

Enhancing biological and biomechanical fixation of osteochondral scaffold: a grand challenge

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Abstract

Osteoarthritis (OA) is a degenerative joint disease, typified by degradation of cartilage and changes in the subchondral bone, resulting in pain, stiffness and reduced mobility. Current surgical treatments often fail to regenerate hyaline cartilage and result in the formation of fibrocartilage. Tissue engineering approaches have emerged for the repair of cartilage defects and damages to the subchondral bones in the early stage of OA, and have shown potential in restoring the joint's function. In this approach, the use of three-dimensional scaffolds (with or without cells) provides support for tissue growth. Commercially available osteochondral (OC) scaffolds have been studied in OA patients for repair and regeneration of OC defects. However, some controversial results are often reported from both clinical trials and animal studies. The objective of this article is to report the scaffolds clinical requirements and performance of the currently available OC scaffolds that have been investigated in both animal studies and in clinical trials. The findings have demonstrated the importance of biological and biomechanical fixation of the OC scaffolds in achieving good cartilage fill and improved hyaline cartilage formation. It is concluded that improving cartilage fill, enhancing its integration with host tissues, and achieving a strong and stable subchondral bone support for overlying cartilage are still grand challenges for the early treatment of OA.

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Introduction

In joints, the articular cartilage, calcified cartilage, and subchondral bone form a bio-composite system, referred to as the osteochondral (OC) unit, which has the unique capability of transferring loads during joint motion¹. Repetitive overloading to this unit could result in cartilage damage and changes in the subchondral bone, leading to mechanical instability of the joints and loss of joint function^{2,3}. If left untreated, the OC defects will lead to the development of osteoarthritis (OA)², where the composition and structure of this unit undergo significant alterations¹. During the process of OA, thinning and degradation of articular cartilage, joint-space narrowing, osteophytes formation and subchondral bone remodelling⁴⁻⁶ take place. Cartilage destruction results from an unbalanced relationship between matrix synthesis by chondrocytes and matrix degradation⁵. Other pathological processes including microfractures, microedema or microbleeding within the subchondral bone could lead to subchondral bone defects such as subchondral cyst formation⁴. If the OC defect has progressed to the stage where the patient's quality of life has significantly reduced and non-surgical treatments are no longer effective, then a joint replacement has to be performed. This major surgical procedure often does not restore the full function of joints and have high long term complication rates.

Between 2003 and 2013, there were 1.296m joint replacements performed in England and Wales including 620,400 hip procedures and 676,082 knee procedures predominantly for OA (over 93%)⁷. OA is a major contributor to functional impairment and reduced independence in older adults⁸ and represents an enormous socioeconomic challenge⁹. Regeneration of the tissues affected by OA in early, mid or late stages of the disease can enhance the quality of life and delay or avoid the need for total joint replacement, thereby reducing the socioeconomic costs.

1.1

Progression of OA and available treatments

In early stages of OA, there is an increase in water content of cartilage, resulting in swelling of the matrix and an increase in metabolic activity of chondrocytes (**Error! Reference source not found.**). These changes are accompanied by the appearance of surface fibrillations characterized by microscopic cracks in the superficial zone of the articular cartilage. In the

subchondral bone, increased remodelling of the cortical bone plate usually leads to increased porosity¹. In this stage, pain and stiffness dominate the other symptoms, and the goal of the treatments is therefore to reduce pain and physical disability and some attempt to control structural deterioration in the affected joints^{6,10}, using physical therapy¹¹, analgesics and NSAIDs⁹. Intra-articular injection of long-acting glucocorticoids is an effective treatment of inflammatory flares of OA. Hyaluronic acid has varying effectiveness when used for intra-articular injections for the treatment of OA of the knee¹⁰.

With the progression of OA, loss of cartilage matrix proteoglycans and erosion of the collagen network leads to the development of deep fissures and partial delamination of the cartilage, while in the subchondral bone, cortical plate thickness gradually increases¹ (**Error! Reference source not found.**). In this stage of the disease, where the cartilage defect is still small (area <2-3cm²), microfracture (MF) marrow stimulation is considered a medically necessary treatment. MF is a minimally invasive procedure which seeks to repair cartilage damage through releasing mesenchymal stem cells (MSCs) from the underlying bone which then differentiate to become chondrocytes and create new cartilage. It involves removing the damaged cartilage and then drilling into or otherwise puncturing the surface of the underlying bone in order to allow blood and bone marrow to come through to the bone/cartilage interface, where the MSCs contribute to the formation and repair of the cartilage and bone. However, the regenerated cartilage is mainly fibrocartilage, and is not expected to have the same durability as the articular hyaline cartilage. This type of cartilage is mostly type I collagen, fibrocytes and a disorganized matrix that lacks the biomechanical and viscoelastic characteristics of normal hyaline cartilage¹² and can fail with high shear forces in the joint, leading to an ongoing articular surface irregularity and subsequent secondary arthritic change¹³. This was demonstrated by the high 5 year post-microfracture re-operation rates, which is between 30 and 50 %¹⁴.

Osteochondral (OC) autografts or allografts¹⁴, scaffolds, and focal knee resurfacing implants are among the approaches that have been explored for treatment of small- to mid-sized lesions¹⁵. OC autografts have been proposed to provide an immediate reliable tissue transfer of a viable

OC unit in a single-stage procedure. This procedure exploits the regenerative potential of bone and bone-to-bone healing, since the cartilage has a limited healing capacity¹⁴. For example, Hangody et al. (2010) analysed the results of mosaicplasty (where multiple autografts are used) in 82 athletes with signs of OA. They reported significant improvements after the procedure, although slight radiographic degeneration in one-third of the patients at mid- to long-term follow-up was observed^{14,16}.

Fresh OC allografts provide the surgeon with more freedom regarding the size of the defect that can be treated. Common indications for OC allograft include large, focal chondral defect, osteochondritis dissecans and unicompartmental arthritis¹⁷. However, apart from general complications of open joint surgery, OC allograft transplantation is also associated with a risk of disease transmission from the allograft and subchondral collapse due to inadequate integration. The latter is responsible for a majority of graft-related failures¹⁴.

Tissue engineering (TE) approaches has been developed as a potential solution for repair and regeneration of OC defects. In this approach, scaffolds are designed and fabricated to provide a physical environment to support cellular activities and prompt tissue regeneration. OC scaffolds can be implanted by arthroscopy or mini-arthrotomy and fixed by press-fit. Some cases may require additional fixation through sutures, pins or fibrin glue. Currently, lesion size range from 2 to 8 cm² can be treated using osteochondral scaffolds which are available in predetermined sizes or patches that can be shaped and sized at the time of implantation¹⁴. Commercially available scaffolds such as Chondromimetic (Tigenix NV), MaioRegen (FInceramica), TruFit® BGS Plugs (Smith & Nephew) have been used, with or without cells, in clinical trials for treatment of small cartilage and osteochondral defects (OCDs)($<1.5 \text{ cm}^2$). However, limited success was reported, and none of these scaffolds have achieved satisfactory durable clinical results.

In late-stage OA, chondrocytes clustering and apoptosis are evident in the cartilage. In the deeper zones of cartilage, chondrocytes undergo hypertrophic differentiation and the calcified cartilage expands and advances into the overlying hyaline articular cartilage, with duplication of

the tidemark (**Error! Reference source not found.**). This process is initiated by the penetration of vascular elements into the osteochondral junction. In addition to the development of osteophytes at the joint margins and cysts within the subchondral bone, the subchondral cortical plate becomes flattened and deformed, a process referred to as 'bone attrition'. In this stage of the disease, underlying zones of calcified cartilage and subchondral bone are exposed¹. In these advanced cases joint prosthesis surgery is often required⁹.

To-date, OC tissue engineering approaches have mainly focussed on regeneration of small OC defects mostly in early stages of OA. However, with the right scaffold, treatment of large, late-stage OC defects could become possible. The idea of a “smart” scaffold which provides an appropriate biomechanical environment to support healthy cell growth and promote OC regeneration has been reported as the Holy Grail in the last decades in the treatment of both early and late stages of OA. However, this has been achieved only in early stages of OA, and with limited success. In this paper, we discuss the requirements of an OC scaffold and insights from the studies of OC scaffolds performance, both *in vivo* and in clinical settings, in the light of similar events observed during the development of OA. The effect of biomechanical and biological fixations of the scaffold on the healthy regeneration of OC tissue has become increasingly apparent. The results discussed in this study would provide us with the essential knowledge for the successful development of future clinical OC scaffolds.

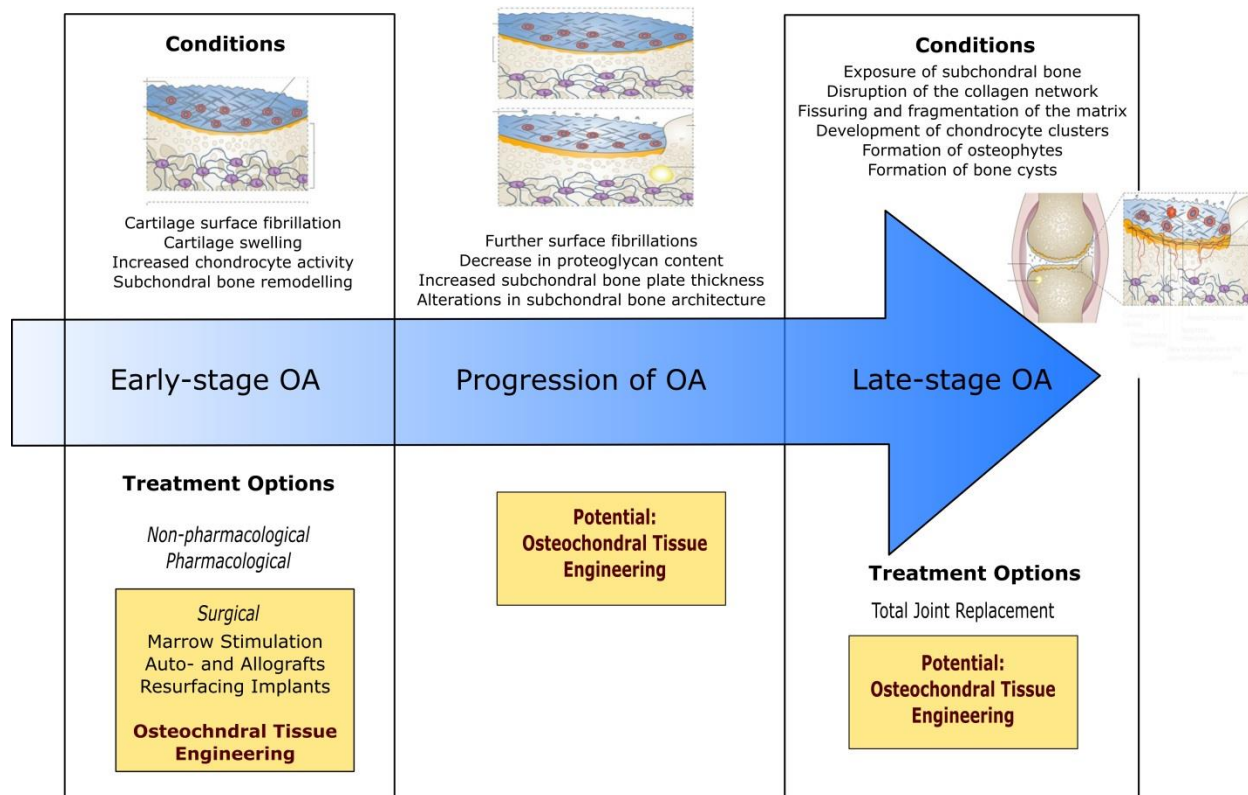


Figure 1 Progression of OA: conditions and treatments in each stage. Pictures of osteochondral units adapted from¹

