few of them have been studied, hence their role is not as well-understood. In general, they consist of a protein core attached to which are monosaccharides and oligosaccharides (Heinegard et al 1995). Some of these molecules appear to help to organise and maintain the macromolecular structure of the matrix. For example, Anchorin CII, a collagen-binding chondrocyte surface protein, may help to anchor chondrocytes to the collagen fibrils of the matrix (Pfaffle et al. 1990).

Cartilage oligomeric protein, an acidic protein, is concentrated primarily within territorial matrix of the chondrocyte and appears to be present only within cartilage and to have capacity to bond to chondrocytes. This molecule may have value as a marker of cartilage turnover and of progression of cartilage degeneration in patients who have osteoarthritis (Sharif et al. 1995).

Figure 1.3: Diagrammatic representation of the macrofibrillar collagen network and aggrecan with hyaluronic acid

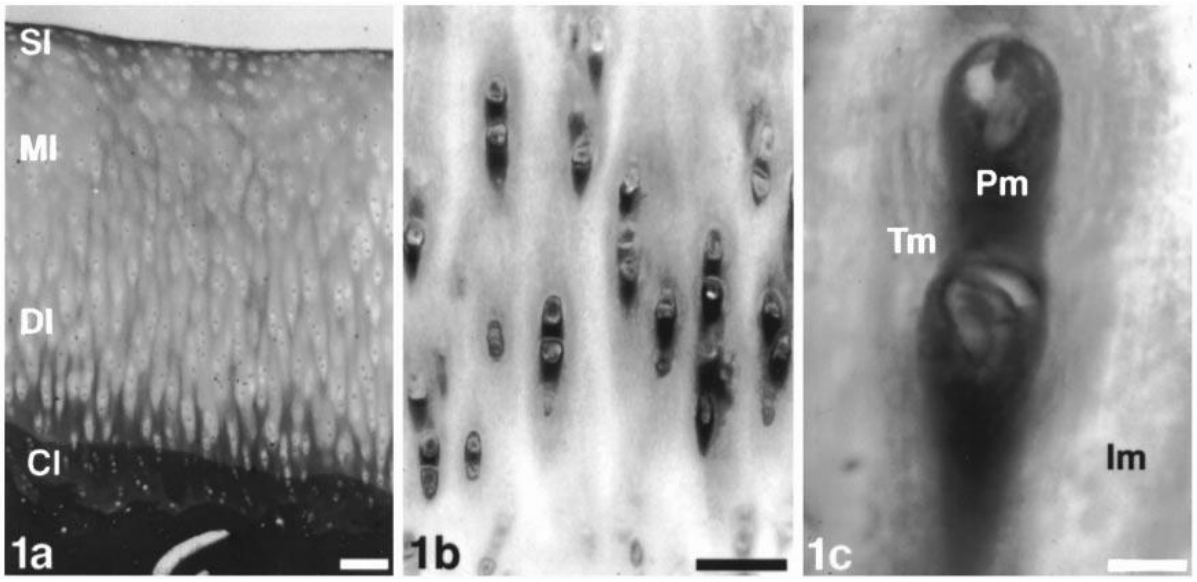


(Modified from Poole AR: Cartilage in Health and Disease. In Koopman W [ed]. Arthritis and Allied Conditions. A Textbook of Rheumatology. Ed 14. Vol 1. NewYork, Lippincott Williams & Wilkins 2260–2284, 2001.)

Structure

From the articular surface to the subchondral bone, the structure and composition varies throughout its' depth (figure 1.4.).

Figure 1.4 The zones of articular cartilage

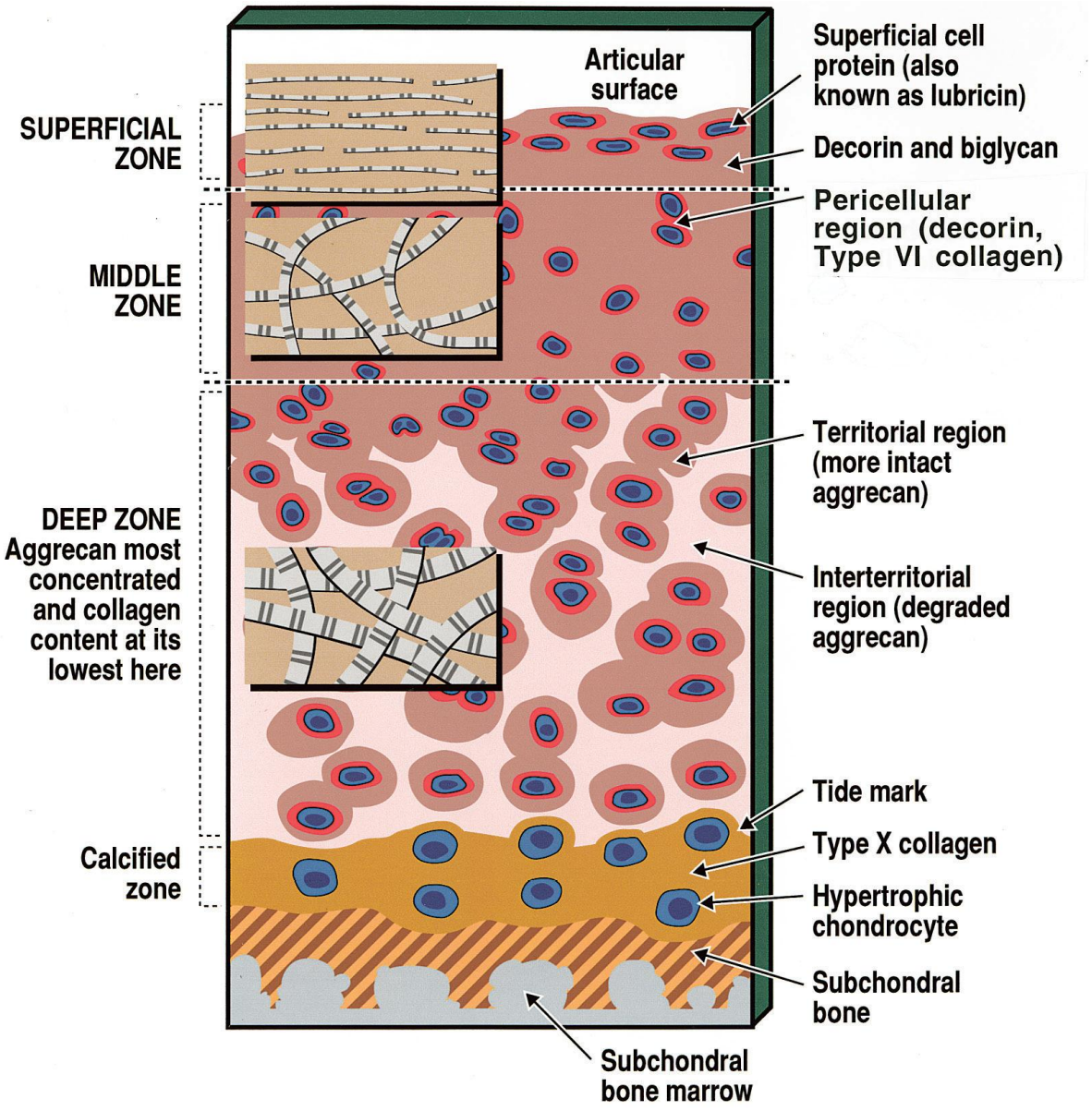


Articular cartilage histology. (*a*) Van Gieson staining for collagen demonstrating superficial (S1), middle (M1) deep (D1) and calcified (C1) layers. (*b*) Haematoxylin and eosin staining illustrating the radial alignment and matrix organisation in the deep layers. (*c*) Detail showing differentiation of the pericellular microenvironment (Pm), territorial matrix (Tm) and the interterritorial matrix (Im). Bars: (*a*) 100 lm, (*b*) 50 lm, (*c*) 10 lm.
Figure taken from CA Poole J Anat. 1997

These differences include cell shape and volume, collagen fibril diameter and orientation, proteoglycan concentration and water content. The cartilage can be divided into 4 zones:

1. Superficial zone
2. Middle or transitional zone
3. Deep zone
4. Zone of calcified cartilage

Figure 1.5: Diagrammatic representation of articular cartilage



Diagrammatic representation of the general structure of human articular cartilage from an adult to show the zones, regions, and relationship with subchondral bone. The insets show the relative diameters and organizations of collagen macrofibrils in the different zones. Some special features of molecular content or properties also are indicated. (Modified from Poole AR: Cartilage in Health and Disease. In Koopman W [ed]. Arthritis and Allied Conditions. A Textbook of Rheumatology. Ed 14. Vol 1. New York, Lippincott Williams & Wilkins 2260–2284, 2001.)

The superficial zone consists of thin collagen fibrils arranged parallel to the surface. The chondrocytes in this layer are elongated and the long axis is also parallel to the surface thereby providing the tensile and shear strength (Akizuki et al 1986). The chondrocytes are covered by a thin film of synovial fluid, called ‘lamina splendens’ or ‘lubricin’ (Buckwalter

& Mankin 1997). This protein is responsible for providing an ultimate gliding surface to the articular cartilage. Preservation of this superficial layer is critical to protect the deeper zones and damage to the superficial zone alters the mechanical properties of cartilage thus contributing to the development of osteoarthritis. Type IX collagen is found in this layer between type II bundles that provide resistance to shear. It is thought that this layer limits passage of large molecules between synovial fluid and cartilage (Alford and Cole 2005). The water content is at its highest level and the proteoglycan content at its lowest.

The chondrocytes in the middle zone are more spherical and the cell density in this zone is lower. The collagen fibres have a larger diameter, are obliquely arranged though have less apparent organization (Buckwalter and Mankin 1997). The proteoglycan aggrecan concentration is higher in this zone. This layer resists compressive forces but also serve as a transition between the shearing forces on the surface and the compressive forces placed on the deeper layers

The deep zone contains the highest concentration of proteoglycans and the lowest concentration of water. Cell density is at its lowest in this zone. The collagen fibres have a large diameter and are organized perpendicular to the joint surface. The chondroytes are also arranged in columns. This arrangement serves to resist compressive loads.

The deepest layer, the zone of calcified cartilage separates the hyaline cartilage from subchondral bone. It is characterised by small cells distributed in cartilaginous matrix encrusted with apatitic salts. The chondrocytes in this zone express hypertrophic phenotype. These cells are unique in the way that they synthesise Type X collagen, responsible for providing structural integrity and provide a ‘shock absorbing’ capacity along with the subchondral bone.

Histological staining with haematoxylin and eosin shows a wavy bluish line, called the tidemark, which separates the deep zone from the calcified zone. The number of tidemarks increases with age as the tissue is remodelled (Buckwalter et al. 1985). This zone was considered inactive until Hunziker noticed that chondrocytes in this zone were able to incorporate sulphate into pericellular and territorial matrix (Hunziker 1992). It was also speculated that following injury, the metabolic activity in this zone becomes temporarily impaired.

Matrix is organized in three different zones in the cartilage:

1. Peri-cellular
2. Territorial
3. Inter-territorial

Pericellular matrix is a thin rim of matrix organized tissue in close contact with the cell membrane (2μm wide). This region is rich in proteoglycans and non-collagenous proteins, like cell membrane-associated molecule anchorin CII and decorin (Buckwalter and Mankin 1997). It also contains non-fibrillar collagen, made of type IV collagen.

Territorial matrix surrounds the pericellular region and is present throughout the cartilage. It surrounds individual chondrocytes or a cluster of chondrocytes including their pericellular matrix. In the middle zone, it surrounds each column of chondrocytes. The collagen fibrils in this region are organised in a criss-cross manner thus forming a fibrillar basket surrounding clustered bunch of chondrocytes, protecting them from mechanical impacts.

Inter-territorial matrix forms the most of the volume of all types of matrices, made up of the largest diameter of collagen fibrils. Fibres are oriented differently in different zones,

depending on the requirement, viz. parallel in the superficial zone and perpendicular in the radial zone. This region is distinguishable from others, by formation of aggregates of proteoglycan molecules.

Collectively, these highly specialized layers produce the superior loading and minimal friction characteristics of hyaline cartilage that makes it particularly difficult to restore or duplicate once it is damaged or lost. Damage to any part of this complex system can disrupt normal biomechanical properties of articular cartilage leading to further degeneration (see box 1).

Figure 1.6: Functions of Hyaline cartilage and matrix

Functions of articular cartilage:
1. Provides a low-friction gliding surface.
2. Acts as a shock absorber.
3. Minimizes peak pressures on the subchondral bone.

Functions of the matrix:
1. Protects the chondrocytes from mechanical loading, thus helping to maintain their phenotype.
2. Storage of some cytokines and growth factors, required for chondrocytes.
3. Determines the type, concentration and rate of diffusion of the nutrients to chondrocytes.
4. Acts as a signal transducer for the cells.

Influence of mechanical factors on structure

The functional and structural properties of articular cartilage appear to be conditioned to stresses to which it is most regularly subjected (Seedhom et al. 1979). Thus, cartilage which is regularly subjected to high levels of stress shows a higher content of PGs (Slowman & Brandt 1986), higher cell volume (Eggli et al. 1988), and is stiffer (Swann & Seedhom 1993) than a cartilage exposed to low stress levels. Both the organization pattern of collagen and content of PGs of the articular cartilage appear to be intimately related to local functional requirements. Areas which are regularly subjected to high levels of shear stress, i.e. patellar surface of femur and femoral condyles, show a higher degree of collagen orientation and a thicker superficial zone than an area which is preferentially subjected to weightbearing, e.g., the tibial plateaus rich in PGs (Arokoski et al. 1999). Thus, the mechanical forces work up and shape the biological properties of articular cartilage. Mechanical forces also seem to be more important than motion in supporting the properties of cartilage, since movement of the joint in the absence of normal loading causes and maintains atrophic changes in cartilage (Palmoski & Brandt 1981).

Injury and Repair

The aetiology of cartilage defects can be classified into traumatic, those due to chondromalacia patellae and osteochondritis dissecans. This thesis will investigate all 3 aetiologies but the majority of patients have injury induced defects.

Acute traumatic joint injury is known to increase the risk for subsequent development of osteoarthritis (Davis et al 1989), but the mechanisms responsible for this process are unclear. Post traumatic osteoarthritis may account for substantial morbidity in a relatively young and active population with many patients showing radiographic signs of osteoarthritis by 10 years after anterior cruciate ligament or meniscal injury (Roos et al. 1995).
Chondral lesions involving the knee are common. Aroen et al. (2004) demonstrated articular cartilage pathology in 66% of patients in a study of 993 consecutive arthroscopies. In a more substantial paper, articular cartilage lesions were discovered in 63% of 31000 arthroscopies (Curl et al. 1997).

Injuries to joints are common in humans, particularly during sporting activities. Knee trauma is common in athletes such as soccer players, reportedly accounting for 20% of all their injuries (Levy et al. 1996). The high incidence can be attributed to the high velocities and repetitive high impact twisting movements that occur when kicking a ball. This places unusual and high stresses on the articular cartilage (Levy et al. 1996).

Chondral lesions secondary to injury are thought to occur through two distinct mechanisms:

1) The largest group arise through abrasive wear, which results in superficial fibrillation. Often at this stage the lesion is asymptomatic until erosion progresses to the subchondral bone.

2) The second type arises due to disruption to the deep cartilage ultra-structure by shear forces.

Ateshian et al. (1994) demonstrated that shear stress is concentrated at the junction of the uncalcified and calcified cartilage, which may produce damage to the cartilage above the tidemark but also the subchondral bone. Levy et al confirmed this when they performed biopsies of the base of the osteochondral defects (OCD)s in soccer players at the delamination border revealing that the calcified cartilage remained with the subchondral bone.

Repair mechanisms

In response to injury, the body reacts in a uniform fashion. Although there are certain characteristics specific to individual tissues and organs, the general pattern requires two essential ingredients. The presence of specific cells is essential not only to clean up necrotic material, but also to synthesize new tissue. These cells are either derived from the replication of cells in situ, or from cells that have migrated from the wound margin or enter the area by blood vessels. The second requirement is a vascular supply. The vascular system supplies many of the cells mentioned and also is a source of many bioactive molecules (such as growth factors, chemotactic, mitogenic, and cytotactic factors, and others) that are needed to create the proper biochemical environment for healing. With these basic ingredients, the classic response to injury is usually described as consisting of three phases.

Classic healing

The phase of necrosis is first, and it begins at the time of injury. There is a variable amount of cellular death, depending on the amount of trauma and devascularisation that takes place. The

cell death that occurs subsequent to the original event increases the overall amount of tissue damage. Coincident with injury, blood escapes from damaged blood vessels, forming a haematoma, and subsequently a clot is produced. Platelets trapped within the clot release various growth factors and cytokines, inducing the migration of pluripotential stem cells into the area, their subsequent proliferation and differentiation, as well as stimulating a vascular invasion. The inflammatory phase is next, and depends almost wholly on the vascular system. Vasodilatation and increased vessel wall permeability give rise to transudation of fluid and proteins, as well as cellular exudation into the damaged area. This results in the formation of a dense fibrinous network, which ultimately contains inflammatory cells, as well as the pluripotential cells that will (in some locations) eventually differentiate into cells capable of replacing the damaged tissue. The remodelling phase is the last, and the longest, of the healing phases. It begins when the fibrinous network is invaded by vascular buds, creating a vascular granulation tissue. In some organs, this tissue matures and contracts, creating a scar. In others, the cells undergo a metaplasia into cells capable of replicating the function and structure of the original tissue.

Limitations of cartilage

The response of cartilage to injury differs from this classic response because of two important features of the structure of cartilage, of which the most important is its avascular status (Mankin 1982). The second and third phases of healing are almost entirely mediated by the vascular system, and thus all the inflammatory and reparative aspects that the vascular system provides are not available to cartilage. In addition, the migration of new phagocytic and pluripotential cells by means of the vascular system is denied to cartilage. Indeed, articular cartilage matrix may contain natural inhibitors of vascular and macrophage invasion, as

well as inhibitors of clot formation. The second difference is that the chondrocytes are literally imprisoned in a mesh of collagen and proteoglycan, unable to migrate to the injury site from adjacent healthy cartilage. Even if they were able to turn their synthetic engines on in an effort to replace damaged matrix, they cannot get to where they are needed. These conditions will be different if the cartilage injury penetrates through the subchondral plate, providing a pathway to the highly vascular bone. In this injury, because of the participation of the vascular system, the repair response is much more similar to that seen elsewhere in the body. Descriptions of the attempt of articular cartilage to heal itself after injury have typically followed two pathways, one detailing the events after a superficial injury to articular cartilage, and the other involving a deep, full-thickness injury through the subchondral bony plate (Buckwalter and Mankin 1997).

Partial thickness injury

The reaction to a partial-thickness injury is the same whether it is tangential or perpendicular to the surface (Ghadially et al. 1977). Immediately adjacent to the margins of the wound there is a zone of necrosis, with ghost cells seen in the chondrocyte lacunae. There is a brief period of mitotic activity and matrix synthesis among the chondrocytes near the injury, but this activity rapidly ceases, with no significant healing (DePalma et al. 1966, Fuller t al. 1972). Cellular proliferation results in small clusters of chondrocytes, seen only in injury and osteoarthritis (Mankin 1974). Studies have demonstrated no progression of healing over time, but importantly, they have also demonstrated that these lesions remain stable and tend to progress only rarely to osteoarthritis (Meachim et al. 1971, Thompson 1975).

Full thickness injury

When injury to a joint surface violates the subchondral plate of bone, the healing process is stimulated by tapping into the potential of the vascular system (Convery et al. 1991, Meachim et al. 1971, Mitchell et al. 1976). The defect is filled with a fibrin clot, trapping cells from blood and marrow. Shapiro et al. (1993) have shown that the source of the repair cells for the entire sequence is from the undifferentiated mesenchymal cells of the marrow. The inflammatory and reparative phases as previously described then produce a maturing, cellular mass. The deeper portions form bone, reconstituting the subchondral plate. The reparative tissue in the cartilage defect undergoes a metaplasia to a hyaline-like chondroid tissue (Shapiro et al. 1993). By 2 weeks, rounded chondrocytes appear and produce substantial amounts of Type II collagen. However, later in the process, there is still significant (20% to 35%) Type I collagen present (Furukawa et al.1980), the proteoglycan content decreases significantly, and the tangential collagen layers of the superficial zone fail to appear. Furukawa et al.(1980) speculate that this altered composition would almost certainly influence the mechanical properties. An important observation by Shapiro et al. (1993) consistent with other experimental studies (Brittberg et al. 1996, Desjardins et al. 1991, Wakitani et al. 1994) of cartilage healing, is that the collagen fibrils of the repair tissue were not well integrated with those of the residual cartilage, rather in some specimens they were separated by a gap. This might lead to vertical shear stresses between the repair and residual cartilage, precipitating micromotion and degenerative changes. Additionally, the chondrocyte lacunae immediately adjacent to the injury site remained empty. Shapiro et al. (1993) noted the appearance of some degenerative changes as early as 10 weeks, and these were more advanced by 24 weeks. Between 6 and 12 months, matrix and cells become more typical of fibrocartilage, and over longer periods of time, surface fibrillation and a cellular areas are present, with subsequent degeneration (Furukawa et al. 1980, Mankin 1982).

Clinical consequences of cartilage injury

The natural history of cartilage injuries is not well understood, but a knowledge of it may help to identify which patients are suitable for treatment. Chondral injuries noted at the time of anterior cruciate ligament reconstruction do not appear to affect clinical outcome at a mean of 8.7 years (Shelbourne et al 2003). Although these defects were small and, in a young population, it may be difficult to extrapolate these findings to patients presenting with symptomatic lesions. In a long-term follow-up of a small group of young patients noted to have chondral defects at arthroscopy, there was a high rate of radiological evidence of osteoarthritis (57%), although most patients had few symptoms (Messner et al. 1996).

Linden published a long-term follow up study on osteochondritis dissecans of the femoral condyles and evaluated 76 knee joints (58 patients) at a mean of 33 years after diagnosis (Linden 1977). Of the 23 patients who were children at the time of diagnosis, only two (9%) had mild osteoarthritis at follow-up. In contrast, osteoarthritis affected 81% of those with adult-onset osteochondritis dissecans, approximately ten years earlier than for primary osteoarthritis.

From this limited information, it is perhaps reasonable to suggest that only symptomatic, chondral defects should be treated as there is no evidence to suggest that patients with asymptomatic lesions will become symptomatic in the future. Osteochondral defects in adults may warrant more aggressive attention because of the high incidence of early-onset osteoarthritis.

Treatment of cartilage injuries

Debate still persists about the best treatment for symptomatic chondral defects. The focus of this thesis is Autologous Chondrocyte Implantation (ACI) but the next section will discuss the efficacy of the different surgical techniques that may be used to address these lesions.

Debridement

Cartilage in and around a symptomatic chondral defect is abnormal. Mechanical overloading results in increased matrix metalloproteinase production (Blain et al. 2001, Honda et al. 2000) which has a damaging effect on the opposing surfaces and surrounding cartilage. Simple excision of this damaged cartilage has been shown to improve symptoms for five years or more (Hubbard 1996). In this study, it was recommended that patients should be selected on the basis of a chondral defect combined with local tenderness. His aim at surgery was meticulous removal of all unstable cartilage and to debride the calcified layer sufficiently for new tissue to form in the base. In this prospective randomised trial, only isolated medial femoral condylar defects were selected and arthroscopic lavage was used as the control. The debridement group had significant improvement when compared with lavage as measured by the Lysholm score. Results gradually deteriorated over the five year period. Studies of debridement in osteoarthritis, as opposed to discrete chondral defects, reach conflicting conclusions (Moseley et al. 2002, Jackson et al. 2003). Opinion is divided as to whether arthroscopic debridement has any place in the treatment of established osteoarthritis although this debate does not apply to the treatment of localised, symptomatic chondral defects.

Microfracture

This procedure was introduced by Steadman et al (Steadman et al 1997, 2001, 2003, 2003, Miller et al 2004) over 20 years ago and is a technique in which accurate debridement of all unstable and damaged articular cartilage is performed, down to the subchondral bone plate while maintaining a stable perpendicular edge of healthy cartilage. An arthroscopic awl is used to make multiple holes in the defect 3 to 4 mm apart, and ensuring the subchondral plate is kept intact. After this microfracture, the defect is filled with so-called super clot, said to be the optimal environment for pluripotential marrow cells to differentiate into stable tissue (Steadman 2001). The rehabilitation protocol is an important part of the microfracture procedure. Early mobility of the joint with continuous passive motion is advocated in conjunction with reduced weight-bearing for an extended period. Microfracture is a modification of the Pridie drilling technique (Pridie 1959). Microfracture, drilling and debridement (abrasion) may all be considered as marrow stimulation techniques, where the chondral lesion is exposed to material moving from the bone cavity through the subchondral plate. This layer is unsealed by removing the lower, calcified layer of articular cartilage and by making holes which penetrate the subchondral plate. Advantages of microfracture over drilling might include reduced thermal damage to subchondral bone and the creation of a rougher surface to which repair tissue might adhere more easily. It is also easier to penetrate a defect perpendicularly with a curved awl during an arthroscopic procedure as compared with a drill. There are currently no published studies which compare microfracture with drilling. According to Hunziker, marrow-stimulation techniques have acceptable clinical results up to five years and decline thereafter (Hunziker 2002), although Steadman et al published outcomes of microfracture to show that at seven years 80% of patients rated themselves as improved (Steadman 2003). They also found that patients aged less than 35 years improved more than those aged between 35 and 45 years. The patients in this study were retrospectively

selected from a larger group and had relatively small chondral defects, with a mean size of 2.8 cm^2, although no histological results were presented. However, Knutsen et al described 20 biopsies after microfracture and noted that 11.4% had predominantly hyaline cartilage and 17.1% a mixture of fibrocartilage and hyaline cartilage within them (Knutsen et al. 2004). There is much interest in microfracture as a treatment of chondral injuries in professional sports players. In a series of 25 National Football League American football players, Steadman et al reported that 76% of players returned to their sport by the next season although this reduced to 36% at a mean follow-up of 4.5 years (Steadman et al. 2003).

Mosaicplasty

Mosaicplasty, or osteochondral cylinder transplantation, was first described in 1993 and is a technique that has been widely advocated (Matsusue et al. 1993, Bobic 1996, Hangody et al.1997, 2003, Morelli et al. 2002). In this procedure, osteochondral plugs are taken with a cylindrical cutting device and used to fill an articular cartilage defect. Plugs are usually taken from the peripheries of both femoral condyles at the level of the patellofemoral joint and introduced as a mosaic to fill the defect. Different sizes of plug can be used in order to maximise filling of the defect. The technique is usually undertaken as an open procedure, although it is possible to perform it arthroscopically. Advantages of this technique are that defects can be filled immediately with mature, hyaline articular cartilage and that both chondral and osteochondral defects can be treated in the same way (Hangody et al 2004). However, donor site morbidity is a concern and Hangody and Fules recommend that the area to be treated is limited to between 1 and 4 cm^2. There are also technical difficulties in restoring the surfaces of both cartilage and bone to produce a smooth, convex joint surface. The thickness of the donor cartilage may differ from that of the area to be treated and reconstitution of the important subchondral layer may not occur. In addition, lateral

integration rarely occurs raising the concern that synovial fluid may penetrate through the subchondral layer and cause cyst formation. Perpendicular access to the cartilage surface by cylinder cutters is required for this technique and this makes treatment of defects of the tibial plateau difficult. Bentley et al advised against using mosaicplasty (especially on the patella) (Bentley et al. 2003). The largest single series of mosaicplasty to date is that of Hangody and Fules (2003) who reported the results of operations on 597 femoral condyles, 76 tibial plateaux and 118 patellofemoral joints at up to ten years post-operatively. Good or excellent results were reported in 92%, 87% and 79% of patients who underwent mosaicplasty of the femoral condyle, tibial plateau and patellofemoral joint respectively. This paper does not give the mean time to follow-up and did not discuss the survival of osteochondral grafts in those patients with the longest follow-up. The long term durability of this technique has been questioned and a recent randomised controlled trial comparing mosaicplasty with ACI has found ACI to be significantly better at a minimum of 10 years follow-up (Bentley et al 2012).

Perichondrial grafts

This technique, which was described by Homminga et al, uses autologous strips of perichondrium fixed to the subchondral bone with fibrin glue (Homminga et al 1990). The long-term results for 88 patients with a mean follow-up of 52 months showed good results in only 38% using the Hospital for Special Surgery score (Bouwmeester et al. 1999). In a histological analysis of 22 biopsies taken after perichondrial grafting, tissue with a hyaline morphology of over 50% was found in only six biopsies (27%).

Carbon fibre

Fine spaces between the fibres of carbon-fibre rods direct the regeneration of tissues on to the surface of a joint. Carbon-fibre matrix is more commonly used in the patella but was reported by Meister et al. to give good results in only 41% (Meister et al. 1998). No systematic histological study has been reported but, in failed implants, poor quality fibrous tissue with carbon fibre fragmentation is seen over the pads. The main disadvantage of carbon rods is the introduction of a nonabsorbable material deep to the subchondral bone. In early osteoarthritis, Brittberg, Faxen and Peterson had 83% success in 37 patients who were studied prospectively (Brittberg et al. 1994). This may, therefore, be the best indication for the use of carbon fibre, where there are degenerative changes present and when knee replacement would be the next form of treatment.

Osteotomy

Osteotomy is usually reserved for early unicompartmental osteoarthritis. Three studies are of relevance when studying articular cartilage healing. Wakabayashi et al observed that cartilage healing principally occurred only when cartilage loss was down to bone (Wakabayashi et al. 2002). In this study, osteotomies were performed and the knees were arthroscopically reassessed a year later. A better Outerbridge score was found in those joints where there was full-thickness cartilage loss before osteotomy (Wakabayashi et al. 2002). This might appear strange but is consistent with the effect of debridement where bone can heal but cartilage does not. Schultz and Gobel looked at four groups of patients who underwent a Coventry-style tibial osteotomy (Schultz et al. 1999). All had a follow-up arthroscopy and biopsy which demonstrated thicker tissue and improved histology when Pridie drilling or abrasion arthroplasty was combined with the osteotomy. An improved walking distance and knee extension in these groups was also claimed. Wakitani et al cultured autologous bone marrow

stem cells to add to the tibial plateau after osteotomy in 12 patients and compared these with a control group of 12 patients who received an osteotomy alone (Wakitani et al. 2002). Cultured stem cells were suspended in a collagen gel and covered with a patch of periosteum. Better histology was obtained at a year but there was no significant clinical benefit in the short term compared with the control group. Evidence from Peterson et al in Gothenberg, using autologous chondrocyte cell implantation in isolated chondral defects, predicted good long-term results at seven years for those who had good results at two years after surgery (Peterson et al. 2002). Combined cell therapy with osteotomy may be a logical way to develop better long-term results in unicompartmental arthritis.

Periosteal grafts

Periosteum has the potential for both chondrogenesis and osteogenesis (Ritsila et al. 1994) and its use has been described in a number of publications (Alfredson et al. 1999, Angermann et al. 1998, Hoikka et al. 1990, Korkala 1988). Lorentzon et al reported promising results in treating patellar lesions in a study of 26 patients with a mean follow-up of 42 months (Lorentzon et al. 1998). They showed that 17 patients had excellent and eight had good results. Only one patient had a poor outcome. Interestingly, they combined grafting with drilling of the defect bed and thus allowed marrow elements to contribute to the repair. To date, these clinical results are comparable with other techniques for patellofemoral lesions. Alfredson and Lorentzon (1999) reported on the post-operative benefits of continuous passive motion in a study of 57 patients with patellar defects who were treated with periosteal grafts. Of the 38 patients who used continuous passive motion post-operatively, 76% had an excellent or good result at a mean follow-up of 51 months. Of the 19 patients who did not have continuous passive motion post-operatively, 53% had an excellent or good result at a mean follow-up of 21 months. One study from Finland reported good clinical results with periosteum after four years (Hoikka et al. 1990). However, by 12 years

after treatment all the patients had a poor clinical result. Calcification of the grafts has been mentioned as a problem in the long term. In the Chinese literature, periosteal grafting in association with a silicon membrane, which was removed at six months, was described as a treatment for large cartilaginous defects in 37 patients (Yang et al. 2004). The follow-up was a mean of 10.5 years (7 to 15). In this heterogeneous group of patients with significant pathology, the clinical results at follow-up were described as excellent in 11 patients, good in 18 and poor in eight.

Autologous chondrocyte implantation

The technique of autologous chondrocyte implantation (ACI) was first performed by Peterson et al. in Gothenburg in 1987 and was the first application of cell engineering in orthopaedic surgery. Brittberg et al presented the results of 23 patients with a mean follow-up of 39 months. Good or excellent clinical results were reported in 70% of cases (88% of femoral condylar defects). Of the biopsies from treated femoral condylar lesions, 11 of 15 had a hyaline-like appearance (Brittberg et al. 1994). A more recent publication from the same group showed durable results up to 11 years following the treatment of osteochondral lesions (Peterson et al 2002). Encouraging results of ACI have also been reported by other authors (Kon et al 2009, Bartlett et al 2005, Saris et al 2009). In studies where histological analysis has been performed, it is apparent that ACI is capable of producing tissue which is hyaline-like in some specimens (Roberts et al 2003). However, the best repair tissue is not morphologically or histochemically identical to normal hyaline cartilage, and fibrocartilage may be found in a proportion of samples. A variation of the ACI technique using culture-expanded bone marrow stem cells has the advantage of not requiring an additional arthroscopic procedure in order to harvest articular cartilage. This technique has been used in conjunction with high tibial osteotomy (Wakitani et al. 2002).

Determinants of outcome after ACI

Krishnan et al investigated the prognostic indicators in a 199 patients who had underwent collagen covered ACI in a 4 year period (Krishnan et al 2005). They discovered that older patients (above 40) had significantly worse outcome than those younger than 40. The improvement in Modified Cincinnati Score (MCS) was greater for those patients with a higher pre-operative score as opposed to those with a low score. Other poor prognostic indicators include knee symptoms for greater than 2 years, patients with 3 or more previous

operations, or multiple defects. The site of the defect also influenced results, with lesions in the patella and the medial femoral condyle doing significantly worse than lesions in the lateral femoral condyles. Interestingly, the size of lesion was not shown to have an effect on the MCS.

In a study of 55 patients, De Windt et al determined the prognostic value of patient age, defect size and age, and location on the clinical outcome 3 years after treatment of OCDs treated with either ACI or microfracture (De Windt et al 2009). Medial femoral condyle lesions had better clinical scores when compared with defects on the lateral femoral condyle. Logistic regression analysis determined that clinical outcome was better in patients younger than 30 years old or defects less than 24 months old. This study also discovered that defect size did not predict outcome, a finding mirrored by other studies (Krishnan et al 2005, Niemeyer et al 2008, Zaslav et al 2009). However, in another study of 17 patients, the clinical results were significantly better in lesions greater than $3cm^2$ (Selmi et al 2008).

The above findings are not exclusively related to the technique of ACI. Mithoefer has shown that patients in a younger cohort, with defects less than $2cm^2$ and shorter duration of symptoms (less than 12 months) were associated with higher clinical scores and return to sports (Mithoefer et al 2006). Age of the patient was also found to influence outcome after microfracture (Knutsen et al 2007).

Goals of this thesis

The general consensus in the literature is that certain patient and defect characteristics have an influence on outcome after ACI in the treatment of OCD in the knee;

- Older patients are said to have worse outcome.
- Patients who have undergone multiple operations prior to ACI also do not experience good results.
- Lesions in the patella tend to have worse results following surgery than lesions in the femoral condyles.

The results when investigating other factors have yet to be elucidated;

- Whether medial or lateral femoral condyles lesions do better is a contentious issue.
- The size of the defect does have an effect in some studies but not in others
- Multiple lesions in the same knee compared with single defects have mixed results
- The significance of a longer duration of symptoms prior to definitive surgery in the form of ACI/MACI is unclear as is the question 'when is it too long to wait for ACI/MACI'

This thesis will add to the body of evidence already present regarding the influence of patient demographics and defect characteristics on outcome following ACI. I also aim to definitively answer the above unanswered questions. Perhaps more importantly, the studies in this thesis will investigate factors that can be manipulated before or after surgery (e.g. body mass index (BMI), the smoking status and physical activity) to optimise results following surgery and increase the health status of the patients. This aspect of studying prognostic indicators has not

been previously reported. An attempt will also be made to ascertain why failures occur in certain cohort of patients.

When assessing prognostic factors in any non-randomised study, there is inevitably interplay of these factors affecting the measured outcome. If these factors are not taken into account then conclusions made on the basis of univariate analysis are invalid. This thesis will also assess the methodology and data analysis of studies on autologous chondrocytes implantation and with the use of a statistical model devise an approach to correctly identify prognostic factors affecting outcome.

Chapter 2: Methods

Introduction

In this chapter, the analyses performed on the different cohort of patients will be outlined. The properties of the Modified Cincinnati Score (MCS) will also be discussed and why it was used as a tool to assess efficacy of surgery. Finally, details of the statistical approach will be discussed, the key points in performing a randomised controlled trial, and the use of multiple linear regression and the generalised linear model when a RCT cannot be performed.

Outline of Patients

This thesis is comprised of a number of different types of studies. The patients in each study will be outlined below. There is a certain amount of overlap of patients in each of the chapters. This was deemed necessary in order to increase the number of patients for data analysis to answer the relevant question in each chapter in an attempt to reduce the probability of a type I statistical error.

Chapter 3

The first study is a randomised controlled trial (Chapter 3). 247 consecutive patients that were enrolled into the trial formed the basis of the data analysis. The operations were performed between July 2002 and July 2005. Prior to the start of this thesis, a multicentre randomised controlled trial (RCT) was in effect. The null hypothesis stated that there was no difference in outcome following autologous chondrocyte implantation using a synthetic type

I/III collagen porcine membrane (ACI-C) and ACI using a collagen bilayer with chondrocytes implanted in the middle; matrix-carried autologous chondrocyte implantation (MACI). The RCT involved over 55 surgeons in 36 centres across the entire of England and Wales. It became apparent that a large number of surgeons were not filling the data capture forms adequately and there were large amounts of missing data. Therefore, these patients were excluded from the analysis. Chapter 3 includes all patients that were operated between July 2002 and July 2005 in the Joint Reconstruction Unit at the Royal National Orthopaedic hospital, Stanmore. The first 91 patients' 1 year results were published in 2005 (Bartlett et al. 2005) and from this cohort 56 patients were included in this chapter.

Chapter 4

Chapter 4 analysed the results of both ACI and MACI in the treatment of osteochondral lesions in the patella-femoral joint. The results of all patients (215) treated from November 2001 to January 2006 were included in this study. However, 35 patients had concomitant procedures such as patellar realignment and so they were excluded from the data analysis (as we could not conclude whether any improvement in function was the result of chondrocyte implantation or the realignment procedure. In this cohort, there were a large number of patients that were not randomised; hence this was a prospective cohort study. Of the 180 patients that were included in this chapter, 80 patients were also analysed in chapter 3.

Chapter 5

This case-control study reviewed the radiographs of 200 patients and correlated findings with the Modified Cincinnati Score (MCS). All the procedures were performed between July 1998 and July 2008. Thirty four patients from this chapter were also part of the cohort from chapter 3.

Chapter 6

A telephone and postal questionnaire was sent to 150 patients who had their operation performed between January 2001 and 2006 to retrieve missing data regarding smoking status, weight, height and physical activity. One hundred and twenty two patients responded and of these 48 were part of the cohort of patients in chapter 3.

The Modified Cincinnati Score

The Cincinnati Knee Rating System is one of the more commonly used specific instruments for assessing knee injuries. It was developed in 1983 (Noyes & Matthews et al. 1983, Noyes & Mooar et al. 1983) and consists of multiple parts; symptoms, sports activity and activities of daily living, along with clinical parameters. This system has been modified over the years (by utilising the subjective components) to the Modified Cincinnati Score (MCS). The MCS only requires patient input to provide a score ranging from 6 to 100 (best function) as well as a functional subscores on pain, swelling, giving way, walking, stairs, running and overall activity levels (see table 2.1). Following surgery, if scores fall within certain ranges, the result could be deemed:

Excellent (MCS > 80)

Good (MCS = 55-80)

Fair (MCS = 30-54)

Poor (MCS < 30)

Though the origin of this modification is unclear, the MCS in recent times is the version most often used in presentations and in the literature (Agel et al 2009, Greco et al 2010).

Table 2.1: The Modified Cincinnati Score

CATEGORY	SCORE
PAIN	
No pain, normal knee, performs 100%	**20**
Occasional pain with strenuous sports or heavy work, knee not entirely normal, some limitations but minor and tolerable	**16**
Occasional pain with light recreational sports or moderate work activities, running, heavy labour, strenuous sports	**12**
Pain usually brought on by sports, light recreational activities or moderate work. Occasionally occurs with walking, standing or light work	**8**
Pain is a significant problem with simple activity such as walking, relieved by rest, unable to do sports	**4**
Pain present all the time. Not relieved by rest	**0**
SWELLING	
No swelling	**10**
Occasional swelling with strenuous sports or heavy work. Some limitations but minor and tolerable	**8**
Occasional swelling with light recreational sports or moderate work activities. Frequently brought on by vigorous activities, running, heavy labour and strenuous sport	**6**
Swelling limits sports and moderate work. Occurs infrequently with simple walking activities or light work (approx 3 times a year)	**4**
Swelling brought on by simple walking activities and light work. Relieved by rest	**2**
Severe problem all the time, with simple walking activities	**0**
GIVING WAY	
No giving way	**20**
Occasional giving way with strenuous sports or heavy work. Can participate in all sports but some guarding or limitations present	**16**
Occasional giving way with light sports or moderate work. Able to compensate but limits vigorous activities, sports, or heavy work not able to cut or twist suddenly	**12**
Giving way limits sports and moderate work, occurs infrequently with walking or light work	**8**
Giving way with simple walking activities and light work. Occurs once per month, requires guarding	**4**
Severe problem with simple walking activities, cannot turn or twist while walking without giving way	**0**
OVERALL ACTIVITY LEVEL	
No limitation, normal knee, able to do everything including strenuous sports or heavy labour	**20**
Perform sports including vigorous activities but at a lower performance level: involves guarding or some	**16**

limits to heavy labour	
Light recreational activities possible with rare symptoms, more strenuous activities cause problems. Active but in different sports; limited to moderate work	**12**
No sports or recreational activities possible. Walking with rare symptoms; limited to light work	**8**
Walking, ADL cause moderate symptoms, frequent limitations	**4**
Walking, ADL cause severe problems, persistent symptoms	**0**
WALKING	
Walking unlimited	**10**
Slight/mild problem	**8**
Moderate problem: smooth surface possible up to approx 800m	**6**
Severe problem, only 2-3 blocks possible	**4**
Severe problem; requires stick or crutches	**2**
STAIRS	
Normal, unlimited	**10**
Slight/mild problem	**8**
Moderate problems only 10-15 steps possible	**6**
Severe problem; requires banister support	**4**
Severe problem; only 1-5 steps possible	**2**
RUNNING ACTIVITY	
Normal, unlimited; fully competitive, strenuous	**5**
Slight mild problem; run half speed	**4**
Moderate problem 2-4 km	**3**
Severe problem only 1-2 blocks possible	**2**
Severe problem only a few steps	**1**
JUMPING OR TWISTING ACTIVITY	
Normal, unlimited, fully competitive, strenuous	**5**
Slight to mild problem; some guarding but sport possible	**4**
Moderate problem; gave up strenuous sports, recreational sports possible	**3**
Severe problem; affects all sports; must constantly guard	**2**
Severe problem; only light activity possible (golf, swimming)	**1**

The subjective components of the Cincinnati Knee Rating System and the MCS have been shown to be reliable, valid and responsive (Barber-Westin et al. 1999, Marx et al. 2001, Agel et al. 2009, Greco et al. 2010). As a measure of responsiveness, the standardized response means (SRMs) are often calculated by dividing the change in score by the standard deviation of the change in score. Pearson product correlations can then be calculated to compare different questionnaires as a measure of construct validity. Agel et al calculated SRM of the MCS to be 0.9 and this compared favourable to the longer version of the Cincinnati System of 0.8 (Kirkley et al. 1998). The responsiveness was much lower for the generic instrument Short Form-12 Questionnaire, where the SRM was 0.37 (Hurst et al. 1998).

The original Cincinnati Knee Rating System was developed to be observer-administered and has a format that requires significant skills to be completed by patients. The MCS however, is self administered and patient-related aspects are taken into account. This is important as it has been shown that observer-based assessments score significantly better than patients' self-administered questionnaires due to observer bias (Hoher et al. 1997). Furthermore, patient-relevant outcome measures are now promoted in general health care, orthopaedics, and sports medicine, and they should be considered the primary outcome measure in clinical trials (Altman et al. 1996, Clancy et al 1998, Roos 2000).

The International Cartilage Repair Society (ICRS) have recommended the use of the Modified Cincinnati Score as the main tool for assessment of knee function in patients involved in cartilage repair clinical research. The committee members of this society include prominent Orthopaedic surgeons and basic scientists who are leaders in cartilage repair research and include the likes of Lars Peterson and Matt Brittberg. More recently, the ICRS has recommended the use of the SF-36 questionnaire, and patients being recruited into the

RCT from 2009 have to complete this questionnaire pre-operatively and annually after their surgery (personal communication Prof G Bentley).

Statistical Approach

A well designed randomised controlled trial (RCT) remains the gold standard for hypothesis testing, as systematic bias due to unknown confounding variables is avoided. However, RCTs are difficult and expensive to set up and run and that is why there are comparatively few in the orthopaedic literature. The enrolment of patients and the allocation process in RCTs can be modified if certain prognostic variables are known before the initiation of the study, such that there are equal numbers of patients with the known factor in each treatment group. This is termed blocking or stratification but cannot be performed when the study has a small number of patients (Altman and Bland 1999). The most effective way to ensure there are equal number of patients in subgroups when randomising in small trials is minimisation which was first described in 1975 and has now become the most accepted way of allocation (Pocock and Simon 1975, Altman and Bland 2005). In the minimisation process, after randomisation of the first few patients each subsequent allocation will be determined by which of the treatment arms would provide a better equilibrium of the explanatory variables in question. This form of randomisation is best performed with the aid of computer software due to its complexity (Altman and Bland 2005) but is certainly useful in making the researcher think about confounding variables from the outset and ensuring adherence to the study.

There are several features that are pertinent to the design and conduct of a good randomised trial. From the very beginning, it is important to have a scientific question in mind, i.e. a

hypothesis-driven study. This will allow a clear focus on how to go about answering the question and a study protocol to be developed.

Choosing the right patient population is the next stage in the planning of a RCT. If this not planned properly, the generalisability of the study may be undermined and worse still the results may be invalid if there is sampling bias. Hence it is important to have clear inclusion and exclusion criteria so that the sample population is appropriate for the research question in mind. In chapter 2 the allocation was simply random without any stratification or minimisation all patients fulfilling the inclusion and exclusion criteria. Once the target population has been decided, the next question that needs to be answered is how many patients need to be recruited into the study. Sample size calculations are important to ensure that the chances of type II statistical errors are minimised. It also important not to recruit too many patients in a RCT as this will lead to waste of valuable resources.

Appropriate outcome measures need to be selected to assess the efficacy of the treatment. Following surgery, these are usually in the form of questionnaires and functional scores. A variety exist in the orthopaedic literature, and it is important to choose one that is appropriate to the research question (i.e. is reliable and valid with no ceiling or floor effects).

Randomisation and concealment is the cornerstone of a RCT. The randomisation procedure can be performed in a variety of ways, but probably the most common is using a computer random number generator (Petrie 2006). Once patients have been truly randomised, it is important for the treatment allocation to be concealed from the investigator in order to prevent bias. A widely accepted way of randomising is to produce computer generated set of random allocation in advance of the study which are then sealed in consecutively numbered opaque envelops (Kendall 2003), which was performed in this RCT. The chief investigator in

the study was blinded to the treatment allocation. When functional scores were collected, the research fellow was also blinded to the treatment received.

A critical aspect of clinical research is quality control. This is most often overlooked during data collection, which then leads to errors because of missing or inaccurate data. This has been an issue in all of the studies in this thesis. Ideally, quality control should begin in the design phase of the study when the protocol is drafted and re-evaluated in a pilot study. The appraisal of the pilot study will allow a thorough assessment of all the key features of the RCT; the sampling strategy, method and tools for data collection and subsequent data handling. Any adjustments to the methodology can then be finalised. An operations manual and also formalised training is very useful when there are several investigators (especially in a multi-centre study). It would also be very useful for the chief investigator to have regular meetings with other investigators to ensure quality data management and appropriate delegation of responsibility and supervision of tasks. Other investigators should be motivated to collect all the relevant data, as time goes by this occurs less and less. This can be avoided by using research nurses to perform and manage these tasks and by regular team meetings.

In conclusion, a well-designed RCT when assessing two different forms of treatment probably represents the most powerful way of assessing new innovations and certainly has the capacity to change surgeons' practice. However, poorly designed and conducted RCTs can be dangerous because of their potential to influence practice based on inaccurate data. Early involvement of a Clinical Trials Unit is advisable to ensure sound methodology when writing the study protocol and their continued involvement will ensure quality control

throughout the whole process. A pilot study if recommended and if successfully implemented will confirm whether the research goals are practical and achievable. Finally, the dissemination of findings is critical and the publication in a peer review journal should follow the CONSORT guidelines.

When assessing prognostic factors in any other type of study, there is inevitably interplay of these factors affecting the measured outcome. If these factors are not taken into account then conclusions made on the basis of univariate analysis may be invalid. If we want to push forward the boundaries of orthopaedic research in non-randomised trials, the data analysis has to be performed efficiently and appropriately so as to utilise the maximum amount of the data obtained from clinical scores (for example) and obtain an answer to the research question, even if the study is retrospective and provides a lower level of evidence. Some studies in the literature on ACI have failed to demonstrate any benefit over other techniques and few have demonstrated a lasting benefit so far. Theoretically, a number of factors can contribute to failure and a scientific approach to elucidate these variables has not been reported. After critically appraising the literature, it has become apparent that due to lack of appropriate statistical methods, the validity of conclusions in many studies is questionable.

The Generalised Linear Model

Multiple linear regression and multiple logistic regression are methods of statistical analysis that account for and quantify the relative contribution of each confounding variable on outcome. Multiple linear regression is used when the dependent variable is continuous (e.g. a scoring system) and multiple logistic regression is used when the outcome is binary (e.g. success or failure) (Petrie 2006). Both methods of analysis can be used to identify explanatory variables associated with the dependent variable and hence promote an understanding of the underlying process and both are types of Generalised Linear Model. Generalised linear models are a general class of mathematical models which extend the usual regression framework to cater for responses which do not have Normal distributions. In a Generalised linear model the response or dependent variable (y) is assumed to be a member of the exponential family (be this either normal, binomial, Poisson etc) and some function (the link function) of the mean response is predicted by a linear response equation of the predictors. The link function is incorporated to define the transformation required for fitting a linear model for non-normal data. Multiple linear regression is a particular type of Generalised Linear Model (GLM) where it is assumed that the response is normal, and that the mean response can be predicted by a linear function with the identity link function (no transformation function needed). Therefore, the GLM is a step forward from multiple regression as it allows the analysis of data in a mathematical model when the distribution of the data is not normal via the link function and transformation of x or y (Petrie and Sabin 2006).

With the formulation of a complex equation, the model allows us to predict the dependent variable taking into account all the explanatory variables. As with any mathematical model, certain assumptions have to be satisfied in order for the analysis to be accurate which usually

involves the residuals. The residual is the difference between observed dependent variable (y) and the corresponding fitted dependent variable or predicted variable (Y) for each explanatory variable (x). The assumptions underlying the multiple linear regression model are (with the first two being the most important)

A linear relation between x and y exists

The observations are independent

The residuals should be normally distributed and the mean should be zero

The residuals should have a constant variance

The x variable can be measured without error (Ananth and Kleinbaum 1997)

GLM can be used when the factors being assessed are not linear and this is a key advantage. In multiple regression, it is assumed that age and health status has a linear relationship but that is not the case. For example, the difference in health status in an adult aged 30 and an adult aged 40 is likely to be less than the difference in adults aged 60 and 70.

A more detailed description of the statistical tests will be given in the methods section of each chapter. However, broadly speaking, the dataset contains the MCS before surgery and at 6, 12 and 24 months after surgery. In chapter 3, there is also MCS latest which is the latest available MCS. The paired t-test was used to confirm whether there was statistically significant improvement in knee function following surgery. This was done to compare results from this thesis with other published data. The independent t-test was used to determine if there was a statistically significant difference between ACI and MACI in improving knee function. Where there were more than 2 groups being analysed,

analysis of variance (ANOVA) was used. Kaplan-Meier survival analysis was performed for time to event variables, for example the time to the joint replacement or osteotomy surgery. Finally the chi-squared test was used to compare categorical data. To assess prognostic factors, the generalised linear model was used and these will be discussed in more detail in each chapter.

Chapter 3:
Autologous Chondrocyte Implantation using Type I/III Collagen Membrane (ACI-C) vs Matrix-Carried Autologous Chondrocyte Implantation (MACI)

Introduction

As discussed earlier, a variety of surgical treatment options exist for the treatment of osteochondral defects (OCDs) in the knee. A number of techniques endeavour to replace the defect with hyaline cartilage (such as osteochondral allografts and autologous osteochondral grafts or mosaicplasty). To date, none have been proven to provide a long term and reproducible improvement from pre-operative function and concerns such as disease transmission and donor site morbidity remain.

The origins of ACI can be tracked back to 1965, following the isolation of adult articular chondrocytes in suspension (Smith 1965). Three years later Laurence and Smith demonstrated that isolated epiphyseal articular chondrocytes survived when implanted into fractures in rabbits (Laurence and Smith 1968). Further advancement in chondrocyte implantation occurred when Bentley and Greer repaired chondral defects in an animal model using autologous epiphyseal and articular chondrocytes (Bentley et al 1971). Chondrocytes were taken from immature rabbits as it was previously shown that chondrocytes from immature rabbits had the potential to divide but chondrocytes from mature rabbits did not (Mankin 1962).

The next logical progression in the development of ACI was to be able to expand the number of chondrocytes without losing their ability to produce Type II Collagen. Green described a technique of culturing chondrocytes after they had been isolated from the extracellular matrix by enzymatic digestion (Green 1977). However, during the expansion, the cells gradually dedifferentiated and lost their ability to manufacture type II collagen. Chondrocytes regained this ability once they were exposed to agarose gels (Benya and Shaffer 1982). Aston and Bentley showed that by growing the chondrocytes at high density the phenotype was preserved and the cells produced type II collagen (Ashton & Bentley,1986). The ability to expand the numbers of adult chondrocytes in a monolayer culture proved a significant advance in the treatment of osteochondral defects using autologous cells. Peterson et al. (1984) reported on the repair of defects in skeletally mature rabbits with autologous or homologous cultured chondrocytes. Cartilage plugs were removed from the mid patellae and enzymatically digested and grown in a monolayer culture for 2 weeks. Following this, the pellets of chondrocytes were implanted into artificially created defects and covered with either a layer of fascia, synovium, blood clot, tendon, or periosteum. Results were encouraging with a total reconstitution of full thickness 3mm defects with hyaline cartilage. Using autoradiography, Grande et al proved that autologous chondrocytes grown in vitro were responsible for the repair tissue in artificially created defects in rabbits (Grande et al 1989).

Brittberg's original technique for ACI involved covering the chondral defect with periosteum harvested from the proximal tibia during the 2nd stage procedure (see operative technique below). Once the defect is covered and the periosteum sutured, the chondrocytes are injected, and the repair process is completed by sealing the periosteum with fibrin glue to ensure there is a watertight seal (Brittberg et al. 1994). Minas and Nehrer (1997) speculated that growth

factors secreted by the periosteum stimulate cultured chondrocytes to divide. Another possible mechanism speculated was that the periosteum and cultured chondrocytes stimulate adjacent cartilage, in the subchondral bone or in the periosteum itself to enter the defect and repair it (O'Driscoll & Fitzsimmons 2001). The latter can probably be disregarded since the results of treatment of chondral defects in both animal models and human subjects have been poor with the use of a periosteal patch alone (Brittberg et al. 1996 and Angermann et al. 1998). Evidence for the dual action of periosteum with cultured chondrocytes was confirmed when dead periosteum was sutured to the rim of the chondral defect in rabbits and chondrocyte suspension was injected into this sealed defect (Lindahl et al 2002). Only 1 out of 8 rabbits developed good repair tissue, thus suggesting that periosteum and chondrocytes work together to repair the defect.

Several concerns were raised with the use of the periosteal flap technique (ACI-P). This so-called first generation technique required a second surgical procedure at the proximal tibia causing additional pain and risks to the patient. In addition, the periosteal flap had to be fixed by sutures 3mm apart thereby damaging healthy cartilage. Further disadvantages are low mechanical stability as well as the unequal distribution of the cultured chondrocytes (especially due to gravity). Furthermore, hypertrophy, delamination, and even graft failure may occur.(Gooding et al 2006)

Second generation techniques involved using a synthetic type I/III collagen porcine membrane (ACI-C). This avoided the removal of the periosteum from the tibia. Though the synthetic membrane still requires suturing to adjacent cartilage, it has been shown that ACI-C has significantly lower rates of graft hypertrophy requiring secondary surgery than ACI-P (Gooding et al 2006 and Wood et al. 2006). A prospective study of 63 patients treated with ACI-C found significant improvements in ICRS and Modified Cincinnati Scores that were

maintained at 3 years with no incidence of graft hypertrophy (Steinwachs et al 2007). The favourable results with ACI-C suggest that the implanted chondrocytes repopulate the defect and synthesize a new cartilage matrix. The periosteal patch acts simply as seal, ensuring the cells stay within the defect.

Concerns regarding unequal distribution of cells, suturing of membrane to adjacent healthy cartilage and also the possibility of cell leakage beyond the periosteal flap or collagen membrane led to the development of Matrix-carried Autologous Chondrocyte Implantation (MACI; Verigen, Leverkusen, Germany). MACI has a similar porcine derived type I/III collagen membrane. One surface has a roughened appearance because of widely spaced collagen fibres, between which chondrocytes are seeded. The other side has a smooth surface due to the high density of collagen fibres (Zheng et al 2003). The MACI membrane can be secured to the defect directly with fibrin glue and therefore does not require additional cover. There is no need for additional sutures or harvesting of periosteum. The procedure can be performed faster and with less extensive exposure (Bentley, BOA 2007).

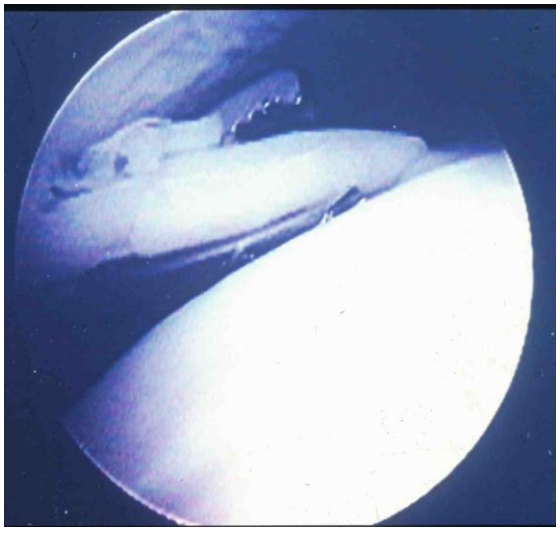
A concern regarding the MACI technique is the number of chondrocytes present in the collagen bilayer. It is estimated that there are 1 million cultured chondrocytes per mm^2. In the ACI-C technique there are approximately 5 million cells per ml of suspension. Le Baron and Atahnasiou performed a study seeding a manufactured scaffold with chondrocytes. They reported that scaffolds with less than 10 million cells/ml resulted in poor cartilage formation (Le Baron et al 2000). Puelacher et al. noted that scaffolds seeded with 20-100 million cells/ml resulted in cartilage formation when implanted subcutaneously into nude mice (Puelacher et al. 1994). However, normal articular cartilage contains only 10,000 cells/$mm^{2.}$ Possible reasons for the need for such high concentrations of implanted chondrocytes may be because a proportion of implanted cells undergo apoptosis once they are implanted into the

defect or perhaps it is the physical crowding of cells that is needed for them to redifferentiate into type II secreting chondrocytes. In their recent review, the Swedish group have advocated using 30 X 10^6 cells/ml in the clinical setting. Despite these reservations several studies have shown that MACI is as efficacious as the 1^{st} and 2^{nd} generation techniques (Bartlett et al. 2005, Cherubino et al. 2003, Brittberg 2003, Zeifang et al. 2010).

Operative Technique

Both ACI-C and MACI are two-stage procedures and have been previously described (Bentley et al. 2003). In the first stage, an arthroscopy is performed to assess the site, size and containment of the chondral lesion, together with the competence of the menisci and cruciate ligaments. If suitable, approximately 300mg of cartilage is harvested from the non-weight bearing areas of the medial or lateral trochlea together with 100mls of venous blood (figure 3.1). The chondrocytes are sent for culture in the patient's serum and several passages of the cells are expected to increase the number of chondrocytes by 20 to 30 fold. After 3 to 5 weeks, the patient is re-admitted for the second (implantation) stage. An open arthrotomy is performed and the lesion is exposed. The lesion is debrided to a healthy and stable edge and care is taken to avoid subchondral bone bleeding (this is supplemented with the use of topical adrenaline).

Figure 3.1: Harvesting of cartilage graft

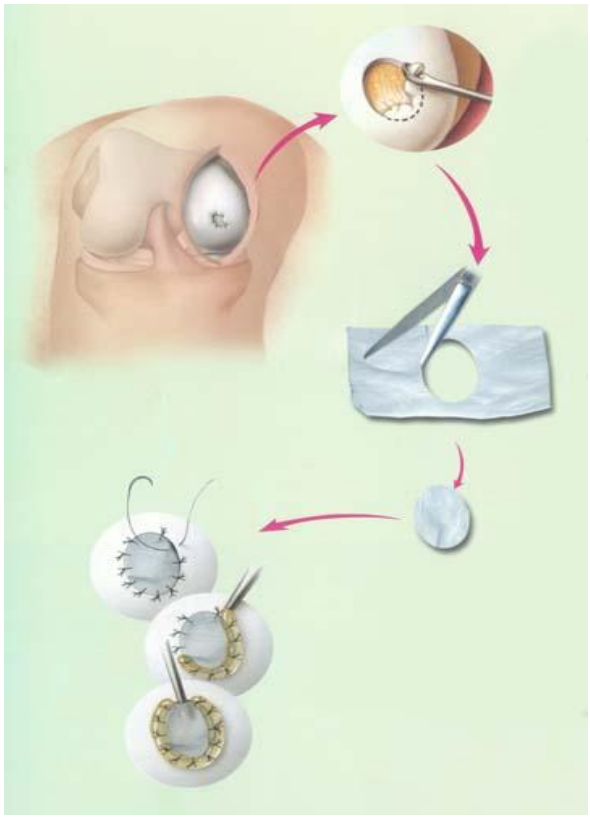


Arthroscopic photo courtesy of Prof Bentley

ACI-C

The collagen membrane is cut to the size and shape of the defect and sutured to the rim of the defect with 6/0 Vicryl sutures approximately 3mm apart (see figures below). Fibrin glue is used to ensure a water-tight seal. A fine catheter is use to inject normal saline to confirm the suture line is water-tight. The suspension of the cultured chondrocytes is then injected to fill the defect. The catheter is removed and the final suture is placed.

Figure 3.2: Overview of ACI-C (Second Stage)



Courtesy of Verigen Corporation

Figure 3.3: Debrided cartilage defect seen intra-operatively

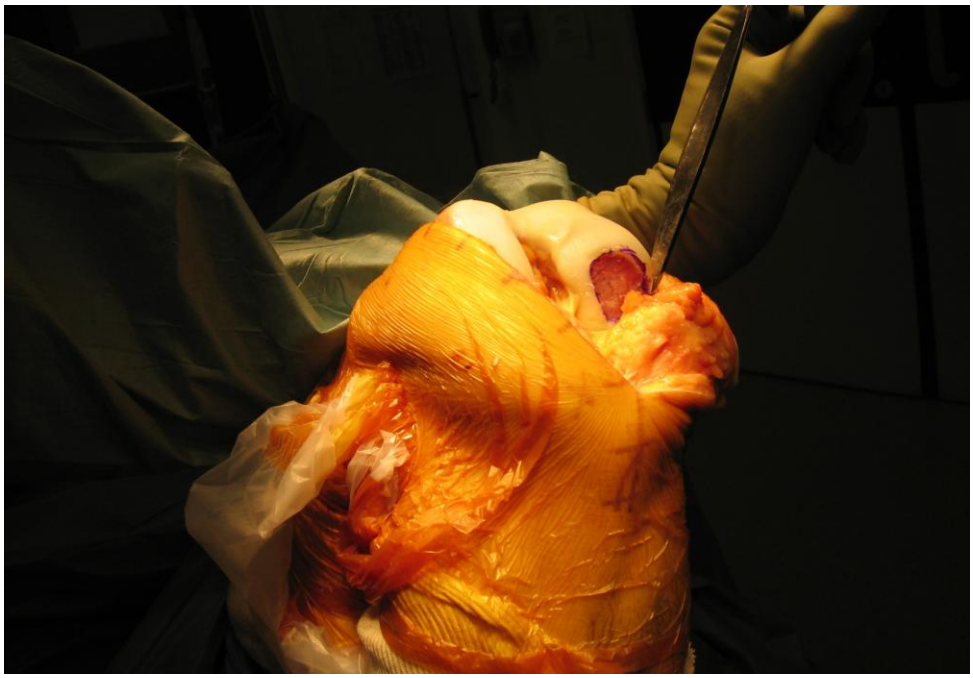
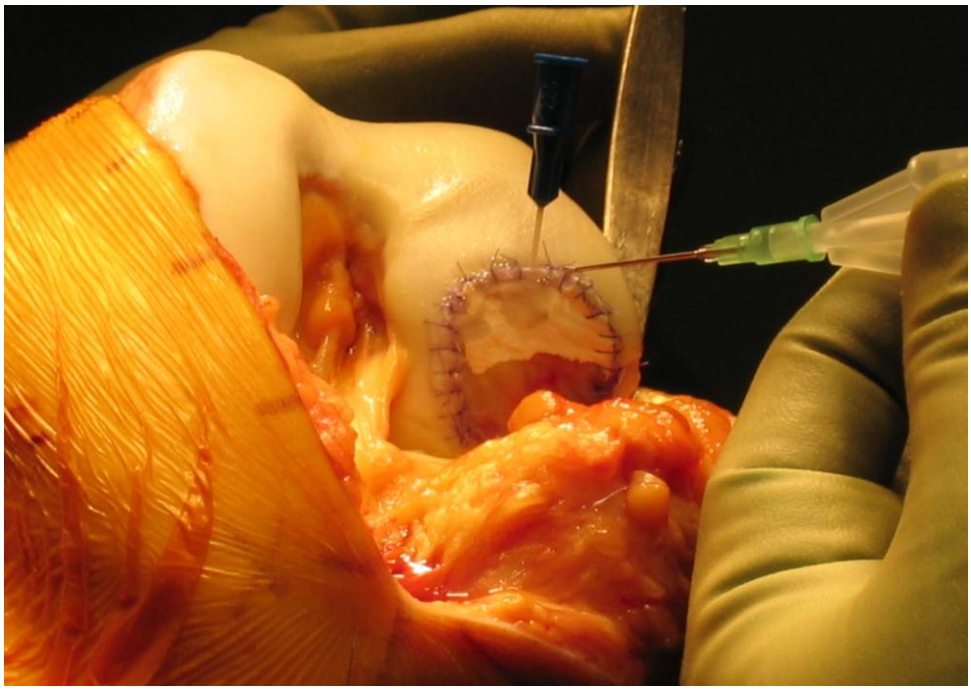


Figure 3.4 The collagen type I/III membrane is sutured to the defect and the cultured chondrocytes are injected

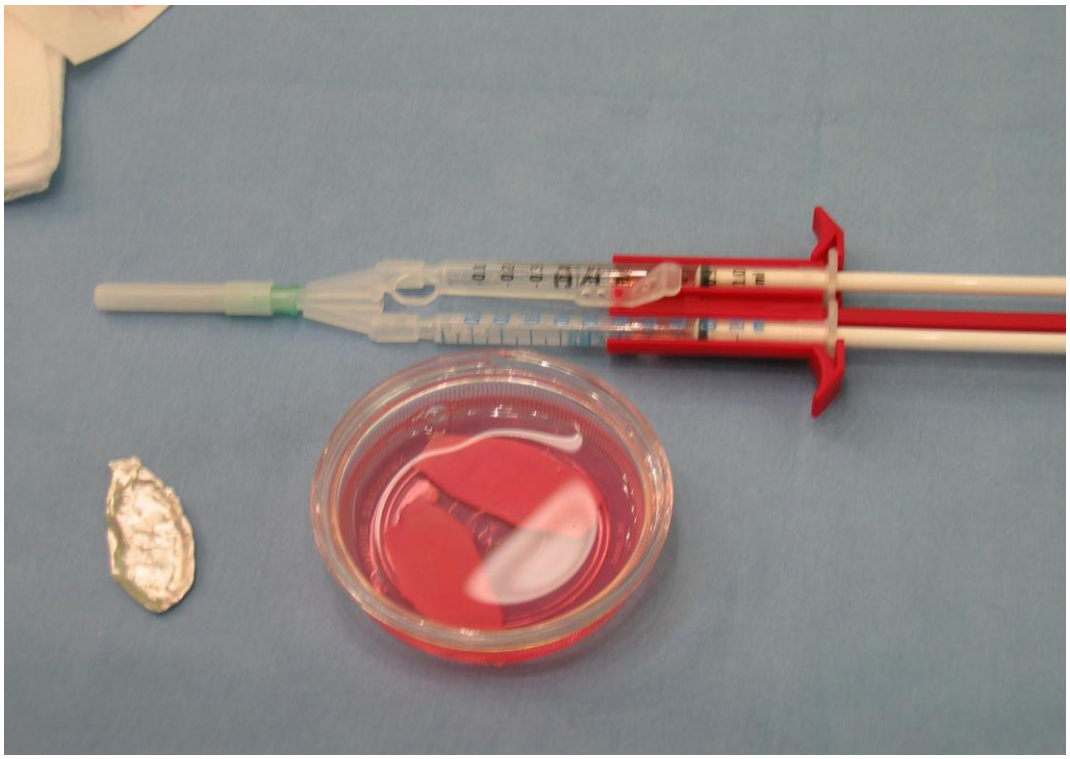


Figures Courtesy of G. Bentley

MACI

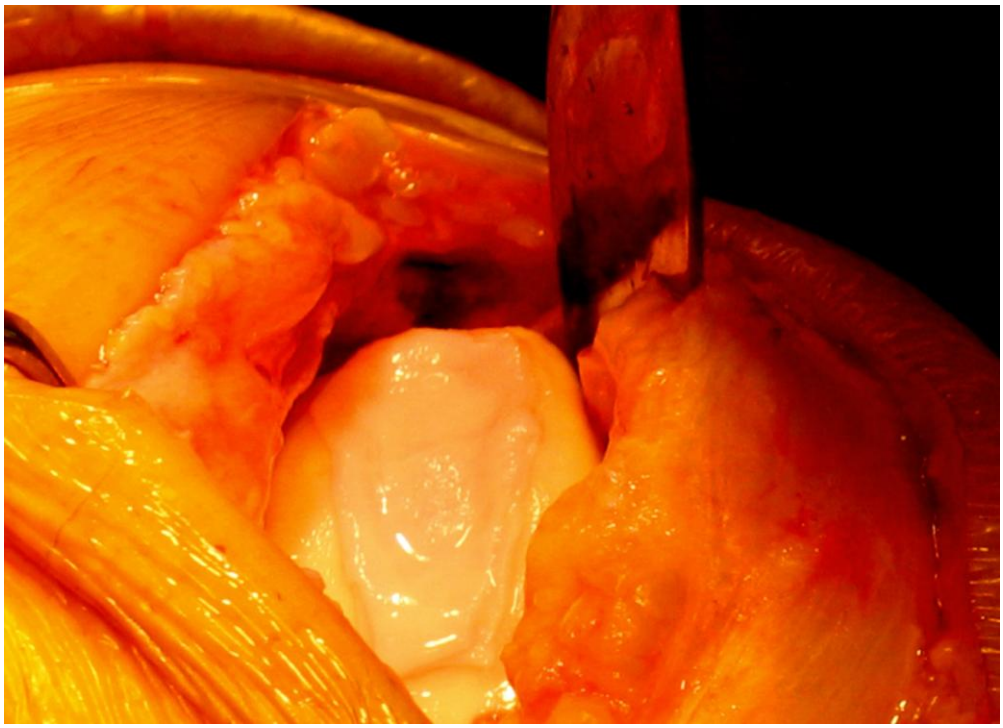
The lesion is templated using a sterile foil from a suture pack (figure 3.5). The MACI matrix is cut to size. Fibrin glue is injected onto the base of the debrided defect and the cut MACI membrane is placed over the defect whilst maintaining firm digital pressure for 2 minutes (figure 3.6). Graft stability is assessed by putting the knee through a full range of motion. If necessary, additional vicryl sutures can be placed to provide further stability.

Figure 3.5: Template cut to size and syringe containing fibrin glue



Courtesy of G. Bentley

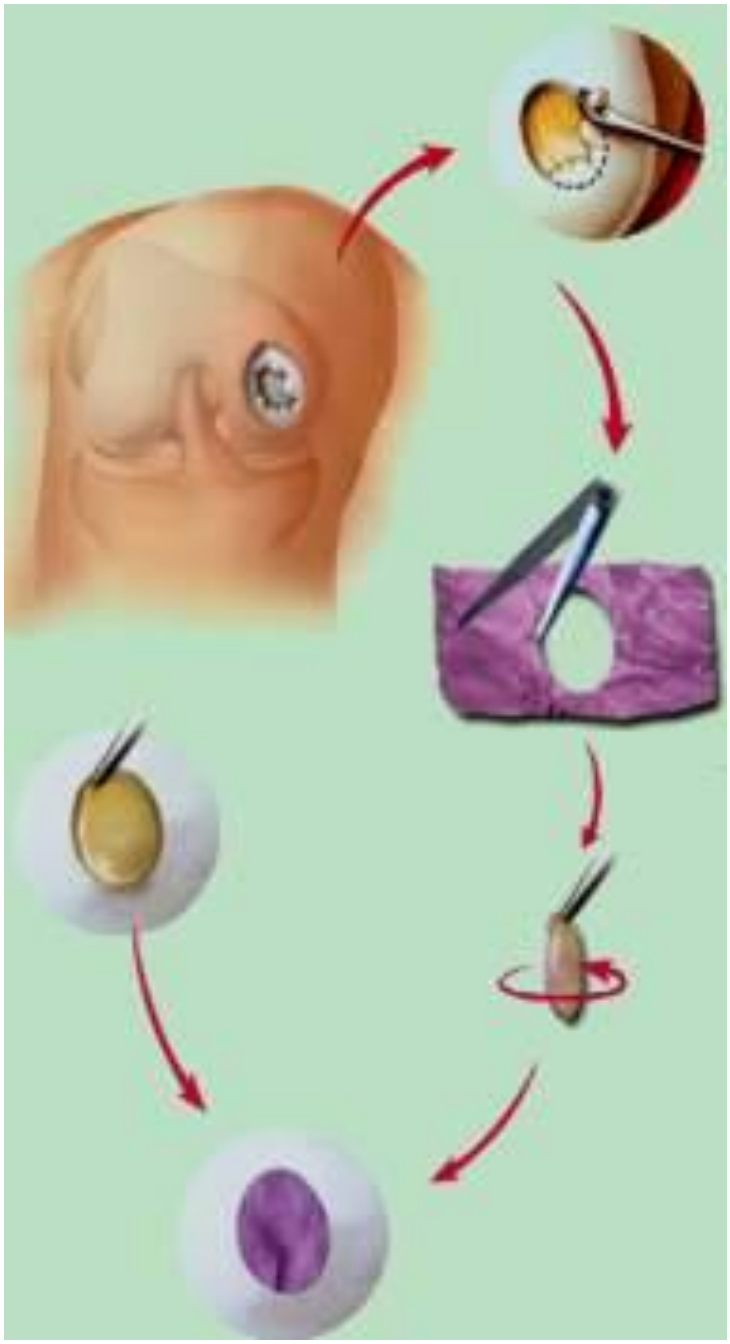
Figure 3.6: Final appearance of MACI graft



Courtesy of Mr. John Skinner

Following both procedures the knee is closed and dressed with a sterile dry dressing. The knee is held in full extension with a plaster-of-Paris backslab. From the first post-operative day patients are encouraged to weight-bear with the aid of crutches. After 2-3 days, the backslab is converted to a lightweight cylinder cast and this removed after another 10 days. Upon cast removal, active physiotherapy is commenced. The standardised regime also consists of, gym-rowing, swimming and cycling after 6 weeks. Patients are advised to avoid pivoting or impact loading. Light jogging is permitted after 6 months and return to contact sports after 1 year.

Figure 3.7: Overview of MACI



Courtesy of Verigen Corporation

Aims and Objectives

A small, prospective randomised study compared ACI-C (44 patients) with MACI (47 patients). It concluded that there was no difference in clinical, arthroscopic and histological outcome one year following implantation (Bartlett et al. 2005). This study was underpowered and hence the inconclusive findings may not be accurate. Another study attempted to identify factors that may affect outcome following ACI-C (Krishnan et al 2006). It concluded that younger patients with higher pre-operative Modified Cincinnati Scores (MCS) and shorter duration of length of symptoms had better outcomes. Single defects located in the trochlea or lateral femoral condyles also tended to do better. The statistical analysis may not be entirely accurate and will be discussed later.

The aim of this chapter is to compare the efficacy of ACI-C with MACI two years after surgery in a prospective randomised controlled study. A power analysis has been performed to minimise type II statistical errors. A secondary aim will be to identify patient and defect characteristics that could have an adverse effect following surgery.

Patients and methods

The South East Multi-Centre Research Ethics Committee and the Joint Research and Ethical Committee of the Royal National Orthopaedic Hospital Trust gave its approval before commencing this study (study number MREC 02/01/73). This is a parallel group randomised controlled trial assessing two different forms of autologous chondrocyte implantation; ACI-C versus MACI. The inclusion criteria for surgery included:

- Persistent knee pain/swelling/giving way attributable to a lesion of the articular cartilage in the knee
- Patients aged between 15 and 50 years
- An osteochondral defect larger than $1cm^2$
- Patient able to comply with rehabilitation programme following surgery.

Absolute contra-indications included:

- Defects smaller than $1cm^2$
- The presence of osteoarthritis
- The presence of rheumatoid arthritis

In addition patients also suffering from joint instability (secondary to Anterior Cruciate Ligament rupture) and joint mal-alignment could still have ACI but these problems had to be addressed simultaneously during the second stage procedure. The patients were assessed clinically in the outpatients department and if eligible were listed for an arthroscopy procedure. At the time of the arthroscopy, if the lesion was suitable for ACI or MACI a

sealed envelope would be opened to determine what kind of intervention the patient would undergo and the first stage procedure would be performed, hence enrolling the patient into the trial.

Outcomes

The primary outcome measure in this study was The Modified Cincinnati Score (MCS). The scores were taken pre-operatively at the time of the clinical consultation when the decision was made to perform the first arthroscopy. The functional assessments were repeated six and twelve months following surgery and yearly thereafter. One year following surgery, an arthroscopy was performed and The International Cartilage Repair Society (ICRS) score was used to gauge the macroscopic appearance of the repair tissue. If possible a biopsy of the graft was undertaken to determine the quantity of hyaline cartilage in the repair tissue. The ICRS scores and the biopsy results formed the secondary outcome measures.

Randomisation

An independent observer used a random number generator from the website www.randomgenerator.com to perform block random allocation. This produced a reference number with an assignment of either ACI or MACI, and these tags were placed in an opaque sealed envelope which were sequentially numbered. The random number generator produced blocks of 10 ACI and 10 MACI tags. The envelopes were kept in theatres in a specified drawer and were opened by the operating surgeon at the time of the first stage procedure to determine which treatment arm the patient would be enrolled into. The patient was blinded to the treatment received. Following the 2nd stage procedure, the patients were assessed by an independent observer who was also blinded to the treatment allocation.

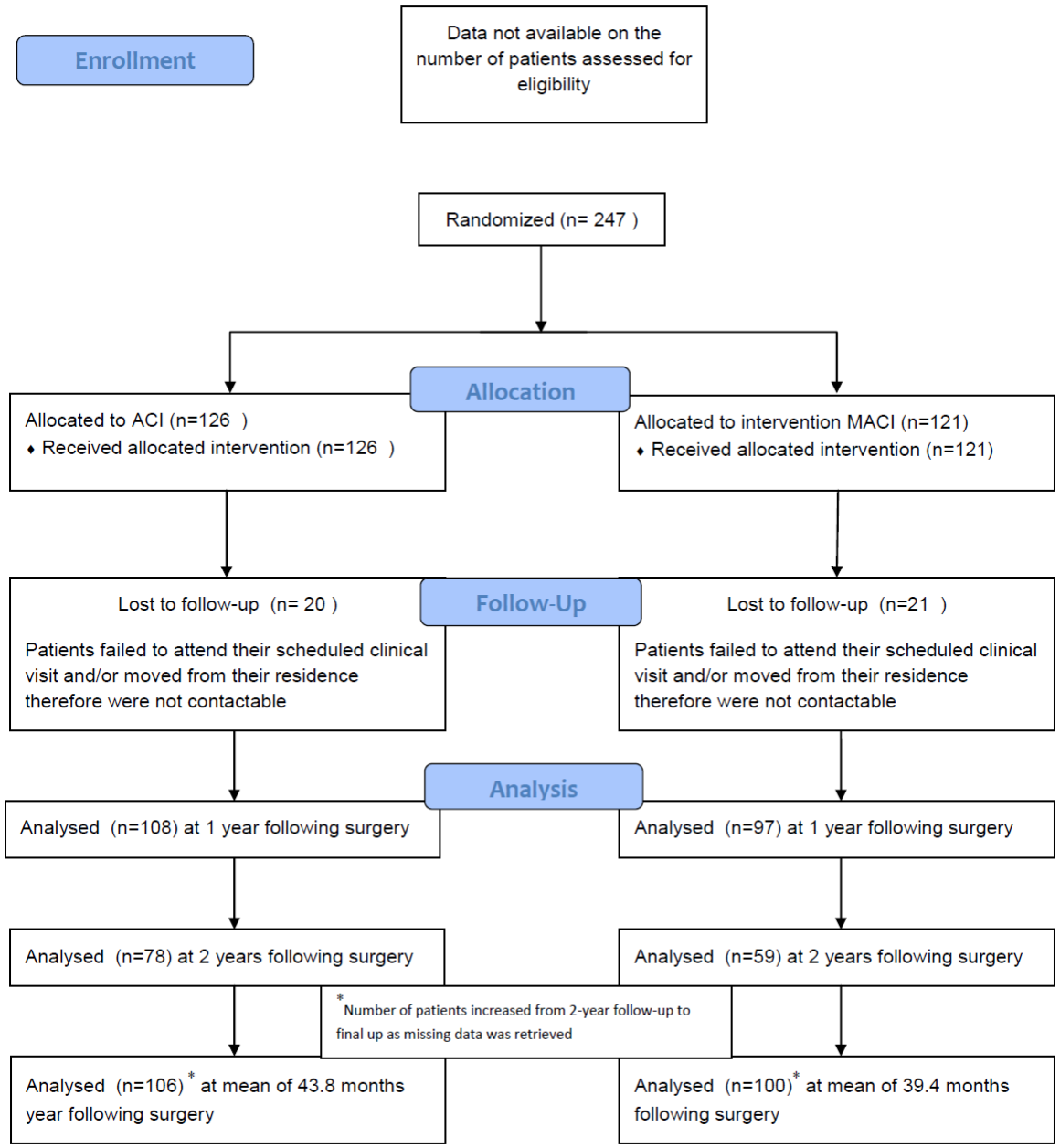
CONSORT Statement

Between July 2002 and July 2005, 247 patients underwent ACI for isolated symptomatic osteochondral defects of the knee. They were all treated at the Royal National Orthopaedic Hospital in one department (The Joint Reconstruction Unit) by three different surgeons. 126 patients had ACI-C and 121 patients had MACI (see figure 3.8). There are fewer patients at the 2 year interval compared to the MCS at latest follow-up. This is because an effort was made to contact patients who were failing to attend their regular annual review to obtain their latest MCS. As a result there, the number of patients increased by 28 in the ACI group and 41 in the MACI group.

Statistical analysis

With $\alpha = 0.05$ and $\beta = 0.2$ (hence a power of 80%) and a standard deviation of 16.9 (taken from previous small study), a total of 200 patients would be required to detect a difference of 10 points in the modified Cincinnati scale. Statistical analyses were performed using SPSS version 18.0. The independent *t*-test was used to compare the improvement in knee scores between patients who had ACI and MACI. The Fisher's exact test was used to compare the proportion of excellent and good results achieved in both groups. The level of significance was set at p=0.05.

Figure 3.8: CONSORT Statement



A generalised linear model was used to assess which factors had the greatest effect on outcome. The residuals were tested for normality and this was found to be the case (personal communication Tim Morris, Statistician in the Clinical Trials Unit, Medical Research Council).

The dependent variable was the latest MCS (MCS latest). The predictive factors in this model were

- the type of surgery (i.e ACI or MACI)
- the aetiology of the OCD
- the anatomical site of the defect
- the number of previous operations prior to the first stage procedure
- the sex of the patient

The predictive covariates were

- the MCS pre-operatively (MCS 0)
- the duration of symptoms,
- the age of the patient at the time of the second stage surgery
- the size of the lesion (in mm^2)

Results

The patient and lesion characteristics in the two treatment groups are summarised in table 3.1. The age distribution of the patients is displayed in figure 3.9. Tables 3.2 displays the anatomical distribution of the chondral lesions and table 3.3 shows the reason for the cause of the OCD.

Table 3.1: Patient and lesion characteristics

	ACI **n=126**	**MACI** **n=121**
Age	33.4 +/- 0.8 n=126	33 +/- 0.8 n=120
Percentage males	53.2%	57%
Length of symptoms (months)	101.2 +/- 7.6 n=122	101 +/- 8.7 n=115
Number of previous operations	2.16 +/- 0.13 n=124	2.4 +/- 1.98 n=120
Size of lesion (mm^2)	522 +/- 28.5 n=122	520 +/- 24.9 n=116
Modified Cincinnati Score pre-operatively (MCS 0)	46.3 +/- 1.5 n=123	47 +/- 1.64 n=116

Figure 3.9: Age distribution of patients

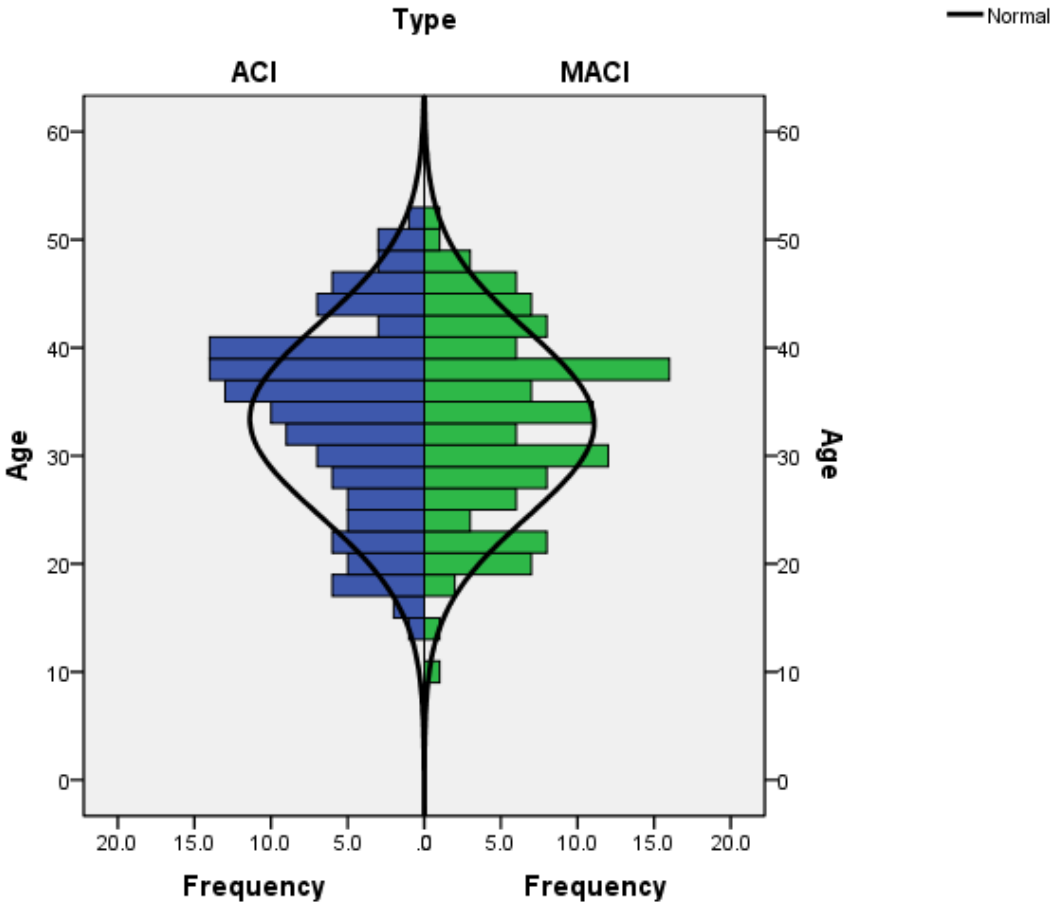


Table 3.2: Anatomical site of chondral lesions according to treatment groups

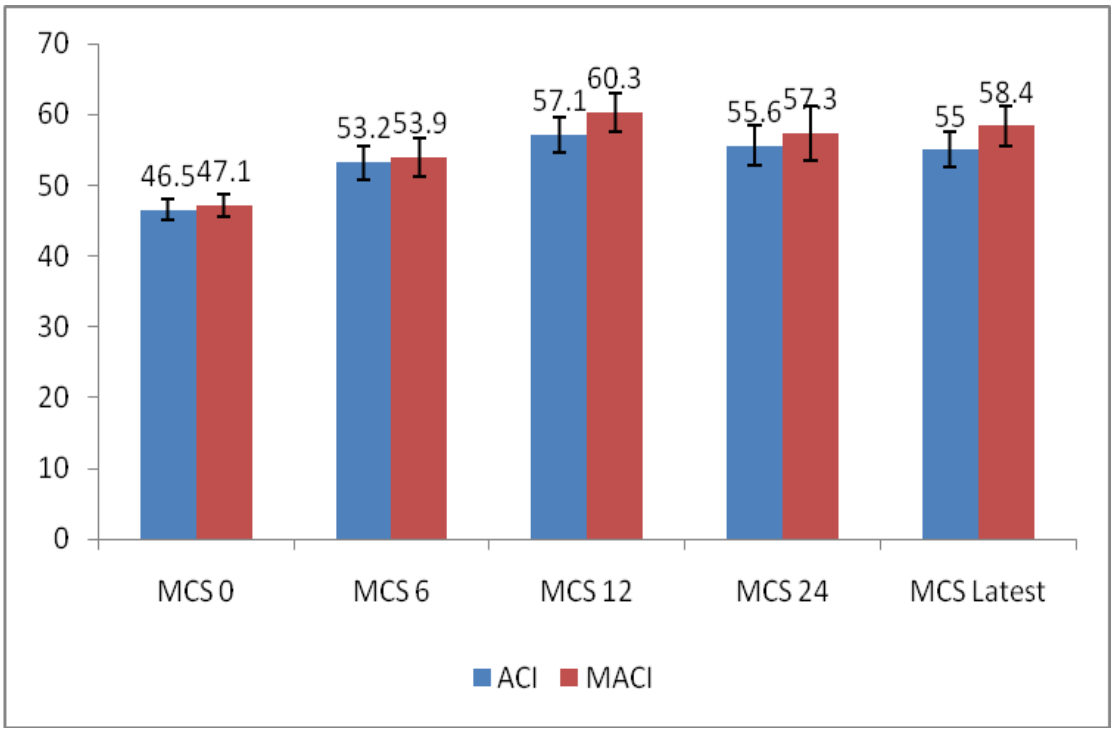
Anatomical site	ACI	MACI
Femur (trochlea, MFC, LFC)	76 (61.8%)	78 (65%)
Patella single defect	28 (22.8%)	25 (20.8%)
Patella multiple defects	19 (15.4%)	17 (14.2%)
TOTAL	123	120

Table 3.3: Aetiology of lesions according to treatment groups

Anatomical site	ACI	MACI
Trauma	53 (48%)	64 (60.4%)
Other	32 (29%)	30 (28.3%)
Failed previous procedures	26 (23%)	12 (11.3%)
TOTAL	111	106

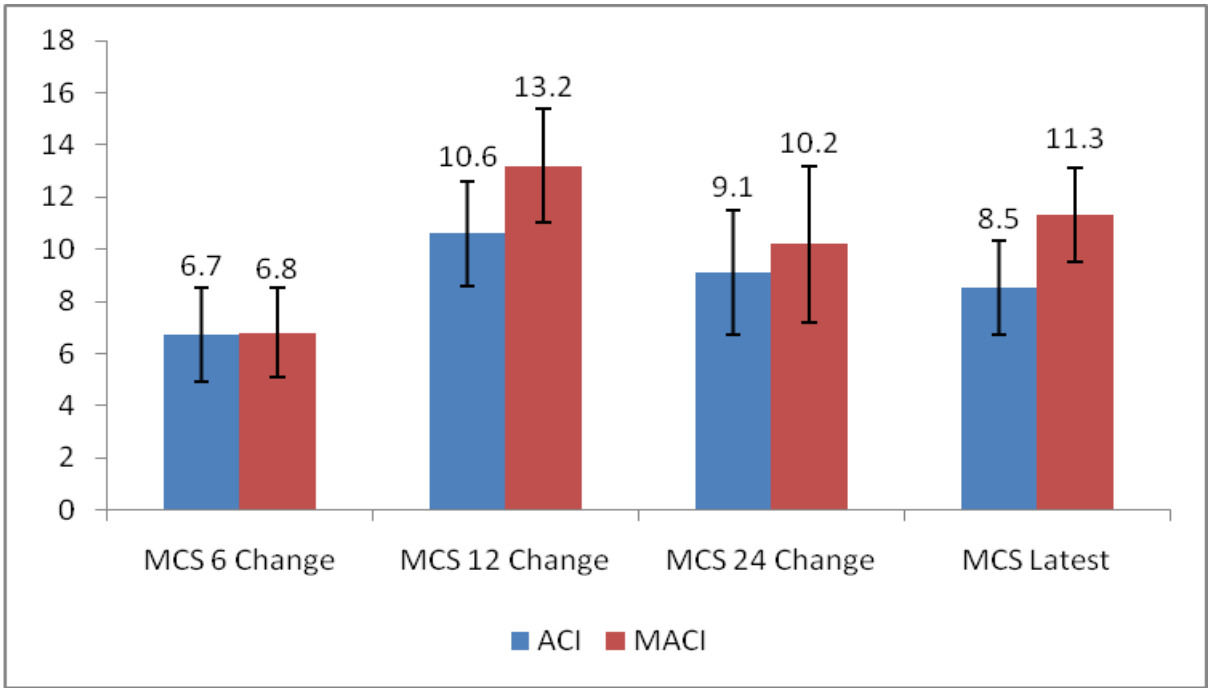
The Modified Cincinnati Scores (MCS) before and after surgery are shown in figure 3.10 for descriptive purposes. The reason for displaying this graph is to compare the absolute functional scores with other published work. The ACI group were followed up for slightly longer compared to the MACI group (39.4 months compared with 43.8 months) but this was not statistically significant (p=0.12).

Figure 3.10: The Modified Cincinnati Scores



The numbers displayed are the mean MCS with standard error bars

Figure 3.11 Change in Modified Cincinnati Scores (MCS) from baseline



Numbers represent mean change from pre-operative status at 6, 12, and 24 months and latest follow-up with standard error bars

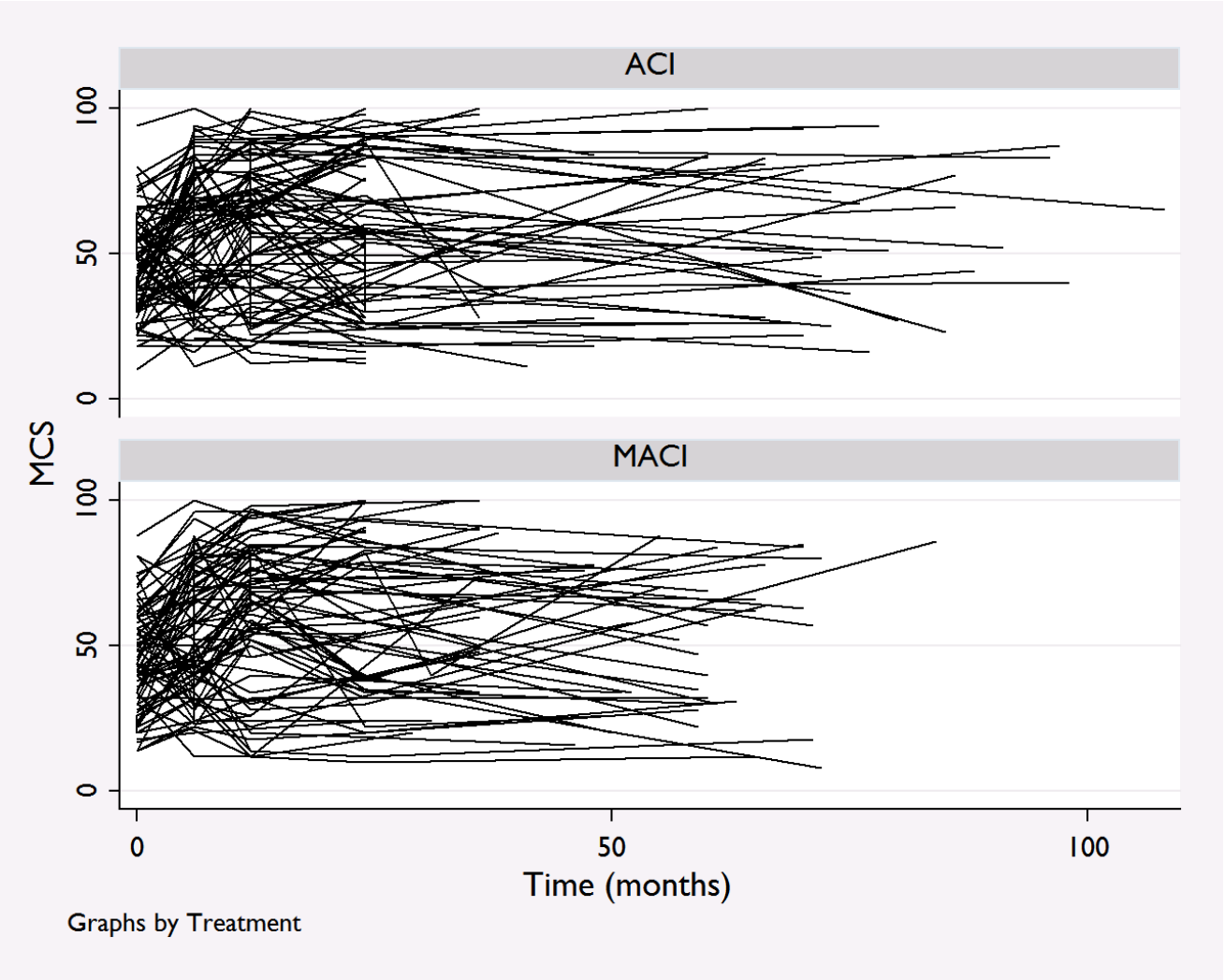
The graph above demonstrates similar improvements in MCS when comparing ACI and MACI groups at all time intervals. The independent t-test revealed no statistical significance between the two groups with respect to their latest MCS (p= 0.3) which was the score of interest.

Patients were grouped according to whether they had an excellent or good result according to the MCS one and two years following surgery and at the latest follow up. This form of analysis was executed to enable comparison of results from this study with other studies which do not use the same outcome measures but report good or excellent results. An excellent result is a MCS of 80 or more, a good result is a score between 55 and 79 inclusive, a fair result is a score between 30 and 54 and a poor result is score below 30 (Bentley et al 2003).

The proportion of excellent and good results did not differ according to the treatment they had (see table 3.4). One year following surgery, 64 patients (59.2%) had achieved good or excellent results in the ACI group compared to 61 patients (62.3%) in the MACI group. At two years, the proportion of good/excellent results had deteriorated, with 52.6% (41 patients) in the ACI group and 55.9% (33 patients) in the MACI group. At the time of latest clinical review (mean of 41.8 months), the results had deteriorated further; the proportion of good/excellent results were only 47% in the ACI group and 53% in the MACI group.

Table 3.5 shows that at the time of the 2 year review, a large number of patients failed to attend their clinical appointments. The two year results were only available for 55.5% of patients (137 patients had 2 year results from a total of 247 patients). An attempt was made to contact patients who had not attended their appointments to ascertain their clinical status and how well their knee is functioning. Therefore, at the latest follow up (at a mean of 41.8 months) results were available for 206 (83.4%) patients. This is why there are a greater number of patients in the final time interval in the CONSORT statement than the previous time interval (MCS 2 years after surgery)

Figure 3.12: Timelines for patients undergoing ACI and MACI



The figure above demonstrates the trend in MCS at each time interval for every single patient segregated according to the type of surgical procedure they received. The general trend is that patients tend to get better after surgery but it appears to take at least 1 year. If patients have improved then they tend to maintain that level of function and have a slow steady decline after 3-4 years.

Table 3.4 – Proportion of Excellent and Good Results in ACI and MACI patients

Time	Outcome	ACI	MACI	p value
1 year	Excellent	21 (19%)	28 (29%)	0.67
	Good	43 (40%)	33 (34%)	
	Fair	24 (22%)	23 (24%)	
	Poor	20 (19%	13 (13%)	
	Total	**108**	**97**	
2 years	Excellent	20 (26%)	15 (25%)	0.73
	Good	21 (27%)	18 (31%)	
	Fair	22 (28%)	19 (32%)	
	Poor	15 (19%)	7 (12%)	
	Total	**78**	**59**	
Latest Follow Up	Excellent	27 (25.5%)	28	0.41
	Good	23 (22%)	25	
	Fair	29 (27%)	33	
	Poor	27 (25.5%)	14	
	Total	**106**	**100**	

The p values are derived from performing the Fisher's exact test to compare proportion of good and excellent results according to the Modified Cincinnati Score (MCS).

An analysis of patients who attended their two year clinical outpatient visit and those patients who did not was performed (table 3.5). There were no significant differences in age, length of symptoms, number of previous operations or the size of the lesions between patients who attended their two year clinical visit and those that did not. However, there was a significant difference in the MCS before surgery in the two groups (patients who attended their follow up had significantly lower score). This an important observation as a lower MCS pre-operatively suggests that the post-operative MCS is going to be lower (see section on generalised linear model).

Table 3.5 – A comparison of results in patients with 24 month follow up data and those without

	No Follow up N=117	Follow up N=122	p value
Age	32.6 +/- 0.76	33.7 +/- 6.8	0.3
Length of symptoms (months)	109.4 +/- 9.6	93.5 +/- 6.6	0.1
No. of previous ops	2.2 +/- 0.16	2.3 +/- 0.16	0.5
Size of lesion	528.7 +/- 28.2	512.6 +/- 25.9	0.68
MCS 0	49.1 +/- 1.56	44.3 +/- 1.58	0.03

p values are derived by comparing the means in the two groups using the independent t-test

Further procedures

Sixteen patients required further procedures (Table 3.6) at a mean of 40.3 months.

Table 3.6 – Major re-operations

Procedure	**ACI**	**MACI**
Revision ACI/MACI	2	3
Patellectomy	1	1
High Tibial Osteotomy	1	2
Unicompartmental or Patellofemoral Replacement	4	0
Total Knee Replacement	2	0
Total	**10**	**6**

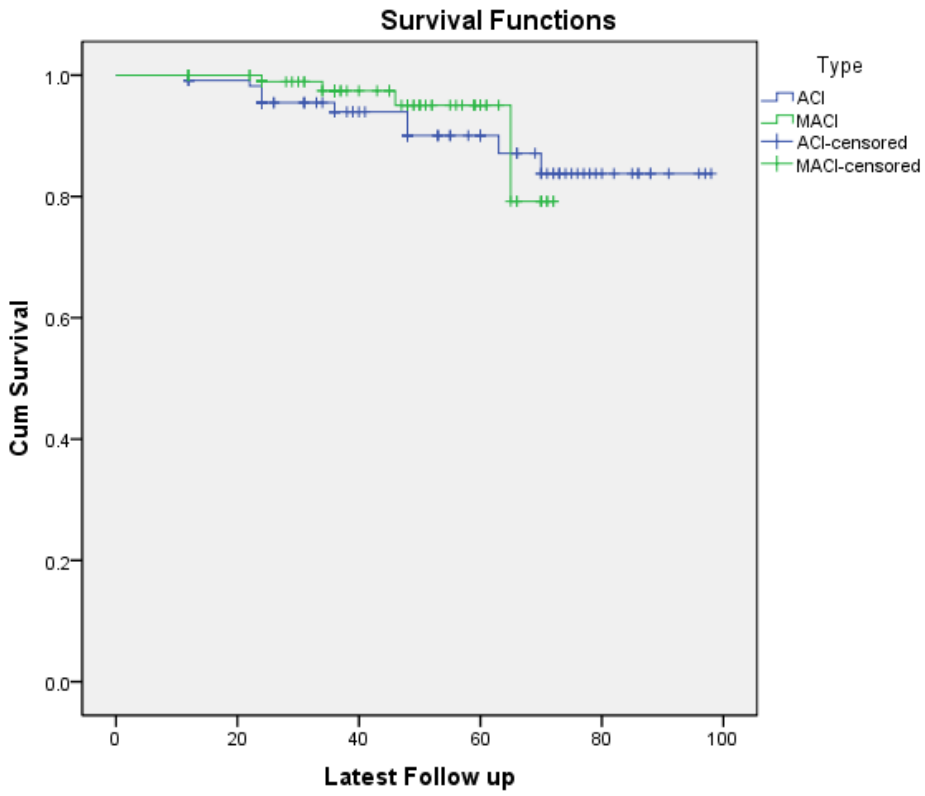
Kaplan-Meier survivorship analysis was performed from the date of the 2nd stage operation to the end-point of re-operation for the above indications (therefore the analysis is on the time to revision surgery). The log-rank test was used to compare whether there was a significant difference in re-operation rates between ACI or MACI groups (figure 3.13). The mean survival times (according to the above end-points) were 89.7 months in the ACI group and 68.7 months in the MACI group. This was not statistically significant (p=0.5). The 5 to 8 year survival figures are displayed in the table 3.7.