Osteochondral tissue engineering

2

Tissue engineering (TE) is a discipline that applies the knowledge of materials science, cell biology and bioengineering to construct tissue templates and restore the function of an injured tissue (Figure 2). It may involve a cell-free approach by using a scaffold only, or it may involve taking the cells from the patient, seeding the cells onto a scaffold and culture this whole in a bioreactor system, then transplanting it back into the patient once the tissue has matured. In either processes, the three-dimensional porous scaffold plays an important role in supporting the (seeded/resident) cells growth and guiding new tissue formation¹⁸. Due to the unique structure and property of OC tissue unit, the concept of the simultaneous regeneration of articular cartilage and underlying bone (OC defect) to develop a well-defined tissue-to-tissue interface¹⁹ has drawn considerable attention, especially as a technique for promoting superior cartilage integration and a treatment for OC defects as often observed in osteoarthritic joints²⁰.

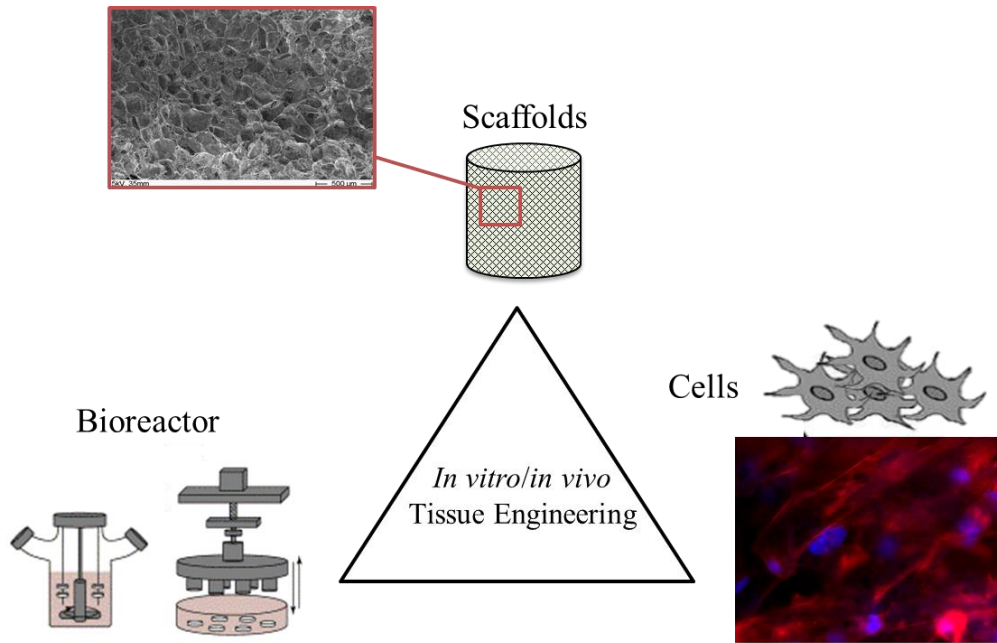


Figure 2 Three key ingredients of tissue engineering; adapted from ^{20 21},

In general, cartilage-to-cartilage interfaces do not integrate well because of the dense avascular nature of cartilage and scarcity of cells; however, it is often observed that host bone integrates well with the grafted bone tissue and other implant materials. To improve the integration of the engineered cartilage tissue with the host tissue, an OC implant can be used where the bony region serves as an anchor for the implant. Thus a successful OC scaffold needs to address both regions concurrently. The cartilage and bone regions of the OC composite scaffold require different physical and mechanical properties to mimic the gradient mechanical property, structure and functionality of the OC unit. A major challenge is thus generating the natural gradation in porosity, composition and biomechanical properties associated with both tissues (i.e. bone and cartilage) as well as the integration of the two types of tissues ²².

2.1

Scaffold design considerations: mimicking the nature

The OC tissue is composed of cartilage and subchondral bone, each with their own specific hierarchical structure and biological property²³. Therefore, to design a biomimetic scaffold an understanding of the OC unit, including its composition, structure and function is essential.

Cartilage – bone junction

Articular cartilage- the top layer of an OC unit- is vital for facilitating a smooth motion within joints and absorbing impact. It consists of chondrocytes embedded in an extracellular matrix (ECM) mainly comprising collagen (60% dry weight²⁴, 90-95% type II²⁵), proteoglycans and non-collagenous proteins. The structure and composition of cartilage is organised on two levels, which are determined according to the distance from the cartilage surface and in relation to its distance from the cells. Typically, articular cartilage is divided into four zones based on the distance from the surface: superficial, middle, deep and calcified zones²⁵. The latter is directly below the deep zone containing hypertrophic chondrocytes embedded in a densely-mineralised matrix which constitutes the OC interface²⁶. Calcified cartilage is separated from the deep zone by a discrete band of mineralised cartilage called “tidemarks”. This line represents the mineralization front of the calcified cartilage and provides a gradual transition between the two dissimilar regions of cartilage (non-calcified and calcified). Immediately below the calcified zone lies subchondral bone plate- a bony lamella (cortical endplate, 1-3mm thick²⁷), which is separated by a “cement line” from the calcified cartilage. Together with the supporting trabeculae and subarticular spongiosa, they form the subchondral bone unit⁴. While the tidemark is crossed by collagen fibrils between the articular cartilage and calcified cartilage resulting in a strong link between these two zones, the cement line marks the separation of the cartilage and underlying bone. It is presumed to be a region of weakness since no collagen fibers are continuous between the calcified cartilage and subchondral bone plate^{4,28}. The steep stiffness gradient between cartilage and subchondral bone unit may be one of causes of cartilage delamination from the bone due to shear stresses⁴. Different magnitudes of strain, internal pressure and fluid flow are developed in each of the osteochondral layers during loading²⁹, the convex joint surfaces can be exposed to large lateral forces which may lead to a variety of shear-induced lesions in the osteochondral region²⁸. Therefore, one of the important considerations in designing bi-/multi-layered scaffolds³⁰ for OC TE is to avoid abrupt and large changes in mechanical properties of different layers.

2.1.2

Role of subchondral bone in maintenance of cartilage

Subchondral bone is essential in function and maintenance of articular cartilage. From biomechanical point of view, the joints can withstand about 2.5–5 times of the body weight

caused by the dynamic loading generated during walking. Subchondral bone enhances the load bearing capacity: normal subchondral bone attenuates about 30% of the loads through joints; only 1–3% is attenuated by cartilage⁴. Cartilage and bone act in concert by performing a biomechanical function of the joints, the former as a bearing and the latter as a structural girder and shock absorber³¹. As such, simultaneous repair and regeneration of cartilage and subchondral bone is a key concept in osteochondral tissue engineering.

It is hypothesised that the thickness of cartilage is dependent on the joint congruency⁴ and local stresses³². A joint with a high congruency has thin cartilage, whereas a joint with a low congruency is covered by a thick layer of cartilage so that it can more easily deform thereby increasing the load-bearing area and decreasing the stress per unit area^{4,33}. It is intuitive that heavily stressed regions have thicker cartilage. As for the subchondral bone, regional differences in mineralisation can also be recognized, and greater density is usually found in the more heavily loaded regions of the joint surface^{4,32}. Therefore, it can be suggested at places within the joint where the stress is assumed to be greatest, the subchondral bone mineralisation is higher and the cartilage is thicker⁴. Therefore, design of the scaffold may need to be adjusted in terms of the thickness and mineralisation/stiffness of layers according to the location of the defect within the joint.

The subchondral bone also plays an important role from nutritive point of view. The subchondral bone plate has a high number of vessels and hollow spaces invading the cement line into the calcified cartilage, and they are mainly concentrated in heavily stressed zones, providing a rich blood supply to subchondral bone and nutrients to the cartilage⁴. Whereas the superficial zone of cartilage is mainly dependent on diffusion via synovial fluid as its nutritive source, the subchondral circulation may make a significant contribution to the nutrition in deep and calcified cartilage^{34,35}. In fact it has been shown that that more than 50% of the glucose, oxygen and water requirements of cartilage are provided by perfusion from these subchondral vessels^{36,37}. The abrogation of contact between the subchondral bone and cartilage leads to

degeneration of cartilage in the long run³⁸. This emphasizes the importance of subchondral bone regeneration and vascularisation in OC tissue engineering.

Highlighting the significance of vasculature in bone formation is the fact that the metabolically active cells are no more than 100 µm away from a capillary for supply of oxygen and nutrients^{39,40}. Often, this poses a problem for tissue engineering (especially cell-free scaffolds – because in the *in vitro* construct this can be alleviated by using bioreactors), since the resident cells may not be able to migrate deep into the scaffold due to diffusion constraints of oxygen and nutrients, only cells close to the surface are able to survive. However, the mineralisation at the periphery of the scaffold actually block further diffusion and mass transfer to the interior of the scaffold, leading to growth of only thin cross-sections of tissue (<500 µm)⁴¹. This needs to be taken into account when designing a scaffold for OC tissue engineering, for example by devising internal channels⁴² in the OC scaffold.

Scaffold structure and properties

2.2 When the calcified zone of cartilage is surgically removed, such as in the case when the basis of a chondral or OC defect is prepared prior to microfracture or scaffold implantation, the bone marrow which contains mesenchymal stem cells (MSCs) may enter the debrided defect via the blood vessel or channels⁴. Controlling the cellular behaviour of bone marrow mesenchymal stem cells (BMMSCs) is vital for achieving correct type of regenerated tissue (e.g. hyaline cartilage as opposed to fibrocartilage). The scaffold provides physical environment to support BMMSCs growth and plays a vital role in controlling BMMSCs fate⁴³ through scaffold-cell interactions to regulate cells phenotype, cytoskeleton spreading, proliferation, gene expression and ECM secretion through metabolic activity, cell–matrix and cell–cell contact⁴⁴.

The microenvironmental factors affecting stem cell behaviour⁴⁵ include scaffold surface characteristics (e.g. wettability and charge – cell attachment), material (cell attachment and differentiation), microstructure (porosity, pore size and shape – cell adhesion, migration and differentiation) and stiffness (cell differentiation).