Table 3.7 – Survivorship figures according to Kaplan-Meier Analysis

Time	ACI	MACI
5 years	94% +/- 2.5	95% +/- 2.9
6 years	90% +/- 3.6	91.1% +/- 4.8
7 years	90% +/- 3.6	*75.9% +/- 10.6
8 years	83.8% +/- 5.4	Not available
Mean survival time(with 95% Confidence Intervals)	89.7 months (95% CI 84.8% to 94.6%)	68.7 months (95% CI 66.3% to 71.1%)
Overall (combining ACI & MACI groups together)	90.3 months (95% CI 86.5 to 94.1%)	

The probability of survivorship (i.e. not requiring revision surgery) is shown as a percentage with the standard error of mean. *only 83 month survivorship figure available for the MACI group

Figure 3.13: Kaplan-Meier Survivorship Analysis of ACI and MACI



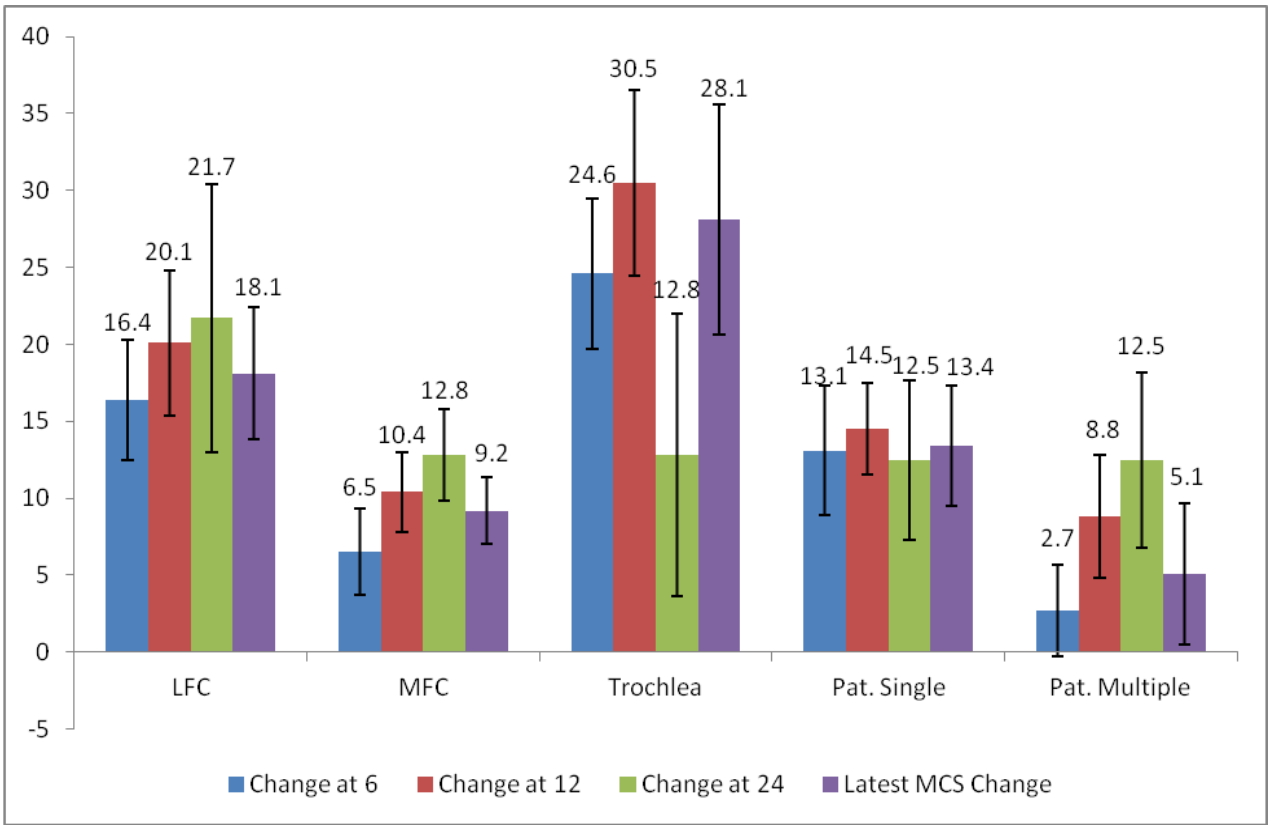
Predictors of Outcome

Since there were no differences in outcome when comparing ACI-C with MACI, the patients were grouped together when analysing predictors of outcome. This enabled greater number of predictive factors to be taken into consideration in the statistical analysis.

Site

The graph below displays the change in the Modified Cincinnati Score (MCS) from baseline (pre-operatively) to the various time frames after surgery listed below. .

Figure 3.14: Improvement in MCS from baseline according to site of defect



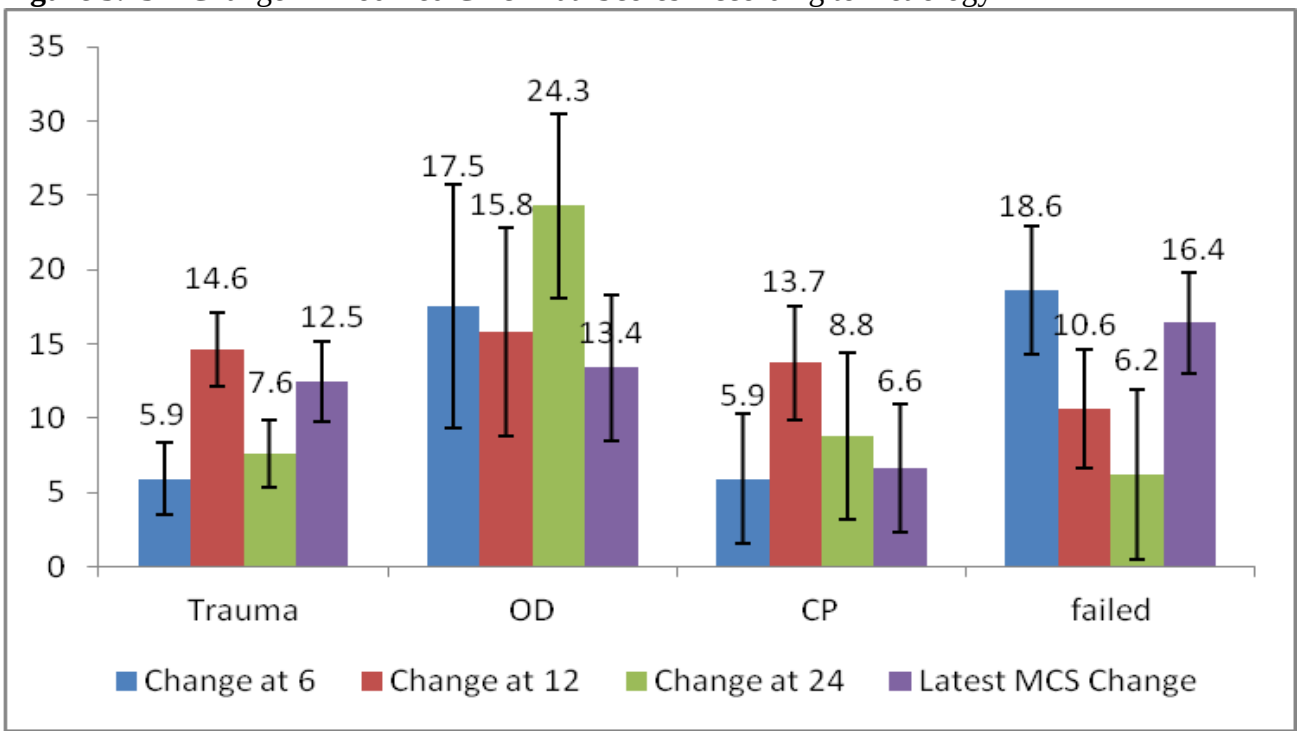
Graph displays mean values with standard error bars. LFC = lateral femoral condyle, MFC = medial femoral condyle, Pat single = single patella lesion, Pat. Multiple = multiple patella lesions

The standard error of the means for the all the groups were quite high (especially at 12 and 24 months after surgery). Therefore statistical analysis was only performed at the final time point which represents the greatest number of patients (change in MCS at the latest follow-up). Analysis of variance (ANOVA) revealed a statistically significant difference between the groups in terms of improvement of the MCS from baseline to mean follow-up of 41.8 months ($p=0.038$).

Aetiology

The graph below displays the change in MCS from the pre-operative score to 6, 12, 24 months after surgery and at the time of latest follow-up (mean 41.8 months) according to the aetiology of the osteochondral defects. Patients who had a previous mosaicplasty, carbon fibre rods, or an ACI-P were grouped together and termed 'Failed'. The other three groups (Trauma, Chondromalacia Patellae (CP) and Osteochondritis Dissecans (OD)) had had previous arthroscopies and debridement procedures.

Figure 3.15 – Change in Modified Cincinnati Scores According to Aetiology



Graph displays mean values with standard error bars

There are quite large standard error bars (especially at the 12 and 24 month time intervals). Therefore, very little conclusions can be made regarding efficacy of surgery at 12 and 24 months after surgery according to aetiology. This is because there was a lot missing data as mentioned earlier in the chapter and some of the data was recovered. Hence, the error bars in

‘Latest MCS Change is smaller. ANOVA revealed no statistically significant difference in improvement in MCS between the groups at the time of latest follow-up (p=0.5).

All patients experienced similar improvements from baseline to the time of final follow-up expect patients with chondromalacia patellae (the mean increase in MCS was approximately half that of the others) but was not statistically significant (analysis of variance test (ANOVA) revealed p=0.5).

It is important to realise that by separating the group of patients into this sort of cohort means that the patients are no longer randomised. Therefore, there may be other variables that may be contributing to the differences in outcome. Table 3.8 shows the results of ANOVA analysis on patient and lesion characteristics that may also have an effect on outcome.

The number of previous operations varies significantly in this cohort of patients. It is reasonable to assume that in the group termed ‘Failed previous operations’ they are going to have had a greater number of previous operations. It was also interesting to note that in this cohort of patients the size of the lesion was greater.

As one would expect the OD group was younger and when compared directly to the ‘Failed group’ was statistically significantly younger (though ANOVA analysis revealed $p > 0.05$ when taking into account all four groups).

In order to address these compounding variables a generalised linear model was utilised.

Table 3.8– Descriptive statistics of patient and lesion characteristics according to Aetiology

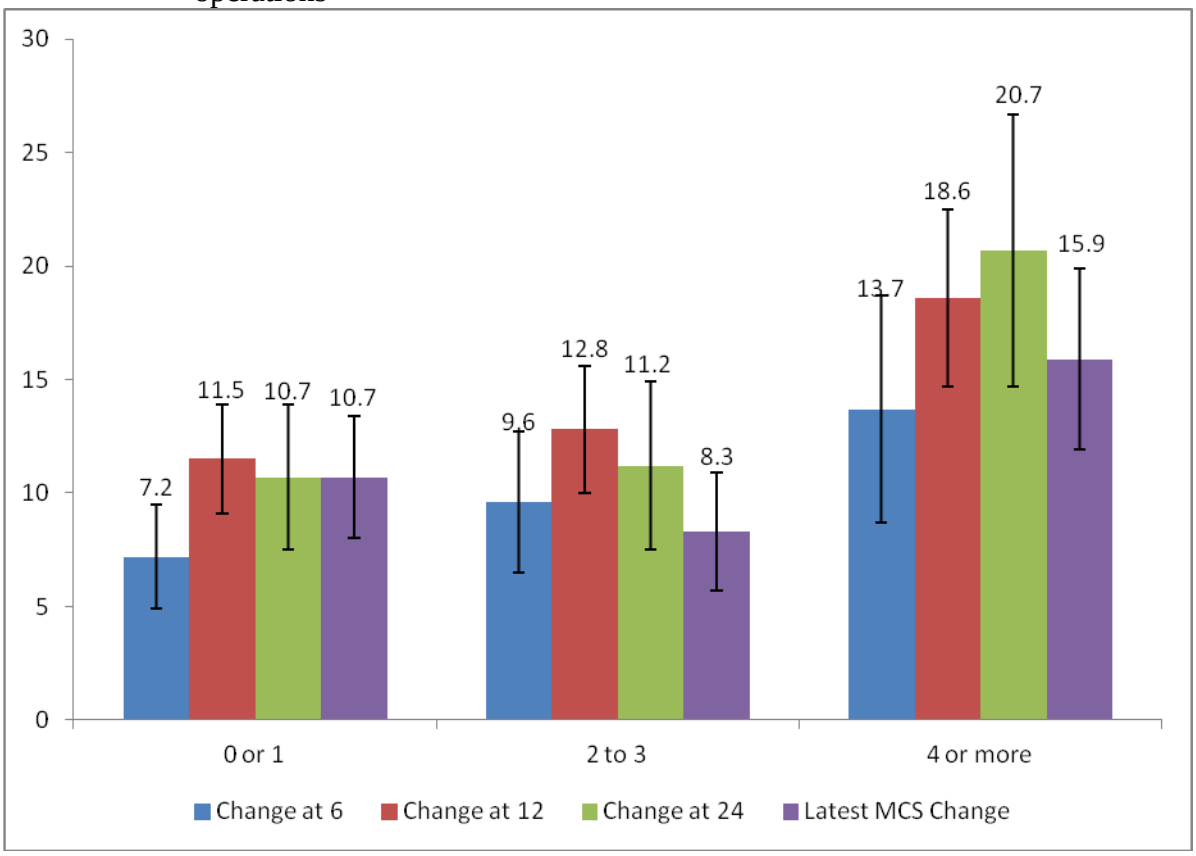
		N	Mean	Std. Error	95% Confidence Interval for Mean		p value (derived from ANOVA)
					Lower Bound	Upper Bound	
Age	Trauma	116	32.9	0.8	31.26	34.60	
	OD	24	28.6	1.9	24.70	32.55	
	CP	38	32.8	1.4	29.95	35.63	0.07
	Failed	38	34.7	1.2	32.20	37.22	
	Total	216	32.7	0.6	31.55	33.93	
Length of symptom	Trauma	113	91.6	7.8	76.10	107.00	
	OD	23	115.8	24	66.02	165.54	
	CP	37	96.8	11.4	73.75	119.82	0.15
	Failed	36	127.2	15.4	95.96	158.38	
	Total	209	101.3	6	89.43	113.13	
No. of previous ops	Trauma	116	2.2	0.1	1.94	2.51	
	OD	24	2	0.4	1.08	2.92	
	CP	38	2.1	0.2	1.66	2.60	***0.002***
	Failed	37	3.4	0.4	2.65	4.17	
	Total	215	2.4	0.1	2.14	2.63	
Size (mm^2)	Trauma	114	499.6	26.7	446.6	552.5	
	OD	23	548	60.3	422.9	673.1	
	CP	38	489.3	39	410.4	568.3	***0.05***
	Failed	36	646.3	59.8	524.9	767.8	
	Total	211	528.1	20.3	487.9	568.2	

CP = Chondromalacia Patellae, Osteochondritis Dissecans = OD, Failed = patients with failed previous mosaicplasty, microfracture or previous ACI

Number of previous operations

The graph below displays the change in Modified Cincinnati Scores (MCS) from baseline according to the number of previous operations the patients had received prior to their first stage procedure.

Figure 3.16: Change in Modified Cincinnati Score according to number of previous operations

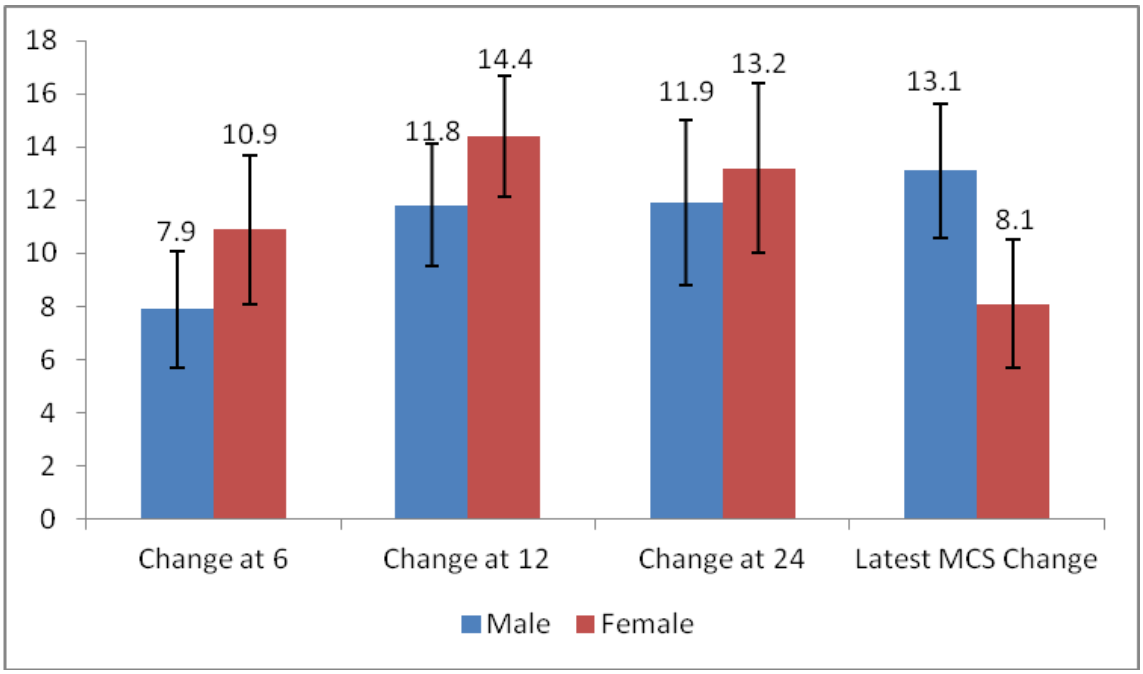


All three groups of patients experienced significant improvement in symptoms and ANOVA revealed no difference in outcome between the three groups at the time of latest follow-up.

Sex

The graph below displays the change in MCS from baseline at 6, 12, 24 months and the time of latest follow-up (mean 41.8 months) in males and females.

Figure 3.17: Change in MCS from baseline in males and females



The independent t-test revealed no statistically significant difference in efficacy of surgery according to the change in the latest MCS between men and women.

Generalised Linear Model (GLM)

In this section there will be 2 models performed. The first model will contain the latest MCS as the dependent variable. The covariate will be the MCS before surgery (MCS 0). The type of surgery (ACI vs MACI) will be the co-factor. The table below displays the results. The second GLM will analyse all the other possible predictors of surgery.

The residuals for this GLM and the following GLM which incorporates all other confounders were plotted and tested for normality. This was found to be the case; hence multiple linear regression testing was performed (Personal communication from Tim Morris, statistician at the Clinical Trials Unit, MRC).

Table 3.9: Results of the GLM to determine efficacy of surgery

Parameter	Magnitude of Effect	Std. Error	95% Confidence Interval		
			Lower	Upper	Significance
ACI	-2.95	3.2	-9.25	3.34	0.36
MACI	0[a]	.	.	.	.
MCS 0	0.6	0.094	0.41	0.78	<0.01

0[a] represents the reference category for comparison of other categorical data within the group.

The GLM shows that the MCS before surgery has a significant effect on the MCS after surgery, whereas the type of surgery (i.e. ACI or MACI) does not. The 'Magnitude of Effect' column displays the data for continuous and categorical factors differently. For continuous data (MCS 0), a one point increase in MCS 0 is likely to increase the latest MCS by 0.6 points. When comparing categorical data there must be a reference category and in this case it is MACI. The model predicts that ACI patients are likely to have a MCS at the latest follow-

up that is 3 points inferior to MACI patients independent of the MCS before surgery (though this is not statistically significant).

As there were no statistically significant differences in outcome between ACI-C and MACI, the patients were grouped together in order to increase the number of patients in which prognostic factors could be analysed. In total there were 169 patients that had all of the previously mentioned factors listed in the database. Tables 3.10a shows the breakdown of the number of patients for each of the categorical variables. Table 3.10b displays the mean for each of the continuous variable. In an effort to increase the efficiency of statistical analysis and in order to analyse more confounding variables the site of the lesion was grouped in to four categories where patella single and multiple lesions were one category. Furthermore, in the aetiology section chondromalacia patella and osteochondritis dissecans were grouped together and termed ‘other’.

Table 3.10a: Categorical Variable Information

Factor		N	Percent
Type of surgery	ACI	90	53.3%
	MACI	79	46.7%
	Total	169	100.0%
Sex	Male	89	52.7%
	Female	80	47.3%
	Total	169	100.0%
Previous operations	>4	29	17.2%
	2-3	60	35.5%
	0-1	80	47.3%
	Total	169	100.0%
Sites	LFC	26	15.4%
	Trochlea	22	13.0%
	Patella	37	21.9%
	MFC	84	49.7%
	Total	169	100.0%
Aetiology	Trauma	85	50.3%
	OD	18	10.7%
	CP	34	20.1%
	Failed	32	18.9%
	Total	169	100.0%

LFC = lateral femoral condyle, MFC = medial femoral condyle, Pat single = single patella lesion, Pat. Multiple = multiple patella lesions, CP = Chondromalacia Patellae, and OD= Osteochondritis Dissecans

Table 3.10b: Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Last MCS	169	8	100	56.6	25.1
Covariates	MCS 0	169	10	88	45.8	16.4
	Age (years)	169	15	52	33	8.8
	Length of Symptoms (months)	169	11	504	100	87.6
	Size (mm^2)	169	96	1575	535	302

Table 3.11 - Results of Generalised Linear Model

Parameter	Magnitude of effect	Std. Error	95% Confidence Interval		Hypothesis Test
			Lower	Upper	Sig. (p-value)
Covariates					
Type of surgery					
ACI	-2.8	3.5	-9.6	4	0.4
MACI	0[a]	.	.	.	.
Aetiology					
Failed	-2.8	5.1	-12.8	7.1	0.6
Other	-1.4	3.8	-8.8	6	0.7
Trauma	0[a]	.	.	.	.
Site					
LFC	-0.8	5.3	-11	9.6	0.9
Trochlea	13.3	5.3	3	23.7	0.01
Patella	0.4	4.4	-8.2	9.1	0.9
MFC	0[a]	.	.	.	.
Sex of patient					
Male	9.8	3.78	2.43	17.2	0.01
Female	0[a]	.	.	.	.
No. Previous Ops					
4 or more	3.91	4.9	5.7	13.5	0.4
2-3	-1.4	3.8	8.8	6	0.7
0-1	0[a]	.	.	.	.
Co-factors					
MCS 0	0.5	0.1	0.25	0.7	<0.001
Age	-0.5	0.2	-0.9	-0.1	0.01
Size	-0.006	0.001	-0.02	0.005	0.3
Length of symptoms	-0.002	0.02	-0.04	0.04	0.9

Other aetiologies included osteochondritis dissecans and Chondromalacia Patellae,

LFC – Lateral Femoral Condyle, MFC – Medial Femoral Condyle,

MCS 0 is the pre-operative Modified Cincinnati Score

0[a] represents the reference category for comparison of other categorical data within the group.

Table 3.11 shows that the only statistically significant factors affecting outcome were the pre-operative MCS, age and sex of patients and site of the lesions. The latest MCS was on average 13 points greater in patients with lesions in the trochlea compared to patients with lesions in the medial femoral condyle ($p=0.01$).

Male patients did significantly better compared to female patients when all other confounding variables were taken into consideration.

The size of the lesion or length of symptoms did not affect outcome when taking into account all the other variables. The strongest predictor of the latest MCS was the MCS before surgery. From the model above, it can be concluded that a single point increase in MCS 0 is likely to increase the MCS 24 by 0.5 points. Therefore if the starting MCS was 10 points higher, then the MCS 24 is likely to be 5 points higher. This relationship is statistically significant ($p<0.001$). There is also an inversely proportional relationship between age and the latest MCS. An increase in age by 1 year is likely to decrease the MCS 24 by 0.5 points ($p=0.01$).

Discussion

Treatment by both ACI and MACI resulted in significant improvements to the Modified Cincinnati Score (MCS) a year following surgery and this improvement was maintained at the latest follow up of 41.8 months. There was no significant difference between the two techniques in terms of the final MCS or the improvement in MCS from the pre-operative status. Furthermore, there was also no difference in the two groups in terms of achieving excellent or good results. This is in keeping with an earlier study which compared ACI vs MACI at 1 year (Bartlett et al. 2005). The mean MCS in the ACI-C group (n=44) was 59 (mean increase 17.5 points) and in the MACI group (n=47) was 64.1 (mean increase 19.6 points). However, this difference was not statistically significant. Analysed another way, 72.3% of patients reported a good or excellent outcome in the MACI group compared with 59.1% in the ACI-C group. These results are similar to the results presented in this study at one year (64% in the ACI group and 61% in the MACI group). In this study, the overall increase in the MCS was 10.6 and 13.2 in the ACI and MACI groups respectively at 1 year. Similar increases in the MCS were experienced by the patients at 2 years and at the last follow up. The increase in MCS from pre-operative status was much higher in the cohort of patients reported by Bartlett et al (2005). The starting MCS was lower in the study by Bartlett et al. compared to this study and this may explain the difference.

There are few randomised trials comparing two different techniques of cartilage transplant. Gooding et al (2006) compared ACI using a periosteal flap (33 patients) with ACI using a synthetic collagen membrane (ACI-C) such as the one used this in this study (35 patients). The clinical results were similar in the two groups, though a significant number of patients in the ACI-P required a re-operation for graft hypertrophy. This difference in the re-operation rates resulted in the trial being stopped prematurely.

More recently, 21 patients were randomised to have either ACI-P or MACI using a resorbable scaffold of polyglactin 910 and poly-p-dioxanon (Zeifang et al 2010). Knee function was assessed according to the International Knee Documentation Committee (IKDC) score at 24 months. Secondary outcome parameters used were the SF-36, Lysholm and Gillquist and Tegner Activity scores. The mean IKDC score changed from 51.1 to 72 in the ACI-P group and from 52 to 76.6 in the MACI group at 12 months and this improvement was maintained at 2 years. There was no significant difference in the IKDC with respect to the treatment received. The only scoring system that demonstrated a significant difference between the two treatment groups was the Lysholm and Gillquist scores which favoured the ACI-P group. Comparison with our results is difficult due to the use of different scoring systems. MRI scoring to evaluate cartilage repair had shown that there was a significant difference in favour of MACI at 6 months but not at 12 and 24 months. This is confirmed by another study which showed complete attachment of MACI grafts in 14 out of 16 patients at a mean of 34.7 days (range 22 to 47 days) (Marlovits et al 2004). For a randomised study it was interesting to read that the ACI-P group contained 10 males and the MACI group consisted of 6 men and 5 women. The number of patients in this study is questionable even though they had performed sample size calculations and stated that their study had 80% power. In their discussion they have admitted that the standard deviation of 3.5 for IKDC scores used to calculate the sample size required was grossly underestimated and hence their sample size calculations were incorrect. There are two other weaknesses of the study; firstly, there were no women in the MACI group and secondly there were no lateral femoral condyle lesions in the ACI-P group. As this is a randomised study with few patients these discrepancies are likely to have occurred by chance, but results from this study have to be interpreted with caution.

In a prospective cohort study, 20 patients received two different types of MACI; 10 patients had a MACI graft with a synthetic collagen membrane (similar to the one used in this study) and 10 patients had a MACI graft based on hyaluronic acid (Welsch et al 2010). They reported that the collagen MACI had higher quality superficial repair tissue according to an MRI scoring system though this did not translate clinically as there were equal proportion of good or excellent outcome (80%) in both treatment groups according to the Brittberg Score. Once again, due to the use of different scoring systems, a comparison between this study and ours is difficult. Feruzzi et al (2008) had demonstrated superior results with the use of MACI on a hyaluronic acid scaffold implanted arthroscopically (50 patients) compared with ACI-P (48 patients) implanted in the traditional manner (open arthrotomy). In both groups, there was statistically significant increase in IKDC scores at 6, 12, 18, 24, 36, 48 and 60 months following surgery. Between the two groups, the arthroscopic MACI group had significantly better scores than ACI-P until 18 months. At 24 months the scores were slightly higher in the arthroscopy group but was not statistically significant. However, complication and re-operation rates were significantly lower in the arthroscopy group (9 complications in the open series versus 2 in the arthroscopy group, $p=0.008$). The authors' speculated that the use of hyaluronic acid as a scaffold and its adhesive properties could explain no graft delamination or loose-body formation. The design of this study has a fundamental flaw. It can not conclude that arthroscopic implantation is better than the open technique since there were two different types of ACI used. It would have been more appropriate to have used MACI in the open series.

MACI was shown to be efficacious in two other studies assessing traditional versus accelerated approaches to post-operative rehabilitation. Sixteen patients were randomised to have an accelerated weight-bearing compared whereas 15 patients had delayed weight-

bearing following surgery (Wondrasch et al 2009). Clinical assessment was performed using the IKDC, the Knee Osteoarthritis and Outcome Score (KOOS) and the Tegner score. Compared to the pre-operative status, all clinical scores showed an improvement in both treatment groups with no statistically significant differences. These were supported by a larger randomised controlled trial of 62 patients randomised to have traditional versus accelerated weight-bearing following MACI (Ebert et al 2008). In their study it was shown that accelerated weight bearing resulted in significantly lower knee pain (according to KOOS).

The rehabilitation following surgery at our Institute is early weight-bearing with the support of crutches. This is based on the theory that long periods of unloading lead to degeneration of articular cartilage, possibly having an adverse effect on the transplanted cells,. Non-weight-bearing and could lead to joint structures (such as menisci, capsular structures and ligaments) adapting to a non-physiological situation which could compromise healing cartilage. The MACI technique allows the grafted cells to be directly held on the defect and minimizes the risk of cell leakage thereby allowing earlier weight-bearing.

The ultimate goal of ACI is to restore function in relatively young, fit and active patient population. The secondary more long-term goal is to avoid the development of secondary degenerative changes in the affected knee that may require a more radical surgical procedure such as tibial osteotomy or partial/total joint arthroplasty. This is the first study to analyse the effectiveness of ACI in avoiding the need for the above procedures. The 7-year survivorship following ACI-C was 90% and following MACI was 75.5% (this difference was not statistically significant). Zaslav et al (2009) demonstrated 75% survivorship of ACI grafts at 48 months (though they used different end-points). The 10-year survivorship in 72 patients was 83.3% with end-point being any operation to remove the implanted ACI graft (Moseley

et al 2010). The criticism of this study could be the lack of a negative control and also the end-point used. It would be ethically incorrect for symptomatic chondral defects of the knee not to have any treatment and hence the lack of negative control. Most joint registries use revision surgery as an end-point when performing survivorship analysis, and so our study adds a useful comparison to joint arthroplasty.

More recently, Filardo et al. (2014) have reported a 10.4% failure rate with the use of a hyaluronan-based MACI at a mean of 77.4 months follow-up. In their review of 142 patients with lesions in the trochlea and femoral condyles, they reported more favourable outcomes in male patients and younger patients which agrees with our results. They also observed better results in patients with shorter duration of symptoms in patients with traumatic lesions, small lesion size (for OCD) and no previous surgery. Perhaps the difference in their results and ours is that they specifically analysed results in a separate cohort of patients i.e. size of lesion in patients with OCD or duration of symptoms in traumatic lesions. The advantage of analysing results in a GLM is that it takes into consideration other variables, hence, is a more accurate form of analysis.

The literature suggests that both ACI and MACI are equally effective in treating full thickness chondral defects in the knee and seem to be better than other techniques for larger defects (Bentley et al 2003, 2012, Visna et al 2004, Saris et al 2008, Saris et al 2009). The results from our Institute do not seem to be as good as those reported from other studies (Brittberg et al 1994, Peterson et al 2000, 2002, Ferruzzi et al 2008, Zeifang et al 2010, Ebert et al 2008, Wondrash et al 2009, Welsch et al 2010, Behrens et al 2006, Moseley et al 2010). The use of different scoring systems makes direct comparison of results extremely difficult. Nevertheless, the proportion of good and excellent results does seem to be lower at our Institution compared to others. This may be because patients are made to wait longer for

cartilage transplant procedure and have had many other procedures prior to ACI/MACI as a result National Institute for Health and Care Excellence (NICE) guidelines which prohibits the use of cartilage transplantation as first line treatment for chondral lesions.

The initial attrition rates in this study were among the highest in the literature for randomised studies assessing ACI. Horas et al. (2003) reported a 5% attrition rate in his study on ACI vs mosaicplasty, while Knutsen reported 0% loss to follow up in his studies on ACI vs Microfracture (Knutsen et al. 2004, 2007). In other studies rates varied from 0 to 28% (Dozin et al 2005, Behrens et al 2006, Visna et al 2004, Saris et al 2009). In total 205 patients (out of 247) had returned for their 1 year visit representing an attrition rate of 17%. However, 2 years following surgery, only 137 patients had returned for their clinical visit, representing an attrition rate of 44.5%.

The general consensus is that in studies with high attrition rates, there is a differential drop out with those lost to follow-up not doing as well as those attending clinical visits (Sprague et al 2003, Kristman 2004, Dumville et al 2006, Fewtrell et al 2008). Schulz and Grimes (2002) argue that loss to follow-up of 5% or lower is usually of little concern, whereas a loss of 20% or greater means that readers should be concerned about the possibility of bias; losses between 5% and 20% may still be a source of bias (Ferguson et al 2002). In a simulation study using 1000 computer replications of a cohort of 500 observations, the authors found seriously biased estimates of the odds ratios with high levels of loss to follow-up (Kristman et al 2004). Murray et al. demonstrated that patients who had total hip or knee replacements and were lost to follow-up had a worse outcome than those who continue to be assessed (Murray et al 1997). The results from this study suggest otherwise. Patients who did not attend their 2 year clinical visit had significantly higher starting MCS than those who did attend (Table 3.5).

There are two possible explanations for this trend. Firstly, the patients being treated are a much younger cohort than those needing joint replacement, hence they are more mobile and are more likely to change residence during the rehabilitation period. The second and probably more likely explanation is that in this Institute we treat many tertiary referrals, so patients may have to travel quite far. If patients have experienced a significant improvement in their symptoms then they may not want to attend a clinical visit 2 or 3 years later just to be asked some questions and be examined, even though they consented to do so at the beginning of the trial.

A large proportion of patients refused to undergo repeat arthroscopy at 1 year to assess the quality of graft and fewer still had a biopsy of the graft performed. It was therefore decided not to include the ICRS grade of the graft or the biopsy results in the analysis as fewer than 20% patients had these and this may not represent the true patient population. Briggs et al had demonstrated that 8 out 14 ACI grafts had hyaline-like cartilage repair, but all 14 grafts contained chondrocytes which had the potential to express type II collagen (Briggs et al 2003). Furthermore, the same group analysed the maturity of graft (according to the timing of the biopsy) to determine if this affected the histological as well as clinical and functional scores. They had suggested that biopsies taken at 1 year may not be a true representation of the amount of hyaline cartilage formation in the long-term as grafts are continuing to mature even at 36 months (Gikas et al 2009). However, it was difficult enough to persuade patients to undergo an arthroscopy and biopsy a year after surgery let alone 3 years! Nevertheless, a statistical model such as the GLM has not been reported in the literature, and it would have been very interesting to establish if hyaline cartilage formation following surgery was a significant prognostic factor independent of the other aforementioned variables.

Conclusion

The results from this randomised trial show that there is no statistically significant difference between ACI and MACI when treating OCD defects of the knee. In this cohort of patients, the pre-operative MCS was the strongest predictor of outcome at 2 years. If patients had previous treatment in the form of mosaicplasty or previous ACI/MACI (i.e. failed aetiology) then they are predicted to have inferior results following revision surgery. Patients with lesions in the patella are also predicted to have significantly worse results. The question therefore has to be asked whether we should be performing such expensive surgery if the lesions are located in the patella or if it is revision surgery. Only cost-utility studies over the long term can answer these questions.

Chapter 4:
The Treatment of Patellofemoral Osteochondral Defects

Introduction

The patellofemoral joint consists of the patella, the femoral condyles and the trochlea groove. The patella is a sesamoid bone which re-directs the forces of the quadriceps to the distal part of femur. Hence, it functions as a lever arm, thereby increasing the efficiency of the extensor mechanism (Hungerford et al. 1979). The patella has a medial and lateral facet which articulates with both of the femoral condyles. In addition, approximately three-quarters of individuals have a third articulating facet on the medial ridge of the patella that articulates with the medial femoral condyle at 120^0 of flexion-the so-called "odd"facet (Goodfellow et al 1976).

The flexion-extension pathway of the patello-femoral joint is complex. There is no contact between the patella and the trochlear groove in full extension. At initiation of flexion the inferior pole of patella comes into contact with the trochlea. As the knee continues to flex to 90^0 the patello-femoral contact point moves more proximally. Flexion beyond 120^0 results in only the medial and lateral aspects of the patella making contact with the femoral condyles (Mihalko et al 2007). As a result of the great pressures throughout the patello-femoral joint, the human body has adapted so that the articular cartilage of the patella is the thickest of any in the body (Grelsamer & Weinstein 2001).

In early flexion, there is a small compressive force across the patellofemoral joint (PFJ). However, the compressive forces increase as flexion increases. On the patella there are therefore three main forces:

1. The pull of the quadriceps
2. The tension of the patella tendon
3. Joint reactive force of the patello-femoral joint.

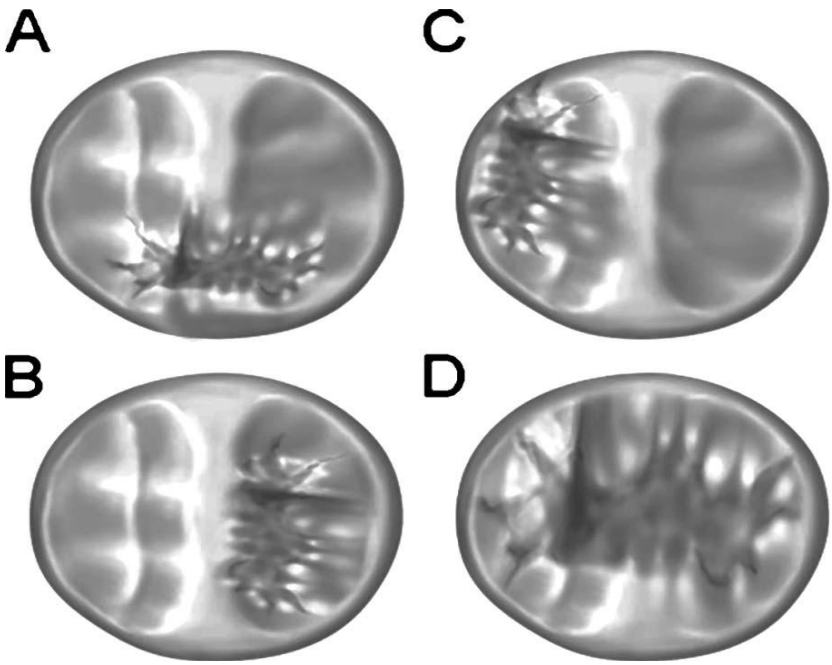
The estimated force through the patello-femoral joint varies from 1.5 times the body weight at 30^0 of flexion to 6 times the body weight at 90^0 of flexion (Grelsamer & Weinstein 2001, Scuderi 1995). After 90^0 of flexion the quadriceps tendon comes into contact with the distal femur and this is thought to dissipate the contact forces (Huberti and Hayes 1984).

Disorders of the patello-femoral joint represent a considerable therapeutic challenge due to the unique anatomy and biomechanics. The organisation of static forces (ligamentous and osseous elements) and dynamic factors (neuromuscular) contribute equally to its functional capacity (Saleh et al 2005). The medial and lateral patello-femoral and patello-tibial complexes form the main soft tissue static stabilisers of the joint (Arendt et al 2002). Patello-femoral function is also dependent on limb alignment, which includes varus or valgus tibio-femoral alignment as well as femoral anteversion. An imbalance of forces is likely to result in malalignment of the patellofemoral joint, and the question then rises as to the role of malalignment in chondral defects in the PFJ and subsequent osteoarthritis (OA) (Post et la 2002). With respect to the patella, the most prevalent area of chondral disease is on the lateral facet (Fulkerson & Hungerford 1990). The fact that the lateral facet becomes overloaded more commonly than the medial side suggests some degree of patella tilt or malalignment is more common than we realise. Certainly, when the literature regarding knee arthroplasty is

reviewed this is the case. In a series of 72 knees managed with patellofemoral arthroplasty 85% required a re-alignment procedure at the time of arthroplasty (Cartier et al 1990). A similar principle should be applied to the treatment of osteochondral lesions in the patella so that if a malalignment problem exists, this should be corrected simultaneously whilst addressing the chondral lesion.

Fulkerson first described the correlation of the site of patella defects with outcome following re-alignment (distal) osteotomy (see figure below).

Figure 4.1 Fulkerson's classification of chondral defects in the patella



This schematic diagram (which is looking at the under-surface of the patella) represents the four chondral defect locations of the patella according to Fulkerson's classification.16 (A) Type I is articular injury to the inferior pole of the patella. (B) Type II is articular injury to the lateral facet of the patella. (C) Type III is articular injury to the medial facet of the patella (frequently associated with a trochlear defect). (D) type IV is articular injury to the proximal pole (Type IVa) or a panpatellar injury (Type IVb). When associated with maltracking,
Type I and Type II injuries have a predictably good outcome with anteromedialization tibial tubercle osteotomy whereas Type III and Type IV injuries have poor outcomes, especially when associated with a trochlea defect.
(With kind permission from T. Minas)

Osteochondral defects in the Patellofemoral Joint

The aetiology of defects in this compartment of the knee is similar to the other compartments with one key difference. Chondromalacia patellae (CP) is a distinct clinical entity in which there is anterior knee pain and softening or breakdown of articular cartilage on the "odd" and medial facet of the patella though changes can occur in the lateral facet especially if there is tightness of the lateral patellar retinaculum as described by Ficat (Ficat and Hungerford 1977). CP was initially thought to result from malalignment of the unstable patella as it articulates with the distal femur (Wiberg 1941). This notion has largely been superseded by the concept that CP is secondary to trauma to the superficial chondrocytes resulting in release of proteolytic enzymes and enzymatic breakdown of articular cartilage and occurs frequently with no evidence of malalignment (Hinricsson 1939, Bentley and Dowd 1983). Two thirds of patients affected by symptomatic chondromalacia patellae are female (Bentley 1970) and symptoms affect two distinct groups. Some are inactive teenage girls and the second group are highly active teenagers of both sexes (Bentley & Dowd 1983). The first group experience symptoms after long periods of knee flexion e.g. when driving or sitting at a desk which is commonly relieved by mobilising the knee joint. The second group are usually active whose symptoms are aggravated by sport and relieved by rest. Outerbridge (1961) characterised cartilage damage in CP into four grades:

Grade I	Softening and swelling or fibrillation/fissuring in an area < 0.5cm
Grade II	Fissuring or fibrillation/fissuring in an area of 0.5-1cm
Grade III	Fibrillation ("crabmeat appearance") or fibrillation/fissuring in an area 1-2cm (figure 4.2)
Grade IV	Erosive changes and exposure of subchondral bone or fibrillation in an area > 2cm (figure 4.3)

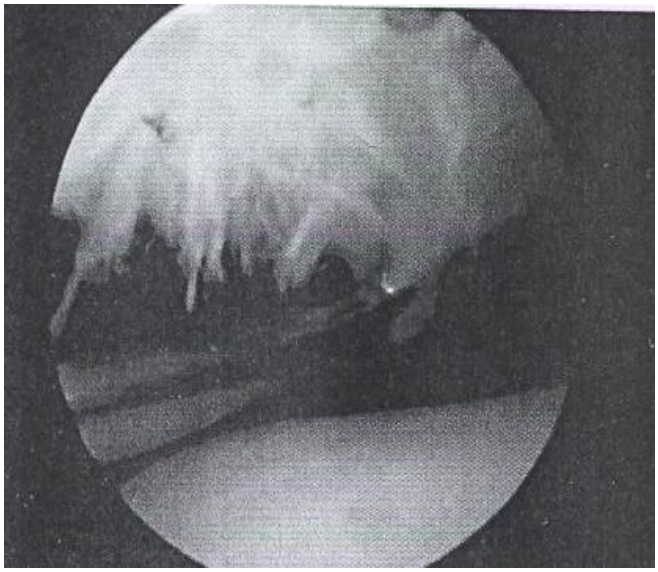


Figure 4.2: Retropatellar fibrillation

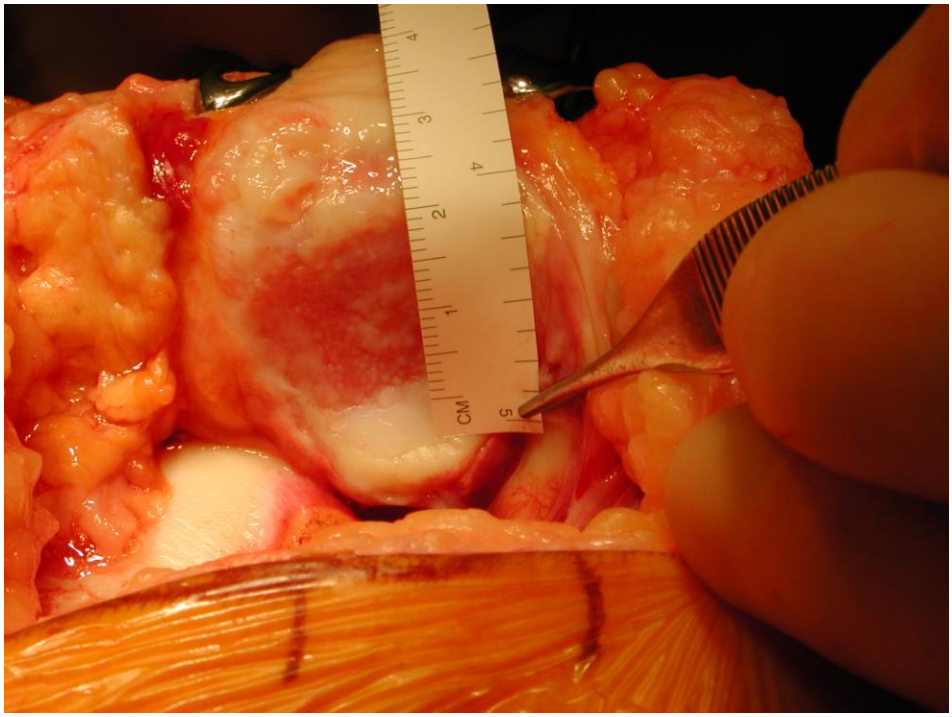


Figure 4.3: Full thickness retro-patellar chondral defect (Figures courtesy of G. Bentley)

Non-operative management

This mainly consists of physiotherapy to improve quadriceps strength. Specific focus on the vastus medialis muscle is also undertaken particularly if there is patella maltracking. It is important to improve function with physiotherapy but at the same time ensuring that the damaged patello-femoral articulation is not overloaded. Recently, more emphasis has been placed on overall body balance and stability. Such core strengthening focuses on abdominal muscle control, trunk balance and limb control. The expectation is that with improved alignment and balance there will be decreased pressure on the PFJ.

The patellar (McConnell) taping technique may be useful in cases of severe lateral patella translation and tilt. This depends on the integrity of the patient's skin to withstand repeated applications. Several braces have also been designed to shift the patella medially and off-load the lateral facet. This form of therapy largely depends on patients' co-operation and determination to put on the brace daily. Finally water-based exercises are useful, particularly in obese patients as joint forces are reduced during these exercise programs.

Surgical procedures to correct re-alignment

Arthroscopic lateral retinacular release

This procedure is frequently utilised in combination with chondroplasty to address lateral alignment and patellar tilt problems with lateral facet chondral damage. Release of the lateral retinacular structures will decrease the pressures on the lateral facet, whilst chondroplasty may provide temporary pain relief.

Proximal soft tissue re-alignment

This procedure is performed when the aim is to unload the lateral facet and improve patellar tracking. A midline incision is performed from the superior pole of patella to medial aspect of tibial tubercle. The lateral patellofemoral ligament and retinaculum is released. The vastus lateralis lower fibres are also released and the release is continued to the tubercle. The vastus medialis is elevated from underlying capsule approximately 10cm from its insertion. It is then advanced to the free edge of the vastus lateralis creating a sleeve around the patella.

Four years following surgery, a 91% patient satisfaction rate was reported by Insall et al (Insall et al. 1979). The outcome following this procedure is not predictable and when there are widespread arthritic changes the failure rate can be high as 62% (Fulkerson 2005). The focus is purely on the relieving pressure off the lateral facet. Therefore, any disease of the trochlea or medial facet may result in increased pain. Soft tissue alignment procedures in combination with other procedures to address chondral damage is indicated in skeletally immature patients (Mihalko et al 2007). In mature patients, success following this procedure has been reported when there is minimal arthritic changes and a Q angle of < 10 (Hughston & Walsh 1979).

Distal Re-alignment

Tibial tubercle transfer is mainly indicated in patients with discrete chondral damage in the patella or trochlea which can be unloaded. An absolute pre-requisite for this procedure is healthy cartilage onto which patella loading can be transferred. Several osteotomies can be performed:

Anterior/Elevation Osteotomy of Tibial Tubercle

This is also known as the *Maquet procedure* and is indicated in distally based disease in the patella. Although effective in younger patients with disease confined to the distal pole, it does not address maltracking (Maquet 1963) and has been abandoned.

Antero-medial Tibial Tubercle Transfer

This is also known as *Elmslie-Trillat procedure.* This mainly indicated in patients with large Q angles, lateral tracking of the patella with or without instability and grade 1/II patella lesions (Brown et al. 1984).

More recently a modification of the Roux-Goldthwaite distal re-alignment has been employed successfully, in which a lateral release, medial quadriceps reefing and medial transfer of the lateral two thirds of the patellar tendon is performed (Bentley 1989).

Anteromedial Tibial Tubercle Osteotomy

This is known as Fulkerson procedure. It involves the transfer of the tubercle to a more anterior and medial location. It is effective in diminishing or eliminating load on the distal and lateral aspects of the patella (Fulkerson & Becker 1990).

Treatment of pure chondral lesions

The general consensus in the literature is that the clinical results from treating cartilage lesions in the medial and lateral femoral condyles are considerably better than treating patellar defects. Microfracture has been shown to lead to inferior results when utilised in the patella (Kreuz & Steinwachs et al 2006, Kreuz & Erggelet et al 2006). Similarly, there are a number of studies reporting on the use of mosaicplasty but there are only a few that have

reported on the results of the patella (Bentley et al 2003, Hangody et al. 2003, Karataglis et al 2006). These studies have indicated that osteochondral plugs transferred to the patella are not as successful as transfer to the femoral condyles. Bentley et al. had shown the arthroscopic appearance was fair to poor in all patients treated with mosaicplasty in the patella (Bentley et al 2003). Hangody's group reported 79% good to excellent results when treating patella and trochlea lesions though this is still lower than 92% success rate when treating femoral condyle defects. The primary reason for this discrepancy has been postulated to be due to difference in articular cartilage thickness between trochlea (donor) and patella (host). The structural organization of the cartilage in the trochlea may not be suited for the biomechanical environment in the patellofemoral joint where shearing forces are more prevalent (Bentley et al 2003).

Both ACI and MACI have been compared in a small randomised trial of 91 patients. Of the 44 patients that had received ACI, fourteen lesions were located in the patellofemoral joint. Seventeen out of 47 patients that had received MACI had lesions in the PFJ. The proportion of good and excellent results were similar 54.5% versus 52.5% (p=0.72) (Bartlett et al. 2005). In another study, the frequency of good/excellent results were significantly higher when treating trochlea and lateral femoral condyle lesions compared with single facet patella and multiple patella lesions (84% versus 66% and 54.2%, p=0.05) (Krishnan et al. 2006).

Several other studies have reported satisfying results with different forms of autologous chondrocytes implantation in treating cartilage lesions in the trochlea, medial and lateral femoral condyles, whilst patella lesions are less predictable (Pascal-Garrido et al 2009, Niemeyer et al 2008, Minas et al 2005, Peterson et al 2002, Brittberg et al 1994). The possible explanation for the inferior results associated with treating patella lesions is the greater shearing forces in the PFJ compared to the lateral and medial compartments. This is a

less favourable environment for the differentiation of transplanted cells than the hydrostatic forces that is more prevalent in the region of the femoral condyles (Fitzgerald et al 2006, Torzilli et al 2006, Grodzinsky et al 2000).

Aims & Objectives

In most of the reported literature, patients with patella defects account for only a small proportion of the total patient population. Hence, it has been very difficult to analyse any prognostic factors that may have an effect on the outcome of treating this difficult cohort of patients. The purpose of this chapter is to identify such factors.

Patients and Methods

The study was approved by the Joint Research and Ethics Committee of the Royal National Orthopaedic Hospital (RNOH) Trust. The primary indication for surgery was persistent pain which was attributable to an articular cartilage lesion of the knee. The inclusion and exclusion criteria was the same as that outlined in Chapter 3 (ACI vs MACI, page 76). All the patients were operated in the Joint Reconstruction Unit at the RNOH between 2001 and 2004. As not all patients were randomised, this was a prospective cohort study. The study group comprised 215 patients. Thirty-five patients had other procedures at the time of the cartilage transplant such as patella re-alignment or anterior cruciate ligament (ACL) reconstruction. Hence, they were excluded from analysis. Of the remaining 180 patients, 84 had an ACI procedure and 94 had a MACI procedure. The primary outcome measure was the Modified Cincinnati Score (MCS). The patients were scored pre-operatively (MCS 0) and at 6 months (MCS 6), 12 months (MCS 12) and 24 months (MCS 24) after surgery.

Statistical Analyses

Statistical analyses were performed using SPSS version 18.0. Paired t-tests were used to compare knee scores before and after surgery. The independent t-test was used to compare outcome between two different groups of patients. Analysis of variance (ANOVA) was used to assess difference in outcome between 3 or more groups. Fisher's Exact Test was used to compare the proportion of excellent and good results achieved in each group and also to compare re-operation rates. The level of significance was set at $p=0.05$.

A generalised linear model was used to assess which factors had the greatest effect on outcome. The highest number of patients available for post-operative scores were in the MCS 12 group, hence in the model the dependent variable was MCS 12. The predictive factors in the model were type of operation (ACI versus MACI), aetiology, site of defect, and the number of previous operations. The predictive covariates were age of the patient, length of symptoms and MCS 0.

Results

The length of symptoms prior to the index procedure was longer in the ACI group and though this was not statistically significant the p-value was quite low (p=0.06) (table 4.1). On average ACI patients had to wait 2 years longer than MACI patients and this may well be a confounding variable. There were no other significant differences between ACI and MACI groups in terms of patient or lesion characteristics (see Table 1).

Table 4.1: Patient and lesion characteristics

	ACI n=84	**MACI n=92**	**p value**
Age	35.2 +/- 1	33 +/- 0.9	0.97
Percentage males	58	60	>0.05
Duration of symptoms (months)	104 +/- 11	80 +/- 7.3	0.06
Number of previous operations	2.1 +/- 0.2	2 +/- 0.2	0.7
Size of lesion (cm^2)	5.1 +/- 0.3	4.5 +/- 0.3	0.15
Modified Cincinnati Score pre-operatively (MCS 0)	43.8 +/- 1.7	43.8 +/- 1.8	0.99

Bivariate correlation showed that there was no significant relationship between length of symptoms and the change in MCS following surgery (Table 4.2). Interestingly, there was no a significant negative correlation between the number of previous operations and the change in functional score 12 and 24 months after surgery. As expected, MCS 0 correlated significantly with the change in MCS 12 months following surgery.

Table 4.2: Correlations between length of symptoms and change in functional scores

		Length of symptoms	MCS change 12	MCS change 24	No. of ops previously
Length of Symptoms	Pearson Correlation	1	-0.08	-0.1	0.2*
	Sig. (2-tailed)		0.4	0.4	0.04
	N	154	93	53	151
No. of ops Previously	Pearson Correlation	0.2*	-0.09	-0.2	1
	Sig. (2-tailed)	0.04	0.4	0.1	
	N	151	102	58	180
MCS 0	Pearson Correlation	-0.2*	-0.4**	-0.4*	-0.2*
	Sig. (2-tailed)	0.02	<0.001	0.01	0.025
	N	143	103	57	169

Table 4.3 shows that the distribution of lesions in the patello-femoral joint were similar in each group ($p>0.05$ for each anatomical site according to Fisher's exact test). There were a higher proportion of trauma patients in the MACI group (refer to table 4.4)) ($p=0.1$) and consequently a lower proportion of 'failed previous procedures' in the MACI group which was statistically significant ($p=0.02$). This group of patients had undergone a previous mosaicplasty or chondrocyte implantation (table 4.4).

Table 4.3: Anatomical site of chondral lesions according to treatment groups

Anatomical site	ACI	MACI
Trochlea	21 (24%)	21 (21.6%)
Multiple lesions (in PFJ)	20 (23%)	20 (20.6%)
Single facet patella	45 (53%)	57 (58.8%)
TOTAL	86	97

Table 4.4: Aetiology of lesions according to treatment groups

Aetiology	ACI	MACI
Trauma	53 (49%)	64 (61%)
Osteochondritis dissecans	13 (12%)	11 (10%)
Chondromalacia patellae	17 (15%)	19 (18%)
Failed previous procedure	26 (24%)	12 (11%)
TOTAL	109	106

Figure 4.4 shows the knee function between the two groups prior to surgery and at all time frames following surgery. It displays functional scores for descriptive purposes and allows comparison of absolute scores with other published work. Figure 4.5 shows that MACI was three times more effective than ACI at improving MCS, and even with such low numbers in the sample the p-value was statistically significant according to the independent t-test ($p=0.038$).

As with the previous chapter, statistical analysis was performed to ascertain the proportion of excellent and good results in order to compare the results from this thesis with other published work. There was not a statistically significant difference in the proportion of good or excellent results in the two groups (Table 4.5). However, when the improvement in MCS from pre-operatively to 2 years following surgery was analysed there was a significant difference.

Figure 4.4: Functional results with the modified Cincinnati Score (MCS)

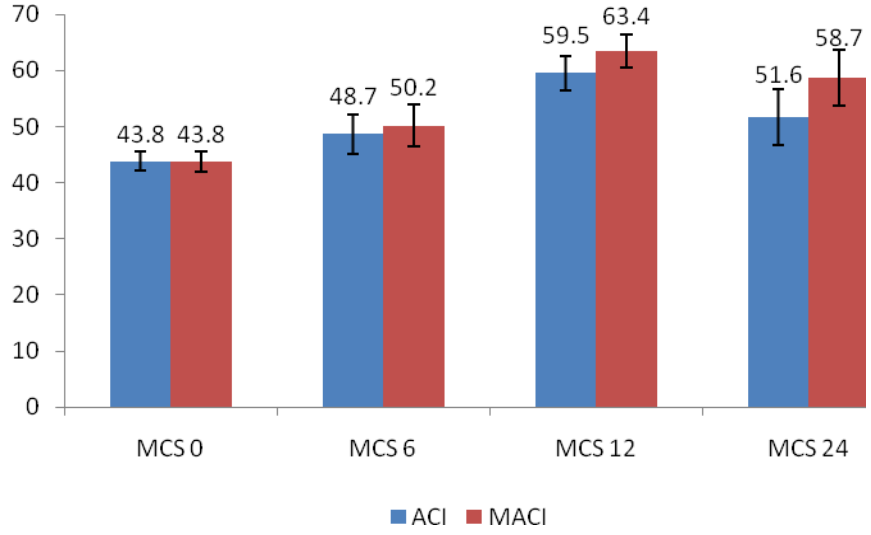


Figure 4.5: Change in MCS

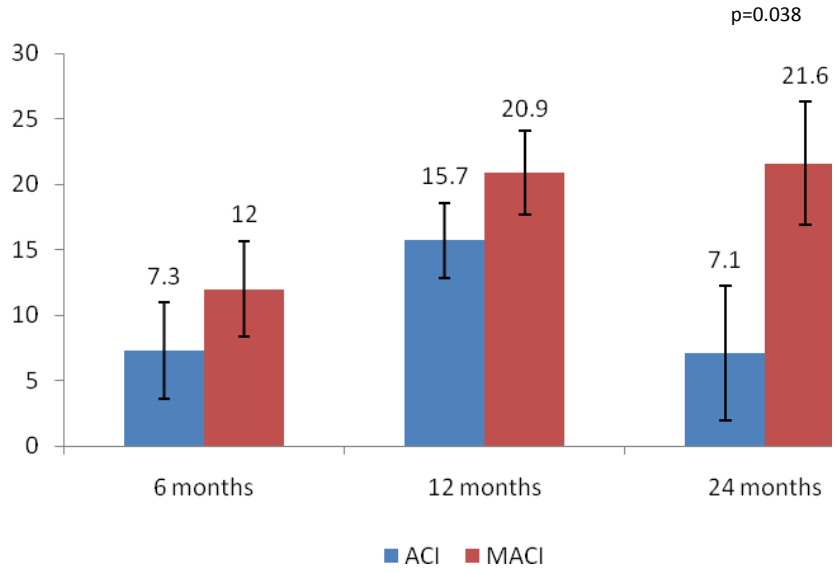


Table 4.5: Proportion of excellent and good results in ACI and MACI patients with lesions in the patellofemoral joint.

Time	Outcome	ACI	MACI	p value
6 months	Excellent	9 (22.5%)	8 (23%)	0.45
	Good	12 (30%)	9 (26%)	
	Fair	16 (40%)	15 (43%)	
	Poor	3 (7.5%)	3 (8%)	
	Total	**40**	**35**	
12 months	Excellent	12 (21.5%)	18 (31%)	0.4
	Good	26 (46%)	26 (45%)	
	Fair	11 (20%)	9 (15.5%)	
	Poor	7 (12.5%)	5 (8.5%)	
	Total	**56**	**58**	
24 months	Excellent	8 (25.8%)	5 (17.5%)	0.45
	Good	8 (25.8%)	7 (24%)	
	Fair	7 (22.6%)	7 (24%)	
	Poor	8 (25.8%)	10 (34.5%)	
	Total	**31**	**29**	

The p values are derived from performing the Fisher's exact test to compare proportion of good and excellent results according to the Modified Cincinnati Score (MCS).

Table 4.6: Complications and further procedures

Procedure	**ACI**	**MACI**	**p-value**
Manipulation under anaesthesia	9	2	0.03
Graft hypertrophy	7	3	0.2
Unplanned arthroscopy	3	3	-
Further realignment procedure	2	0	-
Graft delamination	0	1	-
Total	**21/86 (24.4%)**	**9/94 (9.6%)**	**0.009**

The reoperation rate was higher in the ACI group and this was statistically significant according to Fisher's exact test (p=0.009) (table 4.6). The main reason for the high reoperation rates were for knee stiffness which required a manipulation under anaesthesia. There were also higher rates of graft hypertrophy in the ACI group but this was not significant.

Predictors of outcome

The next section will analyse whether the site of the lesion specifically in the patello-femoral joint, the aetiology of the defect and the number of previous operations influences outcome following surgery. The ACI and MACI patients were grouped together and the mean MCS at each time frame was determined for each factor.

Site

Figure 4.6 illustrates the MCS according to the site of the lesion in all patients (i.e. ACI and MACI patients grouped together). Unfortunately, it was not specified whether the single lesion was in the medial or lateral facet of the patella in 56/160 patients (35%). It was for this reason that single defects in the patella were grouped together (whether they were in the medial facet or lateral facet or not specified). Once again the graph displays the absolute MCS at all time frames for descriptive purposes.

Figure 4.7 displays the change in MCS from pre-operative to 6, 12 and 24 months after surgery. ANOVA revealed a significant difference in efficacy of surgery when treating trochlear lesions compared to single and multiple patella lesions one year following surgery ($p=0.01$).

Figure 4.6: Modified Cincinnati Scores (MCS) According to site of lesion in all patients

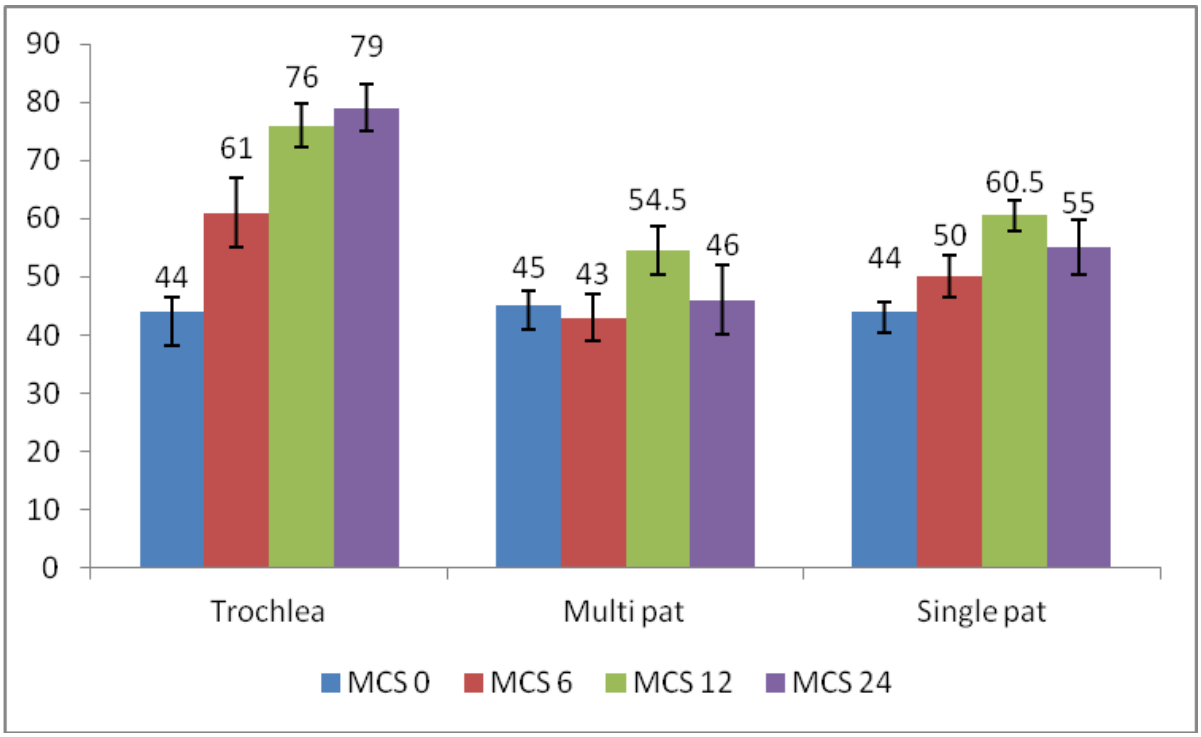
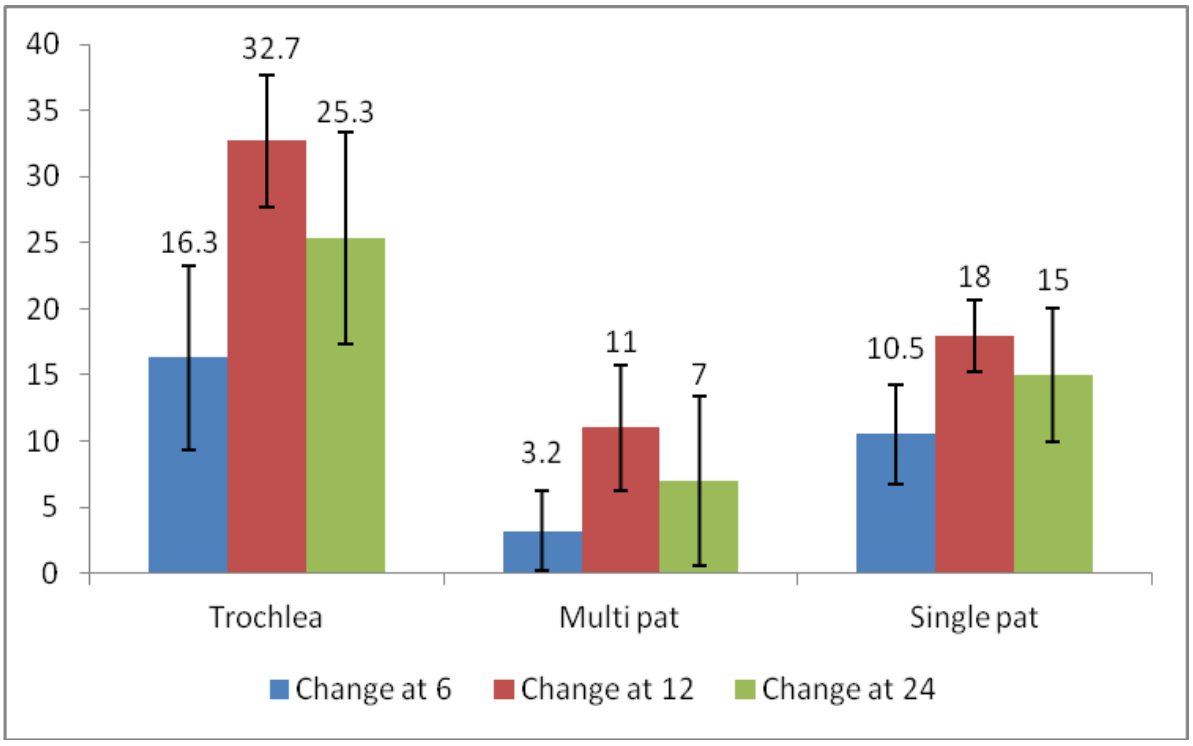


Figure 4.7: Change in MCS from baseline according to site of lesion in all patients



Aetiology

Figure 4.8 illustrates the MCS according to the aetiology of the lesion in all patients. Patients with a failed previous procedure had a much lower MCS 0 than patients being treated for traumatic lesions or patients with chondromalacia patellae (CP). Other diagnoses included osteochondritis dissecans or early osteoarthritis. Figure 4.9 shows the change in MCS from baseline according to aetiology. There were no significant differences between the groups at any of the time frames. All groups experienced am improvement in the MCS from baseline at all time frames, though the improvement in MCS declined after 1 year. One way ANOVA was performed to determine if there was a significant difference in the change in MCS from baseline to 1 year post-operatively (MCS 12). There was no statistically significant difference between the groups (p=0.3).

Figure 4.8: The MCS according to aetiology in all patients

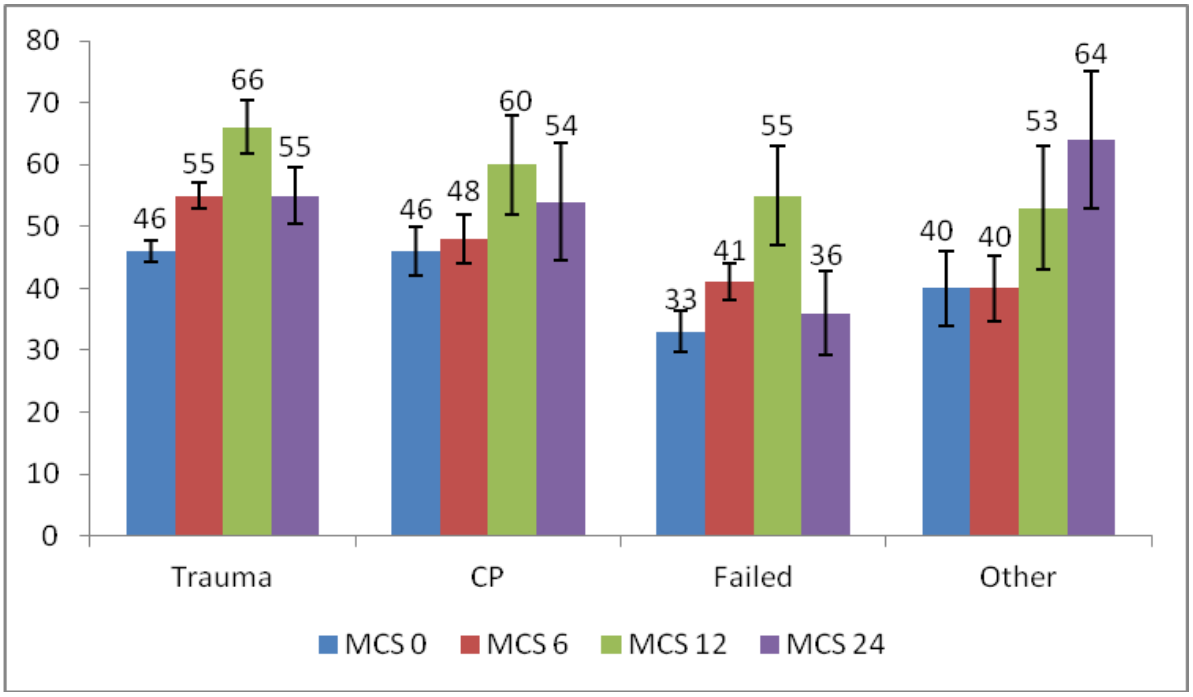
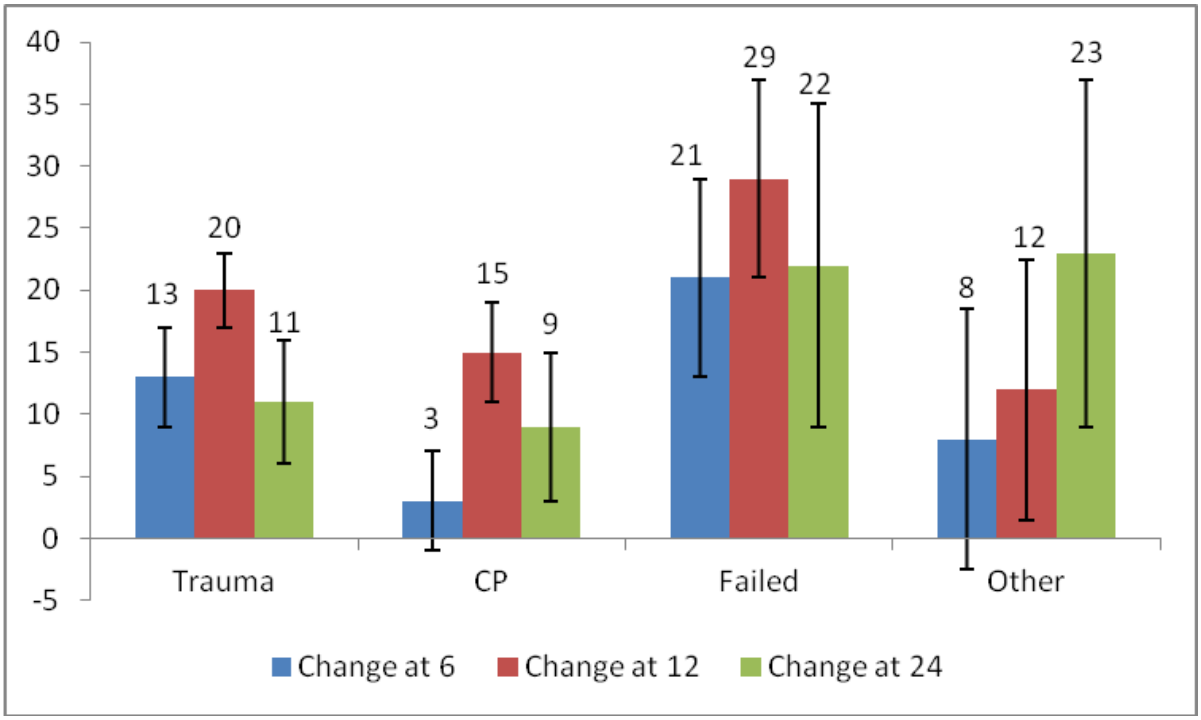


Figure 4.9: The change in MCS from baseline in all patients



Number of previous operations

Figure 4.10 illustrates the MCS according to the number of previous operations. Patients who had 4 or more previous operations had a lower MCS 0. Figure 4.11 demonstrates the change in MCS from baseline. What is clearly evident is that at the time latest follow-up, patients who have less than 4 operations prior to their index procedure (1st stage ACI or MACI) experience greater improvement in their knee function (as measured by MCS) than those have had 4 or more (ANOVA, p=0.03).

Figure 4.10: The MCS in patients according to the number of previous operations

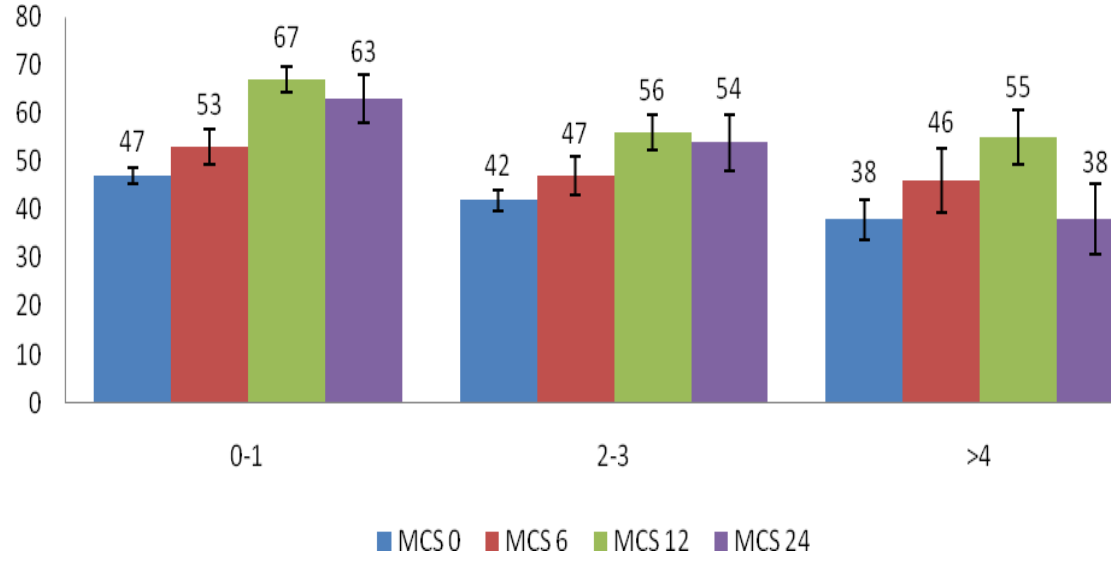
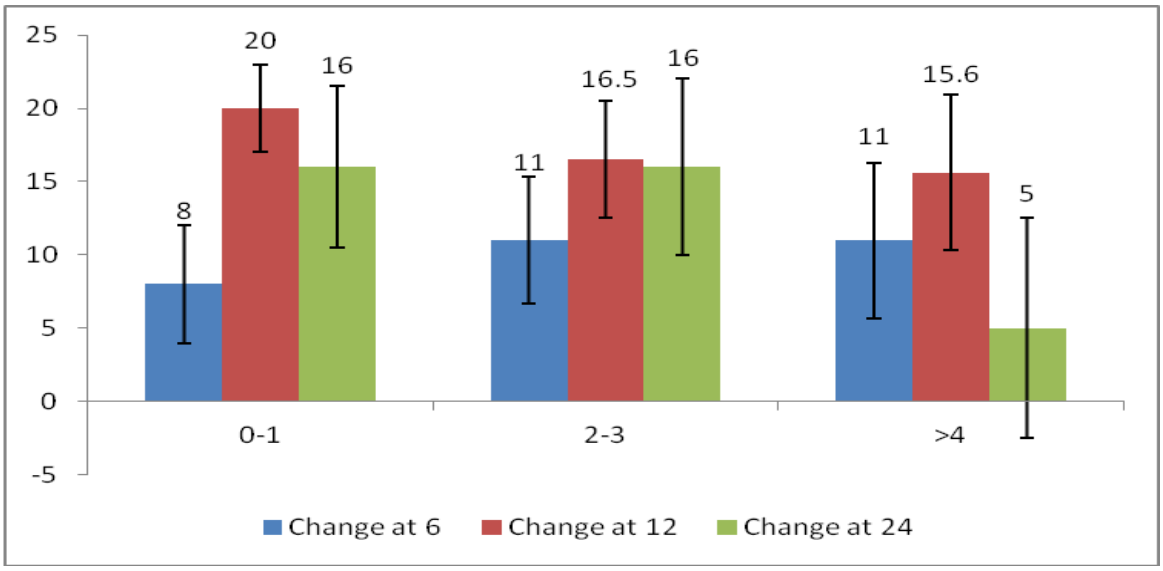


Figure 4.11: The change in MCS from baseline according to number of previous operations



Generalised Linear Model (GLM)

92 patients (with documented MCS one year following surgery) had a complete set of records which could be utilised in this model (this was the dependent variable). Once again, statistical analysis involved the generation of two GLMs. In the first GLM the categorical variables included sex of patient and type of surgery and the continuous variables included MCS 0 and age of patient (i.e. the significant factors from the previous chapter). This was done to ascertain if MACI was truly better than ACI in improving knee function after taking into consideration all other significant variables. The second GLM will include other factors such as aetiology, number of previous operations, site of lesion and duration of symptoms.

Table 4.7: Results of the limited GLM

Parameter	Magnitude of Effect	Std. Error	95% Confidence Interval		
			Lower	Upper	Sig.
Co-variates					
Male	7.9	3.9	0.2	15.7	0.04
Female	0[a]	.	.	.	.
ACI	-4	3.9	-11.7	3.6	0.3
MACI	0[a]	.	.	.	.
Continuous					
Age	.002	.0029	-.004	.007	.536
MCS 0	.445	.1245	.201	.689	.000

0[a] represents the reference category for comparison of other categorical data within the group.

In this model the only significant factors were being a male patient and the pre-operative MCS (MCS 0).

Table 4.8 displays the categorical data information and table 4.9 the continuous variable information in the more comprehensive model. Table 4.10 shows the results of the statistical analysis. The number of previous operations were categorised into either less than 4 or more than 4 as the results in figure 4.11 showed that the improvement in MCS were similar in patients if they had 0-1 previous operations or 2-3. Furthermore, patients with other diagnoses were grouped together with patients with chondromalacia patellae as 'Other'.

Table 4.8: Categorical Variable Information

			N	Percent
Factor	**Type**	ACI	50	53.2%
		MACI	42	46.8%
		Total	92	100.0%
	Aetiology	Others	44	48%
		Failed procedure	11	12%
		Trauma	37	40%
		Total	92	100.0%
	Site	Trochlea	13	14.1%
		Multi pat	23	25%
		Single pat	56	60.9%
		Total	94	100.0%
	Sex	Male	41	44.6%
		Female	51	55.4%
		Total	92	100%
	Previous Ops	4 or more	14	15.2%
		Less than 4	78	84.8%
		Total	94	100.0%

Table 4.9: Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	MCS 12	92	14	100	61.8	22
Covariate	Age	92	16	52	33.7	9.1
	MCS 0	92	10	74	43.6	15.7
	Duration symptom	92	2	360	92.5	82.2

Table 4.10: Results of the Generalised Linear Model

Parameter	Magnitude of Effect	Std. Error	95% Confidence Interval		Sig.
			Lower	Upper	
Type of surgery					
ACI	-4.2	4	-12.1	3.9	0.3
MACI	0[a]	.	.	.	.
Sex					
Male	5.4	4.4	-3.3	14	0.2
Female	0[a]	.	.	.	.
Aetiology					
Other	-2	4.6	-11	6	0.6
Failed previous operation	-1	7.2	-15	13	0.9
Trauma	0[a]	.	.	.	.
Site					
Trochlea	14	6	3	25	0.02
Multiple lesion	-1.1	4.9	-10.6	8.6	0.8
Single lesion	0[a]	.	.	.	.
Previous Operations					
4 or more	-4	5.5	-14.6	7.2	0.5
Less than 4	0[a]	.	.	.	.
Continuous Variables					
MCS 0	0.4	0.15	-0.06	0.6	0.02
Age	-0.4	0.2	-0.8	0.06	0.09
Duration of symptoms	-0.05	0.03	-0.1	0.001	0.05

0[a] represents the reference category for comparison of other categorical data within the group.

When taking all confounding variables into account, there does not appear to be a difference between ACI and MACI in improving knee function after cartilage repair.

Lesions in the trochlea achieve better results than in the patella (single or multiple) and this variable has the most significant effect on outcome. Interestingly, the duration of symptoms prior to surgery did affect MCS 12, whereas the number of previous operations did not have a significant effect on outcome. In the previous statistical analysis (table 4.2), the correlation between length of symptoms and MCS 12 ($r=-0.3$, $p=0.001$) was greater and more significant than the number of previous operations and MCS 12 ($r=-0.22$, $p=0.02$). This further underlines the importance of the generalised linear model to analyse variables that may affect outcome. In the previous chapter duration of symptoms did not have an effect on outcome. Perhaps waiting longer for cartilage repair when the lesion is in the patella is more detrimental

There was a negative correlation between age and MCS 12. Although the p value was low it failed to reach statistical significance. In the chapter on ACI vs MACI, age did have an effect on outcome. Perhaps fewer patients in this chapter has led to a type II statistical error.

Discussion

In this chapter, it has been shown that both ACI and MACI are effective in significantly increasing knee function 12 and 24 months following surgery. There were no statistically significant differences in the pre- and post-operative scores in the two groups. When the paired data was investigated (i.e. analysing the increase in MCS in patients that had both pre- and post-operative scores) there was a significant difference in the increase in MCS 24 months following surgery. The MACI group experienced an increase of 21.6 points whereas the ACI group experienced an increase of only 7.1 point. A confounding variable in this analysis is that the MACI group had a shorter duration of symptoms. This issue was addressed in the Generalised Linear Model (GLM) and will be discussed later.

Only one study has compared ACI and MACI. In a cohort of 91 patients, 28 patients had patellar lesions of which 11 had ACI and 17 had MACI. There were no excellent results in the ACI group and 4 in the MACI group, but with such few numbers, it was not statistically significant (Bartlett et al. 2005). Taking the whole group together (ACI and MACI) there were no differences in outcome when comparing patella lesions with medial femoral condyle lesions. However Krishnan et al. (2006) showed that the proportion of excellent and good results in the trochlea and medial femoral condyle was 84.2% and 84% respectively and this compared favourably to patella single defects (66%) and patella multiple defects (54.2%).

Several other studies have assessed the efficacy of different types of ACI in the patellofemoral joint alone. 32 patients with chondral lesions in the patellofemoral joint (22 in the patella and 10 in the trochlea) were treated with a Hyaluronan based scaffold seeded with autologous chondrocytes much like MACI (Gobbi et al. 2006). The mean subjective International Knee Documentation Committee (IKDC) score increased from 43.2 before

surgery to 73.6 two years after surgery ($p<0.0001$). Once again trochlea lesions obtained better results than patella lesions ($p=0.001$). Although patients with osteochondritis dissecans experienced improvement in IKDC scores this was not significant.

Henderson et al reported on the results of 1st generation ACI techniques (with a periosteal patch) in 44 patients with patella lesions (Henderson et al. 2006). The patients were divided into 2 equal groups; group A (n=22) had ACI with patella realignment and group B (n=22) had patella ACI and normal patella tracking. Group A experienced greater increase in MCS, better SF-36 function and physical component scores and better overall IKDC scores than group B. They speculated that the difference in results may be a result of the unloading effect of the osteotomy, since patellar tracking is normal in the two groups after realignment. Gross suggested that when performing patella ACI an unloading osteotomy should be also be performed if the lesion is in a compartment subjected abnormal load, much like a high tibial osteotomy for lesions in the medial femoral condyle and varus aligned knee (Gross 2002).

In the heterogeneous group of 45 patients reported by Minas et al 62% had distal patellar re-alignment in the form of Fulkerson's anteromedialization (Minas and Bryant 2005). Furthermore, the best functional outcome was in patients with isolated lesions in the patella or trochlea rather than multiple lesions (i.e patella lesion + femoral condyle lesion or multiple patella lesions). Similar results were reported by Pascual-Garrido et al (2009) in 52 patients. The authors' divided their cohort of 52 patients into three groups; 1) isolated ACI treatment, 2) ACI with realignment procedure, and 3) ACI with realignment procedure with history of failed microfracture procedure. There were no statistical differences between outcomes in patients with a history of a previous failed microfracture compared with those with no such history. Patients undergoing anteromedialisation tended toward better outcomes than those

without realignment. However the re-operation in the whole cohort was high at 44% (Pascual-Garrido et al 2009).

In the initial report of ACI by Brittberg et al., a successful outcome in patella lesions only occurred in 2 out of 7 patients (Brittberg et al 1994). Later reports by the same group reported good or excellent results in 11/17 patients at 2 years (Peterson et al 2000) and 13/17 patients at 10 years (Peterson et al 2002**)** because they address patella realignment at the time of chondrocyte implantation. It is clear from these studies that outcome after ACI in the patella is improved with patella realignment procedures if there is patella maltracking. The question rises as to whether outcome can be improved following patella ACI with concomitant anteromedialisation procedures in pure type II patella lesions or laterally based trochlea lesions. The only way to address this question would be to perform a randomised trial with one group having patella ACI in the lateral facet, normal tracking, and anteromedialisation procedure and another group having patella ACI in the lateral facet, normal tracking and no realignment procedure. This is why the conclusions by Henderson et al do not seem accurate as they are comparing results in two different groups of patients (Henderson et al. 2006).

Predictors of outcome

This study has highlighted differences in outcome according to site of the lesion in the PFJ, as well as aetiology and number of previous operations. The GLM also highlighted site of lesion as the most significant predictor of outcome, such that both lateral and medial facet lesions are predicted to have a MCS 12 which is approximately 20 points inferior to trochlea lesions. In this model, patients with failed previous procedures were predicted to have a MCS 12 that was 14.9 points less than patients with traumatic lesions though this did not reach statistical significance. Similarly, patients that had 2-3 operations had a MCS 12 that was 10.5 points less than patients with 0-1 previous procedures (p=0.02). However, patients that had 4 or more procedures had 11.3 points less than the 0-1 group, though this was not significant (p=0.07). MCS 0 was the only continuous variable that predicted MCS 12.

These results are similar to one of the largest case series of purely patellar chondral defects treated with ACI (Niemeyer et al. 2008). 70 patients were treated with either ACI or MACI (depending on surgeon preference) and the mean follow-up was 38.4 months. They reported good or excellent outcome in 67.1% of patients with no difference in outcome between ACI and MACI. They discovered defects in the lateral facet had better post-operative scores than defects in the medial facet or both facets. This is one of the few studies to utilise multivariate analysis to assess prognostic factors; defect location (p=0.02) and age (p=0.048) significantly affected outcome whereas defect size (p=0.068) just failed to reach statistical significance. Other factors such as number of previous procedures, BMI, pre-operative sports activity and length of follow up did not have a statistically significant effect on outcome. Analysing their results further, the mean defect size in the lateral facet was 4.4cm^2 whereas lesions involving both facets, the mean size was 6.2cm^2 (nearly 50% more). Therefore the observed difference in outcome could be explained by location as well as size. This is where the GLM in the

analysis would be extremely useful as it would allow quantification of the effect the size and location of the lesion on outcome whilst taking into account the interaction of these two variables. Another a key flaw in their analysis was that they had not taken into account the pre-operative functional scores of the patient when performing multivariate analysis.

Summary

The results from this study are similar to those reported in literature when treating osteochondral defects of the patello-femoral joint. The results suggest that MACI is more efficacious than ACI when treating chondral lesions in the patella. The overall re-operation rate is also significantly lower in MACI patients ($p=0.009$) and this may reflect the technically challenging nature of performing ACI in patella lesions. Once again numerous operations prior to chondrocyte implantation should be avoided to obtain the best results. If patients have had a failed previous chondrocyte implantation or mosaicplasty on the patella then ACI should probably be avoided and MACI should be considered.

Chapter 5:
Radiographic Evidence of Osteoarthritis

Introduction

The diagnosis of osteoarthritis (OA) is made on the basis of radiographic and clinical findings. The radiographic criteria were first proposed by Kellgren and Lawrence (1957) and this will be discussed in detail later. The American Rheumatism Association described OA as 'a heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone and joint margins' (Altman et al. 1986). Table 5.1 displays how OA is classified and is reproduced from the original paper (Altman et al 1986). The signs and symptoms described by patients with OA of the knee are well established. Evidence from published literature is divided as to the natural history of cartilage defects in the knee in patients without OA (Amin et al 2005, Cicuttini et al 2005, Wang et al 2005, Hunter et al. 2006). However, generally, it is accepted that in patients with symptomatic cartilage defects in the knee, it is likely that patients will develop the full clinical spectrum of knee OA and in an effort to avoid performing arthroplasty in relatively young patients cartilage repair surgery should be performed initially (Biswal et al 2002).

Table 5.1: Classification for subsets of osteoarthritis

I. Idiopathic
  A. Localized
    1. Hands: e.g., Heberden's and Bouchard's nodes (nodal), erosive interphalangeal arthritis (non-nodal), scapho-metacarpal, scaphotrapezial
    2. Feet: e.g., hallux valgus, hallux rigidus, contracted toes (hammer/cockup toes), talonavicular
    3. Knee
      a. Medial compartment
      b. Lateral compartment
      c. Patellofemoral compartment (e.g., chondromalacia)
    4. Hip
      a. Eccentric (superior)
      b. Concentric (axial, medial)
      c. Diffuse (coxae senilis)
    5. Spine (particularly cervical and lumbar)
      a. Apophyseal
      b. Intervertebral (disc)
      c. Spondylosis (osteophytes)
      d. Ligamentous (hyperostosis [Forestier's disease, or DISH])
    6. Other single sites: e.g., shoulder, temporomandibular, sacroiliac, ankle, wrist, acromioclavicular
  B. Generalized: includes 3 or more areas listed above (Kellgren-Moore, see ref. 18)
    1. Small (peripheral) and spine
    2. Large (central) and spine
    3. Mixed (peripheral and central) and spine

II. Secondary
  A. Post-traumatic
  B. Congenital or developmental diseases
    1. Localized
      a. Hip diseases: e.g., Legg-Calvé-Perthes, congenital hip dislocation, slipped capital femoral epiphysis, shallow acetabulum
      b. Mechanical and local factors: e.g., obesity (?), unequal lower extremity length, extreme valgus/varus deformity, hypermobility syndromes, scoliosis
    2. Generalized
      a. Bone dysplasias: e.g., epiphyseal dysplasia, spondylo-apophyseal dysplasia
      b. Metabolic diseases: e.g., hemochromatosis, ochronosis, Gaucher's disease, hemoglobinopathy, Ehlers-Danlos disease
  C. Calcium deposition disease
    1. Calcium pyrophosphate deposition disease
    2. Apatite arthropathy
    3. Destructive arthropathy (shoulder, knee)
  D. Other bone and joint disorders: e.g., avascular necrosis, rheumatoid arthritis, gouty arthritis, septic arthritis, Paget's disease, osteopetrosis, osteochondritis
  E. Other diseases
    1. Endocrine diseases: e.g., diabetes mellitus, acromegaly, hypothyroidism, hyperparathyroidism
    2. Neuropathic arthropathy (Charcot joints)
    3. Miscellaneous: e.g., frostbite, Kashin-Beck disease, Caisson disease

* DISH = diffuse idiopathic skeletal hyperostosis.

Whilst it has been demonstrated that knee cartilage defects are prevalent in patients with radiographic evidence of OA (Link et al 2003), it has not been fully established whether asymptomatic cartilage defects are a risk factor for OA or indeed cartilage loss (Squires et al 2003). Cartilage volume loss in the knee has been shown to be associated with worsening of knee symptoms (Wluka et al. 2003). Furthermore, those with knee OA who are in the highest tertile of knee cartilage loss have a 7-fold increased risk of requiring knee arthroplasty within 4 years. Cicuttini et al., further demonstrated that the presence of asymptomatic chondral defects in the medial compartment identifies healthy individuals most likely to lose knee cartilage and subsequent development of knee OA (Cicuttini et al. 2004, 2005). In their longitudinal study, knee cartilage volume was estimated with T1-weighted fat suppressed magnetic resonance imaging (MRI) in 86 volunteers at base-line and at a 2-year follow up visit. The reduction in total cartilage volume from the tibia and femora were statistically significantly higher (19-25% greater) in volunteers with cartilage defects than those without ($p<0.05$).

Davies-Tuck et al. also reported that cartilage defects in patients with symptomatic OA tends to progress over a two year period and factors associated with progression were increasing age and baseline tibial bone area (Davies-Tuck et al 2007). Cartilage defects were graded 0-4 according to MRI and overall, 81% of defect scores increased, 15% remained unchanged and 4% decreased. It was interesting to note that the cartilage defects scores increased in all compartments ($p<0.001$) except the lateral tibial compartment which remained largely unchanged.

The treatment of patients with mild to moderate arthritic changes in the knee and the presence of a symptomatic chondral defect is complicated by the limited treatment options. Furthermore, the patients tend to be younger than those with OA requiring arthroplasty and,

therefore, the expectations are much higher in terms of return to function and physical activity. Non-operative measures such as non-steroidal anti-inflammatory drugs, steroid and hyaluronan intra-articular injections may provide symptomatic relief, but do not prevent disease progression.

Arthroscopic debridement of chondral lesions in patients with OA was widely performed as it was relatively quick and minimally invasive with few complications. However, this procedure has been proven to be not very effective. In the landmark paper by Moseley et al. (2002) 180 patients with OA of the knee were randomised into three treatment groups; arthroscopic debridement, arthroscopic lavage or placebo surgery (patients received skin incision, simulated debridement without insertion of an arthroscope). Paired t-tests revealed no difference in knee pain and functional scores before surgery and at any of the time frames after surgery in any of the three groups. Furthermore at no point was there a difference in scores post-operatively in the three groups or a difference in the change in scores.

There are few studies assessing cartilage repair techniques in patients with widespread arthritic changes. Bae et al. evaluated the clinical, radiological, histological and second-look arthroscopic findings in 49 knees (46 patients) that had undergone microfracture (Bae et al. 2006). The mean age of the patients was 57 and the mean defect size was 3.9cm^2. At mean follow-up of 2.3 years, good to excellent results (as measured by Baumgaertner's functional score) were obtained in 89% patients. Radiographic evaluation had revealed that the joint space had widened 1.1mm on an antero-posterior view and1.4mm on the lateral views which were statistically significant. Second look arthroscopy had revealed healing rates of 91-99% if the defects were less than 3cm^2. Only 18 cases were evaluated histologically and all had appeared to have significant amount of fibrocartilage in the regenerate tissue. According to analysis by Western Blotting, the amount of type II collagen varied from 20-70%. There are

several problems with this paper which leads me to question the validity of their conclusion that microfracture is efficacious in the treatment of full thickness chondral defects in patients with OA. Firstly, in the 49 cases of microfracture, 41 cases had simultaneous procedures (30 medial menisectomies, 7 excisions of suprapatellar plica excisions, and 4 cases of lateral meniscal surgery). Therefore, the post-operative improvement in functional scores can not all be contributed towards the microfracture performed. Secondly, though there was statistically significant increase in joint space as measured by radiographs, I have to question the clinical significance of 1mm and finally only a small proportion of patients underwent histological (36.7%) and Western Blotting (42.9%) analysis and this may not represent the true patient population.

It is generally accepted that autologous chondrocyte implantation is not efficacious when treating lesions in an arthritic joint, hence patients with widespread OA, or an inflammatory arthropathy are excluded from this treatment. However, the evidence for this is not so robust. One may question the ability of chondrocytes from arthritic joints to expand adequately and then function when implanted to the same level as chondrocytes from non-arthritic joints. In an *ex vivo* model, Stoop et al. reported similar levels of type I and II collagen production in chondrocytes taken from OA patients and chondrocytes taken from patients who are about to undergo ACI (Stoop et al. 2007). However, there was an exponentially higher level of mRNA production of IL-1β from OA chondrocytes compared with 'healthy' individuals. This cytokine is secreted by activated macrophages and is an important mediator of the inflammatory response. Furthermore, IL-1β is known to induce chondrocytes-mediated cartilage degradation and to reduce type II collage expression (Smith et al 1989). Bearing this in mind, the authors then implanted the same cells expanded and seeded on collagen scaffolds into immunodeficient mice to assess their ability to generate hyaline-like cartilage. Eight

weeks after implantation, no cartilage formation was observed in the empty control scaffolds, whereas there was cartilage like tissue formed from cells of OA patients but not healthy individuals when the cell concentration was 1 X 10^6 cells/cm^2 in 1/3 donors. When the concentration was 3 X 10^6 cells/cm^2 all three donors exhibited cartilage formation. However, this concentration is 3 times higher than the current levels used in human ACI.

In another animal model, where OA-like degenerative process was created in a rodent knee by transecting the ACL, a three dimensional ACI was implanted in the 'trochlea' region. Reverse transcription polymerase chain reaction analysis demonstrated the expression of type II collagen in the chondrocytes implanted group, but not in the groups with no chondrocytes present. This finding was also confirmed macroscopically and histologically (Kuroda et al. 2011).

Tom Minas's group in Boston are one of the centres in which ACI is performed in the United States of America and the procedure is performed even in the presence of mild OA (defined as less than 50% joint space loss). In their review of ACI in patients with early OA, they reported 93% survivorship rates at 5 years with the end point being arthroplasty. There was also a mean improvement of WOMAC pain score of 4.9 points (51% improvement) and WOMAC function scores of 15.7 points (53%) (Minas et al 2010). Similar results were reported by others on the efficacy of ACI in treating large complex lesions and kissing lesions (Ossendorf et al. 2011, Kon et al 2011, Kreuz et al 2009, Osendorf et al. 2007).

Aims & Objectives

The aim of this chapter is to investigate whether patients with evidence of early osteoarthritis (OA) on their pre-operative radiographs of the knee were associated with worse outcome following ACI than those without any evidence of OA. It was also determined whether ACI or MACI was effective in reducing the need for arthroplasty which is effectively an indication of end stage disease (OA). The rationale for performing cartilage transplant in patients with osteochondral defects and early OA is that once the cartilage lesion is treated and any unstable flaps of cartilage are removed, the progression of OA should be reduced and, knee function improved.

A secondary aim was to assess the reliability and reproducibility of the Kellgren and Lawrence and the Stanmore Grading system for evaluating severity of OA in knee radiographs (Table 5.2 and figures 5.1 to 5.5).

Patients and Methods

This was a retrospective case-control study of patients who had autologous chondrocyte implantation (ACI) or matrix-carried autologous chondrocyte implantation (MACI).

The pre-operative radiographs of 200 patients that had undergone ACI or MACI from July 1998 to December 2008 were randomly retrieved from archives. They were reviewed by 2 independent observers who were blinded to the outcome of surgery and what kind of surgery the patient had received. The presence of osteoarthritis (OA) radiologically was determined and, if present, was graded according to Kellgren and Lawrence and The Stanmore Grading System (see table 5.2 below). The unweighted Kappa statistic was used to establish levels of agreement when analysing inter-observer variation. The Landis & Koch criteria was used to interpret results:

(Landis and Koch 1977)

- < 0.2 slight agreement
- 0.21 – 0.4 fair
- 0.41 to 0.6 moderate
- 0.61 to 0.8 substantial
- > 0.8 almost perfect

Table 5.2: The Grading Systems Used to Assess OA

Grade	Kellgren & Lawrence	Stanmore Grade
0	No arthritis	No arthritis
1	Doubtful	Joint space narrowing
2	Minimal	Presence of osteophytes
3	Moderate	2 + subchondral sclerosis
4	Severe	3 + early bone loss with or without subchondral cysts
5		4 + joint disorganization + destruction

Figure 5.1: Radiograph showing Stanmore grade I changes – joint space narrowing

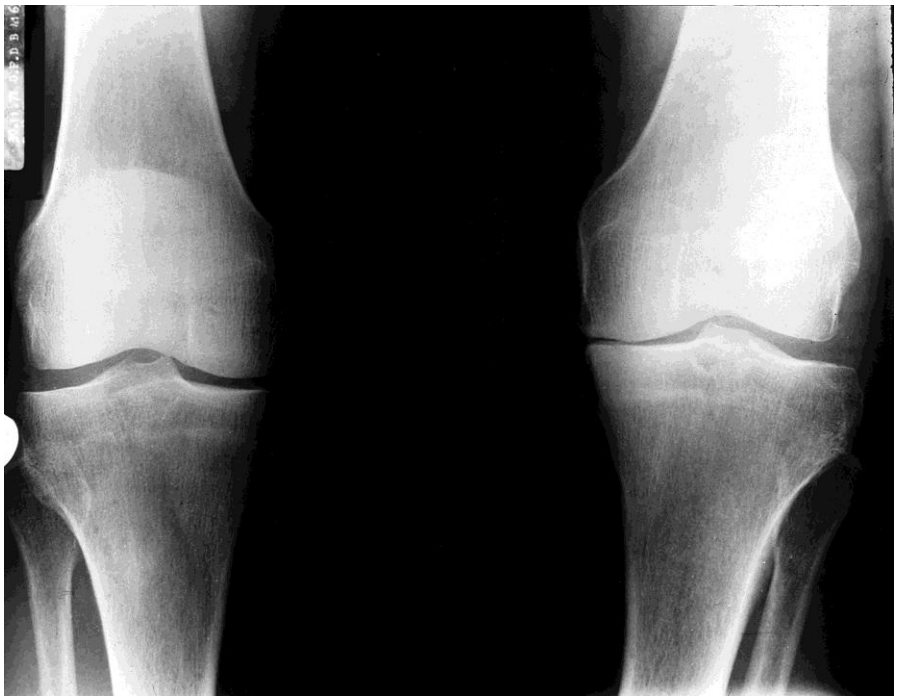


Figure 5.2: Stanmore grade II – Osteophyte formation

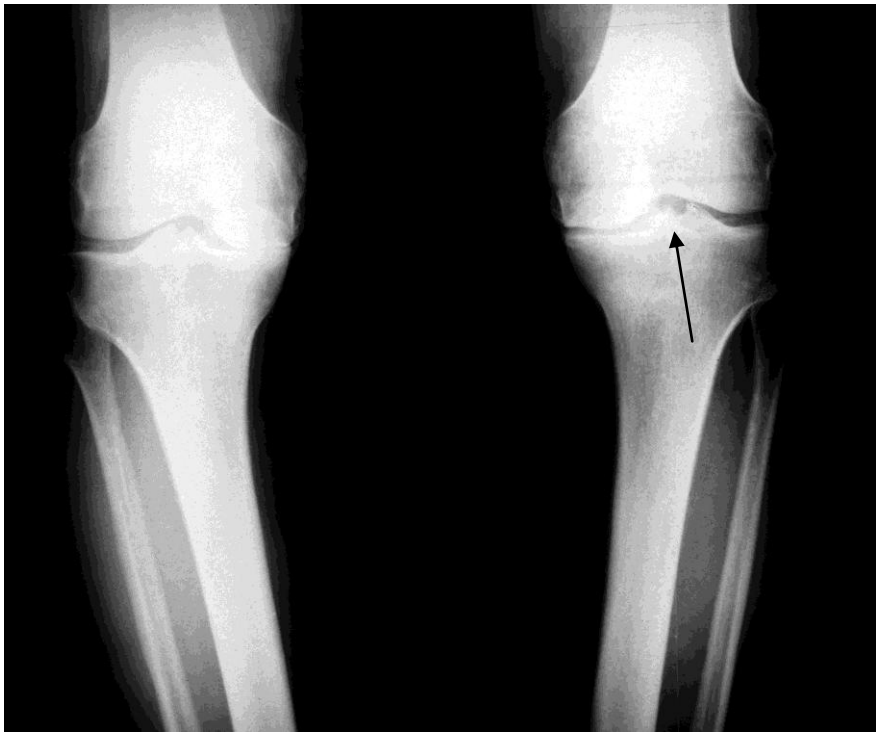


Figure 5.3: Stanmore Grade III – subchondral sclerosis

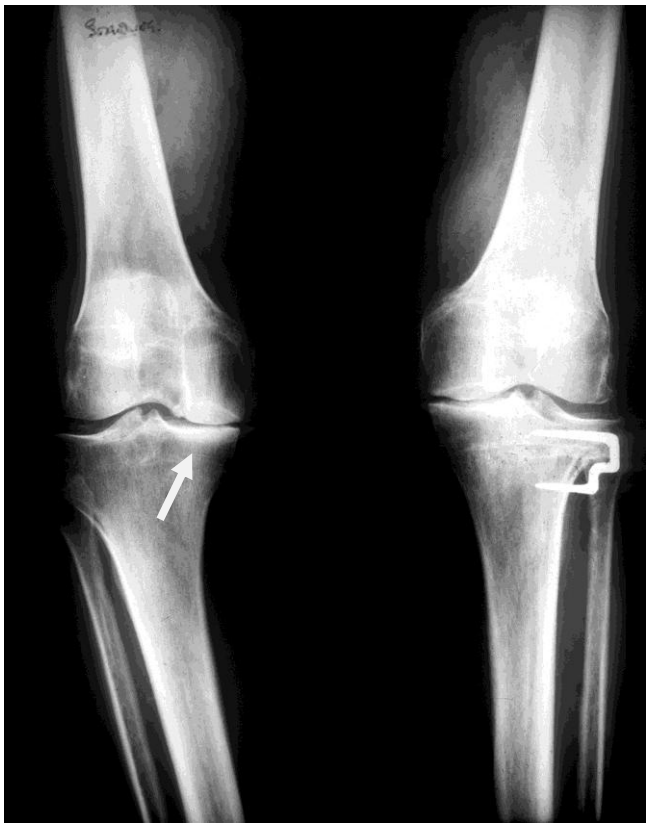


Figure 5.4: Stanmore grade IV – Bone cyst

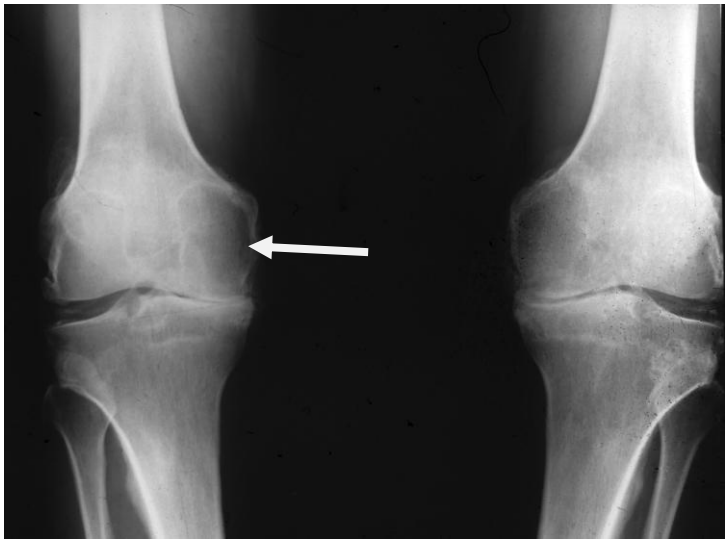
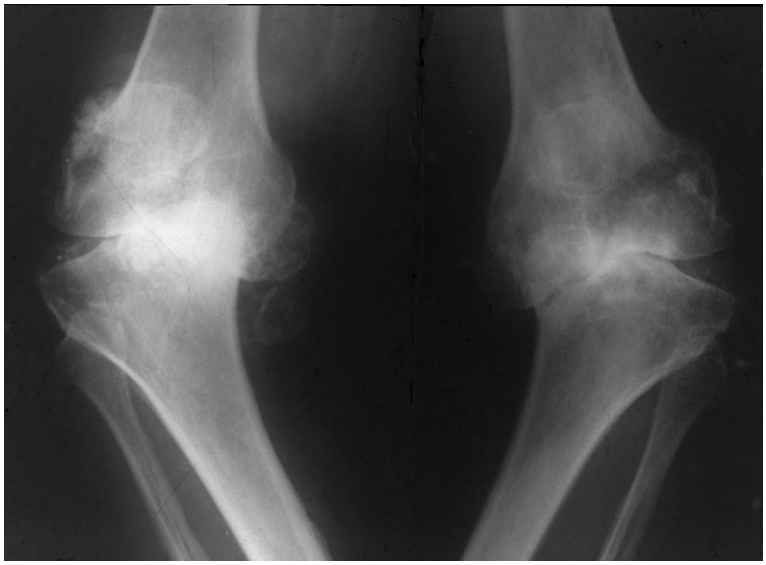


Figure 5.5: Stanmore grade V – joint destruction



Figures courtesy of G. Bentley (personal communication)

Knee function before and after surgery was assessed according to the Modified Cincinnati Score (MCS). An attempt was made to contact all patients who had their radiographs reviewed by telephone or post so that the most up to date MCS was also available, as generally after 2 years patients were failing to attend their clinical appointments.