Scaffold surface and material characteristics affects cell attachment and differentiation

When a scaffold is implanted into an OCD, the biological fluid (e.g. from bone marrow, synovial fluid, etc) from the patient will be in contact with the surface of the scaffold. The surface wettability affects protein absorption and consequently cells attachment to it. Therefore, understanding the molecular mechanism of cell adhesion on biomaterials is important to manipulate the scaffold-cell interaction.

When an anchorage-dependent cell (such as MSCs) comes in contact with a surface, it must adhere to the surface to remain viable, proliferate or differentiate. Attachment to the surface can be non-receptor-mediated via weak chemical bonding, such as electrostatic, hydrogen or ionic bonding; however, this type of adhesion does not guarantee the transmission of signals from the microenvironment to the cells, which is necessary to prompt the secretion of ECM molecules from cells, and without it the cells may go into apoptosis. By contrast, receptor-mediated adhesion through ECM molecules, such as fibronectin or collagen allows signal transmission. These ECM molecules adsorb onto the surface of biomaterial from the surrounding environment and then bind cell integrins through their specific amino acid sequences. These specific amino acids are called ligands, and the minimum adhesion motif on ECM molecules should contain at least three amino acids, which are often symbolised by Arg-Gly-Asp (RGD). On the other hand, glycoprotein integrins comprising one α and one β chain. There are about 16 recognised subunits and eight subunits with various combinations, which results in receptors with preferential binding affinity to certain ECM molecules^{47,48}.

Once ligand binds to the surface, integrins are formed into dot-like or streak-like 'focal adhesions'. In these focal adhesions, integrins communicate with structural and signalling molecules, such as talin, α -actinin, filamin, paxillin or vinculin, which link integrin receptors to cytoplasmatic actin cytoskeleton (Figure 3). External signals are then transmitted from the microenvironment to the nuclei of cells⁴⁹, thereby influencing intracellular transport processes and the secretion of various molecules and determining the cellular activities such as cell proliferation and differentiation or apoptosis⁴⁷. The extent and strength of cell adhesion to a surface affects its migration and its

decision to switch between proliferation and differentiation behaviour^{47,49,50}. For example, cells with large adhesion are usually dormant in migration and proliferation and more active in the expression of differentiation markers⁵⁰. The optimum adhesion of cells generally occurs on a moderately hydrophilic and positively charged scaffold because the adhesion molecules are adsorbed in a favourable geometric conformation, making ligands available to bind with cell receptors⁵⁰. In short, cell attachment requires specific binding proteins to be present on the material. The surface morphology and chemistry of the scaffold regulate proteins adsorption, and influence cells attachment and alignment⁵¹.

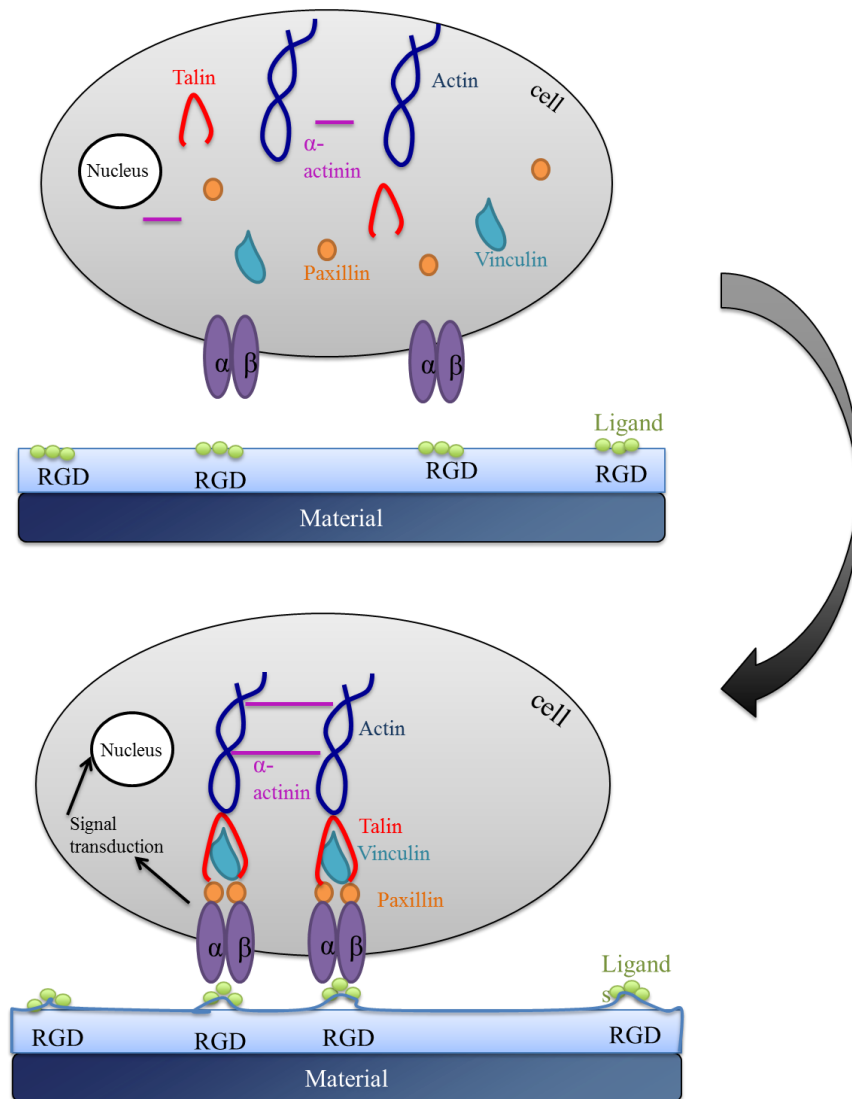


Figure 3 Cell-scaffold interaction, modified from^{48,52}

Biomaterials used in tissue engineering of OCDs are usually categorized into four major groups: natural polymers, synthetic polymers, metallic materials, and inorganic materials such as ceramics and bioactive glasses. Multicomponent systems can be designed to generate composites of enhanced performance². Naturally derived polymers such as collagen, alginate, gelatin and chitosan have the advantage of native biological function, enhancing cellular attachment, proliferation and function^{55,56}. As explained earlier, cells primarily interact with scaffolds via ligands on the material surface. Scaffolds synthesized from natural extracellular materials (e.g. collagen) naturally possess these ligands in the form of RGD binding sequences, whereas scaffolds made from synthetic materials may require deliberate incorporation of these ligands through, for example, protein adsorption⁴⁶. The main disadvantages of these naturally derived biomaterials are batch-to-batch variability and low mechanical strength. With synthetic polymers (e.g. PCL, PLA, PLGA) on the other hand, it is possible to precisely control the mechanical properties and tailor the structure and apply surface modifications. However, they exhibit poor cell adhesion due to their intrinsic hydrophobicity and lack of natural ligand binding sites⁵⁶.

Bioceramics, such as calcium phosphates, are known for their excellent osteoconductivity^{2,57}. The most common types of calcium phosphates for bone TE scaffolds are hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), tricalcium phosphate (TCP), biphasic calcium phosphates and multiphasic bio-glasses⁵⁸. The physical properties of the calcium phosphate ceramics, such as degradation rate, modulus and processability, can be controlled by altering their composition⁵⁹.

2.2.2 Scaffold microstructure affects cell adhesion, migration and differentiation

Once attach to a substrate, the cells need to migrate into the scaffold three-dimensional space, proliferate and differentiate into the appropriate tissue type there. Cell migration requires scaffold to be porous^{60,61} and to have an interconnected pore structure to allow for healthy cellular invasion and growth, nutrition delivery⁵⁴ to the cells inside the scaffold, as well as removal of metabolic waste from the cells. Vascularisation – as explained in the design considerations – is not therefore possible without porosity to allow oxygen and nutrition

diffusion and vasculature formation⁶². In fact, absence of any bone formation on a solid scaffold that lacked porosity demonstrates the importance of this factor in tissue formation⁶³.

Cell migration is also affected by another microstructural factor: pore size. Cells use a bridging mechanism when migrating through a porous scaffold; that is, they use neighbouring cells as support to bridge across pores larger than their diameters. If the pore dimension greatly exceeds the size of a cell, then the cell can only spread along the walls of the pore, instead of bridging a pore, a phenomenon that influences cells' migration ability and speed in general⁵⁴. If pores are too small, cell migration is limited; the cells trying to bridge the small pores block the way for migration into the centre of the scaffold, resulting in the formation of a cellular capsule around the edges of the scaffold. This in turn can limit diffusion of nutrients and removal of waste resulting in necrotic regions within the construct. However, cells travelling through larger pores may migrate slower, but their directional movement allow them to travel further into the scaffold increasing cell migration and scaffold infiltration⁵⁴.

Differentiation of MSCs can be influenced by pore size as well as the pore shape. This can be attributed to the particular cellular events prerequisite in the chondrogenic and osteogenic differentiation processes of MSCs. Chondrogenesis of MSCs is associated with morphologic changes from fibroblast to spherical morphology, in which the fibroblastic morphology is formed through cell–matrix interactions during migration and proliferation, and develops into spherical morphology in the process of condensation. The chondrogenic differentiation of MSCs occurs in highly regulated stages. Aggregation of mesenchymal cells into pre-cartilage condensations is crucial for chondrogenesis. Condensation occurs through cell–cell contacts, which is controlled by the association of cell adhesion molecules of the adjacent cells, formation of gap junctions and changes in the cytoskeletal architecture, subsequently activating intracellular signalling pathways to initiate the transition from chondroprogenitor cells to a fully committed chondrocyte^{44,66,67}. The ability of MSCs to aggregate in the larger pores in a porous scaffold, coupled with proliferation of cells within the scaffold might facilitate chondrogenic

condensation process of MSCs⁴⁴, showing the effect of pore size in controlling MSCs differentiation.

Cell shape is a potent regulator of cell growth and physiology⁶⁹ and many events related to embryonic development (e.g. single and collective cell migration, dorsal closure, etc⁷⁰)⁷⁰ and stem cell differentiation are influenced by cell shape⁴³. An example can be seen in Figure 4, where the effects of cell spreading and focal adhesions are shown on viability, migration, proliferation and differentiation of cells.

In bone and cartilage development, flattened and spherical cell morphologies are the most relevant, where the spherical morphology of chondrocytes is closely related to their chondrogenic potential⁶⁸. A direct comparisons of cell and nuclear shape of BMMSCs shows that a more rounded nuclear shape is associated with the greatest expression of molecular markers associated with chondrogenesis⁷¹ and a change in the cell shape profoundly alters the organization of the actin cytoskeleton and the assembly of focal adhesions⁷². As shown in Figure 4, the extent and strength of cell adhesion to a surface affects its migration and its decision to switch between proliferation and differentiation behaviour^{47,49,50}. For example, cells with large adhesion are usually dormant concerning migration and proliferation and more active in the expression of differentiation markers⁵⁰. It is therefore possible to influence MSCs fate artificially through control of their shape by synthetic extracellular matrices⁴³, such as scaffold pore shape or surface chemistry, and hence influencing differentiation of MSCs to chondrocytes and osteoblasts.