An earlier pilot study enabled us to perform sample size calculations. With $\alpha = 0.05$ and $\beta = 0.2$ (hence a power of 80%), a total of 116 patients would be required (58 in each group) to detect a difference of 10 points in the Modified Cincinnati Score.

Results

The patient and lesion characteristics in the two treatment groups are summarised in table 5.3. The latest MCS was available in 158 patients. Patients with radiographic evidence of OA had experienced a statistically significant greater number of operations prior to ACI or MACI. The duration of symptoms and size of lesions were greater in this group of patients (though the p value was low it was not statistically significant).

Table 5.3: Patient and lesion characteristics

	No OA	**OA**	**p value**
Age	33.6 +/- 1	35.1 +/- 0.8	0.25
Percentage males	52%	56%	0.9
Proportion of ACI procedures	54.5%	41%	0.17
Length of symptoms (months)	81 +/- 12.2	112.6 +/-12.1	0.07
Number of previous operations	2.13 +/- 0.25	3.18 +/- 0.27	0.006
Size of lesion (mm^2)	449 +/- 42.3	566 +/- 45.8	0.06
Modified Cincinnati Score pre-operatively (MCS 0)	51.4 +/- 1.8	40.6 +/- 1.7	< 0.001
Time to follow-up (months)	38 +/- 1	48 +/- 2.9	0.04

The distribution of lesions in the two groups anatomically is shown in table 5.4. The majority of lesions were on the medial femoral condyle. There were no discrepancies between the two groups.

Table 5.4: Anatomical site of chondral lesions according to treatment groups

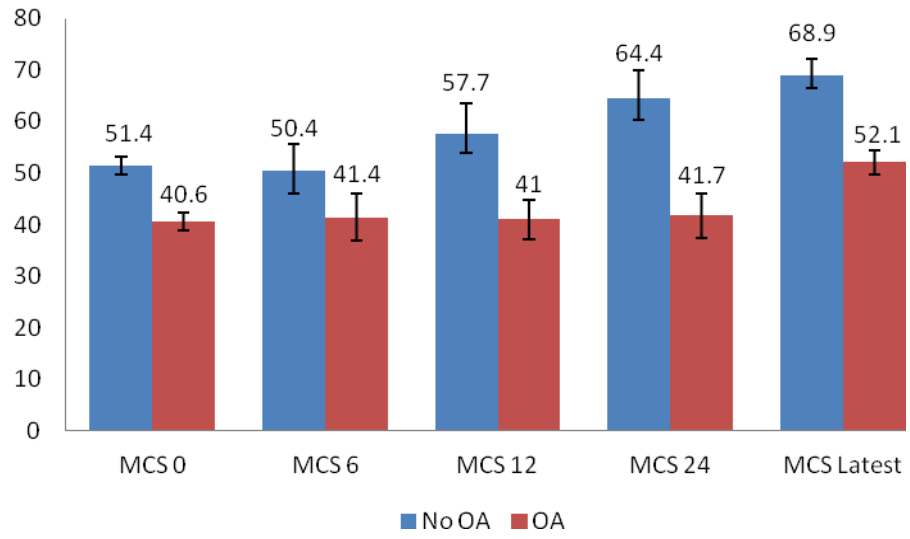
Anatomical site	No OA	OA
Lateral femoral condyle	8 (16%)	4 (10%)
Medial Femoral Condyle	28 (56%)	22 (53.5%)
Trochlea	3 (6%)	1 (2.5%)
Patella single defect	7 (14%)	9 (22%)
Patella multiple defects	4 (8%)	5 (12%)
TOTAL	50	41

The aetiology of the lesions in the two groups is shown in table 5.5. In the OA group a greater proportion of patients had a failed previous mosaicplasty or previous ACI and this was statistically significant (p=0.01).

Table 5.5: Aetiology of lesions according to treatment groups

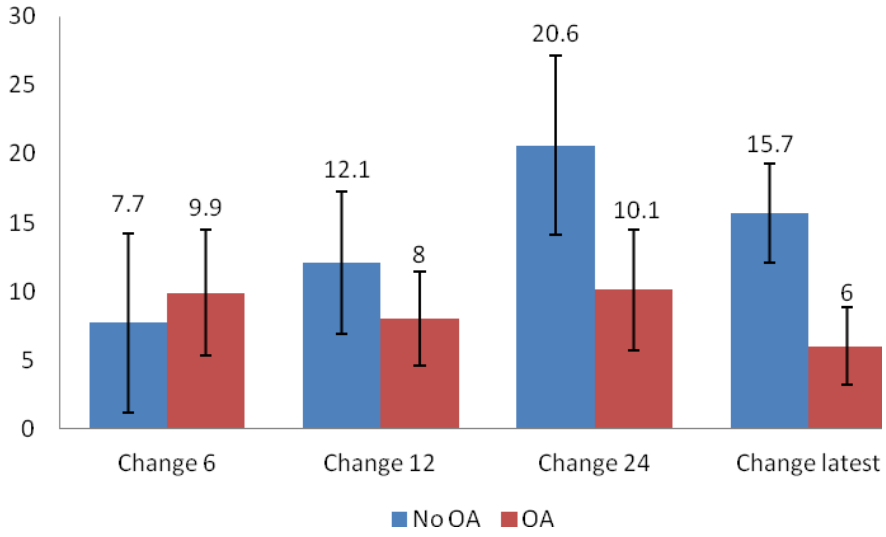
Aetiology	No OA	OA
Trauma	19 (57.5%)	14 (38%)
Osteochondritis dissecans	9 (27%)	2 (5.4%)
Chondromalacia patellae	3 (9%)	3 (8%)
Failed previous procedure	2 (6%)	18 (49%)
TOTAL	33	37

Figure 5.6: The Modified Cincinnati Scores in patients with and without osteoarthritis



The Modified Cincinnati Scores (MCS) before and after surgery are shown in figure 5.6 for descriptive purposes. Figure 5.7 shows that two years after surgery, patients with no radiographic evidence of OA were likely to increase their MCS by twice as much as those with evidence of OA. However, it was interesting to note that patients with OA still experienced a clinically significant 10 point increase in MCS (although this was not statistically significant). At the latest follow up (mean 43 months), both groups experienced significant increases in MCS from the baseline, though the increase in MCS was greater in the No OA group which was significant (independent t-test to compare increase in MCS from baseline between two groups, p=0.01).

Figure 5.7: The Change in Modified Cincinnati Scores in Paired Groups



The proportion of good and excellent results according to MCS is displayed in Table 5.6. The results were significantly better in the No OA group compared with the OA groups at all time frames. Two years following surgery, Good to Excellent results were achieved in 71% patients with no OA compared with 30% with OA (no excellent results). At the time of latest clinical review (mean 50 months) the results were similar (69% versus 40.5%). Of note, the results were better in the OA group at the latest follow-up compared with the 2 year results, but this may simply be because of the greater sample size at final follow-up.

Table 5.6: Proportion of Good and Excellent results according to MCS

Time	Outcome	No OA	OA	p value
1 year	Excellent	11 (37%)	2 (6%)	0.01
	Good	7 (23%)	8 (22%)	
	Fair	2 (7%)	12 (33%)	
	Poor	10 (33%)	14 (39%)	
	Total	**30**	**36**	
2 years	Excellent	10 (42%)	0	0.008
	Good	7 (29%)	7 (30%)	
	Fair	4 (17%)	8 (35%)	
	Poor	3 (12%)	8 (35%)	
	Total	**24**	**23**	
Latest Follow Up	Excellent	33 (53%)	16 (16.5%)	0.0004
	Good	10 (16%)	23 (24%)	
	Fair	11 (18%)	40 (41%)	
	Poor	8 (13%)	18 (18.5%)	
	Total	**62**	**97**	

The p value is generated from performing the Fisher's exact test to compare the proportion of good or excellent clinical outcome according to the MCS in patients with radiographic OA and those without OA.

Further procedures

Table 5.7: Major Re-operations

Procedure	**No OA (n=95)**	**OA (n=84)**
Revision ACI/MACI	2	7
Patellectomy	1	0
High Tibial Osteotomy	3	8
Unicompartmental or Patellofemoral Replacement	1	15
Total Knee Replacement	2	3
Others (including microfracture, mosaicplasty)	0	6
Total	**12 (13.6%)**	**36 (42.9%)**

Kaplan-Meier survivorship analysis was performed from the date of the second stage operation to the end-point of re-operation for the above indications. Table 5.8 shows the 3, 5, 7 and 8 year survivorship figures and figure 5.8 displays the plot. The log-rank test was used to compare whether there was a significant difference in re-operation rates between patients with radiographic evidence of OA and those without**.** As predicted, the survivorship was significantly better at all time frames in the No OA group. Figure 5.8 shows that the overall survivorship was significantly better in the No OA group according to the log rank test (p=0.005). However, it is not known whether it is osteoarthritis that is contributing to the inferior clinical results of survivorship or other discrepancies within the OA group, such as

greater number of previous procedures, larger lesions and longer duration of symptoms.

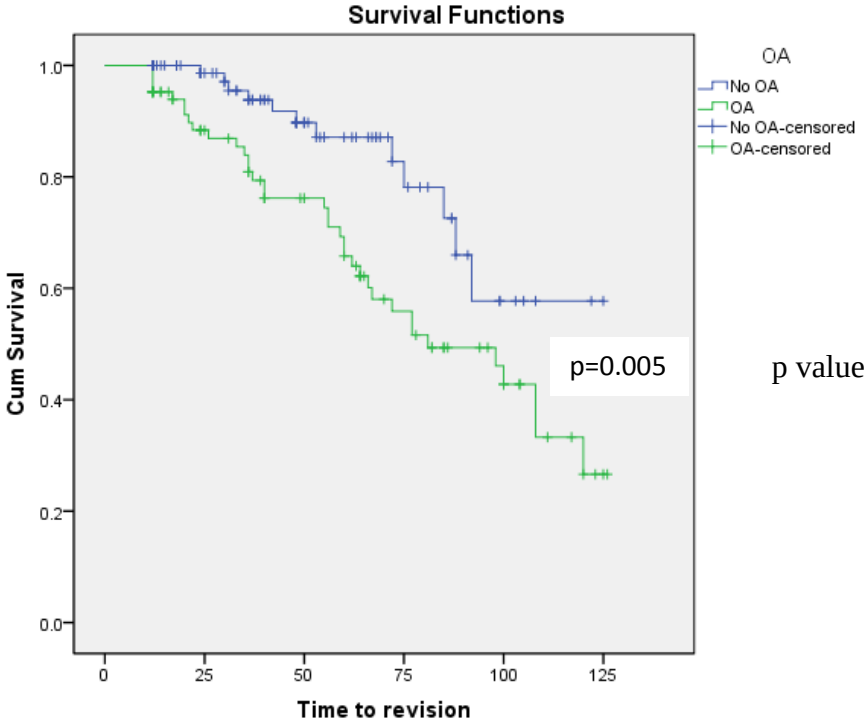
Therefore the Generalised Linear Model was used to account for these variables.

Table 5.8: Survivorship figures according to Kaplan-Meier Analysis

Time	No OA	OA
3 years	93.8% +/- 3	76.2% +/- 5.1
5 years	87.1% +/- 4.7	51.6% +/-6.7
7 years	82.7% +/- 6	49.3% +/-6.7
8 years	78.2% +/- 7.3	26.6% +/- 8.9

The probability of survivorship (i.e. not requiring revision surgery or major re-operation) is shown as a percentage with the standard error of mean.

Figure 5.8: Kaplan-Meier Survivorship Analysis of Patients with OA and without OA



p value derived from log rank test.

Generalised Linear Model (GLM)

The dependent variable in this model was the latest MCS. Once again 2 GLMs were created. In the first GLM, the categorical variables were type of surgery (ACI or MACI) and the presence or absence of OA. The continuous variables were MCS 0 and age. The results can be seen below.

Table 5.9: Categorical Variable Information

		N	Percent
Type	ACI	61	48.0%
	MACI	66	52.0%
	Total	127	100.0%
OA	No OA	50	39.4%
	OA	77	60.6%
	Total	127	100.0%

Table 5.10: Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Latest MCS	127	10	100	56	26.3
Covariate	MCS 0	127	10	88	44.4	18.1
	Age	127	16	55	34.8	8.8

Table 5.11: Results of the Limited Generalised Linear Model (GLM)

Parameter	Magnitude of effect	Std. Error	95% Confidence Interval		Sig.
			Lower	Upper	
ACI	0.8	3.64	-6.4	7.9	0.8
MACI	0[a]	.	.	.	.
No OA	8.8	3.9	1.2	16.4	0.02
OA	0[a]	.	.	.	.
Continuous					
MCS0	0.7	0.1	0.5	0.9	<0.001
Age	-0.7	0.2	-1.1	-0.3	0.001

0[a] represents the reference category for comparison of other categorical data within the group.

Both age and the pre-operative MCS were significant factors. Patients with no OA were likely to have a MCS at latest follow-up that is nearly 9 points greater than patients with OA independent of the other mentioned variables in the model.

In the next GLM, there were four main aetiologies; traumatic, osteochondritis dissecans (OD), chondromalacia patellae (CP) and failed previous ACI/mosaicplasty. As there were so few patients in the OD and CP groups, it was decided to group patients according to whether they had failed previous surgery or not. Other categorical prognostic factors in the analysis included presence of OA, ACI or MACI procedure, and anatomical site of the lesion. The co-variates in the analysis included the pre-operative MCS, age, and duration of symptoms. Table 5.12 displays a breakdown of the number of patients for each of the categorical variables.

Table 5.12: Categorical Variable Information

		N	Percent
Type of surgery	ACI	38	52.1%
	MACI	35	47.9%
	Total	73	100.0%
Presence of OA	No	25	34.2%
	Yes	48	65.8%
	Total	73	100.0%
Previous Failed ACI/Mosaicplasty	No	42	57.5%
	Yes	31	42.5%
	Total	73	100.0%
Site	LFC/Trochlea	12	16.4%
	Patella	24	32.9%
	MFC	37	50.7%
	Total	73	100.0%

ACI = autologous chondrocytes implantation, MACI = Matrix-carried autologous chondrocytes implantation, LFC = lateral femoral condyle, MFC = medial femoral condyle

Table 5.13: Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Covariate	Latest MCS	73	10	100	49.2	26.8
	MCS 0	73	10	88	39.3	17.3
	Age	73	17	49	35.4	8.2
	Duration symptoms (months)	73	6	360	107	87

Table 5.14: Results of the Generalised Linear Model

Parameter	Magnitude of effect	Std. Error	95% Confidence Interval		Hypothesis Test
			Lower	Upper	Sig.
Type of surgery					
ACI	-1.1	4.4	-9.8	7.6	0.8
MACI	0[a]	.	.	.	.
Presence of OA					
No OA	6.1	5.2	-4	16.3	0.2
OA	0[a]	.	.	.	.
Previous failed surgery					
No	0.7	5.9	-12.2	10.8	0.9
Yes	0[a]	.	.	.	.
Site of defect					
LFC/Trochlea	11.9	6.3	-0.4	24.2	0.05
Patella	1.6	5.1	-8.4	11.6	0.75
MFC	0[a]	.	.	.	.
Continuous variables					
MCS0	0.8	0.15	0.5	1.1	<0.001
Age	-1.2	0.27	-1.8	-0.72	<0.001
Duration of symptoms (months)	-0.02	0.03	-0.07	0.04	0.5

Dependent Variable: Latest MCS

LFC=lateral femoral condyle, MFC=medial femoral condyle, MCS 0 = pre-operative MCS

0[a] represents the reference category for comparison of other categorical data within the group.

Table 5.14 shows that the presence of OA has no significant effect on outcome when other variables were introduced into the model. In the first model, the presence of OA predicted an approximate 9 point decrease in the latest MCS. The number of patients with complete data set decreases as more variables are introduced. It may be that by introducing more variables

into the GLM the power of the statistical model decreases and hence an increase in the chance of a type II statistical error.

There was also no difference in outcome between ACI and MACI patients in this model. Interestingly, there was no difference in outcome in patients according to whether they had a failed previous procedure or not. Site was a significant factor. The MCS in patients that had trochlear or lateral femoral condyle lesions were on average nearly 12 points greater than in patients in which the lesions were in the medial femoral condyle. As predicted from previous models, MCS 0 was the strongest predictor of outcome, with a 1 point increase in MCS 0 likely to predict a 0.8 increase in the latest MCS.

Age was also a strong predictor; as the patients' age increases by a year, the MCS was predicted to decrease by 1.2.

Inter-Observer Variation

One hundred and fifteen radiographs were reviewed by two independent observers. The table below displays the results for each individual observation.

The inter-observer variation using the Kellgren & Lawrence Grading system only resulted in a fair agreement (Kappa=0.31). However, when the Stanmore Grading System was used, there was substantial agreement (Kappa=0.72).

Table 5.15: Inter-observer variation

Patient number	K&L Grade Observer 1	K&L Grade Observer 2	Stanmore Grade Observer 1	Stanmore Grade Observer 2
1	2	2	1	1
2	0	0	0	0
3	0	0	0	0
4	3	2	2	2
5	2	2	1	1
6	2	2	1	1
7	1	2	2	2
8	0	0	0	0
9	2	3	2	2
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	2	2	1	1
14	0	0	0	0
15	0	0	0	0
16	0	1	0	1
17	0	0	0	0
18	0	0	0	0
19	0	1	0	1
20	0	0	0	0
21	0	0	0	0
22	0	2	0	1
23	0	0	0	0
24	0	0	0	0
25	0	0	0	0
26	1	1	1	0
27	0	1	0	0
28	0	2	0	1

Table 5.15 continued

Patient number	K&L Grade Observer 1	K&L Grade Observer 2	Stanmore Grade Observer 1	Stanmore Grade Observer 2
29	3	2	2	2
30	0	0	0	0
31	1	2	1	1
32	0	0	0	0
33	0	2	0	2
34	0	1	0	0
35	0	0	0	0
36	0	0	0	0
37	0	0	0	0
38	3	3	3	3
39	0	1	0	1
40	2	3	3	3
41	1	2	1	1
42	1	1	0	0
43	0	0	0	0
44	2	3	2	2
45	1	1	1	1
46	0	1	0	0
47	0	2	0	1
48	2	3	3	3
49	0	1	0	1
50	1	0	1	1
51	1	1	1	1
52	1	2	2	2
53	0	0	0	0
54	0	0	0	0
55	0	2	0	2
56	2	1	1	1
57	0	1	0	0
58	0	1	0	0
59	2	3	2	2
60	0	0	0	0
61	0	0	0	0
62	2	3	3	3
63	0	1	0	0
64	0	1	0	0
65	0	0	0	0
66	0	0	0	0
67	0	0	0	0
68	0	0	0	0
69	0	0	0	0
70	0	0	0	0
71	0	0	0	0
72	0	2	0	1
73	0	0	0	0
74	0	1	0	0

Table 5.15 continued

Patient number	K&L Grade Observer 1	K&L Grade Observer 2	Stanmore Grade Observer 1	Stanmore Grade Observer 2
75	0	0	0	0
76	0	0	0	0
77	0	0	0	0
78	1	0	0	0
79	0	0	0	0
80	0	0	0	0
81	0	0	0	0
82	0	0	0	0
83	0	1	0	0
84	1	2	1	1
85	0	0	0	0
86	3	2	2	2
87	0	0	0	0
88	2	2	1	1
89	0	0	0	0
90	0	0	0	0
91	0	0	0	0
92	0	2	0	1
93	0	0	0	0
94	0	0	0	0
95	0	2	0	1
96	0	0	0	0
97	0	0	0	0
98	0	0	0	0
99	0	0	0	0
100	0	0	0	0
101	0	0	0	0
102	0	0	0	0
103	0	2	0	1
104	0	0	0	0
105	0	0	0	0
106	1	2	2	2
106	0	0	0	0
108	0	2	0	1
109	0	1	0	0
110	0	0	0	0
111	2	1	1	1
112	0	0	0	0
113	0	0	0	0
114	0	0	0	0
115			0	0

Discussion

The initial results from this chapter suggest that Autologous Chondrocyte Implantation (ACI) is not as effective in patients with radiographic evidence of osteoarthritis (OA). The Modified Cincinnati Scores (MCS) were lower in the OA group at all time frames but the greatest discrepancy occurred at the longest interval following surgery (MCS Latest). Furthermore, when the paired data was analysed, although the OA group experienced an increase in MCS from their pre-operative status, this increase was statistically lower than the No OA group. Similarly, there was a greater proportion of good and excellent results at 1, 2 and latest follow up in the No OA group than the OA group.

Using Minas et al classification (Minas et al 2009), simple isolated OCDs situated on the femoral condyles measuring less than $4cm^2$ are termed simple lesions. Defects that are larger than $4cm^2$ on the femoral condyles or any sized lesions in the patella, trochlea, or tibia are defined as complex. Kissing lesions and knees with evidence of early osteoarthritis are termed salvage. Niemeyer reported on the 2-year clinical results of a membrane seeded ACI in 59 patients with OCD in the knee (Niemeyer et al. 2010). They sub-divided their patients into three groups; isolated defects (group A), complex defects (group B), and salvage defects (group C – patients with signs of OA or kissing lesions). A significantly better IKDC score was reported in group A compared with group C patients. However, 11/15 patients in group C reported significant improvement (greater than 10 points) at 24 months following surgery. The overall failure rate (defined as improvement in IKDC score of < 10 points or worsening knee function) in this group was 17% though the failure rate was significantly lower in group A (5.9%) compared with groups B and C (40%). This group claim that although the ACI surgery is inferior in patients with OA compared to those patients with single defects, it

should still be considered, especially in young patients in which arthroplasty surgery is the only other alternative.

Minas et al. specifically analysed the minimum 2-year results of ACI in patients with radiographic evidence of OA. Their definition of early OA was peripheral intra-articular osteophyte formation and/or 0-50% joint space narrowing (Minas et al 2010). Clinically, patients were included in the study if they had normal radiographs but kissing lesions or generalised chondromalacia was present on arthroscopy. It was interesting to note that out of the 328 patients that had 2-year results, 153 had early OA as defined by their criteria. Paired t-test revealed statistically significant improvements in all 5 scoring systems at 2 years following surgery and a mean of 5 years after surgery. Furthermore, they reported 93% survivorship at 5 years with the end point being revision with arthroplasty. However, their re-operation rate was considerably higher at 61%, mainly for the need to address graft hypertrophy, as they were still using autologous periosteum to contain the chondrocytes. The results seem very good in this study, in a cohort of patients that are similar to the ones in this chapter. However, this was a heterogeneous group of patients, as 103 patients had simultaneous procedures performed, including High Tibial Osteotomy (47) and Tibial Tubercle Osteotomy (44). Therefore one cannot state for certain whether the improvement in function was because of surgery to address the chondral defect or the simultaneous procedure performed to correct malalignment and other pathology.

Kreuz et al. also stated that second-generation ACI techniques were efficacious in the treatment of focal degenerative cartilage defects. Nineteen patients with Kellgren and Lawrence grade II osteoarthritis in their pre-operative radiographs were followed up for 4 years (Kreuz et al. 2009). There were statistically significant improvements in all three scoring systems used to assess knee function 6 months following surgery which was

maintained at the time of the latest follow-up. However, 47% patients (9 out of 19) had to have a second operation (usually in the form of arthroscopic debridement for symptoms like grinding, pain and swelling). 21% of patients (4 out of 19) had to have revision surgery or knee arthroplasty. The overall re-operation rate seems particularly high given that periosteum grafts were not used, though the revision surgery rate is similar to results from this study in this difficult cohort of patients.

Nehrer et al (2009) treated 53 patients with a hyaluronan-based scaffold seeded with autologous chondrocytes similar to MACI. These cohorts of patients' knees were composed of 23 simple lesions, 22 complex and 8 salvage lesions. They further divided their patients into those with a primary indication for surgery (42 patients with stable, normally aligned knee, with single OCD and age under 55) and secondary indication (11 patients (including 7 salvage cases) with more complex problems such as osteonecrosis following chemotherapy). They reported significant improvements in three scoring systems from pre-operative status in the primary indication group which was maintained at 7 years. The secondary indication group reported significant improvement at 2 years but the results declined to pre-operative status from 3 years onwards. The results from this chapter are similar. Graph 2 shows that there was significant increase in MCS at 2 years but at the time of latest follow-up, although there was still an increase it was not significant. Furthermore, Kaplan-Meier analysis of simple, complex and salvage cases revealed a 5 year survival of 95%, 75% and 12.5% respectively. Therefore the outcome in patients with early OA in terms of delaying arthroplasty is not good and the results from this chapter mirror this. Once again precaution needs to be taken when interpreting these results as a number of confounding variable were not addressed in this study. Patients with salvage procedure were older. Also, 11 patients had simultaneous procedures (such as HTO or ACL reconstruction) and it is not described how

many of these procedures were performed in each group. Certainly the results from this chapter suggest that age is more of an important determinant than OA (Generalised Linear Model). Two studies have reported on the efficacy of ACI in patients older than 40 and 45 years of age but with no evidence of OA (Kon et al. 2011, Rosenberger et al 2008). They concluded that although the patients' satisfaction and function did improve at mean follow-up period of 4.7 years, the results were significantly inferior to younger patients that the authors' had reported on previously. Once again, a number of confounding variables leads me to question the validity of their conclusions, namely the lack of younger patient cohort!

Hollander et al performed histological analysis of 23 patients' OCDs in the knee treated with Hyalograft C (a MACI type repair procedure) (Hollander et al 2006). Nine out of 14 patients had radiographic evidence of OA. Paradoxically, the repair tissue was mature hyaline cartilage in 36% of biopsies taken from non-arthritic joints and 67% of biopsies from arthritic knees. Even more surprising, was that in the 3 knees that were graded as severe OA radiographically, the repair tissue in 2 was hyaline. These initial results suggest that the presence of OA in the knee does not inhibit the maturation of implanted autologous chondrocytes. Furthermore, the type II collagen and proteoglycan content was significantly higher in biopsies from arthritic joints. These histological and biochemical analysis together imply that the presence of OA does not inhibit tissue regeneration but rather may improve it. Could it be that arthritic, degenerate tissue are primed for repair and require only appropriate cellular cues and the necessary substrate for regeneration? It was unfortunate that these histological and biochemical results were not correlated with the patients' functional results. In addition, no functional studies were performed to determine the mechanical stability of the repair tissue. The key criticism in the data analysis of the patients in this chapter is the lack of histological information. This will be reviewed in the discussion chapter.

Joint haemostasis and the composition of synovial fluid are of key importance in cartilage regeneration, particularly in the presence of osteoarthritis. Periosteum is known to drive chondrogenesis and in a goat model, better repair was seen in OCDs when the lesion was covered in periosteum compared to those that were not (Saris et al 2003). Similarly, synovial fluid improves chondrogenesis after chondrocytes implantation in a rabbit model (Neidel and Schulz 2000). In humans, synovial fluid from patients with acute traumatic OCDs have been shown to stimulate chondrogenesis, whereas, patients with chronic lesions inhibit cartilage proliferation (Rodrigo et al 1995). I suspect that the cytokine milieu in chronic traumatic lesions is considerably different to acute lesions and is one of the reasons why there is a discrepancy in results. The pro-inflammatory status of cytokines in the synovial fluid in patients with osteoarthritis is not beneficial for cartilage repair (Schlaak et al 1996, Scanzello et al 2009).

Inter-observer variability

Kellgren and Lawrence first described the radiological classification for osteoarthritis in the hand (Kellgren and Lawrence 1957). This was later adopted by the World Health Organisation in Rome as the standard for description of osteoarthritis in any joint. Several different versions of the original scoring system exist in the literature though none of them have been validated. The scoring system proposed by us seems logical as it corresponds to the pathological process occurring in the knee. This has not been published previously. The substantial agreement using the Stanmore Grading system (k=0.7) suggests that this is easier to use than the Kellgren and Lawrence Scoring system which only had fair agreement (k=0.3).

Conclusion

The results from this chapter suggest that ACI is not as effective in treating OCDs in the knees of patients with early osteoarthritis as it is in patients without. The initial analysis revealed quite marked differences in results. Even the first GLM, adjusting for some of the confounding variables, patients without OA were predicted to have a MCS which is 8.8 points better than patients with OA. When other confounders were included in the analysis, OA no longer became a significant predictor of outcome. Age was not a significant factor in Chapter 4 but was in Chapter 3. It may be that age in the presence of OA is significant but otherwise not.

Chapter 6:

Smoking, Body Mass Index and Physical Activity

Introduction

The previous chapters have discussed factors that are not modifiable, such as the MCS before surgery or the size of the lesion. This chapter will investigate the effects of three factors which can be influenced before surgery to ascertain whether they have an effect on outcome following surgery; smoking, physical activity and weight of the patient (measured as the body mass index [BMI]).

In a large Finnish prospective study, multivariate analysis revealed higher age, being overweight, smoking and previous knee injuries as significant predictors of incidental knee pain (Miranda et al 2002). Of the 2122 employees in a forestry company, 214 (10%) developed severe knee pain in one year. The odds ratio (OR) of developing knee pain if the BMI was greater than 29 (compared to BMI of 26 – 29.9) was 1.8 (p=0.02). The odds ratio was similar if the employees were smokers or ex-smokers compared to non-smokers (OR=1.6, p=0.01). In this model, previous knee injury was the greatest risk factor for developing knee pain (OR=2.4, p=0.0001). It was interesting to note that in this study, the amount of general physical exercise or the practise of different sports was not related to the one year incidence of knee pain or the persistence of knee pain.

Another epidemiological study examined the relationship between knee osteoarthritis leading to arthroplasty and being overweight, smoking and hormone therapy (Sandmark et al 1999).

Women with a high BMI at the age of 40 had a relative risk (RR) of 9.2 of developing knee arthrosis and men had a RR of 3.2. At the age of 50, the RR was 7.8 for women and 5.9 for men. Smokers were less likely to develop knee osteoarthritis (OA) leading to knee arthroplasty than non-smokers (RR=0.6, 95% confidence interval 0.4 to 1). This effect appeared to be dose dependent as heavy smokers had a stronger inverse relation than light smokers (defined as less than 15 pack years). This 'protective' effect of smoking on knee OA has been described in earlier studies though the mechanisms by which this occurs is yet to be discovered (Felson et al 1989, Davis et al 1990, Hart and Spector 1993). Sandmark and colleagues also assessed the effects of physical work load and participation in sporting activities (divided into 3 classes) on the development of knee OA and found no effect (Sandmark et al. 1999).

In a large epidemiological study of over 320,000 male construction workers in Sweden there was a doubling of the risk for having severe osteoarthritis of the knee with an increase of BMI of 5 Kg/m^2 (Jarvolm et al 2005). Similar to the findings of Sandmark et al., non-smokers had a relative risk of 40% for OA of the hip though this effect was not evident in the development of knee OA.

With these epidemiological studies in mind, the effects of these confounding variables on the outcome following orthopaedic surgery will be discussed in more detail.

1. Smoking

Smoking is a well-established risk factor for cardiovascular and respiratory disease. It also has deleterious effects on wound healing (Sorenson et al 2005, Manassa et al. 2003). More recently, cigarette smoking has been associated with musculoskeletal problems, such as progression of knee osteoarthritis, low-back pain and degenerative disc disease (Amin et al. 2007, Goldberg et al. 2000, Deyo et al. 1989). In orthopaedics, smoking is associated with worse clinic results following rotator cuff repair, ACL reconstruction, hind foot fusion, spinal fusion, hemicallotasis and hip and knee arthroplasty (Glassman et al. 2000, Moller et al. 2003, Ishikawa 2002, W-Dahl et al. 2004, Karim et al. 2006, Mallon et al. 2004)

Cigarette smoke has two phases: a volatile phase and a particulate phase. During the predominant volatile phase, nearly 500 different gases are released (which include nitrogen, carbon monoxide and hydrogen cyanide). In the particulate phase, nearly 3500 chemicals are released and as water is removed the particulate matter that remains, or 'tar', contains the majority of the carcinogens of cigarette smoke (Porter et al. 2001). Nicotine (the addictive component of cigarette smoke) has been implicated in the pathogenesis of a variety of diseases by increasing platelet aggregation, decrease microvascular prostacyclin levels, and, perhaps more importantly in surgery, inhibiting the function of fibroblasts, red blood cells and macrophages (Jorgensen et al 1998, Zevin et al 1998).

Despite these associations, a comprehensive literature review failed to identify any publications on the effects of smoking on the outcome after ACI for the treatment of OCDs of the knee.

2. Body Mass Index

Over the last two decades, the impact of certain risk factors such as ageing and BMI in OA has been actively studied. The general consensus in the literature is that a high BMI raises the risk for the development of knee OA and the relative risk varies from 1.6 to 9.2 (Holmberg et al. 2005, Anandacoomarasamy et al. 2009, Felson 1996, Manek et al. 2003, Manninen et al. 2004, Janssen & Mark 2006, Miranda et al. 2002, Sandmark et al. 1999, Gabay et al. 2008). A more recent study suggested that a high BMI was associated with accelerated rate of joint space narrowing, a radiographic indicator of severity of OA (Benichou et al. 2010).

It is widely believed that obesity increases subchondral bone stiffness, hence transmitting more force to the articular cartilage, suggesting an injury mechanism with obesity as a risk factor (Dequeker et al. 1983, Felson et al. 1988). Ford et al. described a dose-response relationship between BMI and meniscal injury requiring surgery in middle-aged and older adults (Ford et al. 2005). With a reference category of a BMI of 20-22.49, the odds ratio of men sustaining a meniscal injury is 3 if the BMI is 27.5-29.99, 4.8 if the BMI is 30-32.49 and 14.6 if the BMI is greater than 40. Similar results are seen in women with an odds ratio of 24.3 if the BMI is greater than 40 (Ford et al 2005). Furthermore, obese patients (BMI greater than 30) were 2.5 times more likely to develop OA following menisectomy than case matched controls (Englund and Lohmander 2004).

Several studies have indicated excessive weight as being a negative predictor of success of total knee arthroplasty whilst there are number of other studies which have reported no effect (Aglietti and Rinonapoli 1984, Ahlberg and Lunden 1981, Dannenmaier et al. 1985, Stern and Insall 1990, Griffin et al. 1998, Pritchett and Bortel 1991, Winiarsky et al. 1998, Bordinie et al. 2009, Jarvenpaa et al. 2010). Several other studies have reported lower functional scores

in obese patients when compared to matched controls but the absolute improvement from pre-operative status was similar in both groups (Spicer et al. 2001, Foran et al. 2004, Amin et al. 2006, Jarvenpaa et al. 2010)

The results following unicompartmental knee replacement (UKR) in obese patients are clearer. At an average follow up of 40.2 months, a BMI of greater than 32 did predict early failure in patients who had undergone UKR for medial compartment disease (Berend et al. 2005).

Similarly, the revision rate following patellofemoral replacement was significantly greater in obese patients compared to non-obese (van Wagenberg et al. 2009).

To date, there are no studies in the literature assessing the effect of weight or BMI on cartilage repair when treating chondral defects in the knee. What is evident is that the obese patients are more likely to suffer from OA and also at an earlier age. This is postulated to be as a result of increased mechanical stresses the articular cartilage has to endure in weight bearing joints of such patients. Numerous *in vitro* studies have demonstrated that chondrocytes are sensitive to mechanical signals induced by loading and that moderate exercise is beneficial for cartilage constitution (Roos & Dahlberg 2005). However, excessive stresses or static stress disrupts the anabolic/catabolic balance in cartilage (Sharma et al. 2007). It may be that obese patients experience more static stress when involved in physical activity and hence are more predisposed to OA and worse outcome after knee surgery.

3. Physical Activity

The promotion of physical activity has been a recent world-wide public health initiative in developed countries. Increased physical activity is thought to protect against a number conditions such as cardiovascular disease, obesity and osteoporosis. There have been conflicting results from cross-sectional studies examining the effects of physical activity on knee health. Middle aged physical education teachers were reported to have less radiological evidence of OA than aged matched controls (White et al. 1993). Conversely, ex-elite female athletes had higher rates of radiographic OA than a control group (Spector et al. 1996). There are similar discrepancies in longitudinal radiographic studies. Results from the Framingham Heart Study showed that recreational exercise had no effect on the radiological status of older healthy adult knee joints (Felson et al. 2007). However, an Australian prospective study revealed that the average level of physical activity was independently associated with the development of radiographic knee OA (Szoeke et al. 2006). These inconsistent results are probably a result of the fact that the primary outcome measure is radiographic evidence of OA. Whilst some studies have found an increased rate of osteophyte formation in exercising individuals (Spector et al. 1996), others have found no change in joint space (Felson et al. 2007). Furthermore, it is well known that some people with radiographic evidence of OA have no symptoms, and others, with minimal or no OA visible have severe symptoms.

The effect of physical activity on healthy adult cartilage (no previous injury or knee disease) has been assessed by cross-sectional studies utilising MRI (Hanna et al. 2007, Racunica et al. 2007). Both studies indicate vigorous activity (i.e. activity leading to sweating, breathlessness or an increased pulse rate) was associated with increased tibial cartilage volume.

Inactivity has been shown to be detrimental to articular cartilage health and development. Physically less active children have been shown to have less cartilage and gain less cartilage

over 2 years than active children (Jones et al. 2003). This finding has important implications (particularly on the rehabilitation process following ACI), as the cartilage repair process following ACI is similar to cartilage development in children. Inactive adults (e.g. quadriplegics) have suffered from rapid cartilage loss within the initial 12 months of immobility (Vanwanseele et al. 2002). Cartilage receives its nutrition from synovial fluid and movement of the joint is required in order for synovial fluid to move in and out of hyaline cartilage. Hence, the rehabilitation following ACI should take these observations into account (i.e. early range of motion exercises).

Chondral and osteochondral lesions of the knee are especially common amongst those partaking in sporting activity (Smith et al. 1995). Up to a quarter of all knee injuries resulting in haemarthrosis are associated with cartilage damage (Curl et al. 1997). Participation in recreational and competitive sporting activities has been associated with increasing incidence of articular cartilage damage to the knee (Curl et al. 1997, Levy et al. 1996, Messner et al. 1996, Piasecki et al. 2003). The chondral injuries in this cohort of patients often occur with other knee injuries, and have been described in up to 50% of athletes undergoing anterior cruciate ligament (ACL) reconstruction (Curl et al. 1997, Drongowski et al. 1994, Piasecki et al. 2003). Though athletes have been able to return to sporting activity after some form of cartilage repair, over time, knee function and athletic activity has declined (Messner et al. 1996, Mithoefer et al. 2009). Furthermore, in high demanding athletes the risk of developing knee OA following injury can be as high as 12 fold (Drawer et al. 2001, Kujala et al. 1995).

Articular cartilage repair in patients involved in regular sporting activities represents considerable challenge due to forceful, repetitive joint loading in impact sports. The repair tissue has to be able to withstand the significant mechanical joint stresses generated during such sporting activities. Microfracture, mosaicplasty and ACI have all been used in athletes

with varying success (Gobbi et al. 2004, Gudas et al. 2005 & 2006, Kreuz et al. 2007, Kon et al. 2009). Functional scores are widely used to assess efficacy of various cartilage repair techniques, but only a few studies have assessed the ability to return to sporting activity. This is an important parameter of functional outcome in a subset of patients.

With all of the above information in mind, the hypotheses, aims and objectives of this chapter are outlined below.

Aims, Objectives, Hypotheses

1) To compare the efficacy of ACI in smokers and non-smokers

   Hypothesis:

   i) smokers have worse outcome

   ii) there is negative correlation between exposure of smoke and functional outcome following this procedure

2) To analyse the difference in outcome of ACI in patients with ideal BMI and those who are overweight or obese

   Hypothesis:

   i) patients with ideal BMI have the best outcome

   ii) there is a negative correlation between BMI and functional outcome after ACI

3) To determine if there was a difference in outcome in patients who regularly took part in sporting activities prior to their injury. A secondary aim in this cohort of patients was to determine the proportion of patients that returned to sporting to activities after ACI and to what level of sporting prowess they returned to

   Hypothesis: Those involved in sports prior to injury have a better outcome following ACI

There will obviously be interplay with all the three factors listed above. For example, it stands to reason that those with a very high BMI are unlikely to be involved in many sporting activities. For this reason the Generalised Linear Model (GLM) was used to take into account this interplay and still provide information regarding statistical significance of the listed factors independent of each other.

Patients and Methods

In the first form (Form A see appendix) for all new patients entering the ACI vs. MACI trial, there are sections on smoking status as well as height and weight that needed to be documented. Unfortunately, this information was not recorded in a large proportion of patients and they certainly could not be used for statistical analysis. A detailed questionnaire concerning the patients' weight, height and smoking status at the time of the surgery was constructed. The questionnaire (see appendix) also sought to delineate the physical activity profile of the patients prior to their injury and what level of sporting activity they were engaged in two years following surgery. The outcomes measures used to assess success of surgery were the Modified Cincinnati Score (MCS), and the Visual Analogue Score (VAS) before and after surgery. The functional scores were measured at 6, 12 and 24 months after surgery.

The information provided by smokers also included the number of cigarettes smoked and the number of years the patient had smoked for. This enabled us to calculate the number of pack years (see formula below) which is an indication of the lifetime exposure of smoke.

$$\text{Pack years} = \frac{\text{no. of cigarettes smoked per day X no. of years smoked}}{20}$$

The patients' height (in metres) and weight (in kilograms [Kg]) are used to calculate the body mass index (BMI). Patients with a BMI of 20 to 24.9 are categorised as 'ideal body weight'. Those with a BMI of 25 to 30 are overweight and those with a BMI of greater than 30 are obese.

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{Height (metres)}^2}$$

Patients who played a competitive sport at least once a month and train regularly were categorised as taking part in regular physical activity. This also included going to the gym and dancing. The physical activity profile was sub-divided into high, mid and low impact sports according to the type of activity performed:

1. **High-impact sports:** jumping, pivoting, cutting (basketball, netball, volleyball, soccer, gymnastics)
2. **Mid-impact sports:** running and turning (racquet sports, hockey, skiing)
3. **Low-impact sports:** running, cycling, swimming

An earlier pilot study enabled us to perform sample size calculations. With $\alpha = 0.05$ and $\beta = 0.2$ (hence a power of 80%), a total of 90 patients would be required (with twice as many non-smokers as smokers) to detect a difference of 10 points in the Modified Cincinnati Score. Similarly, using the same parameters, 100 patients would be required to identify a difference between those patients who are overweight or obese and those who have an ideal BMI. The questionnaire was sent out to 150 patients who had traumatic osteochondral lesions in the knee treated with either ACI or MACI and the two year results (according to the MCS) were available with the hope that the response rate would be at least 75%. 122 patients responded (81%).

Results – Smokers

The age, BMI, duration of symptoms, number of previous operations and size of lesions were all higher in the Ex-smokers group, though this was not statistically significant (Table 6.1).

Table 6.1: Patient demographics

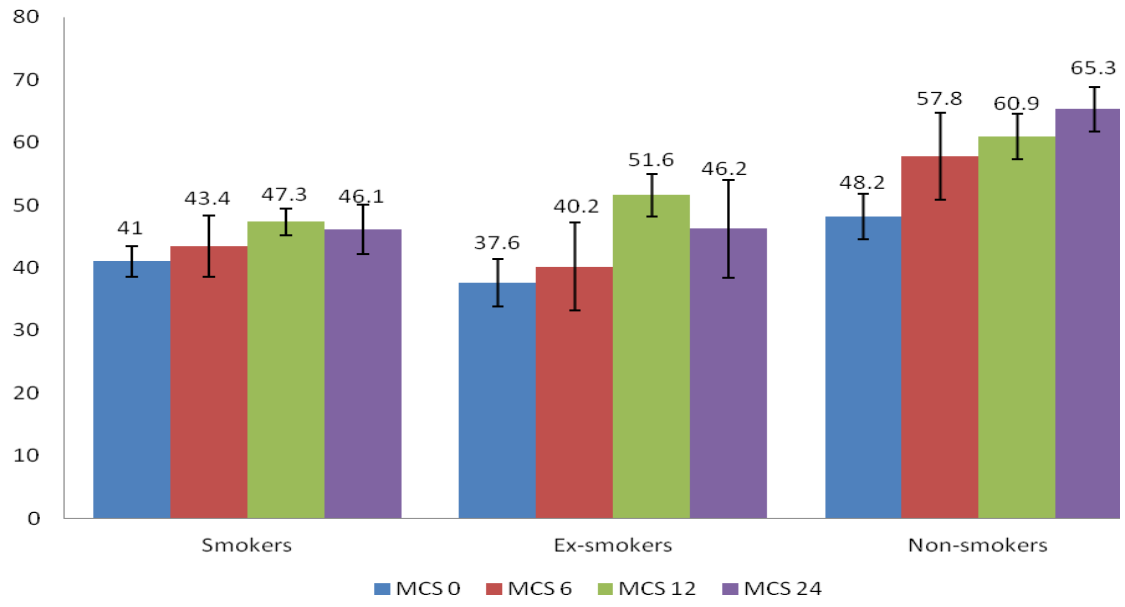
	Smokers (n=48)	Ex-smokers (n=15)	Non-smokers (n=66)	p-value
Age	33.4 +/- 1.2	36.4 +/- 1.9	33.7 +/- 1.1	0.5
Body Mass Index	26.4 +/- 0.6	27.5 +/- 1.4	26.3 +/- 0.5	0.6
Duration of symptoms (months)	102 +/- 10	137.1 +/- 28.6	113.3 +/-11.7	0.4
No. of previous operations	2.4 +/- 0.3	3.3 +/- 0.8	2.3 +/-0.2	0.2
Size of lesion (cm)	5.6 +/- 0.5	6.4 +/- 0.9	4.8 +/- 2.1	0.6

Means displayed with standard error. p-values derived from one-way ANOVA

Figure 6.1 shows the absolute values of the MCS at baseline and at 6, 12 and 24 months after surgery for descriptive purposes. Figure 6.2 shows the change in MCS from baseline to the aforementioned time frames. When directly comparing the graphs, a discrepancy is observed. For example, in figure 6.1 the change in the Modified Cincinnati Score 24 months after surgery in smokers (i.e. MCS 24 – MCS 0 = 5.1) does not match the change in MCS seen in figure 6.2 (change at 24 months = 9.3). This is because in figure 6.2, only the dataset in which patients who have scores pre-operatively and at 24 months after surgery are included in the analysis. The data analysis in figure 6.1 utilised all patients who had MCS at any of the time frames (so some patients may have had scores at 6, 12 and 24 months but not preoperatively and some may have scores pre-operatively but not at 24 months). It is

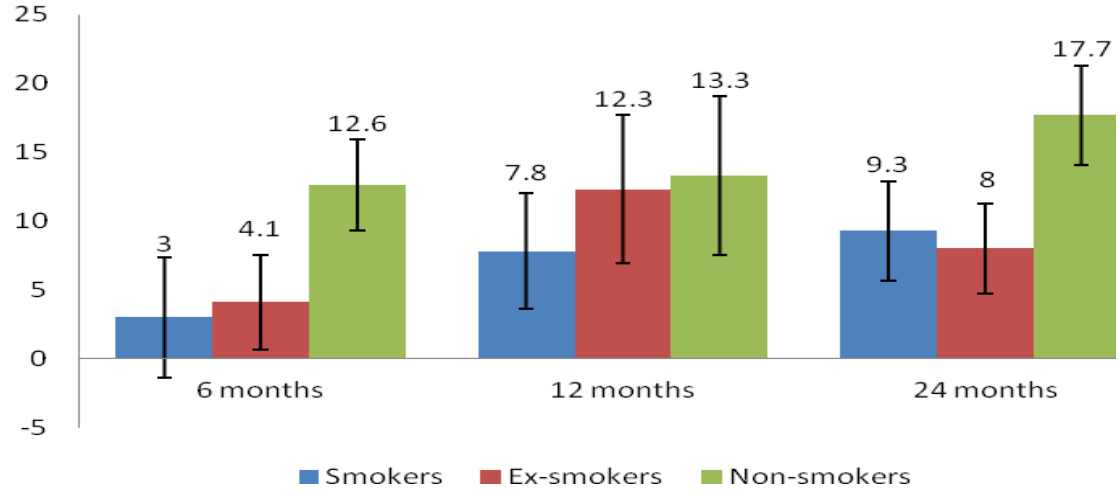
therefore more useful to compare results in figure 6.2, even though number of patients is less and therefore the standard error is higher. ANOVA failed to identify a significant difference between the three groups in terms of change in MCS 24 months after surgery (p=0.2). When a direct comparison between smokers and non-smokers was analysed using the independent t-test was performed, the p-value was lower but still not significant (p=0.09).

Figure 6.1: Functional results with the Modified Cincinnati Score



Graph displays mean values with standard error bars

Figure 6.2: Change in MCS in smokers, non-smokers and ex-smokers



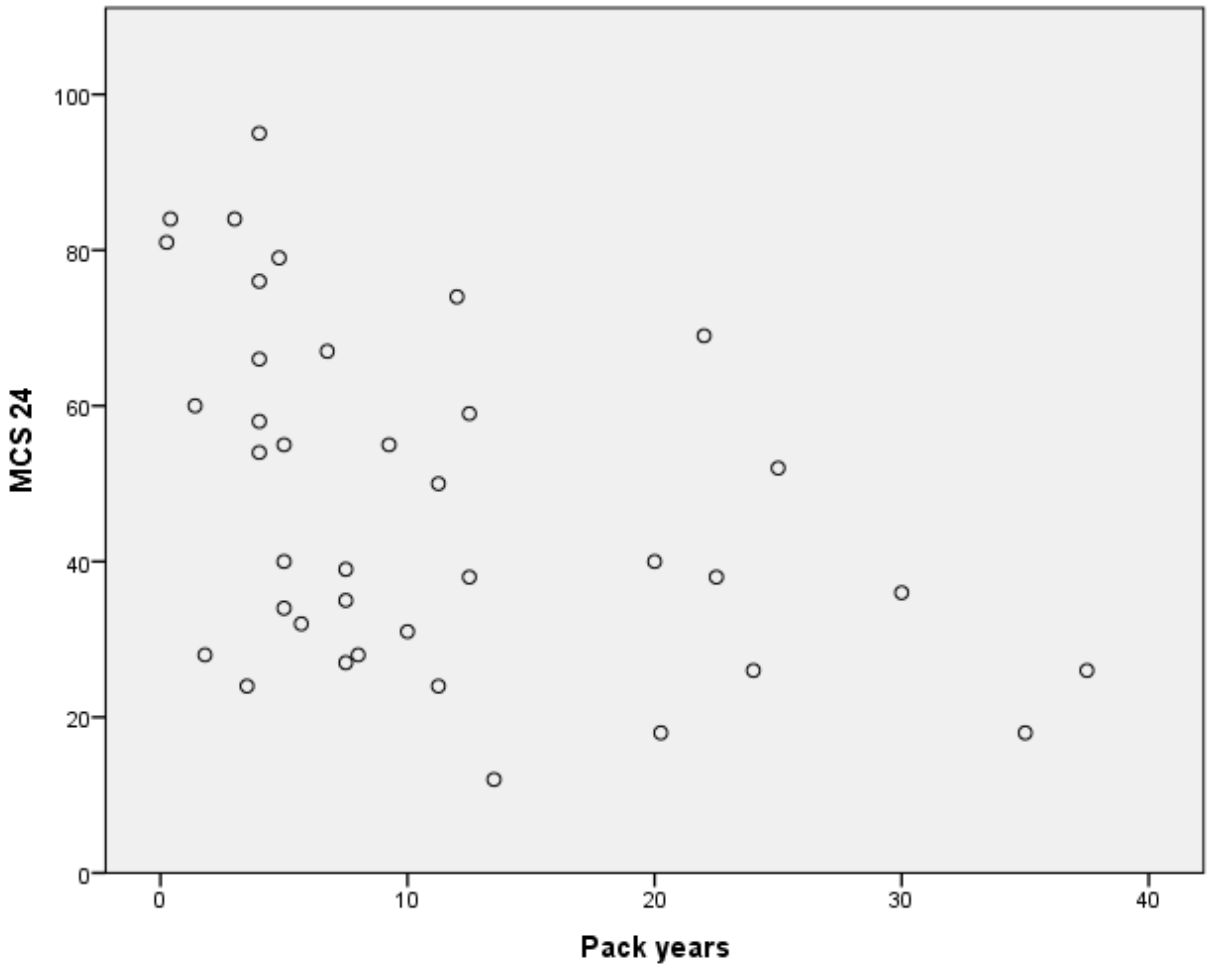
Graph displays mean change in Modified Cincinnati Score from preoperative score with standard error bars

Table 6.2: Excellent and Good Results According to MCS

Time	Outcome	Smokers	Non-smokers	p value
6 months	Excellent	2 (7%)	9 (20%)	0.014
	Good	5 (19%)	17 (38%)	
	Fair	16 (59%)	12 (27%)	
	Poor	4 (15%)	7 (15%)	
	Total	**27**	**45**	
1 year	Excellent	5 (12.5%)	13 (23.5%)	0.002
	Good	9 (22.5%)	25 (45.5%)	
	Fair	15 (37.5%)	11 (20%)	
	Poor	11 (27.5%)	6 (11%)	
	Total	**40**	**55**	
2 years	Excellent	3 (9.5%)	20 (40%)	0.014
	Good	9 (28%)	13 (26%)	
	Fair	11 (34.5%)	13 (26%)	
	Poor	9 (28%)	4 (8%)	
	Total	**32**	**50**	

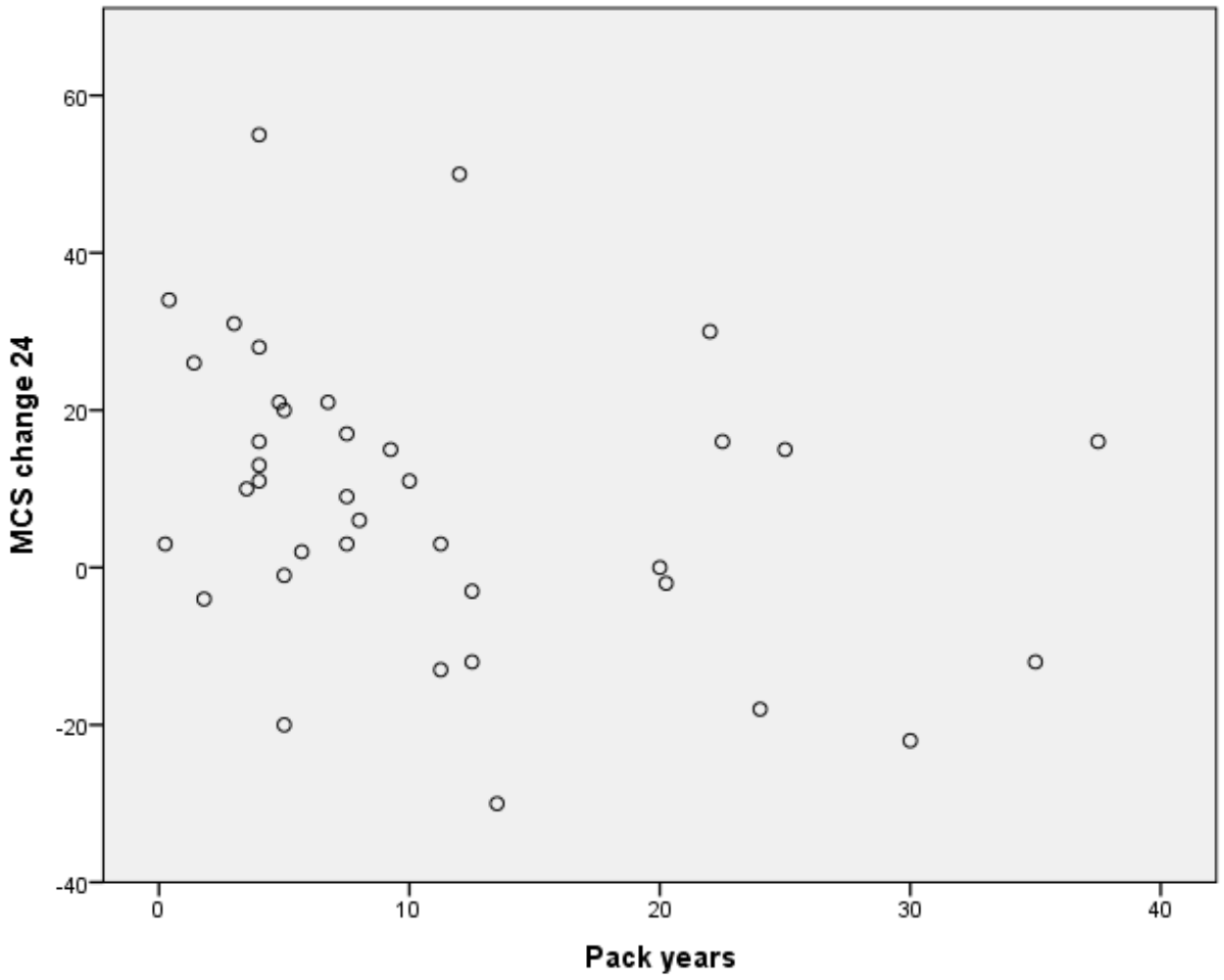
The proportion of good and excellent results (according to MCS) was significantly better in non-smokers compared to smokers (see Table 6.2) at all time intervals after surgery.

Figure 6.3: Relationship between MCS at 24 months and pack years



Linear regression analysis was performed to determine whether lifetime exposure to cigarette (pack years) had an effect on the change in MCS 24 months after surgery. Pack years is calculated by multiplying average number of cigarettes smoked per day by the number of years smoked during the patients' life and dividing that by 20. Figure 6.4 shows that there was a significant negative correlation between the change in MCS 24 months and pack years ($r = -0.3$, $p=0.01$). In these analyses, non-smokers were excluded from analysis (as they did not have pack years to speak of).

Figure 6.4: Relationship between Change in MCS at 24 months and Pack Years



Generalised Linear Model (GLM)

The GLM was used with the dependent variable being MCS 24 and the factors being analysed were the type of surgery, smoking status and MCS before surgery (MCS 0). This essentially serves as a baseline for future GLMs, in which we include other factors into the model. The results are shown below. Smoking did have a significant effect and smokers were predicted to have a MCS that was 13 points worse than non-smokers. The MCS before surgery was also a significant factor in the model.

Table 6.3: Results of the Limited Generalised Linear Model

Parameter	Magnitude of effect	Std. Error	95% Confidence Interval		Sig.
			Lower	Upper	
Type of surgery					
ACI	0.26	4.5	-8.6	9.1	0.9
MACI	0[a]	.	.	.	.
Smoking status					
Smoking	-13.3	5	-23.3	-3.4	0.009
Ex-smoker	-14.5	7.7	-29.7	0.7	0.06
Non-smokers	0[a]	.	.	.	.
MCS 0	0.6	0.13	0.3	0.85	<0.001

0[a] represents the reference category for comparison of other categorical data within the group.

Results – Body Mass Index

Patients who were overweight or obese were older although this was not statistically significant (Table 6.4). Furthermore, these two groups of patients had experienced their symptoms for longer before undergoing the index procedure but this was also not significant. The number of previous operations and size of the lesion was similar in all three groups. The proportion of non-smokers in the three groups was slightly higher in patients with ideal BMI but this was also not statistically significant.

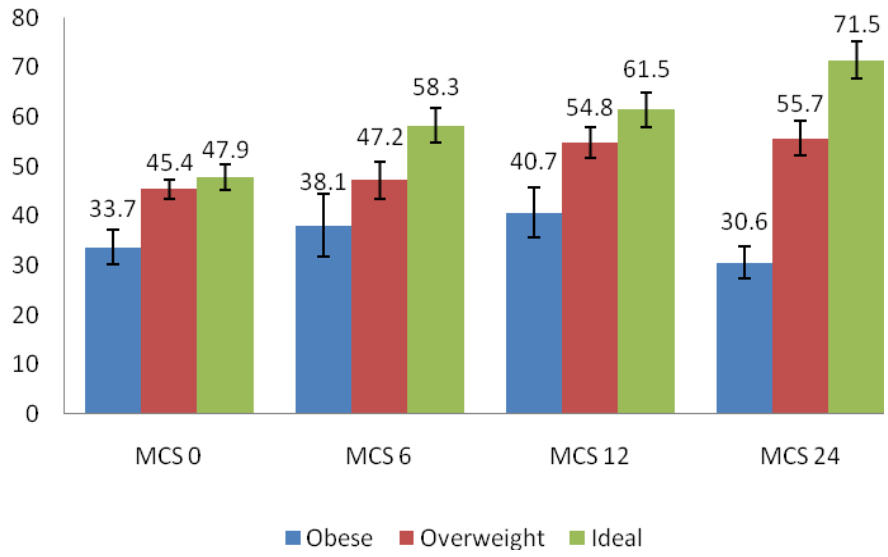
Table 6.4: Patient and lesion characteristics according to BMI

	Ideal Weight (n=53)	Overweight (n=63)	Obese (n=22)	p-value
Age	32 +/- 1.2	35.2 +/- 1	35 +/- 1.7	0.1
Body Mass Index	23 +/- 0.3	27.1 +/- 0.2	33.4 +/- 0.7	<0.001
Proportion of non-smokers	58%	47%	45%	0.5
Duration of symptoms (months)	90.9 +/- 11.8	116.6 +/- 10.7	129.8 +/- 20.9	0.2
No. of previous operations	2.2 +/- 0.2	2.4 +/- 0.2	2.5 +/- 0.4	0.7
Size of lesion (cm)	5.4 +/- 0.5	5.6 +/- 0.4	5.7 +/- 0.7	0.9

Table displays mean value with standard error of mean

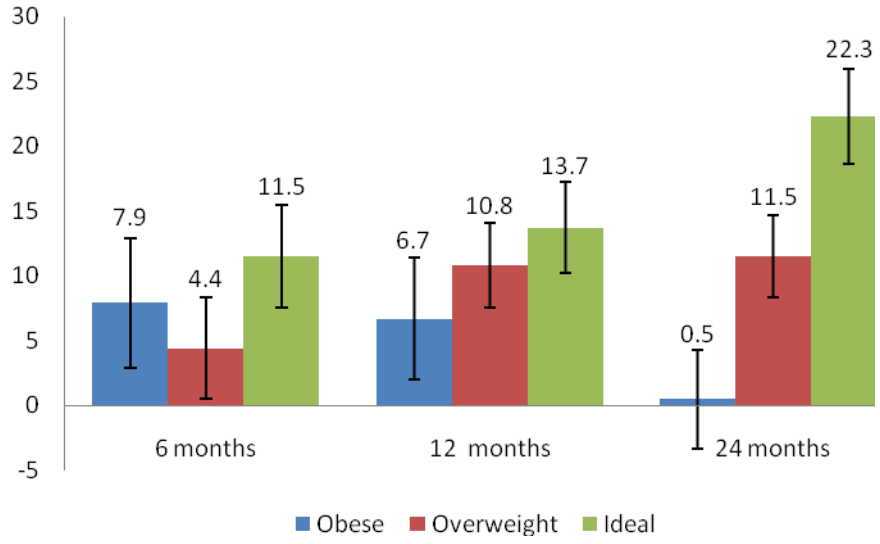
The Modified Cincinnati Scores before and after surgery are shown in figure 6.4 below. Once again this is for descriptive purposes and allows comparison of scores with literature. Figure 6.5 shows the change in MCS from baseline and judges the efficacy of surgery in these groups.

Figure 6.4: Functional scores before and after surgery according to BMI



Graph displays mean MCS with standard error bars

Figure 6.5: Change in Modified Cincinnati Score according to BMI



Graph displays mean MCS with standard error bars

Non-smokers had the greatest improvement 24 months following surgery and analysis of variance (ANOVA) revealed this to be statistically significant ($p=0.002$).

According to the Modified Cincinnati Score, the proportion of good and excellent outcome was higher in patients with ideal BMI than those who were overweight or obese (Table 6.4). This was the case at all time frames after surgery though was only statistically significant at one and two years following surgery. At the 24 month post-operative period, only one obese patient (5.5%) experienced a good or excellent outcome compared with 49% of overweight patients and 82% of patients with ideal BMI. This discrepancy was highly significant. An advantage of analysing results in this way is that it can enable us to compare these results with other studies which do not use the same scoring systems but do categorise results in a similar fashion. The disadvantage is that the effect of surgery is not being measured as we are not comparing pre- and post- operative scores. This will be discussed later in the chapter.

Table 6.5: Proportion of Good and Excellent Results According to the MCS

Time	Outcome	Obese	Over-weight	Ideal Weight	p-value
6 months	Excellent	2 (14%)	2 (6%)	6 (17%)	0.08
	Good	1 (7%)	11 (33%)	14 (39%)	
	Fair	6 (43%)	13 (39%)	13 (36%)	
	Poor	5 (36%)	7 (22%)	3 (8%)	
	Total	**14**	**33**	**36**	
1 year	Excellent	1 (6%)	8 (16%)	9 (22.5%)	0.014
	Good	5 (28%)	18 (35%)	18 (45%)	
	Fair	4 (22%)	18 (35%)	9 (22.5%)	
	Poor	8 (44%)	7 (14%)	4 (10%)	
	Total	**18**	**51**	**40**	
2 years	Excellent	0	8 (18%)	17 (45%)	<0.0001
	Good	1 (5.5%)	14 (31%)	14 (37%)	
	Fair	10 (55.5%)	17 (38%)	4 (10%)	
	Poor	7 (39%)	6 (13%)	3 (8%)	
	Total	**18**	**45**	**38**	

p-values derived from Chi-Square Test

According to the paired t-test, patients with an ideal weight had a statistically significant improvement at the time of final follow-up of 2 years after surgery compared to baseline ($p<0.0001$). Overweight patients experienced similar improvements in the MCS 2 years after surgery, though the p value failed to reach statistical significance ($p=0.07$). ANOVA revealed that there was a statistically significant difference in the change in MCS 2 years after surgery between the three groups, with the greatest improvement occurring in the ideal weight group. ($p=0.002$).

Figure 6.6: Relationship between BMI and MCS at 24 months

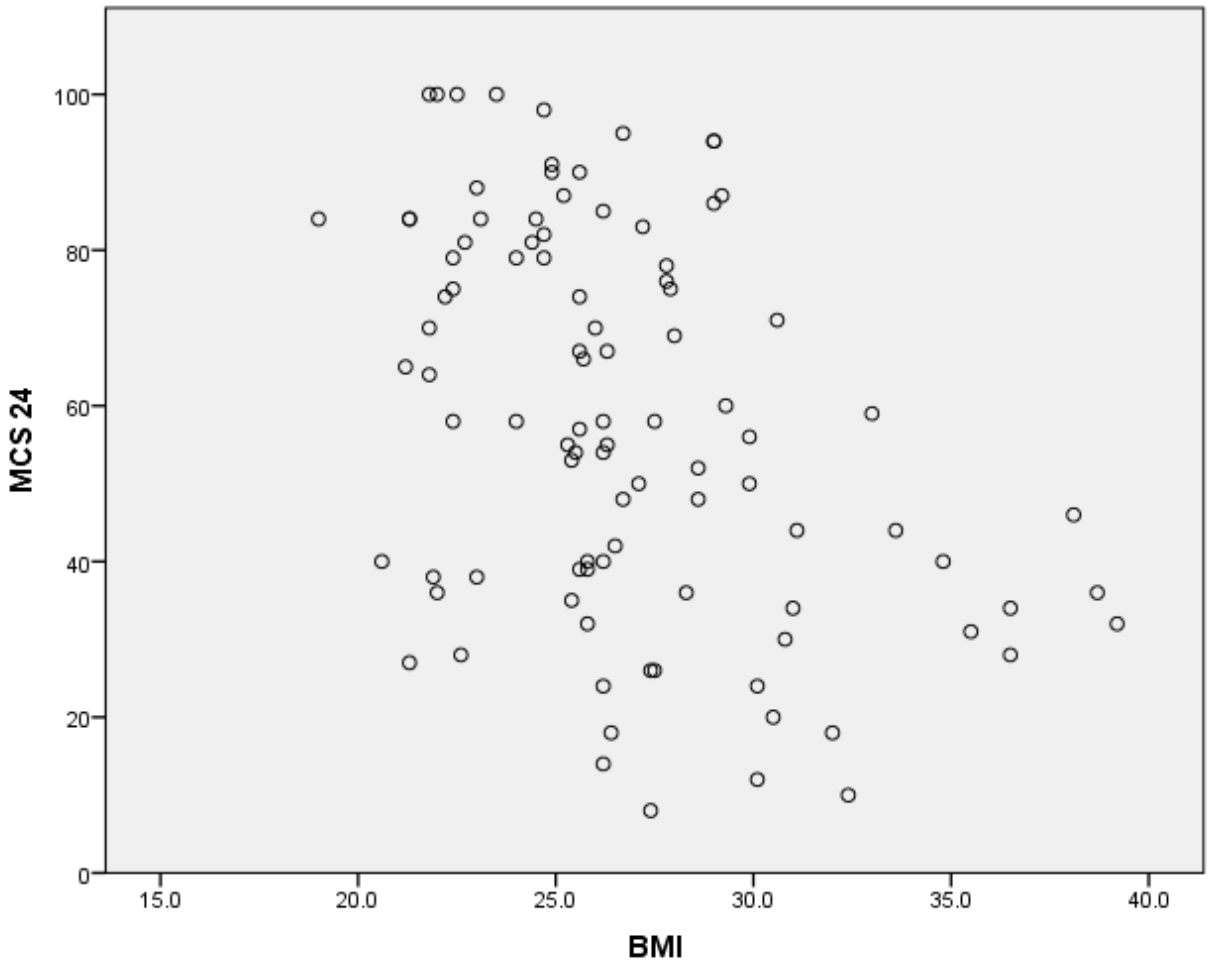
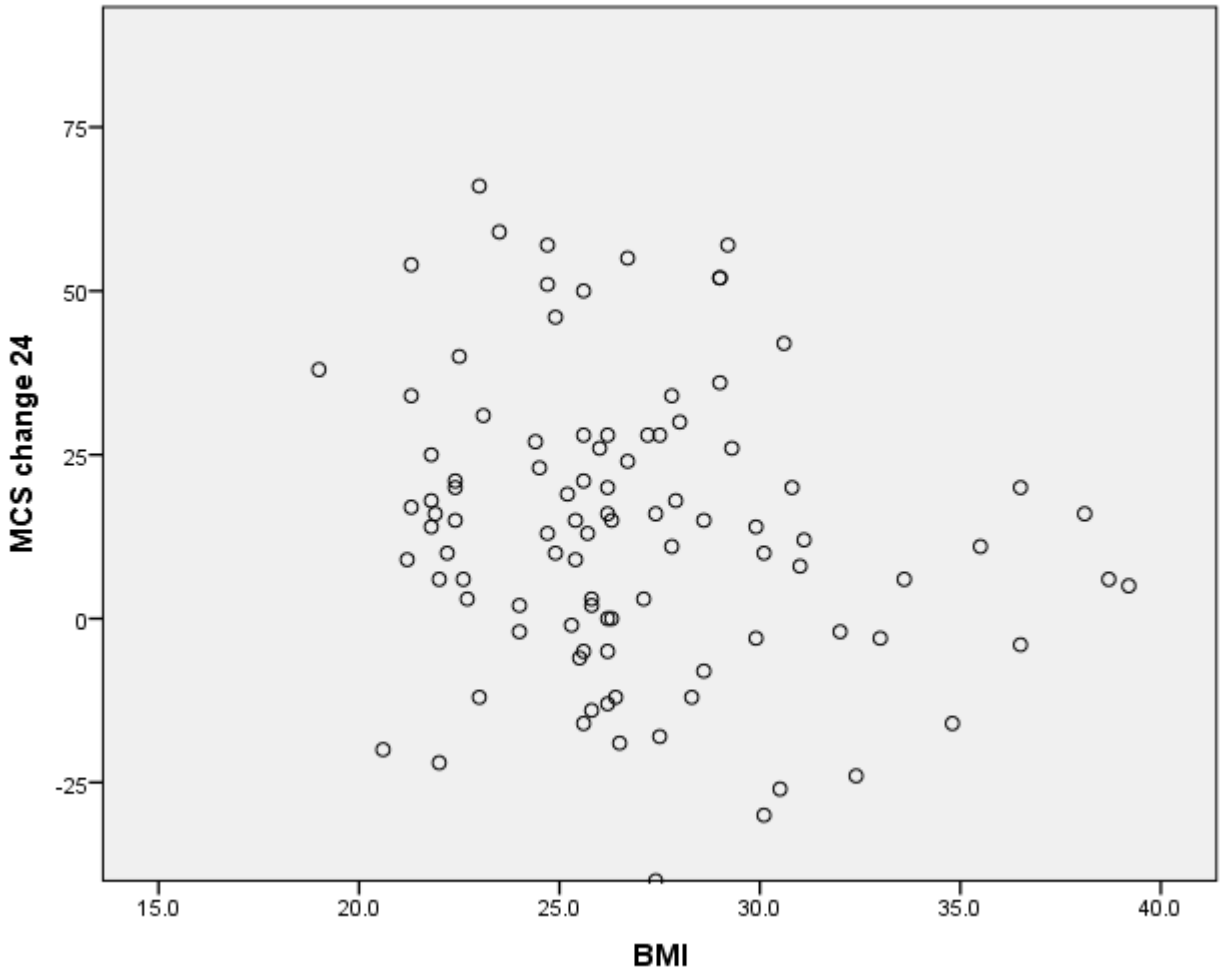


Figure 6.6 displays a statistically significant negative relationship between the BMI and the MCS 24 months after surgery (r = -0.4, p=0.001). However such a relationship did not exist when linear regression was performed using the variables BMI and change in MCS at 24 months. Figure 6.7 illustrates the relationship between BMI and the change in MCS 24 months after surgery.

Figure 6.7: Relationship between BMI and change in MCS 24 months after surgery



Generalised Linear Model (GLM)

The GLM was used with the dependent variable being MCS 24 and the factors being analysed were the type of surgery, BMI and MCS before surgery (MCS 0). This essentially serves as a baseline for future GLMs, in which other factors will be included in the model. The results are shown below. Once again MCS 0 was a strong predictor of the MCS 24 months after surgery, as was BMI, which had a greater magnitude of effect. In this model if the BMI increased by 1, the MCS 24 was predicted to decrease by 1.5.

Table 6.6: Results of the Limited GLM

Parameter	Magnitude of effect	Std. Error	95% Confidence Interval		
			Lower	Upper	Sig.
Type of surgery					
ACI	-0.8	4.2	-9.1	7.5	0.8
MACI	0[a]	.	.	.	.
MCS0	0.6	0.1	0.3	0.8	<0.001
BMI	-1.6	0.5	-2.6	-0.5	0.004

0[a] represents the reference category for comparison of other categorical data within the group.

Results – Physical Activity

ANOVA revealed a significant difference in the mean age between the four groups of patients. Patients involved in high impact sports had experienced their symptoms for shorter duration than the other groups, though this was not significant. All the other patient and lesion characteristics were similar (Table 6.6). There was a significant difference in the proportion of smokers and non-smokers between the four groups with the highest percentage of non-smokers belonging in the 'Mid-impact' and 'High-impact' groups.

Table 6.7: Patient and lesion characteristics in patients with varying degree of physical activity pre-injury

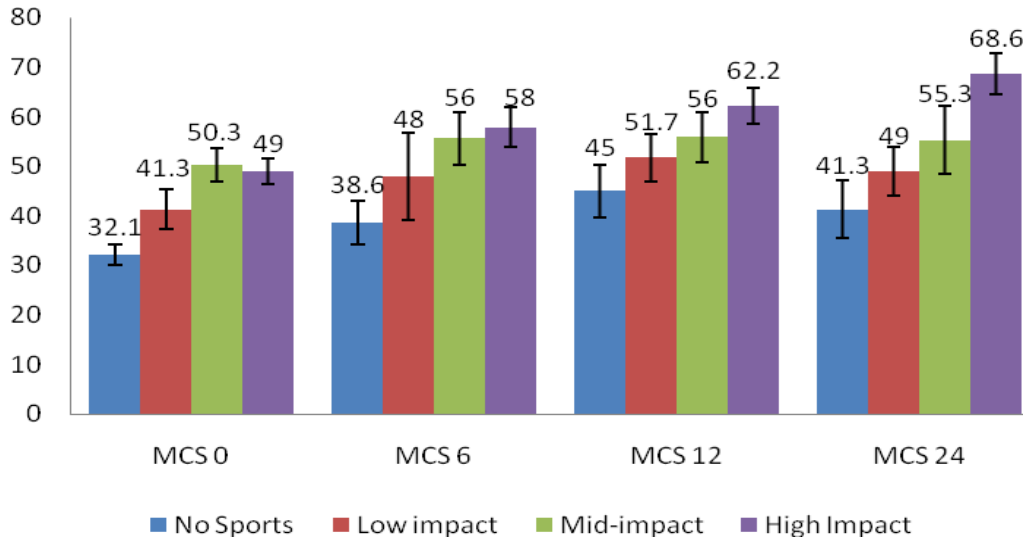
	No Sports (n=25)	Low impact (n=21)	Mid-impact (n=24)	High Impact (n=49)	p-value
Age	36.2 +/- 1.4	34.2 +/- 2.1	35.5 +/- 1.6	31 +/- 1.3	0.04
Body Mass Index	26.5 +/- 1	26.5 +/- 0.9	27.4 +/- 1	26 +/- 0.4	0.6
Proportion of non-smokers	28%	29%	75%	61%	<0.001
Duration of symptoms (months)	117.8 +/- 10.8	134.6 +/- 23.6	129.1 +/- 23.5	92.3 +/- 10.4	0.2
No. of previous operations	2.4 +/- 0.2	2.2 +/- 0.3	2.1 +/- 0.2	2.3 +/- 0.2	0.9
Size of lesion (cm)	5.4 +/- 0.6	6.1 +/- 0.7	5.9 +/- 0.9	5.3 +/- 0.5	0.8

Table displays mean value with standard error of mean

The independent t-test revealed that patients partaking in high impact sports prior to their injury had statistically significantly higher pre-operative MCS ($p<0.001$) and at the final time point 24 months after surgery ($p=0.001$). When analysing the mean increase in MCS, there was not much difference between the four groups (especially at 1 year following surgery).

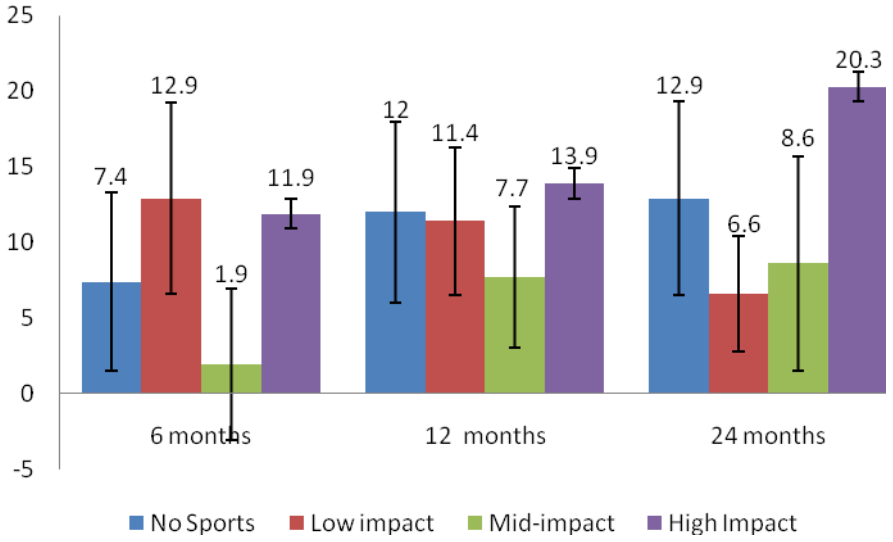
Two years after surgery, the greatest increase in MCS occurred in the High Impact group, though this was not significant (p=0.1) (see figure 6.9).

Figure 6.8: Functional scores before and after surgery according to physical activity



Graph displays mean MCS with standard error bars

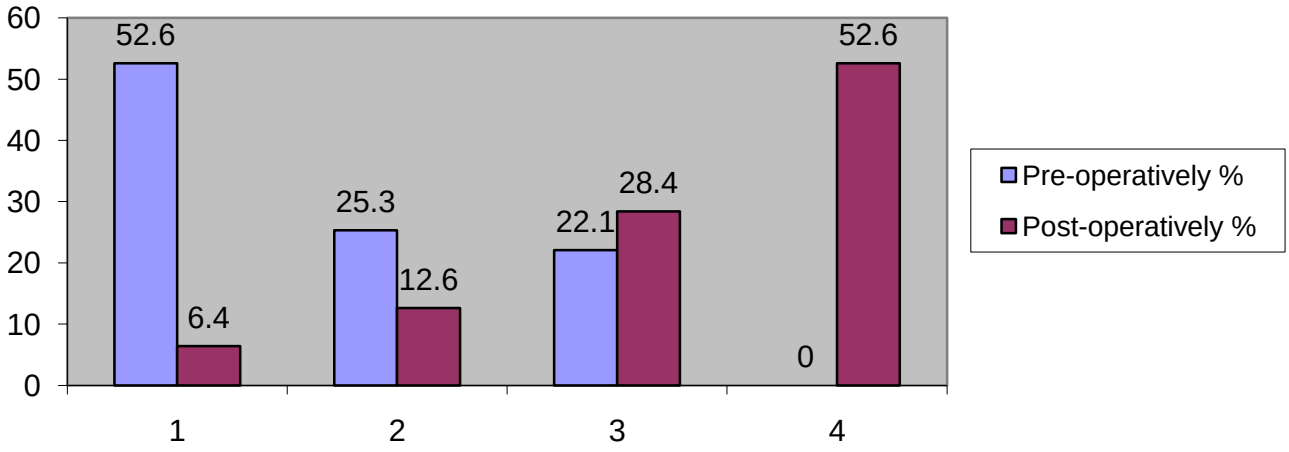
Figure 6.9: Change in Modified Cincinnati Scores according to physical activity



Graph displays mean MCS with standard error bars

Of the 94 patients in participating in sports pre-injury, forty-five (47.9%) have returned to some form of sporting activity. This has mainly been in a low- to mid-impact kind of sporting activities (see figure 6.10). Within the sporting group (n=94), if the patients had to wait for 5 years or longer for surgery then they were less likely to return to sporting activity (26% versus 50%, p=0.025).

Figure 6.10: Return to sporting activity



1) High-impact sports: jumping, pivoting, cutting (basketball, volleyball, soccer, gymnastics)

2) Mid-impact sports: running and turning (racquet sports, hockey, skiing)

3) Low-impact sports: running, cycling, swimming

4) No sports, activities of daily life

Generalised Linear Model

The GLM was used with the dependent variable being MCS 24 and the factors being analysed were the type of surgery, MCS before surgery (MCS 0) and physical activity. This essentially serves as a baseline for future GLMs, in which other factors will be included in the model. The results are shown below. Once again MCS 0 was a strong predictor of the MCS 24 months after surgery. Patients partaking in high impact sports were predicted to have a MCS 24 score that was 13.3 points greater than patients that did not take part any sport.

Table 6.8: Results of the limited GLM according to physical activity

Parameter	Magnitude of effect	Std. Error	95% Confidence Interval		
			Lower	Upper	Sig.
Type of surgery					
ACI	1.3	4.6	-7.7	10.3	0.84
MACI	0[a]	.	.	.	.
Physical activity					
High impact	13.3	6.75	0.09	26.5	0.048
Mid-impact	1.1	7.9	-14.4	16.6	0.9
Low-impact	-2.7	7.3	-17	11.6	0.7
No Sports	0[a]	.	.	.	.
MCS 0	0.6	0.14	0.37	0.9	<0.001

0[a] represents the reference category for comparison of other categorical data within the group.

Generalised Linear Model with all three variables

There were 81 patients in total who had their BMI, smoking status and pre-operative physical activity profile recorded. The distribution of smokers and non-smokers as well as the physical activity profile can be seen in Table 6.8. Table 6.9 displays the mean for each of the continuous variables in this cohort of patients.

Table 6.9: Categorical Variable Information

		N	Percent
Smoker	Smoker	31	38.3%
	Ex-smoker	7	8.6%
	Non-smoker	43	53.1%
	Total	81	100.0%
Sports Type Preop	High impact	34	42.0%
	Mid-impact	14	17.3%
	Low impact	18	22.2%
	No Sports	15	18.5%
	Total	81	100.0%

Table 6.10: Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	MCS24	81	8	100	57.6	25.3
Covariate	BMI	81	19	39.2	26.7	4.32
	MCS0	81	10	94	43.7	17.8

The smoking status, pre-operative physical activity profile, BMI and MCS 0 all had statistically significant effect on outcome 2 years following surgery (Table 12). The most significant variable was BMI and the magnitude of effect was such that each increase in pre-operative BMI could predict a 2.1 points decrease in MCS 24. This effect was more powerful than MCS 0. Using this model, smokers could be predicted to have a MCS 24 that is 12.5 points less than non-smokers. Similarly, those taking part in high impact sports could be predicted to have a MCS 24 that is nearly 12 points greater than those that do not take part in sports at all.

Table 6.11: Results of Generalised Linear Model

Parameter Estimates

Parameter	Magnitude of effect	Std. Error	95% Confidence Interval		Hypothesis Test
			Lower	Upper	Sig.
Smoking Status					
Smoker	-12.5	4.8	-21.9	-3.0	0.01
Ex-smoker	-3.7	8.1	-19.5	12.1	0.65
Non-smoker	0[a]	.	.	.	.
Physical activity					
High impact sports	11.6	6.1	-0.5	23.6	0.06
Mid-impact sports	-1.97	7.4	-16.5	12.5	0.8
Low Impact Sports	-1.67	6.6	-14.6	11.2	0.8
No Sports	0[a]	.	.	.	.
Continuous Variables					
BMI	-2.1	0.5	-3.1	-1.1	p<0.001
MCS0	0.4	0.1	0.1	0.6	0.004

0[a] is used as the reference category to compare other factors against

Generalised Linear Model – Further variables

A generalised linear model incorporating more variables was utilised. These included number of previous operations, duration of symptoms, age of patient and size of lesion (Table 6.11). Only 66 patients included all of these variables. Table 6.12 displays the continuous variable information.

Table 6.12: Further Categorical Variable Information

		N	Percent
Smoking status	Smoker	26	39.4%
	Ex-smoker	5	7.6%
	Non-smoker	35	53.0%
	Total	66	100.0%
No. previous ops	1	19	28.8%
	2	18	27.3%
	3	12	18.2%
	4 or more	17	25.8%
	Total	66	100.0%
Physical activity profile	High impact	26	39.4%
	Low and Mid-impact	26	39.4%
	No Sports	14	21.2%
	Total	66	100.0%

Table 6.13: Further Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	MCS24	66	8	100	57.8	25.1
Covariate	Age	66	18	50	33.5	8.5
	BMI	66	19	39.2	26.8	4.3
	Length of symptoms	66	3	300	106.4	77.9
	MCS0	66	10	94	44.4	17.3
	Size	66	1	15	5.6	3.2

Including these variables in the model has altered the predictors of outcome significantly (Table 6.13). The most profound effect is that MCS 0 no longer predicts MCS 24 which does not seem logical as that is the one parameter which would seem the most likely to be related to functional scores following surgery. From the previous model, the results were similar in mid- and low impact sportsmen and therefore they were grouped together in this model. This still did not alter that fact that physical activity was still a statistically significant predictor of outcome (p=0.02). The duration of symptoms prior to the index procedure was a new found significant factor in determining MCS 24. Table 6.13 shows that for a further month that the patient has to wait for ACI, the MCS 24 is likely to be 0.07 less. This means that if a patient has to wait a year the MCS 24 is likely to be 1 point less. The strength of the relationship between BMI and MCS 24 as well as smoking status and MCS 24 has also strengthened when the interplay of all the above mentioned factors is taken into consideration. This is surprising since the number of patients in the model has decreased and the number of co-variates and predictors has increased from the previous model.

Table 6.14: Results of the new generalised linear model

Parameter Estimates

Parameter	Magnitude of effect	Std. Error	95% Wald Confidence Interval		Hypothesis Test
			Lower	Upper	Sig.
Type of surgery					
ACI	4.3	4.3	-4.1	12.6	0.3
MACI	0[a]	.	.	.	.
Smoking Status					
Smoker	-15.1	4.9	-24.7	-5.5	0.002
Ex-smoker	-8.7	8.8	-25.9	8.483	0.3
Non-smoker	0[a]	.	.	.	.
Previous Operations					
1	8.6	6.5	-4.1	21.4	0.2
2	6.3	6.1	-5.7	18.227	0.3
3	6	6.6	-6.9	18.933	0.4
4 or more	0[a]	.	.	.	.
Physical Activity Profile					
High Impact	11.4	6.5	-1.3	24	0.08
Mid- and Low impact	-2.811	6	-14.6	9	0.6
No Sports	0[a]	.	.	.	.
Continuous Variables					
Age	-0.24	0.3	-0.8	0.3	0.4
BMI	-2.4	0.5	-3.4	-1.3	<0.001
Length of symptoms	-0.07	0.03	-0.1	-0.01	0.02
MCS0	0.12	0.15	0.18	0.4	0.4
Size	0.001	0.007	-0.01	0.015	0.9

0[a] represents the reference category for comparison of other categorical data within the group.

A possible explanation for the lack of effect of MCS 0 on MCS 24 is that there may be a relationship MCS 0 with one of the other continuous variables. If this is the case then two

highly correlated variables in one model may both become insignificant. This is termed collinearity. Table 6.14 displays the results of these correlations.

Table 6.15: Correlations between the continuous variables in the Generalised Linear Model

		Age	BMI	Length of symptoms	MCS0	Size
MCS0	Pearson Correlation	-0.1	-0.3**	-0.21*	1	0.05
	Sig. (2-tailed)	0.2	0.003	0.046		0.6
	N	134	135	122	135	117

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

As expected there was significant relationship between MCS 0 and BMI. There was also a significant negative relationship between MCS 0 and length of symptoms. However, in the Generalised Linear Model, Length of Symptoms is still significantly related to MCS 24 whereas MCS 0 is not. This suggests that the strength of the relationship between MCS 24 and Length of Symptoms is greater than that between MCS 0 and MCS 24.

Discussion

The findings from this study suggest that smoking and having a high BMI prior to surgery can have a deleterious effect on outcome following ACI. Although smokers and obese patients' pre-operative knee function is less (according to the MCS), the improvement after surgery is also lower. Patients involved in high impact physical activity are likely to do better than patients that are not involved in any sporting activity. The results from the Generalised Linear Model confirm that these factors have an effect on outcome independent of each other. The next section will discuss these factors in more detail.

A number of different statistical tests have been used to address similar questions. It was necessary to present the results as seen in figures 6.1 (p.188), 6.4 (p.196) and 6.8 (p.203) as this represents the various groups we are interested in and the difference in their pre-operative and post-operative function (essentially a presentation of 3 different case-controlled studies). This allows a comparison of the results from this study with other studies that have utilised the Modified Cincinnati Score (MCS). For example it can be stated non-smokers have a MCS at 2 years that is comparable to the study in Gothenburg (Peterson et al. 2010) whereas smokers do not. However a number of other studies have not used the MCS and instead have grouped their results as Excellent/Good and Fair/Poor according to different scoring systems. Dividing patients into these 4 categories is a less efficient way of analysing the results but does enable comparison with a greater number of other studies.

Figures 6.2, 6.5 and 6.9 give an idea of the effect the variables have on the response to surgery (i.e. the change in MCS from pre-operative status and how this varies between different groups). This is perhaps more important than the previous graphs as the data is paired and we can sequentially see the efficacy of ACI in different groups of patients (e.g. the

change in MCS 2 years after surgery is almost non-existent in obese patients compared to a 22 point increase in ideal weighted patients). Perhaps the most efficient way of analysing all of these factors is with the use of a generalised linear model. This gives some indication of the magnitude of effect of each of the mentioned variables on outcome after surgery but this is not comparable to other studies and they have not utilised the same statistical model. However, it does allow us to determine whether the variables have a significant impact on outcome independent of each other which is important in any study assessing prognostic factors.

1. Smoking

To the best of our knowledge, this is the first study to assess the effect of smoking on outcome of ACI/MACI. The results suggest that patients who smoke have worse pre-operative function. In addition, they do not experience as great a benefit from this procedure as non-smokers. It is unclear why smoking should affect outcome after ACI. Articular cartilage is avascular and does not rely on constituents in blood for repair. It receives its nutrition and oxygen supply by diffusion from the synovial fluid and subchondral bone (Hofstaetter et al 2005, Lane et al. 1977). The partial pressure of oxygen in synovial fluid (50 to 60 mmHg) (Lund-Olesen 1970) is approximately half than in arterial blood (80 to 100 mmHg) and in osteoarthritic joints the oxygen tension is decreased further (Pfander et al. 2005). In animal models, long term hypoxia down regulated gene expression levels of collagen and growth factors in knee articular cartilage (Hofstaetter et al 2005). Knee articular cartilage also expresses the hypoxia-inducible factor 1α, which helps tissue function at low oxygen tensions.

Smoking has been shown to reduce its expression in other tissues (Michaud et al. 2003) and perhaps a similar mechanism is applicable in cartilage. Smoking has also been shown to delay chondrogenesis in a mouse model of tibial fracture healing. Mice exposed to smoke exhibited less type II collagen in the fracture callus with a delay in the chondrogenic phase of fracture healing (El-Zawawy et al. 2006). Tissue hypoxia may have been a major factor in the impaired production of cartilaginous callus in mice exposed to smoke. Perhaps, decreased or delayed type II collagen synthesis as a result of smoking as well as tissue hypoxia in the synovial joint lead to the deleterious effects of smoking on cartilage repair in our patients.

Several reports have suggested that smokers experience more musculoskeletal pain than non-smokers (Amin et al. 2007, Mallon et al. 2004, Anderson et al. 1998, Karim et al. 2006). In a longitudinal study assessing the degree of cartilage loss in male smokers and non-smokers according to MRI, men who smoked at the baseline of the study had statistically significant higher pain scores (according to the visual analogue score) than non-smokers (Amin et al. 2006). They were also found to have an increased risk for cartilage loss at the medial tibiofemoral joint (odds ratio (OR) 2.3 with 95% confidence interval (CI) 1.0 to 5.4) and the patellofemoral joint (OR 2.5, 95% CI 1.1 to 5.7). Pain constitutes one fifth of the modified Cincinnati score, the other activities being assessed are walking, jumping, running, stairs, overall activity as well as symptoms of swelling and giving way. Therefore our cohort of smokers not only experience greater pain but also global restriction in function. Smokers were also found to have higher levels of pre-operative pain and lower levels of function prior to rotator cuff repair. In this study, smokers experienced less improvement following open rotator cuff repair than non-smokers (Mallon et al. 2004).

Poorer outcomes were reported amongst smokers following anterior cruciate ligament reconstruction (Karim et al 2006). Though pain was not an issue in such cases, smokers were less likely to return to their original level of pre-injury sport and had worse functional knee scores than non-smokers. Animal studies suggest that decreased cellular density and type I collagen expression in injured ligament of mice exposed to smoke provides an explanation for poor healing of knee ligaments (Gill et al 2006).

ACI/MACI is not a universally successful procedure with reported rates of graft failures ranging from 7 to 25%, delamination from 8 to 22% and graft hypertrophy from 0 to 36% (Gikas 2009). An important study has shown several prognostic factors that may be

associated with poor outcome following ACI. These include older patients, patients with lower pre-operative Cincinnati scores, longer duration of symptoms, multiple defects, and multiple procedures prior to the index procedure (Krishnan et al 2006). This case-controlled study has highlighted smoking as a possible cause of failure. Smoking has been shown to be the single most important risk factor for the development of complications after elective arthroplasty of the hip or knee (Moller et al. 2003). In addition wound complications have been shown to be related to smoking habits and smokers are more likely to need further surgery (Espehaug et al. 1997). Observational studies suggest that prolonged abstinence from smoking decreases the risk of many perioperative complications (Moller et al. 2003). Though the ex-smokers group did not experience as greater a benefit from ACI/MACI, the improvement following surgery was still better than the smoking group.

Conclusion

The counselling of patients undergoing ACI/MACI should include smoking not only as a general cardiopulmonary risk, but also that a poorer result can be expected following this procedure. Patients should be encouraged to enrol in smoking-cessation programme and at the very least stop smoking 6 to 8 weeks prior to surgery and during the period of rehabilitation as recommended in the literature (Moller et al 2003, Warner 2006).

2. Body mass index (BMI)

Following any kind of surgery, obese patients have a significantly higher risk of complications, including myocardial infarction, wound infection, nerve injury and urinary tract infection (Bamgbade et al. 2007). There is also a two fold increase in death rate following major surgery. Functional results following orthopaedic procedures are rather more unpredictable. Amin et al demonstrated no difference in Knee Society Scores in 300 consecutive primary total knee replacements when comparing obese with non-obese patients (Amin et al 2006). Conversely, Berend et al reported that patients with a BMI greater than 33.7 was associated with medial bone collapse following total knee arthroplasty (Berend et al 2004) and a BMI greater than 32 predicted higher failure rates after unicompartmental knee arthroplasty (Berend et al 2005). More dramatic failure rates are seen in obese patients who underwent patellofemoral replacement (van Wagenberg et al. 2009). The failure to identify increased revision rates in obese patients who have undergone total knee or hip arthroplasty may be related to the fact that wear of the prosthesis is a function of use not time. McClung et al analysed the relationship between quantitative activity (measured with pedometer) and BMI and demonstrated in 209 individuals that had undergone total hip or knee arthroplasty that a higher BMI was associated with lower activity (McClung et al. 2000).

Results from this study are unequivocal. This is the first study of its kind to analyse results following ACI in obese and non-obese patients. Indeed, a comprehensive literature review failed to identify clinical research on the effect of obesity on any cartilage repair techniques in the knee. However one small study in the German literature did show statistically inferior results in overweight compared with normal weight patients following microfracture for the treatment of OCDs in the talus (Becher et al 2008).

The poor results following ACI in the knee can probably be explained by the increase in mechanical forces across the joint with an increase in patient weight. Several *in vitro* studies have shown that extracellular matrix (ECM) production by chondrocytes is highly sensitive to mechanical signals mediated by loading (Gabbay et al. 2008). Whilst moderate, regular exercise is beneficial for cartilage constitution (Roos & Dahlberg 2005), excessive stresses or static stress is harmful (Sharma & Chang 2007) and this is more likely to be present in obese patients. Static loading has also been shown to increase the synthesis of pro-inflammatory cytokines (Knobloch et al 2008, Sharma et al 2007). A case controlled study has shown a significant relationship between higher BMI and meniscal tears (Ford et al 2005) adding further weight to the argument that obese patients experience greater mechanical forces in the knee that is detrimental to knee function.

The poor outcome in obese patients following ACI cannot be explained by mechanical stress alone. The established relationship between obesity and OA in non-weight bearing joints such as the hand (Attie & Scherer 2009) suggests that adipose tissue may have a role to play in this study also. Adipose tissue has been shown to secrete pro-inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-A) and IL-1 (Trujillo et al 2006). A more recent study has confirmed that infrapatellar fat pad in obese women's knees secreted greater concentrations of IL-6 than subcutaneous adipose tissue (Distel et al 2009). There were also higher secretions of adiponectin and lower levels of leptin. Their results suggest that locally produced pro-inflammatory cytokines produced in the knee may contribute to cartilage damage in obese patients. Such an effect is not age related and may contribute to poor results seen in this study.

A prospective, longitudinal observational study determined whether serum levels of IL-6, TNF-A, and CRP as well as BMI were associated with an increased prevalence of

radiographic knee osteoarthritis (Livshits et al. 2009). Using multiple logistic regression, they demonstrated that a high BMI ($p=0.0003$) and increased levels of IL-6 were independent predictors of the appearance of radiographic OA 5 years from the time these cytokines were measured. Their results further confirm the important role IL-6 may have in propagating OA and that this is independent of BMI. Could IL-6 represent a therapeutic target to prevent cartilage damage?

As well as IL-6, adipose tissue also synthesizes and secretes over a hundred other proteins (such as hormones and growth factors) termed adipokines (Trujillo et al. 2006, Gabay et al 2008). The main adipokine is Leptin, and since its discovery in 1994 (Zhang et al 1994), its role has been extensively studied. Adiponectin and resistin are two further adipokines that are associated with leptin in playing key roles in homeostatic mechanisms such as insulin regulation and inflammation as well as pathologic events. All three adipokines have been implicated in the pathogenesis of chronic inflammatory joint disease such as rheumatoid arthritis (Pottie et al 2006). Leptin has been shown to induce the expression of growth factors, stimulate proteoglycan and collagen synthesis, and increase the stimulatory effect of pro-inflammatory cytokines on nitric oxide production in chondrocytes (Aspden et al 2001). Leptin also has an effect on bone tissue (Takeda et al 2003). Some of the most recent research has demonstrated that leptin behaves differently in lean and obese patients (Pallu et al 2010). Matrix metalloproteases (MMPs) and tissue inhibitors of metalloproteases (TIMPs) are physiologic degradative enzymes and their protein inhibitors respectively. Up regulation of TIMP-2 was markedly increased in leptin treated chondrocytes compared to control in normal and overweight patients only and decreased in obese patients (Pallu et al. 2010). Furthermore, the expression of MMP-13 was greatest from leptin treated chondrocytes from obese patients. These BMI-dependent effects of leptin may change the cartilage repair process following

ACI. The leptin-induced TIMP-2 expression may delay cartilage destruction in non-obese patients whilst promoting cartilage damage in obese patients. The question that remains unanswered is if obese patients lose weight, will leptin behave differently, or have obese patients' bodies adapted to permanently respond in a negative manner?

3. Physical Activity

This study reports significantly better results in patients actively involved in high impact sports prior to their injury than patients not involved in sports at all. The absolute MCS 2 years following surgery was significantly better in the high impact sporting group as was the increase in MCS 2 years after surgery. The high impact group were the only group of patients to experience a statistically significant increase in MCS 6 months after surgery and the scores continued to improve 12 and 24 months after surgery.

Kreuz et al. demonstrated better results of ACI in patients with higher sports activity levels from 6 months following surgery and this discrepancy in results was maintained at 18 and 36 months after surgery (Kreuz et al 2007). However, it was interesting to note that in their study there was no correlation between sports activity levels and pre-operative Cincinnati scores. In their cohort of patients, the 94% returned to some form of sporting activity (though the majority were at a lower level).

In another study, only 15 out of 45 soccer players returned to playing this sport with 12 at the same pre-injury skill level (Mithoefer et al 2005). This was despite their cohort being younger, with a shorter duration of symptoms and less number of previous operations compared to the patients in this study. Furthermore, functional outcome, time to return and return to sports after ACI was related to level of participation in elite soccer players compared with recreational players (84% versus 16%).

A systematic review analysed results from 12 studies assessing microfracture, 7 on ACI and 5 on mosaicplasty all in athletes. Good and excellent results were observed in 67% in the microfracture group, 82% in the ACI group and 93% in mosaicplasty group. The rate to return to sporting activities were 67% in ACI group, 66% in microfracture group and 91% in

mosaicplasty group (Mithoefer et al 2009). However, over the longer-term, the best durability was reported after ACI (96% versus 52% for microfracture and mosaicplasty).

Many animal studies have shown the positive effect of exercise on the healing of articular cartilage defects. Such defects in exercised horses and dogs resulted in greater concentration of endogenous proteoglycan as well as thicker and stiffer cartilage than non-exercised animals (Palmer et al 1995, Jurvelin et al. 1986, Kiviranta et al. 1988, Oettmeier 1992). Further evidence for the protective effects of exercise on articular cartilage has come from studying knockout mice with heterozygous inactivation of Col2a1-genecoding for type II procollagen, which makes cartilage more susceptible to osteoarthritis. In this experiment lifelong wheel running had a protective effect against experimental osteoarthritis (Lapvetelainen et al 2001). Translated clinically, studies have suggested that people who participate in regular physical activity enhance the metabolic activity of cartilage, which may equip articular cartilage with better self-repair mechanism that are otherwise absent in those who are sedentary (Urquhart et al 2007).

Other studies have shown that ACI resulted in 96% of adolescent athletes being able to regularly participate in high-impact, pivoting sports at the recreational level or higher and 60% returned to the same or higher level of athletic activity (Mithoefer & Minas et al. 2005). However, only a third of the patients were able to return to the same level of athletics if their pre-operative symptoms persisted for longer than 12 months. In this chapter, our patients' mean duration of symptoms was 111.4 months and the modal average was 120 months. This discrepancy can be explained by current NICE guidelines which state that any form of cartilage transplant in the UK should not be used for routine primary treatment and all patients receiving such treatment should be enrolled in ongoing or new clinical studies (NICE Guidelines 2005). Consequently, the majority of patients enrolled in this study, have

had several surgical procedures prior to cartilage transplant and have had symptoms for a considerable period of time.

Other forms of surgical treatment for cartilage damage have reported better results in the short-term. Simple debridement and arthroscopic washout of cartilage defects resulted in all 15 soccer players returning to physical activity at a mean of 10.8 weeks (Levy et al. 1996). However, in their cohort the mean defect size was 1.1cm^2 and 26% of patients required further surgical procedures. 2 years following microfracture for the treatment of OCDs in the knee, 44% of athletes were able to return to sporting activity. A younger age group with defect sizes less than 2cm^2 and shorter duration of symptoms (less than 12 months) were associated with a higher rate of return to sports (Mithofer et al. 2006). Good results were obtained with autologous osteochondral grafting, even at 7 years with 21 out of 30 patients returning to sporting activity (Marcacci et al. 2007). However, level I evidence suggests ACI is superior (Bentley et al. 2003), especially in the long term (Bentley et al 2012).