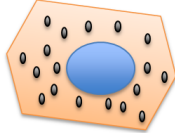
Cell behaviour/ cell spreading	Viability	Migration	Proliferation	Differentiation
	↓	↓	↓	↓
	↑	↑	↑	↓
	↑	↓	↓	↑

Figure 4 Effect of cell spreading/adhesion on subsequent cell behaviour. Blue refers to cell nuclei and black dots represent focal adhesions (adapted from⁴⁷)

There are a number of techniques available to produce porous scaffolds, depending on the scaffold material. Pore-inducing techniques for synthetic polymers include solvent casting in conjunction with particulate leaching, phase separation, gas foaming, melt-moulding and fibre bonding^{39,73}, all of which involve high temperatures, the use of chemicals or pH levels unsuitable for protein-based natural polymers. Consequently, the number of methods to generate pores in natural polymer is quite limited. Two of the most commonly used methods are freeze-drying⁷⁴ and critical point drying.

The use of 3D printing has gained considerable attention in recent years. This technique is especially fitting to generate OC scaffolds, since this tissue has a complex graded structure where biological, physiological, and mechanical properties vary significantly over the full thickness of osteochondral unit⁷⁵. “Solid Free Form” technologies including 3D printing provide us with tools to closely control the design and shape (including the distinct curvatures of joints) in the final products; hence producing tailorable scaffolds have become a reality. Different techniques of 3D printing are extensively discussed in^{76, 77} and³⁹. These include: direct 3D printing, indirect 3D printing⁷⁸ bioplatter printing (using a “bioink” or cell-laden

gels)^{79,80}, fused deposition modelling (FDM)⁸¹, selective laser sintering (SLS)⁸² and stereolithography (SLA).

Scaffold stiffness affects cell migration and differentiation

Scaffold stiffness is another factor affecting stem cell fate^{83,84}. Naive mesenchymal stem cells are shown to specify lineage and commit to phenotypes with extreme sensitivity to tissue-level elasticity⁸³. At the cellular scale, normal tissue cells probe elasticity as they anchor and pull on their surroundings⁸⁴ (Figure 5). The cells response to mechanical properties of their matrix involve a feedback loop, where cells exert contractile forces on the matrix (for example it has been shown that each fibroblast on a scaffold exerts 1nN force to the matrix⁸⁵, which can contract it depending on the stiffness of the matrix). The contractile forces generate strain in the substrate. This strain can be detected by other cells and they respond to this by adjusting their cytoskeleton and overall state^{54,84,86}. Engler et al. (2006) showed that human MSCs favoured differentiation into neuron-like cells on soft substrates, into myogenic lineage on substrates with moderate elasticity and into osteogenic lineage on rigid substrates⁸³ (Figure 5). Similarly, the matrix stiffness can affect cell migration. The contact between fibroblasts, epithelial cells, smooth muscle and the matrix reduces on softer substrates, and cells migrate from softer regions to stiffer regions when subjected to gradient stiffness⁴⁵. Tailoring the stiffness of the scaffold layers to induce chondrogenesis and osteogenesis is an important consideration in achieving the correct type of regenerated tissue.

Regardless of microstructure, the intrinsic resistance of a solid to a stress is measured by the solid's elastic modulus E , which is obtained by the linear slope of stress-strain curve when subjected to a force⁸⁴. A scaffold's mechanical properties, on the other hand, are derived from both its composition (solid elastic modulus), microstructure (%porosity) and topology (connectivity and shape of the pores)^{54,87}. This is why the bulk modulus of a porous scaffold maybe many times lower than that of the local elastic modulus facing an individual cell (e.g.^{88,89}).

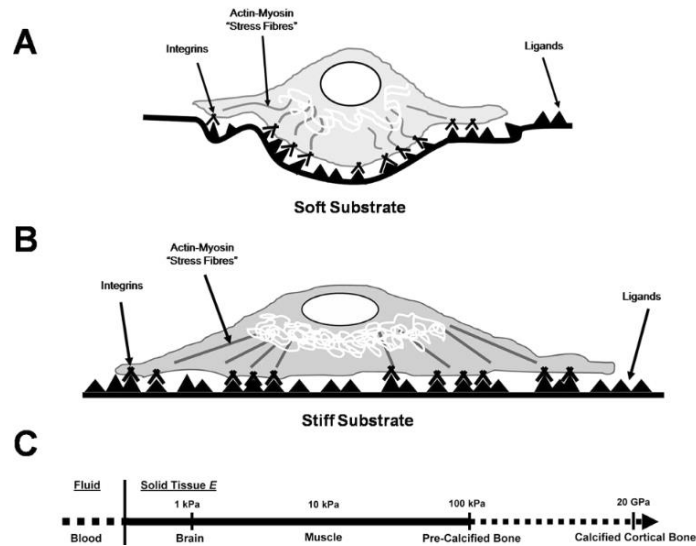


Figure 5 Cell-scaffold interaction in soft (A) and stiff (B) substrates ⁵⁴

Scaffolds can be developed as cell-free matrices, or as tissue-engineered construct before implantation. In the former, the fabricated scaffold is implanted either in a single-step (with no cell) or a two-step (with autologous cells) procedure. In the latter, the scaffold is seeded with autologous cells from the patient in the laboratory, the tissue is grown *in vitro* and the neo-tissue is implanted in the patient (two-step procedure). In this method, utilising a bioreactor could be beneficial. During physiological loading, a range of mechanical stimuli are developed in cartilage such as compressive and shear strain, stress, hydrostatic pressure and fluid flow. Bioreactors help to emulate these conditions *in vitro* by providing environmental, biochemical and mechanical cues to the cells¹⁸. The beneficial effects include differentiation of progenitor cells towards chondro- and osteogenic lineages²⁹.

There are several types of bioreactors suitable for osteochondral tissue engineering, including “rotating” and “perfusion” bioreactors. In the rotating bioreactors, scaffolds are suspended between two cylinders in the cell culture medium and gas flow occurs via a silicone membrane. Although mass transport to the surface of the construct is enhanced by this method, it still only takes place by diffusion within the scaffold. The perfusion bioreactors include perfusion cartridges and perfusion chambers, with the latter being capable of applying 3D mechanical loading to the construct. This method is designed in a way to provide interstitial fluid flow

through the scaffold¹⁸, and is an effective way for initial cell seeding as well as subsequent mass transport⁹¹. Perfusion bioreactors have been used for generation of osteoinductive⁹² grafts and cartilage constructs⁹³, and simultaneous construction of both tissues in a double-chamber (hydrostatic pressure but no mechanical stimulation) perfusion bioreactor⁹⁴.

Performance of OC scaffolds in animal and clinical studies

3 A great number of scaffolds have been fabricated and explored for osteochondral tissue engineering. These scaffolds have been developed specifically to reproduce bone and cartilage either with or without the addition of cells⁹⁵. Of those, only a small number has been advanced into clinical trials⁹⁶.

***In vivo* performance of osteochondral scaffolds: animal model studies**

3.1 OCDs involve articular cartilage and associated subchondral bone. Multilayered osteochondral scaffolds have been developed to mimic the native architecture of the osteochondral tissue unit. There are many research groups around the world that have developed different osteochondral scaffolds from a range of biomaterials and their combinations. Table 1 & Table 2 summarises the typical osteochondral scaffolds that have been evaluated in *in vivo* animal studies (Table 1) and in clinical studies (Table 2).

Gotterbarm *et al* have reported a composite scaffold of collagen I/III for the upper cartilage layer and Tricalcium Phosphate (TCP) as bone section. They incorporated growth factors such as Bone Morphogenic Protein (BMP) and Transforming Growth Factor (TGF- β) in their scaffold and evaluated the performance of the scaffolds in a minipig model. 52-weeks post-operation results showed that the scaffolds were fully degraded. However, only approximately 32% of the defect area was restored with the lamellar trabecular bone. They concluded that although the use of growth factors assisted the rate of resorption, but it did not increase new bone formation. No information regarding the quality of neocartilage was given in this study^{97,98}.

Deng et al ⁹⁹ have designed a scaffold comprising of gelatine-chondroitin sulfate- sodium hyaluronate (GCH) for cartilage and gelatine-ceramic bovine bone (GCBB) for bone compartments. The mechanical properties (modulus) of this scaffold were superior to the scaffold made from collagen and TCP as reported by Gotterbarm ⁹⁸. The bone section showed a modulus of 13.4MPa and a modulus of 5.7MPa for cartilage section. The scaffolds were implanted in large patella OCDs of rabbits with and without addition of cells. It was shown that the scaffolds containing cells encouraged formation of hyaline cartilage at 6, 12 and 24 weeks. In comparison with cell-containing scaffolds, the scaffolds alone prompted formation of a fibrous tissue. Although favourable results were observed in terms of the quality of the tissue, the size of the animal model, as well as the short duration of the study did not give any indications of their long term performance in clinical settings (i.e. human).

In another study¹⁰⁰, a composite scaffold of different types of silk with and without incorporation of growth factors was studied in rats for 8 weeks. A satisfactory biointegration of scaffold with the surrounding tissue was observed. The researchers also observed formation of a neo-osteochondral tissue, with mulberry silk favouring osseous differentiation (collagen type-I) and non-mulberry directing chondrogenic differentiation (proteoglycan with both collagen type-I and collagen type-II). This was very encouraging in terms of achieving the correct tissue type; however, again the animal model and duration of study were the limiting factors.

A polylactic acid poly-ε-caprolactone (PLCL) support structure was fabricated using laser micromachining technology and thermal crimping to create a functionally-graded open pore network scaffold¹⁰¹. This scaffold was evaluated in a rabbit model with and without MSCs. The authors observed no evidence of inflammation or giant cells, and concluded that the acellular constructs performed better than cell-seeded constructs with endogenous progenitor cells homing through microtunnels. However, the duration of study was very short (4 weeks), and although the scaffold is branded as an osteochondral scaffold, no information about the subchondral bone regeneration was given.

Recently, a multi-layered biomimetic scaffold comprising a bone layer of type-I collagen/HAp, an intermediate layer of type-I/type-II/HAp, and a superficial layer of type-I/type-II collagen/hyaluronic acid, was developed and tested in femoral condyles of 8 rabbits. After 12 weeks, it was shown that the International Cartilage Repair Society (ICRS) score of joints treated with the scaffold was higher than the non-treated joints, showing a Grade II cartilage (nearly normal) compared to Grade III (abnormal) in non-treated groups. The level of bone formation was also significantly higher in groups treated with the scaffold ¹⁰². However, looking closely at uCT images (Figure 6(a)), it can be seen that there are areas of incomplete bone regeneration after 12 weeks. The scaffolds were further evaluated in caprine model and compared to a commercial scaffold –TruFit¹⁰³. Complete bone regeneration took place after 12 months (Figure 6).

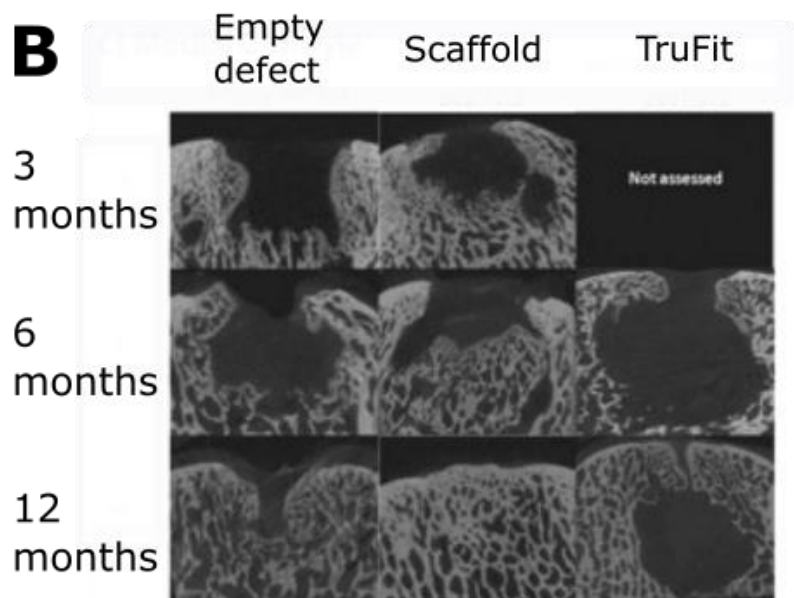
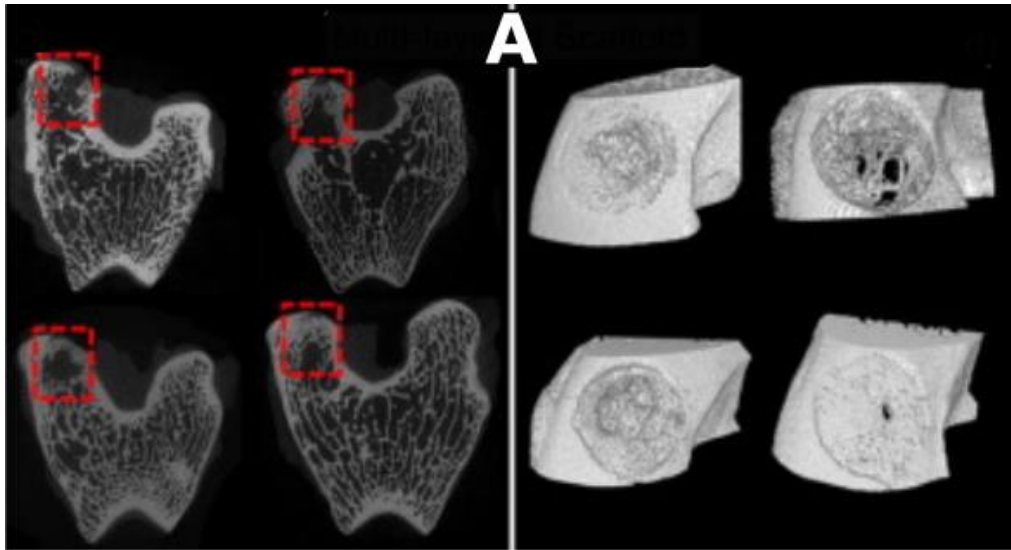


Figure 6(A) Micro-CT analysis at 12 weeks post-surgery showed greater levels of bone repair in the multi-layered scaffold group than the empty defect group¹⁰² (rabbit model). (B) Micro-CT analysis showed improved subchondral bone repair in the multi-layered scaffold group in medial femoral condyle¹⁰³ (caprine model)

3.2

Clinical performance of typical existing OC scaffolds

A search in Clinicaltrials.gov with the key phrase “osteochondral scaffold” resulted in 9 entries, four of which with high relevance to the current review are discussed below.

BiPhasic Cartilage Repair Implants (Exatech, Taiwan): Biphasic osteochondral scaffolds¹⁰⁴ or BiPhasic Cartilage Repair Implants (BiCRI, Exactech Taiwan) are used in a Matrix Assisted Autologous Chondrocyte Implantation (MACI). The biphasic cylindrical plugs (8.5 x 8.5mm) are made from polylactic-co-glycolic acid (PLGA) and PLGA plus b-tricalcium phosphate (TCP) by particulate leaching method. PLGA, which comprises 1.5 mm of the cylinder height, serves as the cartilage layer, and PLGA+TCP serves as the osseous phase. A reservoir, between these two layers is created for double-minced chondrocytes harvested from the graft. The structure of the BiPhasic Cartilage Repair Implant is illustrated in Figure 7. Ten patients with grade III and IV lesions (size less than 20mm) of the knee femoral condyle were treated with this osteochondral scaffold, and assessed using Knee injury and Osteoarthritis Outcome Score (KOOS) and visual analogue scale (VAS) scores at 6 weeks, 3, 6, 12 and 24 months intervals. Magnetic resonance imaging (MRI) scans were performed at 12 months post operation to assess cartilage formation. No serious Adverse Events (AE) was reported but KOOS score increased significantly only after 2 years. It was reported that the interface between the graft and the neighbouring native bone was distinguishable by MRI. Second-look arthroscopy after 12 months showed that in 70% of patients the neo-cartilage was well-integrated, while 30% showed incomplete integration and presence of fibrous tissue. Histology of the biopsy samples stained positive with col-II and Alcian blue showed mostly a hyaline cartilage with viable columnar cells. The study later moved into a phase III clinical trial. However, no results have been published yet. As it was observed from MRI scans of patients after 12 months, the boundary of the bony pit was still distinguishable¹⁰⁴.

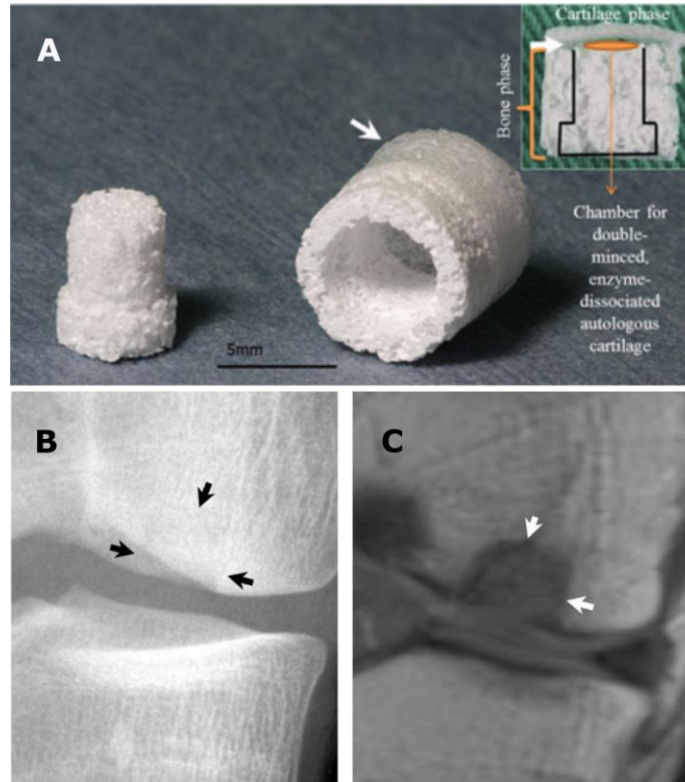


Figure 7 Biphasic cylindrical scaffold; (A) the white arrow indicates the 1.5mm PLGA layer for chondral phase. (B) At 12-month postoperation, the surgically created pit at the grafted site was filled with radiopaquetrabecule. Black arrows indicate contour of the bony pit. (C) MRI (coronal acquisition with T1 sequence) at 12-month still clearly demarcates the bony pit, which is depicted by a signal different from the native cancellous bone. White arrows indicate interface between graft and host bone. (Adapted from ref¹⁰⁴, with permission from Elsevier).

Chondromimetic (TiGenix, Belgium): Chondromimetic implant is intended to serve as a scaffold for cellular and tissue ingrowth in small osteochondral defect. The plug consists of a chondral layer with collagen and glycosaminoglycans (GAG) and an osseous layer with collagen, GAG, and calcium phosphate. The clinical trial was terminated due to slow patient recruitment rate. A preclinical study in goats is available¹⁰⁵ with implantation of scaffolds in 9 goats for 26 weeks. It was shown that hyaline-like cartilage (50% in medial femoral condyle (MFC) defects and 83% in lateral femoral condyle (LFC) defects) with mechanical properties close to that of native cartilage were formed. The efficacy was described as a viable alternative to marrow simulation. Getgood and colleagues compared the performance of Chondromimetic with that of TruFit in critical sized defects of MFC and lateral trochlear sulcus¹⁰⁶. The scaffold was also combined with BMP-7 and rhFGF18 and evaluated in ovine model. Statistical analysis demonstrated significant

improvements in gross repair, with respect to the mechanical properties and histological score, over empty defects when Chondromimetic was combined with rhFGF18¹⁰⁷. However, subchondral bone regeneration was incomplete, and subchondral cysts were formed in the case of combining with BMP-7, as revealed by histological examination shown in Figure 8.

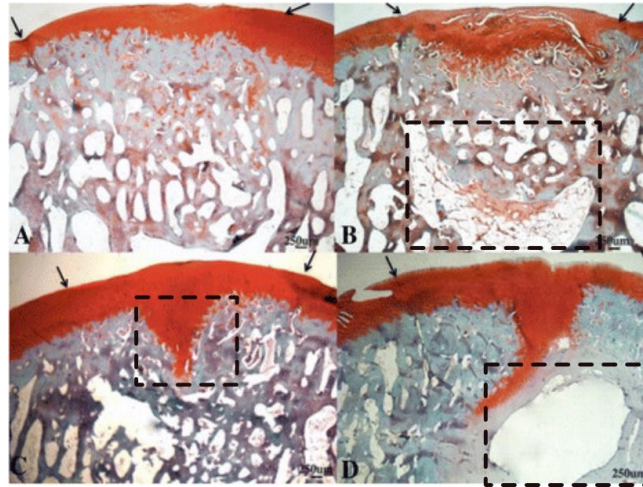


Figure 8 Histological sections stained with Safranin O. A) Empty defect, B) Scaffold alone, C) Scaffold + rhFGF-18, D) Scaffold + BMP-7. In C and D there appears to be a cartilage cleft, with significant proteoglycan staining extending down into the subchondral bone. In D there is a large subchondral cyst. The arrows denote the margins of the defect. Adapted with permission from ref¹⁰⁷ from Springer.