What can be done?

Obesity is an established risk factor for the development and progression of knee osteoarthritis (Felson 1996, Jarvolm et al 2005) and knee pain (Jinks et al 2006). The Arthritis, Diet and Activity Promotion Trial (ADAPT trial) was designed to assess whether long term exercise and dietary weight loss (separately or together) were effective modes of improving knee pain, function and mobility (Messier et al 2004). 316 participants who were overweight(BMI > 28) and over 60 years of age with knee pain, radiographic evidence of OA, and self-reported physical disability were enrolled in the community based trial. Patients were grouped into 4 distinct 18 month interventions: exercise only, dietary weight loss only, dietary weight loss plus exercise, and usual healthy lifestyle (control). 80% completed the study. There were significant improvements in the diet plus exercise group in physical function, 6 minute walk distance, stair climb time and knee pain relative to control. In the exercise group there was a significant increase in the 6 minute walk distance only. The results of this study may not be directly related to the cohort of patients in this study, however the application of weight loss and exercise prior to surgical intervention in the form ACI does seem logical in order to improve outcome. A combination of dietary weight loss and quadriceps strengthening has been shown to significantly reduce knee pain and improve knee function in patients over the age of 45 with a BMI of greater than 28 (Jenkinson et al 2009). These findings were further substantiated in an overview of nine systematic reviews which concluded that exercise interventions for patients with knee OA reduce pain and improve physical function (Jamtvedt et al 2008). Another systematic review reported robust evidence that weight reduction improves self reported disability and reduces pain (Christensen et al 2007). Furthermore, the onset of injury and pattern of injuries over time in overweight and obese patients has been shown to be attributed to BMI and weight reduction may be an

avenue to reduce the risk of injury in sedentary overweight and obese patients (Janney and Jakicic 2010).

Conclusion

The results from this chapter unequivocally identify smoking, a high BMI and low levels of physical activity as risk factors for inferior results following autologous chondrocyte implantation. It was clearly demonstrated that although these factors were related to each other (e.g. there were a greater proportion of smokers in 'non-athletes'), each of the aforementioned variables had an independent effect on outcome. It is difficult to advise patients to increase their activity level following injury. However, they should be advised to stay active and maintain their weight in order to improve outcome.

Chapter 7: Discussion

Methodological Considerations

Most if not all studies in the literature report their results in term of proportions (i.e. percentage of good and excellent results) regardless of the scoring system used to assess patient satisfaction and knee function. Although this allows comparison of results with other studies, there are two main problems with this approach. Firstly, by converting continuous data into binary data, valuable information is lost. Secondly, and more importantly, reporting outcome following surgery as percentage of good/excellent results does not take into account the patients' baseline status (i.e. their knee function before surgery). It has been consistently reported in this thesis that the Modified Cincinnati Score pre-operatively (MCS 0) is the strongest predictor of the latest MCS after surgery, independent of any other variable. Therefore, if patients' scores are high before surgery, the measured MCS after surgery at any time point is also likely to be high. The functional score after surgery is important, but what is more important is how effective the surgery is (i.e. the improvement or increase in MCS), as ultimately that is what we are striving to improve.

In one study, it was reported that ACI patients with high MCS 0 were likely to have a high MCS post surgery (Krishnan et al 2006). It was also stated that patients with longer duration of symptoms had worse outcome and patients with more than 2 procedures prior to ACI did significantly worse than those that had 2 or less procedures. It stands to reason that patients waiting for a long period of time were likely to have had more operations, and also a low

starting MCS. Since any one of these 3 variables could have influenced the final outcome performing simple linear regression without inclusion of said confounding variables is not appropriate. This statistical approach has been widely adopted throughout the Orthopaedic world and not just on reporting on outcome after ACI (Laxdal et al 2005, Gobbi & Francisco 2006, Santaguida et al 2008, Mosely et al 2010, Welsch et al 2010, Dunbar et al 2012).

Multiple linear regression was used by De Windt et al (2009) when assessing prognostic factors determining success of treatment for cartilage defects. Twenty-five patients had undergone ACI and 30 patients had received microfracture and knee function was assessed according to the Knee injury and Osteoarthritis Outcome Score (KOOS). When analysing their results, the continuous explanatory variables were dichotomised such as patient age (less than or greater than 30 years of age) and duration of symptoms (less than or greater than 24 months). This approach has to be questioned as it leads to loss of valuable data and the model used is capable of handling continuous and categorical data. It was interesting to note that patient age showed a high correlation with defect location and was therefore excluded from the multiple linear regression model. Again this has to be questioned as one of the reasons for using this model is its ability to cope with collinearity. Whilst the authors' must be applauded for anticipating the interplay between the prognostic factors, what they did not include was the surgical technique in their regression model. This is of significant importance as the two techniques are very different (microfracture versus ACI). In this thesis ACI and MACI patients were grouped together because fundamentally they are two types of the same technique. However, the surgical technique (i.e. whether the patient had received ACI or MACI) was included as an explanatory variable to see if it had an effect.

Limitations with the Generalised Linear Model

When dealing with the analysis of data in a retrospective study (and especially when assessing prognostic factors in an outcome study), the GLM represents the best way of ensuring the correct results (and consequently conclusions) have been obtained. However, a RCT still remains the best way of answering a clinical research question due to the limitations of the GLM. The key limitation is the number of variables that can be analysed in the model which is dependent upon the sample size. To ensure accurate results, a rough 'rule of thumb' is that the number of variables that should be included in the analysis should not exceed the sample size divided by 10 (Petrie and Sabin 2006). Therefore, if there were 100 patients in a study then the maximum number of explanatory variables that can be included in the analysis is 10. In each of the preceding chapters, the pre-operative variables were similar in the two groups being compared, however, this may not be true in other studies, and hence the analysis may not include all the confounding variables (particularly if there was a small number of patients in the study), leading to inaccurate conclusions being made.

Secondly, there is the issue of collinearity. This is the term given when two explanatory variables in a GLM are highly correlated with each other. Under these circumstances, it is difficult to evaluate the individual effects of each of the explanatory variables and the problem manifests itself by neither explanatory variable having a significant effect on the dependent variable. The solution to this problem is to only include one of the variables. However, this may be difficult if the model includes several variables that are correlated. An example of collinearity occurred in chapter 5. When the GLM was performed and solely analysing the effects of smoking, BMI and physical activity MCS 0 was a strong predictor of MCS 24. When further variables were added to the model (such as duration of symptoms prior to ACI and size of lesion), MCS 0 ceased to be a significant predictor of MCS 24. A simple linear regression was performed to assess the relationship between MCS 0 and

duration of symptoms and size of lesions and it became apparent that a relationship did exist between MCS 0 and duration of symptoms.

Finally and perhaps most importantly, when using the GLM, we are assuming that we know all the possible confounding variables, and if the sample size is large enough are including all of them in the data analysis. This may not always be the case, as it is not always possible to be aware of every possible confounding variable. This is why a RCT is the most accurate method of determining superiority of one treatment over the other. In a well designed RCT, if the randomisation is performed correctly and if there is adequate blinding, then bias due to both known and unknown confounding variables is avoided in the study comparison and should be similar across the randomised treatment groups.

Main Findings

Prognostic factors that may affect outcome may be divided into modifiable and non-modifiable. The randomised controlled trial (chapter 3) revealed no difference in outcome when patients were treated with ACI or MACI. However, when the outcome of ACI vs MACI was analysed in the patello-femoral joint (chapter 4), there appeared to be better outcome if MACI was used. In the PFJ chapter there was a statistically significant superior result when MACI was used in improving MCS 24 months after surgery ($p=0.038$). After taking all confounding variables into account in the GLM, ACI patients were predicted to have MCS that was 7 points less than MACI patients when treating lesions in the patellofemoral joint. The p value was no statistically significant ($p=0.09$). The lack of significance may be due to lack of patient numbers in the GLM (i.e. the study is underpowered). Therefore it can be stated that with respect to the technique of chondrocyte transplantation, there is no statistically significant difference in the outcome when using ACI or MACI.

Chapter 6 analysed other modifiable risk factors, namely BMI, smoking and physical activity. When analysing these factors independently, smoking and a BMI above 30 had a profound negative effect on the MCS 2 years after surgery. It stands to reason that there may be interplay between being overweight, smoking and lack of physical activity. However, when controlling for these variables in the GLM, the negative relationship persevered. It was interesting to note that the improvement in MCS in ex-smokers was similar to smokers 1 year following surgery but not at 2 years. However, the GLM (which is a more powerful form of analysis) revealed no statistically significant differences between non-smokers and ex-smokers 2 years following surgery. There was negative relationship between BMI and MCS 2

years after surgery. When the BMI was categorised into three groups (ideal weight, overweight and obese), patients with ideal weight experienced the maximum benefit following surgery whereas obese patients experienced no benefit. The results in overweight patients were intermediate. This relationship was confirmed in the GLM. After controlling for other confounding variables, a one point increase in BMI predicts a 2.1 point decrease in MCS 2 years after surgery. This relationship was even stronger than MCS 0!

Participating in sports and physical activity is difficult to do prior to surgery as patients are often debilitated with pain. However, the GLM has demonstrated that patients taking part in high impact sports pre-injury experienced significantly better results than patients taking part in no sports, even after controlling for BMI, smoking and other confounding variables.

A consistent finding in this thesis was that the outcome following surgery were significantly inferior in the patella, while the results in the trochlea were the best. This non-modifiable risk factor for poor results is well reported in the literature (Brittberg et al 1994, Minas et al 2005, Krishnan et al 2006, Niemeyer et al. 2008, Pascal-Garido et al 2009). Multiple defects were associated with even worse outcomes. Other non-modifiable factors included aetiology (i.e. patients with previous failed ACI or mosaicplasty had poorer results). Results from chapter 5 identified another non-modifiable risk factor; the presence of early osteoarthritis. There was a significant difference in the absolute latest MCS between the two groups and there was also a significant difference in improvement in the MCS from baseline. Furthermore, the cumulative probability of survival (the endpoint being arthroplasty or osteotomy) at 5 years was 87% for patients with no radiographic evidence of OA compared with 52% for the OA group.

Clinical consequences

A number of factors have been identified that have a significant effect on outcome following ACI. However, the GLM identifies a relationship between a factor and outcome and a positive result does not necessarily mean a causative relationship. Therefore, modifying a factor in a patient may not necessarily improve their outcome.

There does not appear to be a difference in outcome when performing ACI or MACI to treat osteochondral defects in the knee other than in the patella. The forces acting in the anterior knee compartment are different from those in the medial or lateral compartment and this partially explains the inferior results of all cartilage repair techniques (Bentley et al 2003, Hangody et al. 2003, Karataglis et al 2006, Kreuz & Steinwachs et al 2006, Kreuz & Erggelet et al 2006). MACI is technically easier to perform than ACI, especially in the patella, and therefore we should consider performing MACI when treating patella lesions. It was interesting to note that the results of treating trochlear lesions were significantly better than patella lesions, even though they articulate in the same joint. The reason for this is unclear. I can only speculate that the location of trochlear lesions as documented by the surgeons may be inaccurate (i.e. the lesion may be more distal and not actually be in contact with the patella or only a small proportion of the lesion be in contact with the patella in terminal extension). Obviously, there is no way of proving this unless all patients with trochlear lesions are re-scoped.

The question as to whether we should perform ACI in patients with mild OA (e.g. Kellgren and Lawrence grade 1 or 2) remains unanswered. Performing ACI to treat OCD in patients with generalised arthritic changes in the knee is not as efficacious as treating patients with an OCD and no radiographic evidence of OA, but nonetheless, is still shows significant benefit.

As there was a dose dependent effect of smoking on outcome, patients should be encouraged to abstain prior to surgery. The only accurate method of determining whether abstaining from smoking prior to ACI will improve outcome would be to perform a RCT. Smokers could be randomised to either continue to smoke or to abstain smoking for a pre-determined period of time prior to undergoing ACI. To investigate what is the ideal period of abstinence required, it may be prudent to have several different abstinence time frames. Similarly, when treating overweight and obese patients, patients should be encouraged to lose weight prior to surgery. However, from the results of this study it cannot be conclusively stated that losing weight prior to ACI will result in improved outcome. Once again, a randomised controlled trial could answer that question. What can definitely be stated is that obese patients should not undergo ACI as there is no benefit (as measured by the MCS) whatsoever. The results from this study do raise some questions. If ACI was not performed in obese patients then would their knee function deteriorate? If this is the case, then is it acceptable to perform ACI to maintain the current level of function? Once again the only way to answer these questions would be to perform a randomised controlled trial in obese patients with two groups; group 1 would be composed of obese patients with OCD in the knee and no treatment and group 2 would have identical patients in which ACI is performed. Ethical issues regarding non-treatment of patients in pain may prevent a trial such as this from being instigated.

After adjusting for variables such as BMI and smoking, it was shown that patients partaking in high impact physical activity pre-injury had significantly better results than those not taking part in sports at all. Obviously this is not a variable that can be modified but some form of exercise (e.g. simple static quadriceps contractions) would be useful to prevent muscle wasting prior to surgery to improve functional outcome post-operatively and patients should be encouraged to try and stay as active as possible whilst waiting for ACI.

When trying to address the significantly inferior results in patients with known risk factors for adverse outcome, one needs to consider ways in which the surgical technique can be improved or in fact whether the right surgical technique is being used.

Limitations

In each of the chapters a small pilot study was performed to determine the appropriate sample size. This allowed accurate statistical evaluation of each of the factors in the case-controlled studies as well as the randomised controlled trial. However, it became apparent that large sections of patient and lesion details were not being completed by the operating surgeons in the data capture sheets. This resulted in a smaller number of patients with complete dataset available for analysis in the Generalised Linear Model, hence the increased possibility of type II statistical errors in most of the chapters. For example, in chapter 3 (ACI vs MACI) after sample size calculations, an adequate number of patients were recruited to determine a difference between the two forms of treatment (126 patients had ACI and 121 had MACI). However, out of the 247 patients only 100 had a complete set of data recorded (e.g. size of lesion, duration of symptoms, number of previous procedures, etc.). This means that when multiple regression analysis is performed, the study may be underpowered. An attempt was made to retrieve the lost data by inspecting the patients' notes but the missing information (e.g. duration of symptoms, number of prior procedures, size of lesion) was also not recorded there. In the future, all data capture forms should be heavily scrutinized. It was probably the case that such data at the time of collection was deemed unimportant and therefore not recorded.

Another major problem in the randomised study was the high attrition rate. Only 122 patients (49%) attended their 2 year clinic appointment, leading to the risk of follow-up bias. However the fact that similar numbers of ACI and MACI patients were lost to follow up, makes it less likely that this would introduce bias in the comparison between the two types of surgery, even if a successful outcome following surgery influenced whether patients attended their clinic appointment. The reasons for this have been discussed in the relevant chapter.

The Royal National Orthopaedic Hospital is a tertiary referral centre, hence patients from all over the country have to be followed up. Therefore it makes it very difficult for patients to attend outpatient clinics, and the motivation to attend if they are doing well has to be questioned. Most of the missing data in this cohort of patients was retrieved via a postal questionnaire or by telephone. Ideally, the trial team needs to identify patients that have not attended their clinic appointment, and alert the investigator, so that patients' knee status can be updated and attrition rates can be optimised.

Chapter 6 assessed modifiable risk factors. In this cohort of patients, the Modified Cincinnati Score (MCS) was collected prospectively. However, the data regarding weight, smoking and physical activity was collected retrospectively. This therefore represents a source of recall bias. Whilst height will not fluctuate, weight can do. That is why patients were grouped into three groups (ideal weight, overweight and obese) as generally patients' weight will not fluctuate beyond this particular range. Unfortunately the same thing cannot be applied when quantifying smoking. However, in the GLM, smoking status was ordinal (smoker, ex-smoker or non-smoker) and this is unlikely to undergo recall bias as patients can usually remember if they were smoking or not. Obviously it would have been more accurate to record the smoking status prospectively. Similarly, the physical activity profile is likely to suffer from recall bias as it may be harder for someone to remember how active they were two years ago than if they smoked or not. However the discrepancy in physical activity between high impact sports players and no sports being played is high and therefore recall bias between these two groups is going to be lower.

The problems mentioned above highlight how difficult it is to 'police' a RCT, especially one that is a multicentre, multi-surgeon trial. As a research fellow, one can continue to write to

surgeons that enrol patients to fill in the relevant details, but they may not write back with the necessary information. The solution is to use a Clinical Trials Unit (CTU). These specialist units' main remit is to design, conduct, analyse and publish clinical trials as well as other studies and to ensure adequate research nurse support to improve complete data collection. Their capability to provide specialist expert statistical, epidemiological and methodological advice would have been invaluable, but the key activity lacking in this study is the co-ordinated data collection from multiple centres and ensuring there is no loss to follow-up.

Future direction

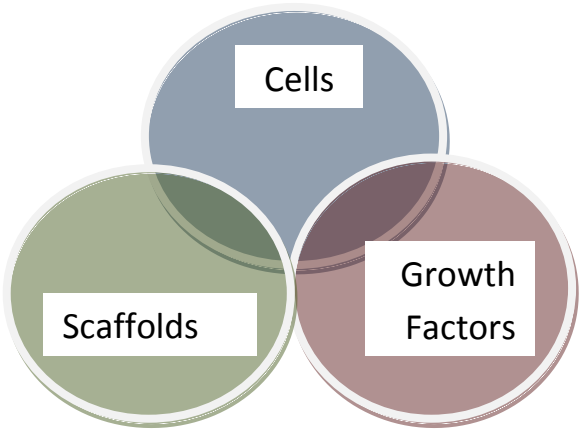
More sophisticated outcome measures

A very small minority of patients agreed to have the 1 year post-operative arthroscopy, and fewer still had biopsy of the repair tissue. A complete histological analysis would have been very useful as we could then determine factors that may influence hyaline cartilage formation and correlate that with clinical outcome. It was shown that with doubling of the time after implantation the likelihood of a favourable histological outcome was increased by more than fourfold up to a time point of 36 months (Gikas et al 2009). Any meaningful analysis of the available biopsy would not be possible as a significant proportion of patients had not undergone the biopsy procedure and therefore the potential for selection bias exists.

Another potential medium for analysis that could have been performed is the synovial fluid, as well as biochemical markers in the patients' blood and urine. Such markers could be correlated with patient and lesion characteristics before and after surgery, and then be used to predict outcome in future patients. Takahashi et al. had demonstrated that urinary pyridinoline had a significant correlation with joint space width and Kellgren and Lawrence (KL) grading on the antero-posterior radiographs of 71 females with knee OA (Takahashi et al 2004). Furthermore, tissue inhibitor of metalloproteinase (TIMP)-1 was the only blood biochemical marker (from three tested) to exhibit a significant relationship with radiographic severity of OA (as judged by KL grade). The main criticism of this paper is that none of the patients' knee function was assessed, hence it could not be determined if there was a relationship between the biochemical markers in question and patients level of pain and knee function. Since we treat patients and their symptoms and not radiographs, the relevance of these findings are questionable.

Development of technique

ACI has evolved considerably since its introduction in 1994. So-called 'third generation' techniques includes the potential for arthroscopic implantation (Feruzzi et al 2008) when treating lesions in the medial femoral condyle. The ultimate goal in treating OCD in the knee is to achieve high success rates in the long term with low complication and re-operation rates. This ultimate goal has 3 key requirements which are linked:



The cells have to be responsive to their environment, and the environment has to optimal to allow chondrogenesis. The question then arises about the type of cells that can be used; both chondrocytes and mesenchymal stem cells (MSCs) possess these characteristics and MSCs will be discussed later. The cytokine milieu and oxygen tension may represent modifiable factors to promote growth once transplantation has occurred. Saris et al compared the results of 'characterised' chondrocytes implantation (CCI) versus microfracture in the treatment of OCD of the knee (Saris et al 2008). This group selected chondrocytes for expansion and transplantation on the basis of expressing a marker predictive of consistent production of hyaline cartilage. This marker profile has been previously described to preserve phenotypic characteristics and differentiation following cell expansion, thereby increasing the

chondrogenic potency (Dell'Accio et al 2001). The results from randomised controlled trial proved that CCI was significantly better than microfracture for the treatment of OCDs of similar sizes (mean = 2.5cm^2). This is in contrast to other studies comparing standard ACI with microfracture (Knutsen et al 2005, 2007, Kon et al 2009). The improved outcome in the study by Saris et al may be attributable to the use of characterised chondrocytes, but no study has compared characterised chondrocytes with chondrocytes prepared routinely. Moreover, the considerable increase in expense during cell selection and expansion prohibits its use, but certainly may represent an improvement in the cell therapy.

The purpose of a scaffold in the context of ACI is to anchor, deliver and orientate cells post-transplantation. There has been considerable evolution in the engineering of biodegradable scaffolds from the original use of the periosteal patch (Brittberg et al 1994). The use of a synthetic collagen membrane has reduced reoperation rates due to hypertrophy (Gooding et al 2006, Gomoll et al 2009), the MACI technique can be performed more quickly (Bartlett et al 2005) and currently the development of gel-like scaffold provides the potential for arthroscopic implantation, thereby further reducing surgical trauma (Selmi et al 2008).

The potential exists to provide instructional cues to cells following transplantation with the use of bioactive factors or reagents. *In vitro* studies have demonstrated that chondrocytes cultured with TGF-β produce increased quantities of type II collagen (Goldberg et al 2005). Growth factors from the TGF-β super family, as well as Insulin Growth Factor (IGF) and Fibroblast growth factor (FGF) have been shown to have a positive effect on chondrogenesis (Skoog et al 1990, Matsumoto et al 1996, Chuang et al 2012), though as yet no work has been conducted on the enhancement of ACI with these factors. The question still remains whether we can enhance proteoglycan and type II collagen synthesis with use of growth factors. Conversely, inflammatory cytokines are present in the synovial fluid of patients with

established osteoarthritis (Distel et al 2009) and are thought to play a role in paracrine inflammation and disease progression. Synovial fluid analysis has not been performed in patients with simple traumatic OCDs, though the presumption is that there may be inflammatory cytokines such as IL-6, IL-1 and TNF present. Targeting these cytokines with monoclonal antibodies may be another way to augment cell therapy by inhibiting matrix breakdown after cartilage injury.

The use of mesenchymal stem cells

An open biopsy of articular cartilage is necessary in order to obtain the chondrocytes necessary for expansion and re-implantation. This exposes the patient to the morbidity of two surgical procedures. Stem cells are an ideal alternative to chondrocytes since they are easily accessible, expandable in culture and multipotent and can be inserted in a single stage. Mesenchymal stem cells (MSCs) have been isolated from many tissues including bone marrow (Pittenger et al. 1999), synovial membrane (De Bari et al. 2004), periosteum (De Bari et al. 2006) and articular cartilage (Douthawaite et al. 2004).

There are two important studies concerning the use of MSCs to engineer cartilage. Caplan's group developed an *in vitro* method of inducing both animal (Johnstone et al. 1998) and human MSCs (Yoo et al. 1998) into the chondrogenic pathway. The second important study was by Wakitani et al. (1994) in which rabbit MSCs were placed in full thickness OCDs in the medial condyle of young mature rabbits. Since then, considerable work has been performed on larger animal models (Liu et al. 2006, Wilke et al. 2007) but to date there are few studies in humans.

Wakitini's group advanced their studies by transplanting autologous culture-expanded bone marrow mesenchymal cells into nine full-thickness articular cartilage defects of the patello-femoral joints (including two kissing lesions) in the knees of three patients. Three weeks before transplantation, bone marrow blood was aspirated from the iliac crest. Adherent cells were cultured with media containing autologous serum. Six months after transplantation, the patients' clinical symptoms had improved and the improvements have been maintained over the follow-up periods (17-27 months). Histology of the first patient 12 months after the transplantation revealed that the defect had been repaired with the fibrocartilaginous tissue.

Magnetic resonance imaging of the second patient 1 year after transplantation revealed complete coverage of the defect, but they were unable to determine whether or not the material that covered the defects was hyaline cartilage.

Haleem et al. used platelet-rich fibrin glue as a scaffold to deliver autologous culture-expanded bone marrow MSCs for cartilage repair. All five patients had significantly improved clinical scores 1 year following surgery. Only two of the five patients had agreed to an arthroscopic assessment of their grafts one year following surgery and they had improved arthroscopic (ICRS) scores from 8/12 to 11/12 (Haleem et al. 2010).

The source of stem cells is an important issue when considering cartilage repair. MSCs from bone marrow appear to have high propensity to cartilage hypertrophy and bone formation (Peltari et al. 2006) and hence may not be the ideal chondroprogenitors. MSCs from synovial membrane appear to have a greater propensity to form cartilage *in vitro* than MSCs from bone marrow and periosteum (Sakaguchi et al. 2005).

Recent work by Bosetti et al. (2011) has identified several growth factors such as bone morphogenic proteins that can induce the differentiation of human bone marrow MSCs into mature chondrocytes, with the additional effect of increased expression of collagen type II and Sox9, the markers for chondrogenesis. However, the more recent discovery of its immunomodulation capacity is just as exciting. Several publications indicate that MSCs and their secretions affect dendritic cells, T-cells, B-cells and Natural Killer cells (Iyer et al., 2008). Therefore the possibility exists of using MSCs to eliminate chronic inflammation from joints.

There has been only one study in humans comparing MSCs and autologous chondrocytes when treating OCDs in the knee. Nejadnik et al. discovered superior scores in the SF-36

physical role section with the use of bone marrow derived MSCs compared with ACI (Nejadnik et al 2010). Furthermore, the ACI group did significantly better if they were aged less than 45. This age-related effect was not seen with the MSC group.

Conclusion

Autologous chondrocytes implantation is still the technique most likely to produce hyaline-like cartilage regenerate when treating osteochondral defects in the knee. When using the absolute Modified Cincinnati Scores, the results from this centre (now involving over 800 patients) are inferior to other centres. This may be because of a challenging cohort of unselected patients who had a long history, had undergone several previous procedures and had a variety of defects. It certainly would be interesting to assess the efficacy of this procedure in patients who have not undergone any other surgical procedure and have had a relatively short duration of symptoms.

The work in this thesis has identified adverse prognostic factors and possible methods to enhance results. Patients can now be adequately counselled regarding prognosis and the generalised linear model can be used to predict a more accurate outcome.

References

Akizuki SA, Mow VC, Lai WM, Pita J, Howell DS. Topographical variations of the biphasic in dentation properties of human tibia1 plateau cartilage. *Trans Orthop Res Soc.* 1986; 11:406-17

Agel J, LaPrade RF. Assessment of differences between the modified Cincinnati and International Knee Documentation Committee patient outcome scores: a prospective study. *Am J Sports Med*. 2009; 37(11):2151-7.

Aglietti P, Rinonapoli E. Total condylar knee arthroplasty. A five-year follow-up study of 33 knees. *Clin Orthop Relat Res.* 1984 Jun; (186):104-11.

Ahlberg A, Lundén A. Secondary operations after knee joint replacement. *Clin Orthop Relat Res.* 1981; (156): 170-4.

Alford JW and Cole BJ. Cartilage Restoration, Part I. Basic Science, Historical Persepective, Patient Evaluation, and Treatment Options. *Am J Sport Med.* 2005; 33(2): 295-306

Alfredson H, Lorentzon R. Superior results with continuous passive motion compared to active motion after periosteal transplantation. A retrospective study of human patella cartilage defect treatment. *Knee Surg Sports Traum Arthroscop.* 199; 7(4): 232-8

Altman DG, **Bland JM**. How to randomise. *BMJ* 1999; 319(7211): 703-4

Altman DG, **Bland JM**. Treatment allocation by minimisation. *BMJ* 2005**;** 330, 9 APRIL 2005: 843

Altman R, Asch E, Bloch G et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of arthritis. *Arth Rheum.* 1986; 29: 1039-1049

Altman R, Brandt K, Hochberg M et al. Design and conduct of clinical trials in patients with osteoarthritis: recommendations from a task force of the Osteoarthritis Research Society. Results from a workshop. *Osteoarthritis Cartilage*. 1996; 4(4):217-43.

Amin S, LaValley MP, Guermazi et al. The relationship between cartilage loss on magnetic resonance imaging and radiographic progression in men and women with knee osteoarthritis. *Arth Rheum.* 2005; 52: 3152-3159

Amin S, Niu J, Guermazi A et al. Cigarette smoking and the risk for cartilage loss and knee pain in men with knee arthritis. *Ann Rheum Dis.* 2007; 66: 18-22

Amin AK, Patton JT, Cook RE et al. Does Obesity influence the clinical outcome at five years following total knee replacement for osteoarthritis. *J Bone Joint Surg. [Br]* 2006; 88:335-340

Amin S, Niu J, Guermazi A et al. Cigarette smoking and the risk for cartilage loss and knee pain in men with knee arthritis. *Ann Rheum Dis.* 2007; 66: 18-22

Amin AK, Patton JT, Cook RE et al. Does Obesity influence the clinical outcome at five years following total knee replacement for osteoarthritis. *J Bone Joint Surg. [Br]* 2006; 88:335-340

Anandacoomarasamy A, Caterson ID, Leibman S et al. Influence of BMI on Health-related Quality of Life: Comparison Between an Obese Adult Cohort and Age-matched Population Norms. *Obesity* 2009; 17: 2114-2118

Anderson HI, Ejlertsson G, Leden I. Widespread musculoskeletal chronic pain associated with smoking. An epidemiological study in a general rural population. *Scand J Rehabil Med* 1998; 30: 185-91

Angermann P, Riegels-Nielson P, Pedersen H. Osteochondritis dissecans of the femoral condyle treated with periosteal transplantation. Poor outcome in 14 patients followed for 6-9 years. *Acta Orthop Scand.* 1998; 69(6): 595-7

Ananth CV and Kleinbaum DG. Regression methods for ordinal responses: a review of methods and applications. *Int J Epidem.* 1997; 27: 1323-33

Arendt EA, Fithian DC, Cohen E. Current concepts of lateral patella dislocation. *Clin Sports Med.* 2002; 21: 499-519.

Aroen LS, Heir S, Alvik E et al. Articular cartilage lesions in 993 consecutive knee arthroscopies. *Am J Sports Med.* 2004; 32, 211–215

Arokoski JP, Hytinen MM, Helminen HJ et al. Biomechaninal and structural characteristics of canine femoral and tibial cartilage. *J Biomed Mech Res.* 1999; 48(2): 99-107

Ashton JE & Bentley G. Repair of articular surfaces by allografts of articular and growth-plate cartilage. *J Bone Joint Surg [Br]* 1986; 68(1): 29-35

Aspden RM, Scheven BA, Hutchinson JD et al. Osteoarthritis as a systemic disorder including stromal cell differentiation and lipid metabolism. *Lancet* 2001; 357: 1118-20

Ateshian GA, Lai WM, Zhu WB et al. An asymptomatic solution for the contact of two biphasic cartilage layers . *J Biomech.* 1994; 27: 1347-60

Attie AD, Scherer PE. Adipocyte metabolism and obesity. *J Lipid Res.* 2009; 50 Suppl: S395-9

Aydelotte MB, Schumacher BL, and Kuettner KE. Chondrocytes from the articular surface and deep zones express different, but stable, phenotypes in alginate gel culture. *Trans. Orthop Res Soc.* 1996; 21: 317

Bae DK, Yoon KH, and Song J. Cartilage healing after microfracture in osteoarthritic knees. *Arthroscopy* 2006; 22: 367-374

Bamgbade OA, Rutter TW, Nafiu OO et al. Postoperative complications in obese and nonobese patients. *World J Surg.* 2007; 31: 556-560

Barber-Westin SD, Noyes FR, McCloskey JW. Rigorous statistical reliability, validity, and responsiveness testing of the Cincinnati knee rating system in 350 subjects with uninjured, injured, or anterior cruciate ligament-reconstructed knees. *Am J Sports Med.* 1999; 27(4):402-16.

Bartlett W, Skinner JA, Gooding CR et al. Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. *J Bone Joint Surg Br.* 2005;87:640-5.

Becher C, Driessen A, Thermann H. Microfracture technique for the treatment of articular cartilage lesions of the talus. *Orthopade* 2008; 37: 198-203

Behrens P, Bitter T, Kurz B and Russlies M. Matrix-associated autologous chodnrocyte transplantation/implantation (MACT/MACI) – 5 year follow up. *Knee* 2006; 13: 194-202

Benichou OD, Hunter DJ, Nelson DR et al. One-year change in radiographic joint space width in patients with unilateral joint space narrowing: Data from the osteoarthritis intiative. *Arthritis Care Res.* 2010; 62(7): 924-931

Bentley G. Anterior Knee Pain, diagnosis and management. *J R Coll Surg Edinburgh*.1989; 34(6): 2-3

Bentley G. Chondromalacia patellae. *J Bone Joint Surg. [Am]* 1970; 52(2): 221-32

Bentley G, Dowd G: Current concepts of etiology and treatment of chondromalacia patellae. *Clin Orthop* 189:209-228, 1983

Bentley G and Greer RB. Homotransplantation of isolated epiphyseal and articular cartilage chondrocytes into joint surfaces of rabbits. *Nature* 1971; 230: 385-8

Bentley G, Biant LC, Carrington RW, et al. A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. *J Bone Joint Surg Br.* 2003;85:223-230.

Bentley G, Biant LC, Vijayan S et al. Minimum ten-year results of a prospective randomised study of autologous chondrocytes implantation *versus* mosaicplasty for symptomatic articular cartilage lesions of the knee. *J Bone Joint Surg [Br]* 2012; 94: 504-9

Bentley G. The contemporary management of articualr cartilage damage. *The Robert Jones Lecture, BOA Congress,* Manchester 2007

Benya PD & Shaffer JD. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell* 1982; 30: 215-24

Berend KR, Lombardi AV, Mallory TH et al. Early failure of minimally invasive unicompartmental knee arthroplasty is associated with obesity. *Clin Orthop Rel Res.* 2005; 440: 60-66

Berend KR, Ritter MA, Meding JB et al. Tibial component failure mechanisms in total knee arthroplasty. *Clin Orthop Relat Res.* 2004; 428: 26-34

Biswal S, Hastie T, Andriacchi TP et al. Risk factors for progressive cartilage loss in the knee: a longitudinal magnetic resonance imaging study in forty-three patients. *Arth Rheum.* 2002; 46: 2884-2892

Blain EJ, Gilbert SJ, Wardale RJ, et al. Up-regulation of matrix metalloproteinase expression and activation following cyclical compressive loading of articular cartilage in vitro. *Arch Biochem Biophys* 2001;396:49-55.

Bland M. Introduction to Medical Statistics. Chapter 17 Multifactorial Methods. 308-324. Oxford University Press 3rd Edition

Bobic V. Arthroscopic osteochondral autograft transplantation in anterior cruciate ligament reconstruction: a preliminary clinical study. *Knee Surg Sports Traum Arthroscop.* 1996; 3(4): 262-4

Bordini B, Stea S, Cremonini S et al. Relationship between obesity and early failure of total knee prostheses. *BMC Musculoskeletal Disorders* 2009; 10: 29-37

Bosetti M, Boccafoschi F, Leigheb M et al. Chondrogenic induction of human mesenchymal stem cells using combined growth factors for cartilage tissue engineering. *J Tissue Eng Regen Med* (2011 Feb 28) (Epub ahead of print)

Bouwmeester P, Kuijer R, Terwindt-Rouwenhorst E et al. Histological and biochemical evaluation of perichondrial transplants in human articular cartilage defects. *J Orthop Res* 1999;17:843-9.

Briggs TWR, Mahroof S, David LA et al. Histological evaluation of chondral defects after autologous chondrocytes implantation of the knee. *J Bone Joint Surg [Br]* 2003;85-B:1077-83.

Brittberg M, Lindahl A, Nilsson A et al. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994; 331: 889–895

Brittberg M, Nilsson A, Lindahl A et al. Rabbit articular cartilage defects treated with autologous cultured chondrocytes. *Clin. Orthop.* 1996; 326: 270-283

Brown DE, Alexander AH, Lichtman DM. The Elmslie-Trillat procedure: evaluation in patellar dislocation and subluxation. *Am J Sports Med.*1984; 12: 104-9

Bruckner P, Mendler M, Steinmann B, Huber S, and Winterhalter KH. The structure of human collagen type IX and its organization in fetal and infant cartilage fibrils. *J. Biol. Hem.* 1988; 263:16911-16917.

Buckwalter JA and Mow VC. Cartilage repair in osteoarthritis. In Osteoarthritis, Diagnosis and Medical/Surgical Management, edited by R. W. Moskowitz, D. S. Howell, V.M. Goldberg, and H. J. Mankin. Ed. 2, pp. 71-107. Philadelphia, W. B. Saunders, 1992.

Buckwalter JA, Kuettner KE and Thonar EJM. Age-Related Changes in Articular Cartilage Proteoglycans: Electron Microscopic Studies. *J Orthop Res.* 1985; 3: 251-7

Buckwalter JA, Pita JC, Muller FJ, and Nessler J. Structural differences between two populations of articular cartilage proteoglycan aggregates. J. Orthop. Res. 1994; 12:144-148.

Buckwalter JA, Roughley PJ, and Rosenberg LC. Age-related changes in cartilage proteoglycans: quantitative electron microscopic studies. Microsc. Res. and Tech 1994; 8:398-408.

Buckwalter JA. Should bone, soft-tissue, and joint injuries be treated with rest or activity? *J. Orthop. Res.* 1995; 13:155-156

Buckwalter JA and Mankin HJ. Instructional Course Lectures, The American Academy of Orthopaedic Surgeons – Articular Cartilage. Part I: Tissue Design and Chondrocyte Matrix Interactions. *J Bone Joint Surg. [Am]* 1997; 79-A(4): 600-11

Buckwalter JA and Mankin HJ Articular cartilage: Part II: degeneration and Osteoarthritis, repair, regeneration and transplantation. *J Bone Joint Surg,* 1997; 79-A: 612–632.

Cartier P, Sanouiller JL, Grelsamer RP. Patellofemoral arthroplasty. 2-12-year follow-up study. *J Arthroplasty.* 1990;5:49-55.

Cherubino P, Grassi FA, Bulgheroni P, Ronga M. Autologous chondrocyte implantation using a bilayer collagen membrane: a preliminary report. *J Orthop Surg (Hong Kong)* 2003;11:10-15.

Chuang CY, Shahin K, Lord MS et al. The cartilage matrix molecule components produced by human foetal cartilage rudiment cells within scaffolds and the role of exogenous growth factors. *Biomaterials.* 2012; 33(16): 4078-88.

Christensen R, Bartels EM, Astrup A et al. Effect of weight reduction in obese patients diagnosed with osteoarthritis: a systematic review and meta-analysis. *Ann Rheum Dis.* 2007; 66: 433-39

Cicuttini FM, Jones G, Forbes A et l. Rate of cartilage loss at two years predicts subsequent total knee arthroplasty: a prospective study. *Ann Rheum Dis.* 2004; 63: 264-268

Cicuttini FM, Ding C, Wluka AE et al. Association of cartilage defects with loss of knee cartilage in healthy, middle-aged adults: a prospective study. *Arth Rheum.* 2005; 52: 2033-2039

Clancy CM, Eisenberg JM. Outcomes research: measuring the end results of health care. Science. 1998 Oct 9;282(5387):245-6.

Convery FR, Akeson WH, Keown GH. The repair of large osteochondral defects. An experimental study in horses. *Clin Orthop.* 1972; 82: 253-262

Curl KJ, Gordon ES, Rushing J et al. Cartilage injuries: a review of 31,516 knee arthroscopies. *Arthroscopy* 1997; 13, 456–460.

Curl WW, Krome J, Gordon ES, Rushing J, Smith BP, Poehling GG. Cartilage injuries: a review of 31,516 knee arthroscopies. *Arthroscopy*. 1997;13:456-460.

Dannenmaier WC, Haynes DW, Nelson CL. Granulomatous reaction and cystic bony destruction associated with high wear rate in a total knee prosthesis. *Clin Orthop Relat Res.* 1985 Sep;(198):224-30.

Davis MA, Ettinger WH, Neuhaus JM et al. The association of knee injury and obesity with unilateral and bilateral osteoarthritis of the knee. *Am J Epidem.* 1989; 130(2): 278-88

Davies-Tuck ML, Wluka AE, Wang Y et al. The natural history of cartilage defects in people with knee osteoarthritis. *Osteoarth Cart.* 2007; 16: 337-342

De Bari C, Dell'Accio F, Luyten FP, () Failure of in vitro-differentiated mesenchymal stem cells from the synovial membrane to form ectopic stable cartilage in vivo. *Arthritis Rheum* 2004; 50(1): 142-50

De Bari C, Dell'Accio F, Vanlauwe J et al. () Mesenchymal multipotency of adult human periosteal cells demonstrated by single cell lineage analysis. *Arthritis Rheum* 2006; 54(4): 1209-21

Dell'Accio F, De Bari C, Luyten FP. Molecular markers predictive of the capacity of expanded human articular chondrocytes to form stable cartilage in vivo. *Arthritis Rheum*. 2001;44:1608-1619.

DePalma AF McKeever CD, Subin SK. Process of repair of articular cartilage demonstrated on histology and autoradiography with tritiated thymidine. *Clin Orthop.* 1966; 48:229-42

Dequeker J, Goris P, Uytterhoven R. Osteoporosis and Osteoarthritis. *JAMA* 1983; 249: 1448-1451

Desjardins MR, Hurtig MB, Palmer NC. Heterotopicm transfer of cryoperserved autogenous articular cartilage in the horse. *Vet Surg.* 1991; 20(6): 434-45

De Windt TS, Bekkers JEJ, Creemers LB et al. Patient profiling in cartilage regeneration. Prognostic factors determining success of treatment for cartilage defects. *Am J Sports Med.* 2009; 37 (Suppl 1): 58-62

Deyo RA, Bass JE. Lifestyle and low-back pain. The influence of smoking and obesity. Spine 1989;14:501–6.

Distel E, Cadoudal T, Durant S et al. The infrapatellar fat pad in knee osteoarthritis. An important source of IL-6 and its soluble receptor. *Arth Rheum.* 2009; 60(11): 3374-3377

Douthwaite GP, Bishop JC, Redman SN et al. The surface of articular cartilage contains a progenitor cell population. *J. Cell Sci* 2004; (117): 889-897

Dozin B, Malpeli M, Cancedda R et al. Comparative evaluation of autologous chondrocyte implantation and mosaicplasty : a multicentred randomised clinical trial *Clin J Sports Med* 2005 ; 15 : 220-6

Drawer S, Fuller CW. Propensity for osteoarthritis and lower limb joint pain in retired professional soccer players. *Br J Sports Med.* 2001; 35:402-408.

Drongowski RA, Coran AG, Wojtys EM. Predictive value of meniscal and chondral injuries in conservatively treated anterior cruciate ligament injuries. *Arthroscopy*. 1994;10:97-102.

Dumville JC, Torgerson DJ, Hewitt CE. Reporting attrition in randomised controlled trials. *BMJ* 2006;**332**:969–71.

Dunbar MR, Griffin DR, Surr G et al. PRE-OPERATIVE FACTORS THAT PREDICT OUTCOME AFTER TOTAL KNEE REPLACEMENT: A SYSTEMATIC REVIEW *J Bone Joint Surg Br* **2012** vol. 94-B no. SUPP II **79**

Ebert JB, Robertson WR, Lloyd DG et al. Traditional versus accelerated approaches to post-operative rehabilitation following matrix-induced autologous chondrocyte implantation (MACI) : comparison of clinical, biomechanical and radiographic outcomes *Osteoarth Cart* 2008; 16: 1131-1140

Eggli PS, Hunziker EB, Schenk RK. Quantitaion of structural features characterizing weight- and less-weight bearing regions in articular cartilage: a stereological analysis of medial femoral condyles in young adult rabbits. *Anat Rec.* 1988; 222(3): 217-227

El-Zawawy HB, Gill CS, Wright RW et al. Smoking delays chondrogenesis in a mouse model of closed tibial fracture healing. *J Orthop Res* 2006; 24: 2150-2158

Englund M and Lohmander LS. Risk Factors for symptomatic knee osteoarthritis fifteen to twenty-two years after meniscectomy. *Arth Rheum* 2004; 50(9): 2811-2819

Espehaug B, Havelin LI, Engesaeter LB et al. Patient related risk factors for early revision of total hip replacements: a population register-based case-control study of 674 revised hips. *Acta Orthop Scand* 1997; 68: 207-15

Eyre DR, Wu JJ, and Woods P. Cartilage-specific collagens. Structural studies. In Articular Cartilage and Osteoarthritis, pp. 119-131. Edited by K. E. Kuettner, R. Schleyerbach, J. G. Peyron, and V. C. Hascall. New York, Raven Press, 1992.

Eyre DR. Collagen structure and function in articular cartilage: metabolic changes in the development of osteoarthritis. In Osteoarthritic Disorders, pp. 219-229. Edited by K. E. Kuettner and V. M. Goldberg. Rosemont, Illinois, The American Academy of Orthopaedic Surgeons, 1995.

Filardo G, Kon E, Andriolo L et al. Clinical profiling in cartilage regeneration: prognostic factors for midterm results of matrix-assisted autolgous chondrocyte transplantation. *Am J Sports Med.* 2014 42(4): 898-905

Fischer AE, Carpenter TA, Tyler JA et al. Visualisation of mass transport of small organic molecules and metal ions through articular cartilage by magnetic resonance imaging. *Mag. Res. Imag.* 1995; 13: 819-26

Felson DT, Anderson JJ, Naimark A et al. Obesity and Knee Osteoarthritis. *Ann Inter Med.* 1988; 109: 18-24

Felson DT, Anderson JJ, Naimark A et al. Does smoking protect against osteoarthritis? Arthritis Rheum. 1989 Feb;32(2):166-72.

Felson DT Weight and Osteoarthritis. *Am J Clin Nutr* 1996; 63(suppl): 430-432

Felson D, Niu J, Clancy M et al. Effect of recreational physical activities on the development of knee OA in older adults of different weights: The Framingham Study. *Arthritis Rheum* 2007; 57: 6-12

Fergusson D, Aaron SD, Guyatt G, Hebert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. *BMJ* 2002;325:652-4.

Feruzzi A, Buda R, Faldini C et al. Autologous Chondrocyte Implantation in the Knee Joint: Open Compared with Arthroscopic Technique. *J Bone Joint Surg [Am]* 2008; 90 Suppl 4: 90-1001

Fewtrell MS, Kennedy K, Singhal A et al. How much loss to follow-up is acceptable in long-term randomised trials and prospective studies? *Arch Dis Child* 2008; 93(6): 458-61

Ebert JB, Robertson WR, Lloyd DG et al. Traditional versus accelerated approaches to post-operative rehabilitation following matrix-induced autologous chondrocyte implantation (MACI) : comparison of clinical, biomechanical and radiographic outcomes *Osteoarth Cart* 2008; 16: 1131-1140

Fischer AE, Carpenter TA, Tyler JA et al. Visualisation of mass transport of small organic molecules and metal ions through articular cartilage by magnetic resonance imaging. *Mag. Res. Imag.* 1995; 13: 819-26

Fitzgerald JB, Jin M, Grodzinsky AJ Shear and compression differentially regulate clusters of functionally related temporal transcription patterns in cartilage tissue. *J Biol Chem* 2006; 281: 24095–24103

Foran JRH, Mont MA, Etienne G et al. The outcome of total knee arthroplasty in Obese patients. *J Bone Joint Surg. [Am]* 2004; 86: 1609-1615

Ford GM, Hegmann KT, White GL et al. Associations of Body Mass Index with Meniscal Tears. *Am J Prev Med.* 2005; 28(4): 364-368

Fulkerson JP & Becker GJ. Anteromedial tibial tubercle transfer without transfer without bone graft. *Am J Sports Med.* 1990; 18: 490-7

Fulkerson JP. Alternatives to patellofemoral arthroplasty. *Clin Orthop Relat Res.* 2005; 436: 76- 80.

Fuller JA, Ghadially FN. Ultrastructural observations on surgically produced partial-thickness defects in articular cartilage. *Clin Orth.* 1972; 86: 193-205

Furukawa T, Eyre DR. Koide S et al. Biomechanical studies on repair cartilage resurfacing experimental defects in rabbit knee. *J Bone Joint Surg [Am]* 1980; 62: 79-89

Gabbay O, Hall DJ, Berenbaum F et al. Osteoarthritis and Obesity: Experimental models. *Joint Bone Spine.* 2008; 75: 675-679

Ghadially FN, Thomas I, Oryshak AF et al. long term results of superficial defects in articular cartilage. A scanning electron microscope study. *J Path.* 1977; 121: 213-17

Gikas PD, Morris T, Carrington R et al. A correlation between the timing of biopsy after autologous chondrocyte implantation and the histological appearance. *J Bone Joint Surg.* 2009; 91-B: 1172-1177

Gikas PD, Bayliss L, Bentley G et al. On overview of autologous chondrocyte implantaion. *J Bone Joint Surg. [Br]* 2009: 91: 997-1006

Gill CS, Sandell LJ, El-Zawawy HB et al. Effects of cigarette smoking on early medial collateral ligament healing in a mouse model. *J Orthop Res* 2006; 24: 2141-2149

Glassman SD, Anagnost SC, Parker A, et al. The effect of cigarette smoking and smoking cessation on spinal fusion. *Spine* 2000; 25(20): 2608-15

Gobbi A, Francisco R. Factors affecting return to sports after anterior cruciate ligament reconstruction with patella tendon and hamstring graft: a prospective clinical investigation. *Knee Surg Sports Traum Arth.* 2006; 14(10): 1021-28

Gobbi A, Nunag P, Malinowski K. Treatment of chondral lesions of the knee with microfracture in a group of athletes. *Knee Surg Sports Traumatol Arthrosc.* 2004;13:213-221.

Goldberg AJ, Lee DA, Bader DL et al. Autologous chondrocyte implantation. Culture in a TGF-β containing medium enhances the expression of chodnrocyte phenotype in passaged human chondrocytes in pellet culture. *J Bone Joint Surg.* 2005; 87-B(1): 128-134

Goldberg MS, Scott SC, Mayo NE. A review of the association between cigarette smoking and the development of nonspecific back pain and related outcomes. Spine 2000;25:995–1014.

Gomoll AH, Probst C, Farr J et al. The use of type I/III bilayer collagen membrane decreases reoperation rates of symptomatic hypertrophy after autologous chondrocytes implantation. *Am J Sports Med.* 2009; 37: 20S-23S

Goodfellow J, Hungerford DS, Zindel M. Patellofemoral joint mechanics and pathology. Functional anatomy of the patello-femoral joint. J *Bone Joint Surg Br.* 1976;58:287-90.

Gooding CR, Bartlett W, Bentley G et al. A prospective randomised study comparing two techniques of autologous chondrocyte implanation for osteochondral defects in the knee: Perisoteum covered versus type I/III collagen covered. *Knee* 2006; 13(3): 203-10

Grande DA, Pitman MI, Peterson L et al. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. *J Orthop Res* 1989; 7: 208-18

Greco NJ, Anderson AF, Mann BJ et al. Responsiveness of the International Knee Documentation Committee Subjective Knee Form in comparison to the Western Ontario and McMaster Universities Osteoarthritis Index, modified Cincinnati Knee Rating System, and Short Form 36 in patients with focal articular cartilage defects. *Am J Sports Med.* 2010; 38(5):891-902

Green WT. Articular cartilage repair. Behaviour of rabbit chondrocytes during tissue culture and subsequent allografting. *Clin Orthop* 1977; 237-50

Grelsamer RP, Weinstein CH. Applied biomechanics of the patella. *Clin Orthop Relat Res.* 2001;389:9-14.

Griffin FM, Scuderi GR, Insall JN et al. Total knee arthroplasty in patients who were obese with 10 years follow-up. Clin Orthop Relat Res. 1998 Nov;(356):28-33.

Grodzinsky AJ, Levenston ME, Jin M et al. Cartilage tissue remodeling in response to mechanical forces. *Annu Rev Biomed Eng* 2000; 2:691–713

Gross AE. Repair of cartilage defects in the knee. *J Knee Surg.* 2002; 15: 167-169

Gudas R, Kalesinskas RJ, Kimtys V, et al. A prospective randomized clinical study of mosaic osteochondral autologous transplantation versus microfracture for the treatment of osteochondral defects in the knee joint in young athletes. *Arthroscopy*. 2005;21:1066-1075.

Gudas R, Stankevicius E, Monastyreckiene E et al. Osteochondral autologous transplantation versus microfracture for the treatment of articular cartilage defects in the knee joint in athletes. *Knee Surg Sports Traumatol Arthrosc.* 2006;14:834-842.

Guerne, P. A.; Blanco, F.; Kaelin, A. Desgeorges, A. and Lotz, M.: Growth factor responsiveness of human articular chondrocytes in aging and development. *Arthrit. and Rheumat*. 1995; 38:960-968.

Hagiwara H, Schroter-Kermani C, and Merker HJ. Localization of collagen type VI in articular cartilage of young and adult mice. Cell and Tissue Res. 1993; 272:155-160.

Haleem AM, Singergy AA, Sabry D et al. The clinical use of human culture-expanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: a pilot study and preliminary results. Cartilage 2010; 1(4) 253-261

Hagiwara H, Schroter-Kermani C, and Merker HJ. Localization of collagen type VI in articular cartilage of young and adult mice. Cell and Tissue Res. 1993; 272:155-160.

Hangody L, Kish G, Karpati Z et al. Arthroscopic autogenous osteochondral mosaicplasty for the treatment of femoral condylar articular defects: a preliminary report. *Knee Surg Sports Traumatol Arthrosc* 1997;5:262-7.

Hangody L, Fules P. Autologous osteochondral mosaicplasty for the treatment of full-thickness defecst of weight-bearing joints: ten years of experimental and clinical experience. *Bone Joint Surg [Am]* 2003;85-A (Suppl 2):25-32.