MaioRegen (Fin-Ceramica Faenza, Italy): MaioRegen is one of the most investigated scaffolds for OC tissue engineering. It was developed as a tri-layered composite with layers of different compositions representing cartilage, calcified cartilage and subchondral bone regions¹⁰⁸, as illustrated in Figure 4A. The upper layer for regeneration of cartilage made from collagen I, a layer of 60% collagen and 40% HAp act as middle layer, and the subchondral section of the scaffold consists of 30% collagen and 70% HAp. The MaioRegen has a reported porosity of 45-65% and porosity-dependent Young's modulus in the region of 1.50-6.85 GPa¹⁰⁸. It has been evaluated in animal studies, as well as several clinical trials cases (see Table 2) and it is currently in its phase 4 clinical trials.

The animal study was conducted on 2 horses, with tri-layer scaffolds implanted in MFC lesions (10mm x 8-10mm). It is reported that newly-formed bone and cartilage-like tissues integrated well with the surrounding host tissue after 6 month period, and formation of tidemark line was

reported¹⁰⁹. However, the newly formed cartilage was fibrocartilage, not integrated hyaline cartilage as expected in the articulating joints. The first clinical trial was completed on 13 patients (15 lesions) on MFC, LFC, patellas and trochleas lesions of grade IV and V with a mean defect size was 2.8 cm² (range: 1.5–5.9 cm²). After a 6 months follow-up period a complete graft attachment in 13 sites and 2 partial detachments were reported. The examination of biopsies, taken from 2 patients who needed additional surgery, revealed that the newly-formed tissue in cartilage compartment was fibrocartilage containing mostly type-I collagen and no proteoglycans¹¹⁰.

Subsequently, the scaffolds were used in another clinical trial on 27 patients (32 defects) with MFC, LFC lesions, patellas, trochleas and tibial plateaus with lesions sized 1.5-6 cm². The 2-5 years follow-up study demonstrated a significant improvement in International Knee Documentation Committee (IKDC) score. The IKDC score increased to 76.5 ± 14.5 (2-year) and 77.1 ± 18.0 (5-year), respectively, from its initial scores of 40.0 ± 15.0. Similarly, the Tegner score increased to 4.0 ± 1.8 (2-year) and 4.1 ± 1.9 (5-year) from its initial score of 1.6 ± 1.1. MRI examination showed significant improvement in Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score and subchondral bone status from 2 to 5 years. At 5 years post-operation, complete filling of the cartilage was observed in 78.3% of the patients, complete integration of the graft in 69.6% of the patients, intact repair tissue surface in 60.9% of the patients, and a homogeneous repair tissue structure was observed in 60.9% of the cases. However, the study did not include negative controls, and due to complex nature of some lesions combined surgeries took place in some cases^{109,111}. Consequently, 23 patients with osteochondritis dissecans (OCD) grade III or IV with average lesion size of 3.5 ± 1.43 cm³ were treated with this scaffold, which resulted in an increase in ICRS and EuroQol Visual analogue scale (EQ-VAS) score; Tegner score increase significantly after 2 years follow-up. However, it did not reach the pre-op levels. MRI data showed that 80% of the lesion was filled, and that scaffold at the bone interface was still detectable after 2 years¹¹². Furthermore, the scaffolds were used for treatment of large OC knee lesions (size 4.35 ± 1.26 cm²) in 49 patients, and the results showed a significant increase in IKDC, VAS and Tegner scores after 2 years compared to

pre-op. Five biopsies were taken and 4 of which were from the failed grafts. Macroscopical examination indicated a well integration of the grafts in all specimens. Histology examinations demonstrated that cartilage region was stained positively for collagen-II and MRI scans showed no signs of edema. It was found that age of the patients, type of defects (OCD better outcome), and level of previous activity affected the clinical outcomes¹¹³. Similar results were observed in 79 patients with medial, femoral and trochlea grade III and IV lesions; IKDC score showed that 82.2% of patients had improved symptoms at 2 year follow-up, with Tegner and MOCART scores increasing significantly at 1 and 2 years compared to pre-operation levels. It was again shown that a better clinical result was obtained in OCD lesions compared to degenerative lesions¹¹⁴.

The MarioRegen scaffold has also been reported with favourable outcome in a series of complex cases, including a 46 years old athletic patient, a 31-year old Olympics level athlete and a 50 years old woman. Due to complexity of lesions in each case, different reconstructive approaches (e.g. alignment correction, autologous OC transplant and microfracturing) were combined. The results after 1 and 2 years follow up were good in terms of MRI showing signals for hyaline-like cartilage and little/no edema of subchondral bone. The patients had lower levels of pain and were able to resume their previous level of activity¹¹⁵⁻¹¹⁷.

Most recently however, a clinical study on 10 patients with OC defects using MaioRegen scaffolds showed a poor OC repair in 1 and 2.5 years postoperative assessments¹¹⁸. In this study, 2 patients were re-operated due to treatment failure and were excluded from the results. None of the patients showed a complete regeneration of the subchondral bone observed from 2.5 years of follow up CT examinations¹¹⁸. This was in contrary to a previous studies that reported 62-72% of patients had complete subchondral bone regeneration after 2 years¹¹⁴. It was further shown that none of the patients had an intact articular surface or complete integration with the surrounding host tissue, as show in Figure 9. Since this report the clinical trials have been discontinued in Denmark and it is advised that MaioRegen be used with caution.

Yan et al have examined the outcomes of clinical trials on 8 patients, and reported that subchondral edema, sclerosis and cyst were observed in most of the cases. A complete integration of the scaffold into the border zone was described; however, T2 mapping data and the zonal T2 index significantly differed in the repair tissue compared to the healthy control cartilage ($P < 0.001$) which indicates a limited quality of the repair cartilage obtained using this scaffold¹¹⁹ as observed in Figure 9.

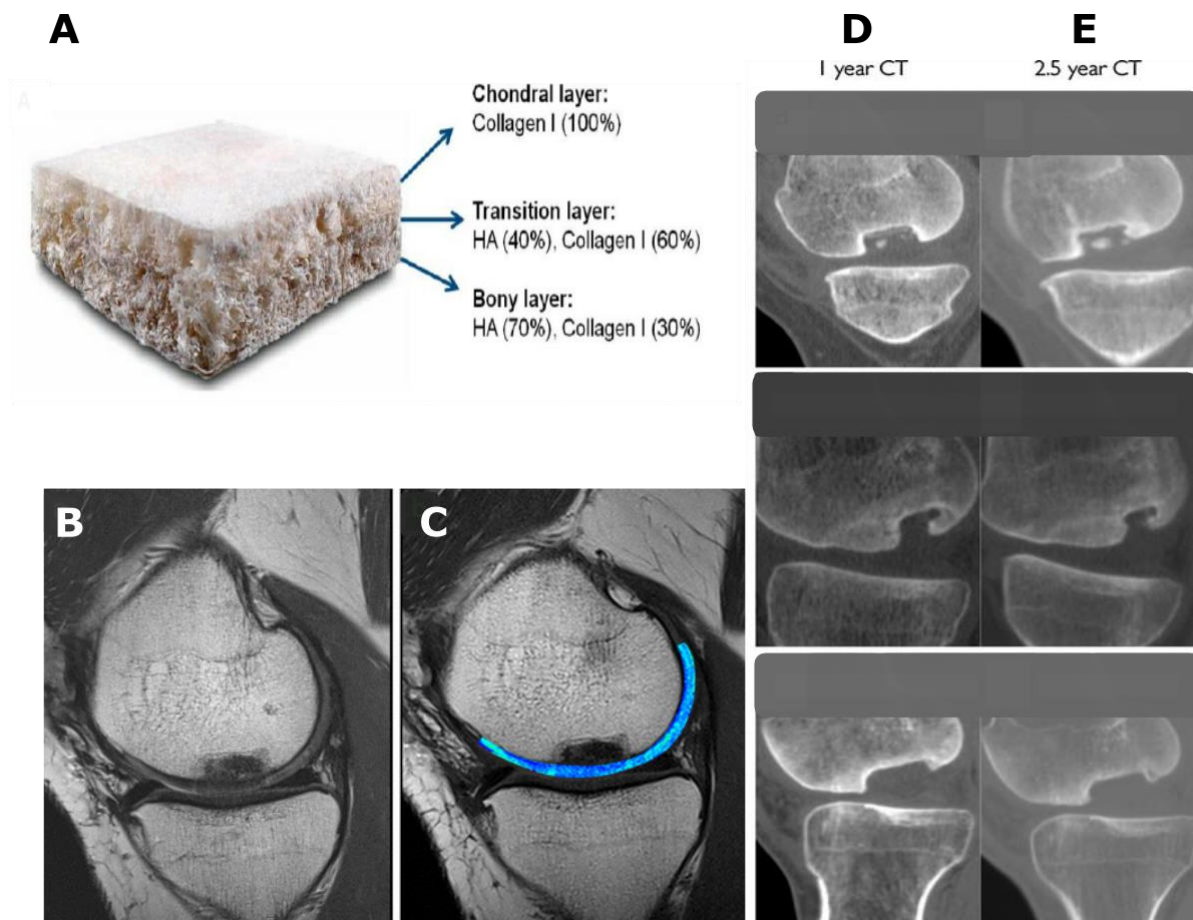


Figure 9: MaioRegen scaffold. (A) shows the three layer in MaioRegen scaffold. (B) and (C) MRI scan of OC lesion repair after 18 months, and bone cysts are observed. (D) and (E) CT scans of three different patients at 1 and 2.5 years after implantation; A clear cylindrical bone cavity is seen in all of the cases. Images adapted from ¹¹⁹⁻¹²¹, with permission from ACS (A) and Springer (B-E).

TruFit (Smith and Nephew): TruFit is another well-explored scaffold for clinical OC treatment, which has produced controversial results. This biphasic plug was originally developed as a backfill for donor sites after autologous OC transplantation; however, it has been used as a

scaffold for OCDs as well. Structurally, the osseous phase of the scaffold consists of calcium sulfate and polyglycolide (PGA) fibres with the chondral phase made of PLGA. Implantation in MFC and lateral trochlear groove showed good histological results of cartilage regeneration after 12 months, followed by encouraging clinical outcomes in small case series. However, later it was observed that in large OCDs that the bone incorporation is delayed leading to instability of the graft and failure. This affects the cartilage –both surrounding and opposing- the plug, which can be damaged because of the direct articulation or increased contact pressure by this instability, and has led to failure or only modest results in the large defects ¹²². The results of studies using TruFit have been contradictory for short/mid-term and long term studies: up to 12 months improvement ^{122,123} have been reported (although Dhollandar ¹²⁴ reports 20% failure); while Joshi observed worsening and 70% failure after 12 months. This coincidence with Verhaegen’s study reported in 2015 ¹²⁵ that no bone ingrowth was observed and instead bone edema and cyst sclerosis were detected ^{123,124,126} by MRI and CT examinations, as shown in Figure 10. Further to questionable results, the scaffold was withdrawn from the global market in 2013.

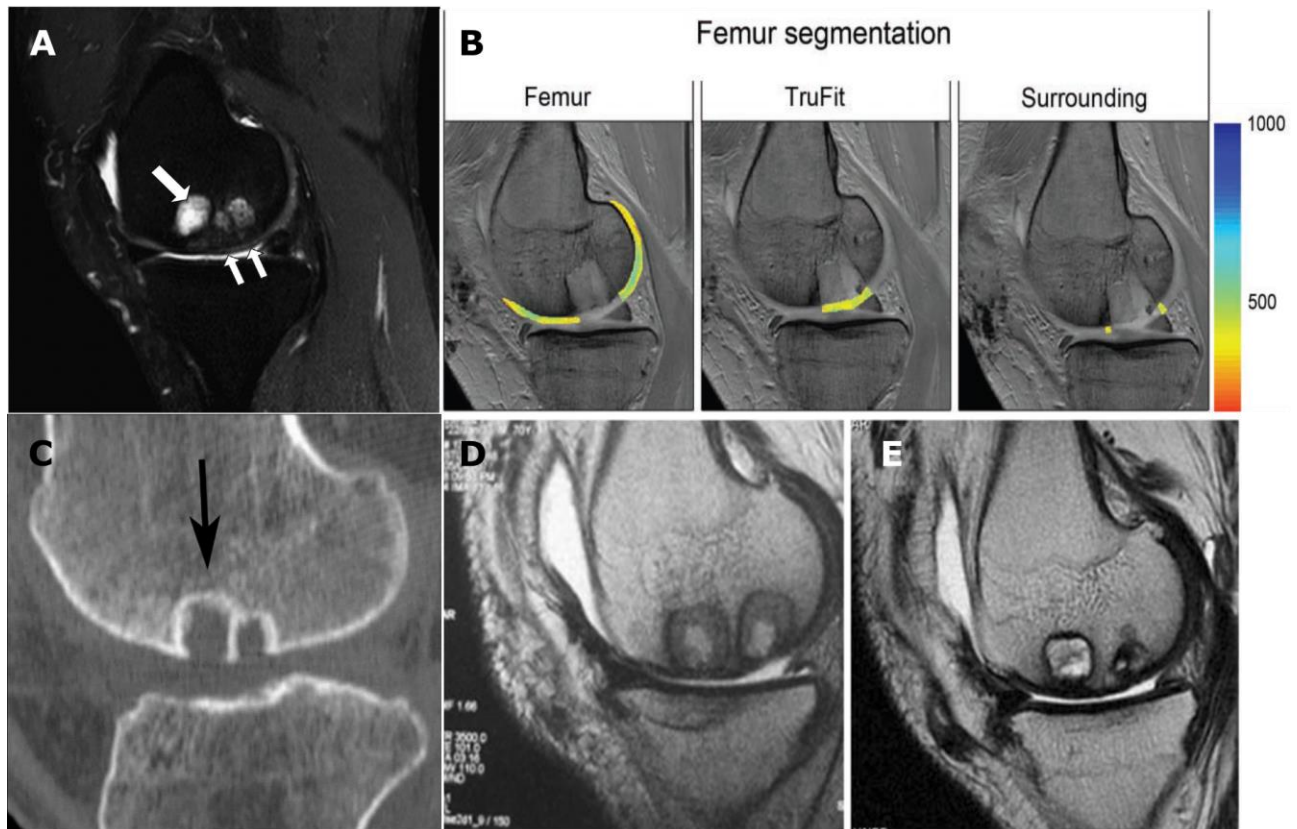


Figure 10 TruFit. (A) MRI (1 T) of an OC lesion of the medial femoral condyle treated with a TruFit plug after 2 years. Despite good scaffold integration and partial filling of the chondral layer (double arrow), subchondral bone changes were clearly seen (arrow) in this MRI sequence¹²⁷. (B) Regions of interest (ROIs). Sagittal magnetic resonance images of postoperative situation when an osteochondritis dissecans lesion of approximately 1.5 cm was treated using 2 TruFit BGS plugs. The bone-plug interface is clearly visible and could be used to define the ROIs. The color bar represents the calculated dGEMRIC index (T1gd), where a high T1gd (1000 ms) is depicted as blue and a low T1gd as red¹²² (C) CT scan failed to show bone ingrowth¹²⁸. (D) MRI at 12 and 48 months show failure of integration¹²⁹

3.3 What have we learnt from the clinical study of the OC scaffolds?

The significance of subchondral bone integration in maintaining a healthy articular cartilage is well established^{118,130} and it was discussed earlier (section 2.1.2) from biomechanical and nutritive perspectives.

In general, during physiological loading, a range of mechanical forces are exerted on cartilage such as compressive and shear stress. These external stresses induce hydrostatic pressure in the cartilage and biofluid flow in and out of the cartilage. The function of subchondral bone is to support the overlying cartilage and protect the underlying cancellous bone from high

stresses. Changes in the properties of the subchondral bone leads to increased strain generated in the cartilage layer thereby initiating/maintaining matrix degradation, which can contribute to initiation/progression of OA¹³¹. Delivery of oxygen and nutrition to different zones of articular cartilage occurs either through diffusion from synovial fluid or through diffusion from micro-blood vessels within subchondral bone depending on the zone of cartilage. Both diffusions are needed to maintain a healthy articular cartilage. Therefore, degeneration of cartilage in long run is expected if the support from subchondral bone is compromised, pointing to a possible reason for failure of healthy regeneration of cartilage as reported in the clinical studies.

To better understand the relationship between cartilage defect and subchondral bone changes, we conducted a study on osteoarthritic femoral heads collected from total hip replacement operations. The cartilage on the femoral head was graded using Outerbridge classification system. A typical femoral head with cartilage graded is shown in Figure 11(A)). The specimens were scanned with a micro-CT and peripheral quantitative CT (pQCT) system to determine the subchondral bone structural changes and volumetric bone mineral density (vBMD, mg/cm³) distribution within the femoral head. A typical micro-CT and pQCT images OA femoral head are shown in Figure 11 (B) & (C), respectively. It was revealed that subchondral bone cysts with varied size existed in the subchondral bone, and these cysts are normally observed at regions of greatest cartilage loss. The cysts formation leads to the changes of loading condition in the joint. As a result, the vBMD increased in the subchondral bone that was dependent on the degree of cartilage degeneration. This is in line with what was observed in other studies regarding the advanced stages of OA¹.

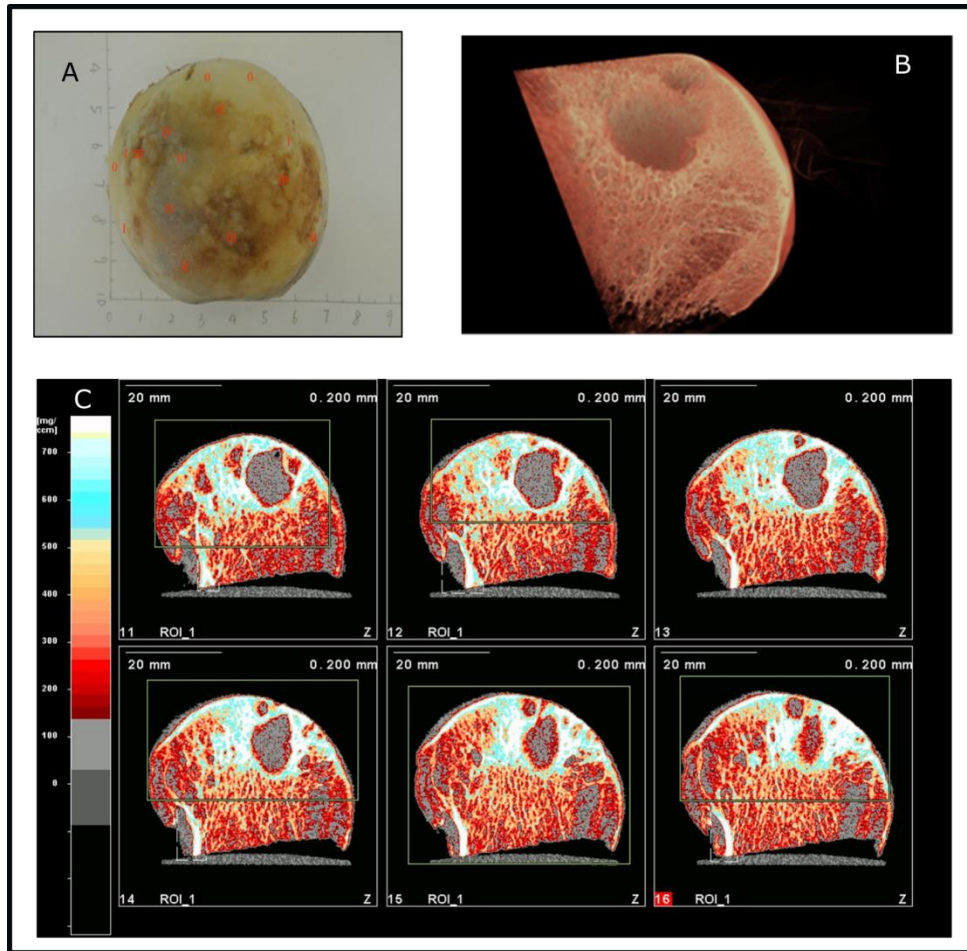


Figure 11 Osteoarthritic femoral head: Visual inspection of OA femoral head showing cartilage and subchondral bone in different stages of disease progression (A), uCT scan showing subchondral bone cyst (B) and pQCT scans showing subchondral bone cysts and bone mineral density distribution surrounding the cysts (C)

The cavities in subchondral bone, which are usually referred to as ‘subchondral bone cysts’, are normally reported in patients with OA. Usually, cysts observed in OA joints are in the range of 0.1-2.5cm in diameter, and appear in multiple. While smaller cysts are detected in the subchondral bone closer to the joint surface, larger cysts typically extend more deeply¹³². There are two main hypotheses about the origin of subchondral bone cysts in OA. The ‘synovial fluid intrusion’ theory suggests that due to cracks in the OC region occurred by repetitive overloading, synovial fluid enters into subchondral bone and leads to formation of these cysts¹³²; while the ‘bone contusion’ theory suggests that the necrotic lesions in subchondral bone, induced by abnormal mechanical stress and subsequent microcracks, edema and focal bone resorption are responsible for the cyst formations¹³³. Subchondral bone cysts are

recognisable in MRI images as areas of fluid signal and in radiographic images as lucent areas with sclerotic rims^{133,134}. The cysts observed in the terminal osteoarthritic cases in our study resembled those of “unfilled bone voids” observed in TruFit^{123,124,128}, MaioRegen and Chondromimetic (see Figure 8, Figure 9 and Figure 10). The “cyst-like” cavities in scaffold developed by Levingstone et al (2016), seem to resolve after 12 months¹⁰³ (see Figure 6).