Hangody L, Ráthonyi GK, Duska Z. Autologous osteochondral mosaicplasty. Surgical Technique. J Bone Joint Surg Am. 2004 Mar;86-A Suppl 1:65-72

Hanna F, Teichtahl AJ, Bell R et al. The cross-sectional relationship between, fortnightly exercise and knee cartilage properties in healthy adult women in midlife. *Menopause* 2007; 14: 1-5

Hardingham TE, Fosang AJ, Dudhia J. Aggrecan, the chondroitin/keratan sulfate proteoglycan from cartilage. In Articular Cartilage and Osteoarthritis, pp. 5-20. Edited by K. E. Kuettner, R. Schleyerbach, J. G. Peyron, and V. C. Hascall. New York, Raven Press, 1992.

Hart DJ and Spector TD. Cigarette smoking and risk of osteoarthritis in women in the general population: the Chingford study. *Ann Rheum Dis.* 1993 Feb;52(2):93-6

Henderson IJP & Lavigne P. Periosteal autologous chondrocytes implantation for patellar chondral defect in patients with normal and abnormal patellar tracking. *Knee* 2006; 13: 274-279

Heinegård D, Lorenzo P, and Sommarin Y. Articular cartilage matrix proteins. In Osteoarthritic Disorders, pp. 229-237. Edited by K. E. Kuettner and V. M. Goldberg. Rosemont, Illinois, The American Academy of Orthopaedic Surgeons, 1995

Hinricsson H: Studies on patellar chondromalacia. An attempt to elucidate its etiology. *Acta Orthop Scand* 10:312-322, 1939

Hofstaetter JG, Wunderlich L, Samuel RE, Saad FA, Choi YH, Glimcher MJ. Systemic hypoxia alters gene expression levels of structural proteins and growth factors in knee joint cartilage. *Biochem Biophys Res Commun* 2005;330:386–94

Höher J, Bach T, Münster A et al. Does the mode of data collection change results in a subjective knee score? Self-administration versus interview. *Am J Sports Med.* 1997; 25(5):642-7.

Hoikka VE, Jaroma HJ, Ritsila VA. Reconstruction of the patellar articulation with periosteal grafts: 4-year follow-up of 13 cases. *Acta Orthop Scand* 1990;61:36-9.

Hollander AP, Dickinson SC, Sims TJ et al. Maturation of Tissue Engineered Cartilage Implanted in Injured and Osteoarthritic Human Knees. *Tiss Engin.* 2006;12(7): 1787-1798

Hollander A, Atkins RM, Eastwood DM et al. Degradation of human cartilage by synovial fluid but not cytokines in vitro. *Ann Rhem Dis.* 1991; 50: 57-8

Holmberg S, Thelin A, Thelin N. Knee osteoarthritis and body mass index: a population-based case-control study. *Scan J Rheumatol* 2005; 34: 59-64

Homminga GN, Bulstra SK, Bouwmeester PS et al. Perichondral grafting for cartilage lesions of the knee. *J Bone Joint Surg [Br]* 1990;72-B:1003-7.

Honda K, Ohno S, Tanimoto K, et al. The effects of high magnitude cyclic tensile load on cartilage matrix metabolism in cultured chondrocytes. *Eur J Cell Biol* 2000;79: 601-9.

Horas U, Pelinkovic D, Herr G et al. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint: a prospective, comparative trial. *J Bone Joint Surg [Am]* 2003; 85: 185-92

Hubbard MJ. Articular debridement versus washout for degeneration of the medial femoral condyle: a five-year study. *J Bone Joint Surg [Br]* 1996;78-B:217-19.

Huberti HH, Hayes WC. Patellofemoral contact pressures. The influence of q-angle tendafemoral contact. *J Bone Joint Surg Am.* 1984; 66: 715-24

Hughston JC & Walsh WM. Proximal and distal reconstruction of the extensor mechanism for patellar subluxation. *Clin Orthop Relat Res.* 1979; 144: 36-42

Hungerford DS & Barry MBS. Biomechanics of the patellofemoral joint. *Clin Orthop Rel Res.* 1979; 144: 9-15

Hunter W. Of the structure and disease of articulating cartilages. *Phil Trans.* 1743; 470-514

Hunter DJ, Zhang Y, Niu J et al. Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. *Arth Rheum* 2006; 54: 1529-1535

Hunziker E. (1992) Articular cartilage structure in humans and experimental animals. In Kuettner KE, Schleyerbach R, Peyron JG, Hascall VC (eds) Articular Cartilage Structure and Osteoarthritis. New York, NY: Raven Press, 183–199.

Hunziker EB. Articular cartilage repair: basic science and clinical progress: a review of the current status and prospects. Osteoarthritis Cartilage 2002;10:432-63.

Hurst NP, Ruta DA, Kind P. Comparison of the MOS short form-12 (SF12) health status questionnaire with th eSF36 inpatients with rheumatoid arthritis, *Br J Rheumatol.* 1998; 37(8): 862-9

Insall J, Bullough PG, Burstein AH. Proximal "tube" realignment of the patella for chondromalacia patellae. *Clin Orthop Rel Res.* 1979; 144: 63-9

Ishikawa SN, Murphy GA, and Richardson EG. The effect of cigarette smoking on hindfoot fusions. *Foot Ankle Int* 2002; 23(11): 996-8

Iyer SS, Rojas M et al. Anti-inflammatory effects of mesenchymal stem cells: novel concept for future therapies. *Expert Opin Biol Ther* 2008; 8: 569-81

Jackson RW, Dieterichs C. The results of arthroscopic lavage and debridement of osteoarthritic knees based on the severity of degeneration: a 4- to 6-year symptomatic follow-up. *Arthroscopy* 2003;19:13-20.

Jamtvedt G, Dahm KT, Christie A et al. Physical therapy interventions for patients with osteoarthritis of the knee: an overview of systematic reviews *Phys Ther* 2008; 88: 123-3

Janney CA & Jakicic JM. The influence of exercise and BMI on injuries and illnesses in overweight and obese individuals: a randomised controlled trial. *Int J Behav Nutr Phys Activ.* 2010; 7: 1-11

Janssen I and Mark AE. Separate and combined influence of body mas index and waist circumference on arthritis and knee osteoarthritis. *Int J Obesity* 2006; 30: 1223-1228

Jarvenpaa J, Kettunen J, Kroger H et al. Obesity may impair the early outcome of total knee arthroplasty. *Scand J Surg.* 2010; 99: 45-49

Jarvolm B, Lewold S, Malchau H et al. Age, bodyweight, smoking habits and the risk of severe osteoarthritis in the hip and knee in men. *Europ J Epidem.* 2005; 20: 537-542

Jenkinson CM, Doherty M, Avery AJ. Effects of dietary intervention and quadriceps strengthening exercises on pain and function in overweight people with knee pain: randomised controlled trial. *BMJ* 2009; 339: 3170-80

Jinks C, Jordan K, Croft P. Disabling knee pain – another consequence of obesity: results from a prospective cohort study. *BMC public health* 2006; 6: 258-65

Johnstone B, Hering TM, Caplan AI et al. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res* 1998; 238(1): 265-72

Jones G, Ding C, Glisson M et al. Knee articular cartilage development in children: a longitudinal study of the effect of sex, growth, body composition and physical activity. *Paed Res* 2003; 54: 230-6

Jorgensen LN, Kallehave F, Christensen E, Siana JE, Gottrup F. Less collagen production in smokers. *Surgery* 1998;123: 450-455.

Jurvelin J, Kiviranta I, Tammi M et al. Effect of physical exercise in indentation stiffness of articular cartilage in the canine knee. *Int J Sports Med.*1986; 7:106-110.

Karataglis D, Green MA, Learmonth DJ. Autologous osteochondral transplantation for the treatment of chondral defects of the knee. *Knee*. 2006;13:32-35.

Karim A, Pandit H, Murray J et al. Smoking and reconstruction of the anterior cruciate ligament. *J Bone Joint Surg [Br]* 2006; 88-B(8): 1027-31

Kellgren JS and Lawrence JS. Radiological assessment of osteoarthrosis. *Arth Rheum Dis.* 1957; 16: 494-502

Kendall JM. Designing a research project: randomised controlled trials and their principles *Emerg Med J* 2003; 20: 164–168

Kirkley A, Adlington J, Wall R et al. The cost-effectiveness of routine pathology consultation in knee arthroscopy. *Arthroscopy*. 1998 Oct;14(7):690-5.

Kiviranta I, Tammi M, Jurvelin J et al. Moderate running exercise augments glycosaminoglycans and thickness of articular cartilage in the knee joint of young beagle dogs. *J Orthop Res.* 1988; 6:188-195.

Knobloch TJ, Madhavan S, Nam J et al. Regulation of chondrocytic gene expression by biomechanical signals. *Crit Rev Eukaryot Gene Expr* 2008; 18: 139-50

Knutsen G, Engebretsen L, ludvigsen TC et al. autologous chondrocytes implantation compared with microfracture in the knee: a randomized trial. *J Bone Joint Surg [Am]* 2004;86-A:455-64.

Knutsen G, Drogset JO, Engebretsen L, et al. A randomized trial comparing autologous chondrocyte implantation with microfracture: findings at five years. *J Bone Joint Surg Am.* 2007;89:2105-2112.

Kon E, Gobbi A, Filardo G et al. Arthroscopic secondgeneration autologous chondrocyte implantation compared with microfracture for chondral lesions of the knee: prospective nonrandomized study at 5 years. *Am J Sports Med.* 2009;37:33-41.

Kon E, Filardo G, Condello V et al. Second generation autologous chondrocytes implantation. Results in patients older than 40 years. *Am J Sports Med.* 2011; 39(8): 1668-1675

Korkala OL. Periosteal primary resurfacing of joint surface defects of the patella due to injury. *Injury* 1988; 19(3): 216-8

Krishnan SP, Skinner JA, Bartlett W et al. Who is the ideal candidate for autologous chondrocyte implantation? *J Bone Joint Surg. [Br}* 2006: 88(1): 61-4

Kristman V, Manno M, Cote P . Loss to follow-up in cohort studies: how much is too much? *Eur J Epidemiol* 2004;**19**:751–60.

Kreuz PC, Erggelet C, Steinwachs MR et al. Is microfracture of chondral defects in the knee associated with different results in patients aged 40 years or younger? *Arthroscopy* 2006; 22:1180–1186

Kreuz PC, Steinwachs MR, Erggelet C et al. Results after microfracture of full-thickness chondral defects in diVerent compartments in the knee. *Osteoarthritis Cartilage* 2006; 14:1119–1125

Kreuz PC, Muller S, Ossendorf C et al. Treatment of focal degenerative cartilage defects with polymer-based autologous chondrocyte grafts: four-year clinical results. *Arth Res Ther.* 2009; 11(2): 1-11

Kujala UM, Kettunen J, Paananen H, et al. Knee osteoarthritis in former runners, soccer players, weight lifters, and shooters. *Arthritis Rheum.* 1995;38:539-546.

Kuroda T, Matsumoto T, Mifune Y et al. Therapeutic strategy of third-generation autologous chondrocyte implantation for osteoarthritis. *Ups J Med Sci.* 2011; 116(2): 107-14

Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33(1): 159-74

Lane JM, Brighton CT, Menkowitz BJ. Anaerobic and aerobic metabolism in articular cartilage. *J Rheumatol* 1977;4:334–42.

Lapvetelainen T, Hyttinen M, Lindblom J, et al. More knee joint osteoarthritis (OA) in mice after inactivation of one allele of type II procollagen gene but less OA after lifelong voluntary wheel running exercise. *Osteoarthritis Cartilage.* 2001;9:152-160.

Laurence M, Smith A. Experiments in chondrocyte homografting in the rabbit. *J Bone Joint Surg [Br]* 1968; 50B: 226-

Laxdal, G Kartus J, Ejerhed L et al. Outcome and risk factors after anterior cruciate reconstruction: A follow-up study of 948 patients. *Arthroscopy* 2005; 21(8): 958-964

Le Baron RG, Athanasiou KA. Ex vivo synthesis of articular cartilage. *Biomaterials* 2000; 21: 2575-87

Levy AS, Lohnes J, Sculley S, et al. Chondral delamination of the knee in soccer players. *Am J Sport Med.* 1996; 24: 634-9

Lindahl A. Novel approaches to future cartilage repair and regeneration . *Royal Society of Medicine (Round Table Series 77)* 2002; 41-45

Linden B. Osteochondritis Dissecans of the femoral condyles: a long term follow-up study. *J Bone Joint Surg [Am]* 1977; 59(6): 769-76

Link TM, Steinbach LS, Ghosh S et al. Osteoarthritis: MR imaging findings at different stages of disease and correlation with clinical findings. *Radiology* 2003; 226: 373-381

Liu H, Kemeny DM, Heng BC et al. The immunogenicity and immunomodulatory function of osteogenic cells differentiated from mesenchymal stem cells. *J Immunol* 2006; 176(5): 2864-71

Livshits G, Zhai G, Hart DJ et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis. The Chingford Study. *Arth Rheum.* 2009; 60: 2037-2045

Lorentzon R, Alfredson H, Hildingsson C. Treatment of deep cartilage defects of the patella with periosteal transplantation *Knee Surg Sports Traum Arth.* 1998; 6(4): 202-8

Lund-Olesen K. Oxygen tension in synovial fluids. *Arthritis Rheum* 1970;13:769–76.

Mallon WJ, Misamore G, Snead DA, et al. The impact of preoperative smoking habits on the results of rotator cuff repair. *J Shoulder Elbow Surg* 2004; 13(2): 129-32

Manassa EH, Hertl CH, and Olbrisch RR. Wound healing problems in smokers and nonsmokers after 132 abdominoplasties. *Plast. Reconstr. Surg.* 2003; 111(6): 2032-7

Mankin HJ. The response of articular cartilage to mechanical injury. *J Bone Joint Surg [Am]* 1982; 64(3): 460-6

Mankin HJ. The reaction of articular cartilage to injury and osteoarthritis. *New Eng J Med.* 1974; 291: 1285-92

Mankin HJ. Localization of Tritiated Thymidine in Articular Cartilage of Rabbits. *J Bone Joint Surg [Am]* 1962; 44A: 688-98

Maquet P. A biomechanical treatment of femoro-patellar arthrosis: advancement of the patellar tendon. *Rev Rheum* 1963; 30: 779

Manek NJ, Hart D, Spector TD et al. The association of body mass index and osteoarthritis of the knee joint. An examination of genetic and environmental influences. *Arth Rheum* 2003; 48(4): 1024-1029

Manninen P, Riihimaki H, Heliovaara et al. Weight changes and the risk of knee osteoarthritis requiring arthroplasty. *Ann Rheum Dis* 2004; 63: 1434-1437

Marcacci M, Kon E, Delcogliano M et al. Arthroscopic autologous **osteochondral** grafting for cartilage defects of the knee: prospective study results at a minimum 7-year follow-up. *Am J Sports Med.* 2007; 35(12): 2014-21

Marlovits S, Striessnig G, Kutscha-Lissberg et al. Ealr postopeartive adherence of matrix-induced autologous chondrocyte implantation for the treatment of full-thickness cartilage defects of the femoral condyle. *Knee Surg Sports Traumatol Arthroscop* 2004; 13: 451-7

Maroudas A and Schneiderman R. "Free" and "exchangeable" or "trapped" and nonexchangeable" water in cartilage. *J. Orthop. Res.* 1987; 5:133-138.

Marx RG, Jones EC, Allen AA et al. Reliability, validity, and responsiveness of four knee outcome scales for athletic patients. *J Bone Joint Surg Am.* 2001 Oct;83-A(10):1459-69.

Matsumoto T, Gargosky SE, Kelley K et al. Characterization of an insulin-like growth factor binding protein-5 protease produced by rat articular chondrocytes and a neuroblastoma cell line. Growth Regul. 1996 Sep;6(3):185-90.

Matsusue Y, Yamamuro T, Hama H. Arthroscopic multiple osteochondral transplantation to the chondral defect in the knee associated with anterior cruciate ligament disruption. *Arthroscopy* 1993: 9(3): 318-21

McClung CD, Zahiri CA, Higa JK et al. Relationship between body mass index and activity in hip or knee arthroplasty patients. *J Orthop Res.* 2000; 18: 35-39

Meister K, Cobb A, Bentley G. Treatment of painful articular cartilage defects of the patella by carbon-fibre implants. *J Bone Joint Surg [Br]* 1998;80-B:965-70.

Messier SP, Loeser RF, Miller GD et al. Exercise and Dietary Weight Loss in Overweight and Obese Older Adults With Knee Osteoarthritis. The ADAPT Trial. *Arth Rheum.* 2004; 5: 1501-10

Messner K, Maletius W. The long-term prognosis for severe damage to the weightbearing cartilage in the knee: a 14-year clinical and radiographic follow-up in 28 young athletes. *Acta Orthop Scand*. 1996;67:165-168.

Michaud SE, Menard C, Guy LG, Gennaro G, Rivard A. Inhibition of hypoxiainduced angiogenesis by cigarette smoke exposure: impairment of the HIF-1alpha/VEGF pathway. *FASEB J* 2003;17:1150–2.

Mihalko WA, Boachie-Adjei Y, Spang JT et al. Controversies and techniques in the surgical management of patellofemoral arthritis. *J Bone Joint Surg Am.* 2007; 89: 2788-2802

Miller BS, Steadman JR, Briggs KK, Rodrigo JJ, Rodkey WG. Patient satisfaction and outcome after microfracture of the degenerative knee. *J Knee Surg* 2004;17:13-17

Minas T, Nehrer S. Current concepts in the treatment of articular cartilage defects. *Orthopaedics* 1997; 20: 525-38

Minas T, Bryant T. The role of autologous chondrocytes implantation in the patellofemoral joint. *Clin Orthop Rel Res.* 2005; 436: 30-39

Minas T, Gomoll AH, Solhpour S et al. Autologous Chondrocyte Implantation for Joint Preservation in Patients With Early Osteoarthritis. *Clin Orthop Rel Res.* 2010; 468: 147-157

Miranda H, Viikari-Juntura E, Martikainen R and Riihimaki H. A prospective study on knee pain and its risk factors. *Osteoarth Cart* 2002; 10: 623-630

Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. *J Bone Joint Surg. [Am]* 1976; 58: 230-33

Mithoefer K, Peterson L, Mandelbaum BR et al. Articular cartilage repair in soccer players with autologous chondrocyte transplantation: functional outcome and return to competition. *Am J Sports Med.* 2005; 33: 1639-46

Mithofer K, Minas T, Peterson L, et al. Functional Outcome of Knee Articular cartilage Repair in Adolescent Athletes. *Am J Sports Med.* 2005; 33: 1147-53

Mithofer K, Williams RJ, Warren RF et al. High-Impact Athletics After Knee Articular Cartilage Repair. A Prospective Evaluation of the Microfracture Technique. *Am J Sports Med.* 2006; 34: 1413-8

Mithoefer K, Hambly K, Della Villa S et al. Return to Sports Participation After Articular Cartilage Repair in the Knee. *Am J Sports Med* 2009; 37 Suppl 1: 167-176

Moller AM, Pederson T, Villebro N et al. Effect of smoking on early complications after elective orthopaedic surgery. *J Bone Joint Surg [Br]* 2003; 85(2): 178-81

Morelli N, Nagamori J, Miniaci A. Management of chondral injuries of the knee by osteochondral autogenous transfer (mosaicplasty). *J Knee Surg.* 2002; 15(3): 185-90

Moseley JB, O'Malley K, Petersen NJ, et al. A controlled trial of arthroscopic surgery for osteoarthritis of the knee. *N Engl J Med* 2002;347:81-8.

Moseley JB, Anderson AF, Browne JE et al. Long Term Durability of Autologous Chondrocyte Implantation *Am J Sports Med* 2010; 38(2): 238-245

Murray DW, Britton AR, Bulstrode CJK. Loss to follow up matters. *J Bone Joint Surg [Br]* 1997; 79(2): 254-7

Nehrer S, Dorotka R, Domayer S et al. Treatment of Full-thickness Chondral Defects With Hyalograft C in the knee. A prospective clinical case series with 2 to 7 years follow-up. *Am J Sports Med.* 2009; 37 (Suppl 1): 81-87

Neidel J, Schulze M. Value of synovial analysis for prognosis of matrix synthesis of transplanted chondrocytes. *Orthopade* 2000, 29:158-163.

Nejadnik H, Hui JH, Choong EP et al. Autologous bone marrow derived mesenchymal stem cells versusautologous chondrocytes implantation: an observational cohort study. *Am J Sports Med.* 2010; 38(6): 110-6

Niemeyer P, Pestka JM, Kreuz PC, et al. Characteristic complications after autologous chondrocyte implantation for cartilage defects of the knee joint. *Am J Sports Med.* 2008; 36(11): 2091-2099.

Niemeyer P, Steinwachs M, Erggelet et al. Autologous chondrocytes implantation for the treatment of retropatellar cartilage defects: clinical results referred to defect localisation. *Acta Orthop Trauma Surg.* 2008; 128: 1223-1231

Niemeyer P, Lenz P, Kreuz PC et al. Chondrocyte-Seeded Type I/II Collagen Membrane for Autologous Chondrocyte Transplantation: Prospective 2-Year Results in Patients with cartilage defects of the knee joint. *Arth.* 2010; 26(2): 1074-82

Noyes FR, Matthews DS, Mooar PA et al. The symptomatic anterior cruciate-deficient knee. Part II: the results of rehabilitation, activity modification, and counseling on functional disability. *J Bone Joint Surg Am*. 1983 Feb;65(2):163-74.

Noyes FR, Mooar PA, Matthews DS et al. The symptomatic anterior cruciate-deficient knee. Part I: the long-term functional disability in athletically active individuals. *J Bone Joint Surg Am.* 1983 Feb;65(2):154-62.

O'Driscoll SW, Fitzsimmons JS. The role of periosteum in cartilage repair. *Clin Orthop* 2001;391(Suppl 1):190-7.

Oettmeier R, Arokoski J, Roth AJ et al. Quantitative study of articular cartilage and subchondral bone remodeling in the knee joint of dogs after strenuous running training. *J Bone Miner Res.* 1992;7(Suppl 2):419-424.

Oh JH, Kim SH, Ji HM et al. Prognostic factors affecting anatomic outcome of rotator cuff repair and correlation with functional outcome. *Arthroscopy* 2009; 25(1): 30-9

Ossendorf C, Kaps C, Kreuz PC et al. Treatment of posttraumatic and focal osteoarthritic cartilage defects of the knee with autologous polymer-based three-dimensional chondrocyte grafts: 2-year clinical results. *Arth res Ther.* 2007; 9(2): 41-51

Ossendorf C, Steinwachs MR, Kreuz PC et al. Autolgous chondrocyte implantation for the treatment of large complex cartilage lesions of the knee. *Sports Med Arthroscop Rehabil Ther Technol.* 2011; 21: 3-11

Outerbridge RE: The etiology of chondromalacia patellae. *J Bone Joint Surg Br* 43:752-757, 1961

Pallu S, Francin P, Guillaume C et al. Obesity affects the chondrocyte responsiveness to leptin in patients with osteoarthritis. *Arth Res Ther.* 2010; 12: 112-21

Palmer JL, Bertone AL, Malemud CJ et al. Site-specific proteoglycan characteristics of third carpal articular cartilage in exercised and nonexercised horses. *Am J Vet Res.* 1995; 56:1570-1576

Palmoski LJ, Brandt KD. Running inhibits the reversal of atrophic changes in canine knee cartilage after removal of leg cast. *Arth Rheum.* 1981; 24(11): 1329-37

Pascal-Garrido C, Slabaugh MA, L'Heureux DR et al. Recommendations and treatment outcomes for patellofemoral articular cartilage defects with autologous chondrocyte implantation. *Am J Sports Med.* 2009; 37 (Suppl 1): 33-41

Pelttari K, Winter A, Steck E et al. Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. Arthrit Rheum 2006; 54: 3254-3260

Peterson L, Brittberg M, Kiviranta I et al. Autologous chondrocytes implantation. Biomechanics and long-term durability. *Am J Sports Med.* 2002; 30(1): 2-12

Peterson L, Minas T, Brittberg M et al. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res*. 2000;374: 212-34.

Peterson L, Menche D, Grande DA et al. Chondrocyte implantation – An experimental model in the rabbit. *Trans Orthop Res Soc.* 1984; 9: 218

Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: a long-term follow-up. *Am J Sports Med.* 2010 Jun;38(6):1117-24. Epub 2010 Feb 24.

Petrie A and Sabin C. Medical statistics at a glance. Chapters 26 – 34. Pages 67 – 91. Blackwell Publishing 2nd Edition. 2006

Petrie A. Statistics in orthopaedic papers. *J Bone Joint Surg [Br]*

Pfaffle M, Borcher M, Deutzmann R et al. Anchorin CII, a collagen-bindingchondrocyte surface protein of the calpactin family. *Prog. Clin. and Biol. Res.* 1990; 349:147-157.

Pfander D, Cramer T, Swoboda B. Hypoxia and HIF-1alpha in osteoarthritis. *Int Orthop* 2005;29:6–9.

Piasecki DD, Spindler KP, Warren TA, Andrish JT, Parker RD. Intraarticular injuries associated with anterior cruciate ligament tear: findings at ligament reconstruction in high school and recreational athletes. *Am J Sports Med.* 2003;31:601-605

Pita JC, Manicourt DH, and Muller FJ. Centrifugal and biochemical comparison of two populations of proteoglycan aggregates from articular cartilage of immobilized dog joints. *Trans. Orthop. Res. Soc.* 1990; 15:17.

Pittenger MF, Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284(5411): 143-7

Poole AR, Rosenberg LC, Reiner A, Ionescu M, Bogoch E, and Roughley PJ. Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. J. Orthop. Res. 1996; 14:681-689

Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics*. 1975; 31(1): 103-15.#

Porter SE, Hanley EN. The Musculoskeletal Effects of Smoking. *J Am Acad Orthop Surg.* 2001; 9: 9-17

Post WR, Teitge R, Amis A. Patellofemoral malalignment: looking beyond the viewbox. *Clin Sports Med.* 2002;21:521-46

Pottie P, Presle N, Terlain B et al. Obesity and osteoarthritis: more complex than predicted! *Ann Rheum Dis.* 2006; 65: 1403-5

Pridie KH. A method of resurfacing osteoarthritic knee joints. *J Bone Joint Surg Br* 1959;41-B(3):618-9

Pritchett JW, Bortel DT. Knee replacement in morbidly obese women. *Surg Gynecol Obstet.* 1991 Aug;173(2):119-22.

Puelacher WC, Kim SW, Vacanti JP et al. Tissue-engineered growth of cartilage: the effect of varying the concentration of chondrocytes seeded onto synthetic polymer matrices. *Int J Oral Maxillofac Surg* 1994; 23: 49-53

Racunica T, Teichtahl AJ, Wang Y et al. Effect of physical activity on articular knee joint structures in community based adults. *Arth Rheum.* 2007; 57: 1261-8

Ritsila VA, Santavirta S, Alhopuro S et al. Periosteal and perichondral grafting in reconstructive surgery. *Clin Orthop Rel Res.* 1994 ; 302 : 259-65

Roberts S, McCall IW, Darby AJ et al. Autologous chondrocyte implantation for cartilage repair : monitoring its success by magnetic resonance imaging and histology. *Arth Res Ther.* 2003 : 5(1) : 60-73

Rodrigo JJ, Steadman JR, Syftestad G et al. Effects of human knee synovial fluid on chondrogenesis *in vitro*. *Am J Knee Surg* 1995, 8:124-129.

Roos EM, Roos HP, Ryd L, Lohmander LS. Substantial disability 3 months after arthroscopic partial meniscectomy: A prospective study of patient-relevant outcomes. Arthroscopy. 2000 Sep;16(6):619-26.

Roos EM, Dahlberg I. Positive effects of moderate exercise on glycosamino-glycan content in knee cartilage: a four month, randomised, controlled trial in patients at risk of osteoarthritis. *Arth Rheum.* 2005: 52: 3507-14

Rosenberger RE, Gomoll AH, Bryant T et al. Repair of Large Chondral Defects of the Knee with Autologous Chondrocyte Implantation in Patients 45 years or older. *Am J Sports Med.* 2008; 36(12): 2336-2344

Sakaguchi Y, Sekiya I, Yagishita K et al. Comparison of human stem cells derived fro various mesenchymal tissues: superiority of synovium as a cell source. Arthrit Rheum 2005 (52) 2521-2529

Saleh KJ, Arendt EA, Eldridge J et al. Operative treatment of patellofemoral arthritis. *J Bone Joint Surg [Am]* 2005; 87(3): 659-671

Sandmark H, Hogstedt C, Lewold S and Vingard E. Osteoarthrosis of the knee in men and women in association with overweight, smoking and hormone therapy. *Ann Rheum Dis* 1999; 58: 151-155

Santaguida PL, Hawker GA, Hudak PL et al. Patient characteristics affecting the prognosis of total hip and knee joint arthroplasty: a systematic review *J Can Chir* 2008; 51(6): 428-436

Saris DB, Dhert WJ, Verbout AJ. Joint homeostasis. The discrepancy between old and fresh defects in cartilage repair. *J Bone Joint Surg Br* 2003, 85:1067-1076.

Saris DB, Vanlauwe J, Victor J et al. Treatment of symptomatic cartilage defects of the knee: characterized chondrocyte implantation results in better clinical outcome at 36 months in a randomized trial compared to microfracture. Am J Sports Med 2009;37(Suppl 1):1-9.

Schultz W, Gobel D. Articular cartilage regeneration of the knee joint after proximal tibial valgus osteotomy: a prospective study of different intra- and extra-articular operative techniques. *Knee Surg Sports Traumatol Arthrosc* 1999;7:29-36.

Schulz KF, Grimes DA. Sample size slippages in randomised trials: exclusions and the lost and wayward. *Lancet* 2002; 359(9308): 781-5

Scuderi GR, editor. The patella. New York: Springer; 1995. p 74.

Seedholm BB, Takeda T, Tsubuku M et al. Mechanical factors and patellofemoral osteoarthrosis. *Ann Rheum Dis.* 1979; 38(4): 307-16

Selmi TAS, Verdonk P, Chambat P et al. Autologous chondrocytes implantation in a novel alginate-agarose hydrogel. *J Bone Joint Surg.* 2008; 90-B(5): 597-604

Shapiro F, Koide S, Glimcher M J. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. *J. Bone and Joint Surg.* 1993; 75: 532-553

Sharif M, Saxne T, Shepstone L, Kirwan J R, Elson C J, Heinegard D, and Dieppe P A Relationship between serum cartilage oligomeric matrix protein levels and disease progression in osteoarthritis of the knee joint. *Brit. J. Rheum.* 1995; 34:306-310.

Sharma L & Chang A. Overweight: advancing our understanding of its impact on the knee and hip. *Ann Rheum Dis.* 2007; 66: 141-2

Sharma G, Saxena RK, Mishra P. Differential effects of cyclical and static pressure on biomechanical and morphological properties of chondrocytes from articular cartilage. *Clin Biomech* 2007; 22: 248-55

Shelbourne KD, Jari S, Gray T. Outcome of untreated traumatic articular cartilage defects of the knee: a natural history study. *J Bone Joint Surg [Am]* 2003;85-A (Suppl 2):8-16.

Skoog V, Widenfalk B, Ohlsén L et al. The effect of growth factors and synovial fluid on chondrogenesis in perichondrium. *Scand J Plast Reconstr Surg Hand Surg*. 1990; 24(2): 89-95

Smith AD, Tao SS. Knee injuries in young athletes. *Clin Sports Med.* 1995; 14: 629-650

Smith RL, Allison AC, Schurman DJ. Induction of cartilage degradation by recombinant interleukin 1 alpha and 1 beta. *Connect Tissue Res.* 1989; 18(4): 307-16

Smith AU. Survival of frozen chondrocytes isolated from cartilage of adult mammals. *Nature* 1965; 205: 782-4

Sorensen LT, Hemmingsen U, Kallehave F et al. Risk factors for tissue and wound complications in gastrointestinal surgery. *Ann. Surg.* 2005; 241(4): 654-8

Spector TD, Harris PA, Hart DJ et al. Risk of osteoarthritis associated with long-term weight bearing sports: a radiological survey of the hips and knees in female ex-athletes and population controls *Arth Rheum.* 1996; 39: 988-95

Spicer DDM, Pomeroy DL, Badenhausen WE et al. Body Mass Index as a predictor of outcome in total knee replacement. *Int Orthop.* 2001; 25: 246-249

Sprague S, Leece P, Bhandari M et al. Limiting loss to follow-ip in a multicenter randomized trial in orthopaedic surgery. *Control Clin Trials* 2003; 24(6): 719-25

Squires GR, Okouneff S, Ionescu M et al. The pathophysiology of focal lesion development in aging human articular cartilage molecular natrix changes characteristic of osteoarthritis. *Arth Rheum.* 2003; 48; 1261-1270

Steadman JR, Rodkey WG, Singleton SB, Britts KK. Microfracture technique for full-thickness chondral defects: technique and clinical results. *Oper Tech Orthop 199*7; 7:300-4.

Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. *Clin Orthop* 2001;391 (Suppl):362-9

Steadman JR, Briggs KK, Rodrigo JJ, et al. Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy 2003;19: 477-84.

Steadman JR, Miller BS, Karas SG, et al. The microfracture technique in the treatment of full-thickness chondral lesions of the knee in National Football League players. J Knee Surg 2003;16:83-6.

Stern SH, Insall JN. Total knee arthroplasty in obese patients. *J Bone Joint Surg Am.* 1990 Oct;72(9):1400-4.

Stoop R, Albrecht D, Gaissmaier C et al. Comparison of marker gene expression in chondrocytes from patients receiving autologous chondrocyte implantation versus osteoarthritis patients. *Arth Res Ther.* 2007; 9(3): 60-69

Swann AC, Sweedholm BB. The stiffness of normal articular cartilage and the predominant acting stress levels: implications for the aetiology of osteoarthrosis. *Br J Rheum* 1993; 32(1): 16-25

Szoeke C, Dennerstein L, Guthrie J et al. The relationship between prospectively assessed body weight and physical activity and prevalence of radiological knee osteoarthritis in postmenopausal women *J Rheumatol* 2006; 33: 1835-40

Tang L-H, Buckwalter JA and Rosenberg LC. Effect of link protein concentration on articular cartilage proteoglycan aggregation. *J. Orthop. Res.* 1996; 14:334-339.

Takahashi M, Naito K, Abe M et al. Relationship between radiographic grading of osteoarthritis. *Arth Res Ther.* 2004; 6(3): 208-212

Takeda S, Elefteriou F, Karsenty G et al. Common endocrine control of body weight, reproduction, and bone mass. *Ann Rev Nutr.* 2003; 23: 403-11

Tanner JM. The essential characteristics of a rating system. *Am J Phys Anthropol*. 1971; 35(3):339-40.

Thompson RC, an experimental study of surface injury to articular cartilage and enzyme responses within the joint. *Clin Orth.* 1975; 107: 239-48

Torzilli PA, Deng XH, Ramcharan M Effect of compressive strain on cell viability in statically loaded articular cartilage. *Biomech Model Mechanobiol* 2006; 5: 123–32

Trujillo ME & Scherer PE. Adipose tissue derived factors: impact on health and disease. *Endocr Rev* 2006; 27: 762-768

Urquhart DM, Wluka AE, Teichtahl AJ et al. The effect of physical activity on the knee joint: is it good or bad? *Brit J Sports Med.* 2007; 41: 546-7

Van Wagenberg JMF, Speigner B, Gosens T et al. Midterm clinical results of the Autocentric II patellofemoral prosthesis. *Int Orthop.* 2009; 33: 1603-1608

Vanwanseele B, Eckstein F, Knecht H et al. Knee cartilage of spinal cord-injured patients displays progressive thinning in the absence of normal joint loading and movement. *Arth Rheum* 2002; 46: 2073-78

Visna P, Pasa L, Cizmar I, Hart R, Hoch J. Treatment of deep cartilage defects of the knee using autologous chondrograft transplantation and by abrasive techniques e a randomized controlled study. Acta Chir Belg 2004;104:709e14.

Wakabayashi S, Akizuki S Takizawa et al. A comparison of the healing potential of fibrillated cartilage versus eburnated bone in osteoarthritic knees after high tibial osteotomy: an arthroscopic study with 1-year follow-up. *Arthroscopy* 2002; 18(3): 272-8

Welsch GW, Mamisch TC, Zak L et al. Evaluation of cartilage repair tissue after Matrix-Associated Autologous Chondrocyte Transplantation using a Hyauronic-based or a Collage-Based Scaffold with Morphological MOCART Scoring and Biochemical T2 Mapping. *Am J Sports Med* 2010; 38(5): 934-941

Wakitani S, Goto T, Pineda SJ et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg [Am]* 1994; 76(4): 579-92

Wakitani S, Imoto K, Yamamoto T, et al. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 2002;10:199-206.

Wang Y, Ding C, Wluka AE et al. Factors affecting progression of knee cartilage defects in normal subjects over two years. *Rheumatology* 2005; 45: 79-84

Warner DO. Perioperative Abstinence from Cigarettes. Physiologic and Clinical Consequences. *Anesthesiology* 2006; 104: 356-367

W-Dahl A, Toksvig LA. Cigarette smoking delays bone healing: a prospective study of 200 patients operated on by the hemicallotasis technique. *Acta Orthop Scand* 2004; 75(3): 347-51

Welsch GW, Mamisch TC, Zak L et al. Evaluation of cartilage repair tissue after Matrix-Associated Autologous Chondrocyte Transplantation using a Hyauronic-based or a Collage-Based Scaffold with Morphological MOCART Scoring and Biochemical T2 Mapping. *Am J Sports Med* 2010; 38(5): 934-941

White JA, Wright V, Hudson AM. Relationships between habitual physical activity and osteoarthrosis in ageing women. *Public Health* 1993; 107: 459-70

Wiberg G. Roentgenographic and anatomic studies on the femoro-patellar joint. *Acta Orthop Scand*; 12:319 –410,1941

Wilke MM, Nydam D, Nixon AJ et al. Enhanced early chondrogenesis in articular defecs following arthroscopic mesenchymal stem cell implantation in an equine model. *J Orthop Res* 2007;25(7): 913-925

Winiarsky R, Barth P, Lotke P. Total knee arthroplasty in morbidly obese patients. *J Bone Joint Surg Am.* 1998 Dec;80(12):1770-4.

Wluka a, Wolfe R, Stuckey S et al. How does tibial cartilage volume relate to symptoms in subjects with knee osteoarthritis? *Ann Rheum Dis.* 2003; 63: 264-268

Wood JJ, Malek MA, Frassica FJ et al. Autologous cultured chondrocytes: adverse events reported to the United States Food and Drug Administration. *J Bone Joint Surg [Am]* 2006; 88(3): 503-7

Wondrasch B, Zak L, Welsch GH et al. Effect of Accelerated weightbearing after Matrix-Associated Autologous Chondrocyte Implantation on the Femoral Condyle on Radiographic and Clinical Outcome After 2 Years. A Prospective Randomized Controlled Pilot Study. *Am J Sports Med* 2009; 37 Suppl 1: 88-96

Yang GY, Lu SB, Wang JF. Long-term clinical observation on the repair of large articular cartilage defects of the hip and the knee with free autogenous periosteum. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2004;18:8-11 (in Chinese).

Yoo JU, Barthel TS, Nishimura K et al. The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells. *J Bone Joint Surg [Am]* 1998; 80(12): 1745-57

Zaslav K, Cole B, Brewster R, et al. A prospective study of autologous chondrocyte implantation in patients with failed prior treatment for articular cartilage defect of the knee: results of the Study of the Treatment of Articular Repair (STAR) clinical trial. *Am J Sports Med.* 2009;37(1):42-55.

Zeifang F, Oberle D, Nierhoff C et al. Autologous Chondrocyte Implantation Using the Original Periostem-Cover Technique Versus Matrix-Associated Autologous Chondrocyte Implantation. A Randomized Controlled trial. *Am J Sports Med.* 2010; 38(5): 924-933

Zevin S, Gourlay SG, Benowitz NL. Clinical pharmacology of nicotine. *Clin Dermatol* 1998;16: 557-564.

Zhang Y, Proenca R, Maffei M et al. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372: 425-32

Zheng Z, Deng Y, Lin XS et al. Induced production of rabbit articular cartilage-derived chondrocytes collagen II on polyhydroxyalkanoate blends. *J Biomater Sci Polym Ed.* 2003;14(7):615-24.

Appendix I:
Data Collection Proforma for ACI-C vs MACI study

The Royal National Orthopaedic Hospital

The UK Articular Cartilage Repair Study

ACI –v- MACI

FORM A – New Patient Form

To be completed once for each new patient enrolled in the study

FORM B – Surgery Form

To be completed for each patient undergoing a cartilage repair procedure (must have had form A completed)

FORM C (Part 1) – Follow Up Form

@ 6 and 12, 24, 36 months post-op

&

FORM C (Part 2) – Follow Up Form

@ 24 and 36 months post-op

To be completed for each follow up arthroscopy procedure (must have had forms A and B completed)

PLEASE RETURN COMPLETED FORM TO:

Prof George Bentley

c/o Nerina Bee

Teaching Centre

RNOH NHS Trust

Brockley Hill

Stanmore

Middlesex. HA7 4LP

UK Articular Cartilage Repair Study

The Royal National Orthopaedic Hospital, Stanmore

FORM A

Surgeon Details

Consultant:	**Completed by:**	**Date:**

Patient Personal Details & Medical History

Patient Name: ……

Hospital Number:…… Gender: ……

Patient Randomisation No…… ACI / MACI (please circle)

Address: ……

……

……

Date of Birth: ……/……/……

Age at the time of MACI/ACI Surgery: ……

Contact Phone Number: .....

Height: .....(cm) **Weight: .....(kg)**

Occupation prior to injury: ..... Occupation now: .....

Smoker: Yes/No/Ex **Allergies: Yes/No If yes please state .....**

Alcohol: Never ☐ **Occasionally** ☐ **Units: < 14** ☐ **15-21** ☐ **> 21** ☐

Opposite Knee: Normal ☐ **Almost Normal** ☐ **Not Normal** ☐

Medication: **NSAIDS** ☐ **Cortiscosteroids** ☐ **Other** ☐

Comorbidity: .....

FORM A

UK Articular Cartilage Repair Study

The Royal National Orthopaedic Hospital, Stanmore

Surgical History

Date of first stage (harvesting): ...../...../.....

Date of Second Stage (implantation): ...../...../.....

Length of Symptoms: .....

Aetiology of Cartilage defect: Traumatic ☐ OCD ☐ Early OA ☐

Chondromalacia Patellae ☐ Other ☐

Side: R ☐ L ☐ **Other Details:** .....

Previous Knee Surgery: Arthroscopy and Removal of Loose Bodies ☐

Drilling/microfracture ☐ Debridement ☐

Diagnostic Arthroscopy ☐ Mosaicplasty ☐

MSP Graft ☐ ACI ☐ Other ☐

No. of previous surgeries on the Index (affected) Knee: .....

Other Details: Lateral Release ☐ Patellectomy ☐

Tibial Tubercle Transfer: **Med** ☐ **Lat** ☐ **Prox** ☐ **Dist** ☐ **Ant** ☐

ACL Recon ☐ **PCL Recon** ☐

Menisectomy: **M** ☐ **L** ☐

Meniscal Repair: **M** ☐ **L** ☐

Osteotomy: **Tibial** ☐ **Femoral** ☐

Other Surgery to same Limb ☐ **Describe:** .....

Indications for Surgery: **Pain with Cartilage Defect** ☐ **Locking** ☐

Giving Way ☐ **Other** ☐

FORM A

UK Articular Cartilage Repair Study

The Royal National Orthopaedic Hospital, Stanmore

Examination Findings & Scoring System (for scoring system please see *overleaf)*

Wasting (6cm above patella) Nil ☙ <2cm ☙ >2cm ☙

Alignment: Normal ☙ Valgus ☙ Varus ☙

Effusion: Nil ☙ Mild ☙ Mod ☙ Sev ☙

Range of Movement (degrees) Flexion: ..... Extension: .....

Fixed flexion deformity: ..... Ext Lag: .....

Crepitus: Nil ☙ Mild ☙ Mod ☙ Sev ☙

Joint Tenderness: Nil ☙ Lateral ☙ Medial ☙ PFJ ☙

Patella Tracking Stable ☙ Unstable ☙ J Sign ☙

Ability to Squat: Normal ☙ Difficult ☙ Unable ☙

Anterior Draw: 0 ☙ 1 ☙ 2 ☙ 3 ☙ **MCL instability:** 0 ☙ 1 ☙ 2 ☙ 3 ☙

Lachman Test: 0 ☙ 1 ☙ 2 ☙ 3 ☙ **LCL instability:** 0 ☙ 1 ☙ 2 ☙ 3 ☙

Posterior Sag: 0 ☙ 1 ☙ 2 ☙ 3 ☙ **Pivot Shift:** Pos ☙ Neg ☙

Visual Analogue Score (0-10): .....

Bentley Functional Rating Score (0-4) .....

Modified Cincinatti Score (1-100) .....

Other Comments:
.....

FORM A

UK Articular Cartilage Repair Study

The Royal National Orthopaedic Hospital, Stanmore

Investigations

Xray Findings **Valgus Angle .....** **Sulcus Angle .....** **(tangential view)**

(AP Standing)

Patella: **Baja** **Alta** **Tracking:** **Normal** **Abnormal**

Defect: **Visible** **Yes** **No** **Depth ..... (mm)**

MRI Scan **Defect size (mm)** **Width .....** **Depth .....**

Defect: **Visible** **Yes** **No** **Depth** **..... (mm)**

Site: **MFC** **LFC** **Pat** **Troch**

FORM B

UK Articular Cartilage Repair Study

The Royal National Orthopaedic Hospital, Stanmore

ACI
Site Size and Depth of Lesion

Type

ACI ☐

MACI ☐

Other ☐ .....

Harvest Site(s)

Type of Cover

Perisoteum ☐

Type I-III collagen membrane ☐

Other ☐

APPROX NO. OF CELLS USED

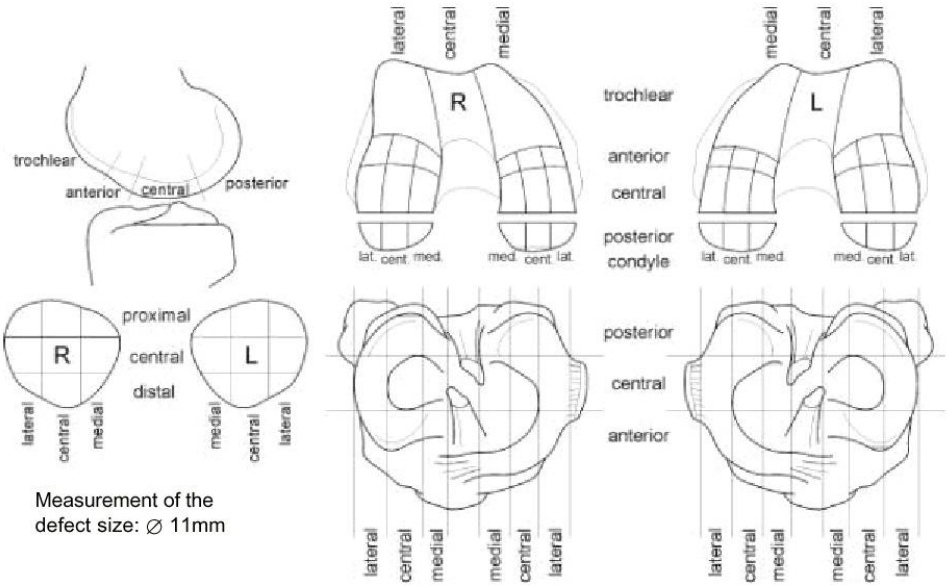
Patient Name .....

Surgeon ..... **Randomisation No**.....

Hospital No. ..... **D.O.B** .....

Intention to Treat: **Yes** ☐ **No** ☐
(i.e. is the patient being treated as randomised)

If No, reason: **Difficult Access** ☐ **Combined Procedure** ☐



Please draw the defects on the above diagram

Defect 1

Location - **Medial Femoral Condyle** ☐ **Trochlear** ☐
Patella – Single Facet ☐ **Patella – Multi Facets** ☐
Lateral Femoral Condyle ☐ **Lateral Tibial Condyle** ☐

ICRS Grade of defect (see over) : **1** ☐ **2** ☐ **3** ☐ **4** ☐ **A** ☐ **B** ☐ **C** ☐ **D** ☐

Outerbridge Grade of defect : **1** ☐ **2** ☐ **3** ☐ **4** ☐

Size of defect pre-debridement (mm) - Height ..... Width .....

Size of defect post-debridement (mm) Height ..... Width .....

5 million ☐

10 million ☐

15 million ☐

More ☐

Notes :

Depth .....

FORM B

UK Articular Cartilage Repair Study

The Royal National Orthopaedic Hospital, Stanmore

Other Structures

Defect 2

Location - **Medial Femoral Condyle** ☐ **Trochlear** ☐

Patella – Single Facet ☐ **Patella – Multi Facets** ☐

Lateral Femoral Condyle ☐ **Lateral Tibial Condyle** ☐

ICRS Grade of defect (see over) **:** 1☐ 2☐ 3☐ 4☐ A☐ B☐ C☐ D☐

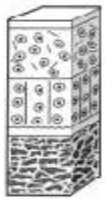
Outerbridge Grade of defect : 1☐ 2☐ 3☐ 4☐

Size of defect pre debridement (mm) **Height ..... Width ....**

	Size of defect post debridement (mm) Height ..... Width .... **Depth .....**
Medial Meniscus	**Intact ☐ Degenerative ☐ Degenerative tear ☐** **Traumatic tear ☐ Absent ☐**
Lateral Meniscus	**Intact ☐ Degenerative ☐ Degenerative tear ☐** **Traumatic Tear ☐ Absent ☐**
Anterior Cruciate Ligament	**Intact ☐ Chronic tear ☐ Acute tear ☐ Lax ☐**
Posterior Cruciate Ligament	**Intact ☐ Chronic tear ☐ Acute tear ☐ Lax ☐**
Loose Bodies	**1 ☐ 2 ☐ 3 ☐ > 3 ☐**
Plica	**Medial ☐ Lateral ☐ Suprapatellar ☐**
Combined Procedures	**ACI and Osteotomy ☐** **ACI and ACL Reconstruction ☐** **ACI and Lateral Release** **Other .....**

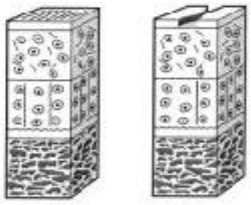
DEPTH OF CARTILAGE DEFECT ASSESSMENT

ICRS Grade 0 - Normal



ICRS Grade 1 – Nearly Normal

Superficial lesions. Soft indentation (A) and/or superficial fissures and cracks (B)



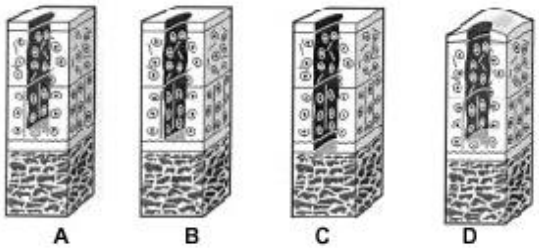
A B

ICRS Grade 2 – Abnormal

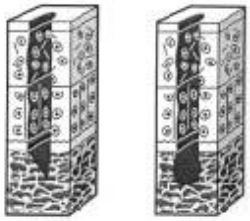
Lesions extending down to <50% of cartilage depth

ICRS Grade 3 – Severely Abnormal

Cartilage defects extending down >50% of cartilage depth (A) as well as down to calcified layer (B) and down to but not through the subchondral bone (C). Blisters are included in this Grade (D)



ICRS Grade 4 – Severely Abnormal



Appendix II:
Data Collection Proforma for Chapter 5: Smoking, BMI and Physical Activity

Form for BMI,

Occupation & Smoking

UK Articular Cartilage Repair Study

The Royal National Orthopaedic Hospital, Stanmore

Patient Name .....

Hospital Number:.....Gender: .....

Height (cm)..... Age at time of 2nd operation.....

Weight (at time of surgery)..... Weight (now).....

Do you have any other medical problems?.....

What mediations do you take?.....

How did you injure yourself.....

Smoker? Y / N / Ex (If ex: when did you stop.....)

If yes/Ex, What do you/did you smoke.....

- **How long did you smoke for.....**
- **How many do you smoke a day.....**
- **Did you smoke before your surgery?.....**
- **Did anyone tell you to stop smoking prior to surgery?.....**
- **If you did stop, how long before the surgery did you stop?.....**

- **Did you start smoking after surgery & if so how long after?……**

Alcohol: Never Occasionally Units: < 14 15-21 >21

How long were you in hospital for after 2nd stage operation?……..

Were there any problems with your wound after the operation?……..

If so what?……Did you require antibiotics?……..

Symptoms Prior To Cartilage Operation (please circle):

Pain	**Swelling**
Stiffness	**Giving way**
Locking	**Grating**

Other (please specify)……

Occupation

Occupation prior to injury……

Occupation prior to operation……

Occupation now……..

Estimated time off work due to symptoms (prior to cartilage operation)……

Estimated time off work due to Cartilage operation……

Did you go to 6th form college?……

After cartilage surgery has there been a modification in your work (if so what?)……..

Occupational Activity (in an average working day)

	Before Cartilage Operation	**After Cartilage Operation**
Sitting for > 2 hours in total		
Standing/walking for > 2 hours (total)		
Kneeling for > 1 hour (total)		
Squatting for > 1 hour (total)		
Getting up from kneeling or squatting > 30 times		
Driving for 4 hours in total		
Walking for > 2 miles in total		
Climbing > 30 flights of stairs		
Lifting/moving weights of +10Kg by hand		
Lifting/moving weights of +25Kg by hand		

Occupational & Psychological Factors

	Before Cartilage Operation	**After Cartilage Operation**	**Better/Worse/Same**
Annual Income			
Work Stress:			
○ Often			
○ Sometimes			
○ Seldom			
○ Never			
Job Satisfaction			
○ Not satisfied			
○ Somewhat			
○ Mostly			
○ Totally			

Physical Activity

Which sports did you play at least 5 times a year (prior to injury)…..

……

At what age did you begin playing these sports……

At what age did you stop……

Have you returned to playing these sports……

How many months after the cartilage operation were you able to return to the following:

- **Walking (for exercise)……..**
- **Swimming……..**
- **Cycling……..**
- **Gym work……**
- **Running……..**