Based on these results a paradigm was developed for cartilage defect progression (**Error! Reference source not found.**):

Damage in the articular cartilage changes the loading pattern on the subchondral bone, which leads to bone remodelling (increase in vBMD and formation of cysts). This affects the physical environment supporting the overlying cartilage, hence enhances the progression of cartilage degeneration. The existing hypothetical model for OA pathogenesis looks at the repetitive joint loading, which causes an initial increase in bone remodeling activity, perhaps as to repair the damage caused by the loading. This increased remodeling is associated with increased vascular invasion of the deep layers of cartilage¹³⁵, which allows access to the cartilage by chondrolytic enzymes. This process has several effects, which include secondary synovial thickening with or without secondary inflammation, loss of B cells from the synovial lining with subsequent additional impairment of enzymatic inhibition. The loss of cartilage integrity caused by the loss of aggrecan, which normally maintains cartilage matrix compressive stiffness, will increase the overload of the joint feeding back to an elevation of bone formation as the joint attempts to adapt to the greater loads. Ultimately, this positive feedback loop will promote the continued loss of cartilage integrity, allowing deterioration to progress to clinically evident OA.²⁷

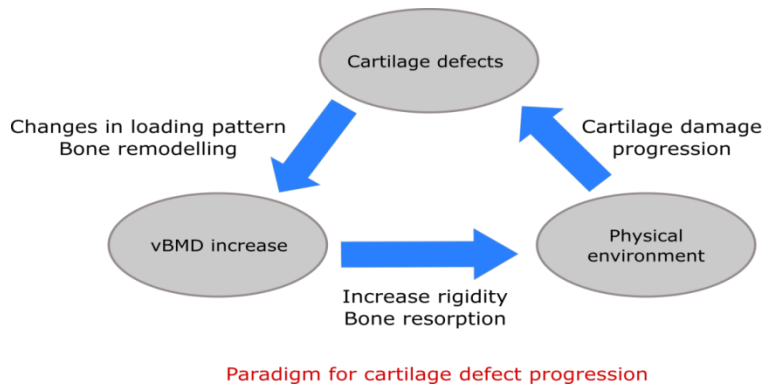


Figure 12 Paradigm for cartilage defect progression

Although the primacy of the onset of articular cartilage degeneration and OA is still debatable¹³⁶, there is no doubt that the subchondral bone plays an important role in progression of the cartilage degeneration. In fact, there is evidence of communication, biomechanically and biochemically, between cartilage and subchondral bone. Where a healthy homeostatic cross-talk leads to regulated bone remodeling and joint maintenance, a catabolic unhealthy cross-talk leads to dysregulated bone remodeling and progressive damage³⁷.

This paradigm of OA progression can also be applied to OC tissue regeneration in TE, as it emphasises the importance of subchondral bone in cartilage regeneration and maintenance. If the scaffold for OC defect repair does not provide a mechanically stable compartment in the region of subchondral bone, the forces on the joint will not be transferred to the walls of the defect to stimulate the cells. When bone marrow cells at the periphery of the defect are unable to regenerate bone, the osseous walls of the defect are resorbed which leads to formation of large cavities and collapse of the surrounding cartilage and subchondral bone¹³⁷. These are the cysts observed in OA bones and in cases the bones treated with the above mentioned scaffolds. The variation in subchondral bone stiffness (e.g. due to existence of cysts) results in interfacial stresses with subchondral bone, as well as varied stress in overlying cartilage when subjected to the dynamic loading. As such, without the subchondral bone healing, the elevated contact stress gradients in normal cartilage near the defects may inhibit normal repair¹³⁸.

Improved biomechanical fixation enhances cartilage fill

As empirically observed in the commercial scaffolds, the dominant factor in scaffold failing to support healthy cartilage regeneration and restore the joint function satisfactorily seems to be the insufficient bone ingrowth and integration with the host tissues. Without a stable^{3.4} biomechanical support, the newly formed cartilage would “collapse”. The “collapsed” cartilage would not be subjected to mechanical stimulation, which is a critical factor for healthy hyaline cartilage formation. As a result, poor cartilage fill and associated fibrocartilaginous repair rather than the hyaline cartilage, as well as poor OC repair are often observed in the clinical trials of a few commercially available OC scaffolds as previously reported.

The authors believed that providing an appropriate physical environment (that includes the generation of an appropriate biomechanical environment and hydrostatic pressure) to support cartilage healing is critical for cartilage fill and hyaline cartilage formation. The researchers at UCL have recently developed a novel biomimetic OC scaffold based on a “sandwich” composite system comprising titanium, PLGA and collagen matrices. The titanium and PLGA supporting frameworks are fabricated by a 3D rapid prototyping technique, and the porous matrix is filled with crosslinked collagen type-I which is spatially graded to form a structural and compositional scaffold.

The biomimetic OC scaffold has been evaluated in sheep condyle model. The *in vivo* sheep study demonstrated that the new bone growth into the titanium matrix at bone section provided a strong mechanical fixation 12 weeks post-operation. This provides a strong support to the overlying cartilage layer leading to the improved cartilage fill compared to a commercially available collagen/hydroxyapatite-based OC scaffold, as demonstrated in Figure 13.

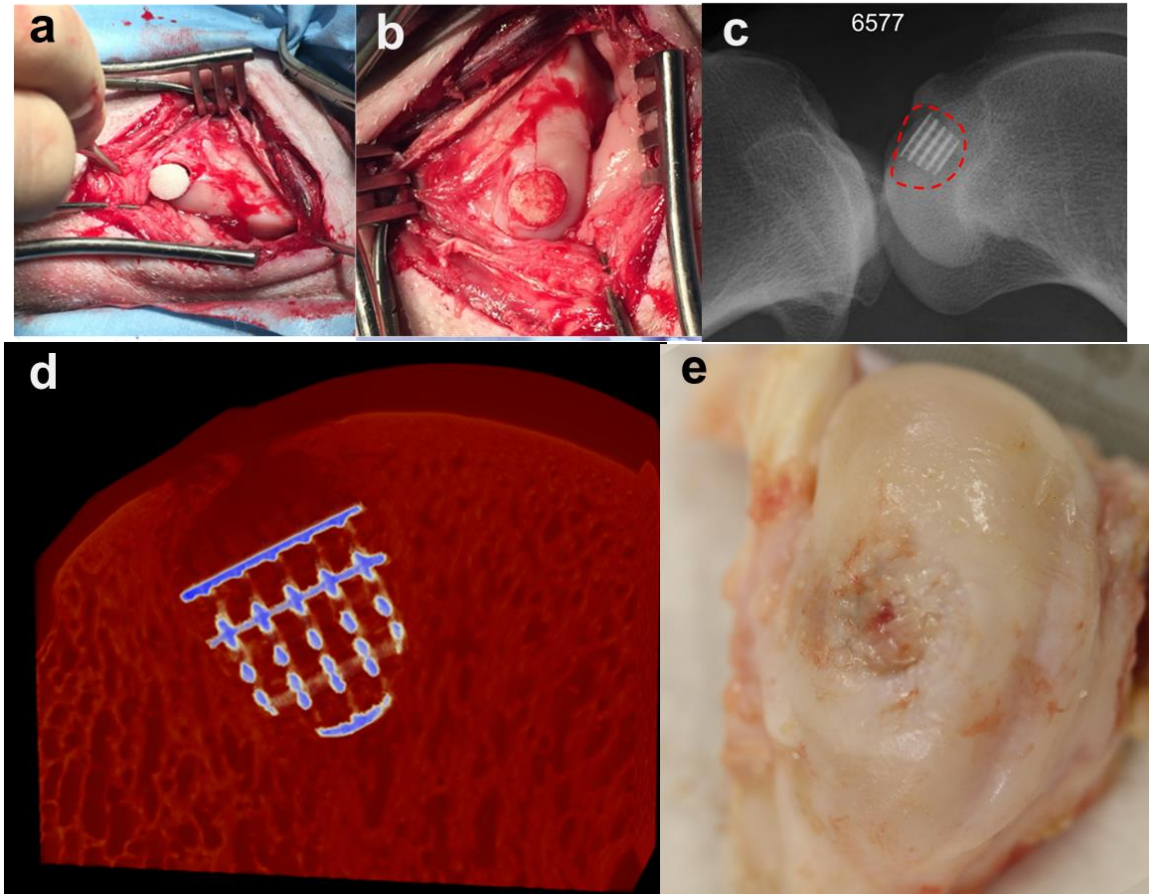


Figure 13: Sheep condyle model was used to evaluate the in vivo performance of the scaffold. Three month results demonstrated that the bone ingrowth into the bone section achieved strong mechanical fixation. This provided an improved physical support to the growth of the overlying cartilage leading to improved cartilage fill.

The scaffold has also been tested in a clinical dog shoulder model where an OCD had occurred due to natural development of OA in the dog. A 10 mm x 10 mm biomimetic scaffold was implanted in the shoulder defect. The 3-months follow up arthroscopic examination revealed the cartilage had regenerated well, matching the curvature of the joint perfectly. Recent reports from the dog owner suggested the dog shoulder function was recovered completely. A glimpse of how this scaffold will perform has been given, with promising results, by Professor Noel Fitzpatrick of the Channel 4 TV series *Supervet*,

This biomimetic OC scaffold has the strength needed to bear the physical load of the joints and its biomechanical structure encourages consistent cartilage fill and a smooth articular surface. It

has the potential to address the unmet clinical need for repair of large OCDs. This functional biomimetic OC scaffold bridges the gap between small OCD treatment and joint replacement. It is hoped that it will provide clinicians with a viable treatment option in situations where the disease has progressed beyond a small defect, but where a full joint replacement could still be avoided. This would lead to tangible and clinically relevant results in a one-step surgical procedure for the treatment of large cartilage and OCDs, relieving pain and improving quality of life by keeping people active.

Perspective summary

⁴ OCDs typically derived by traumatic injuries or OA, involve articular cartilage and associated subchondral bone. These defects are characterised by unbalanced degeneration and regeneration of articular cartilage and bone where the intrinsic repair mechanisms are insufficient. Stopping or delaying progression of OCDs would have significant impact in health care.

The treatment of cartilage and OC defects remains a challenge because treatments to date have failed to achieve a complete restoration of the joint cartilage surface and its properties. Many new technologies, such as OC tissue engineering and stem cell therapies, have been studied and applied to the repair of OC defects. The goal of a tissue engineering approach is to repair the defect in the joint and restore its function in order to delay or remove the need for a joint replacement.

Numerous OC scaffolds have been developed by different research groups around the world, and there are many commercially available products. However, few of these products promote satisfactory durable regeneration of large OC defects. The authors believe that the subchondral bone and adjacent cartilage form a functional unit. OC scaffold that simultaneously support the regeneration of cartilage and subchondral bone is critical for the successful repair of cartilage and OC defects. Lessons learnt from the clinical trials and animal studies suggest that an

improved biomechanical fixation of the OC scaffold would provide an appropriate physical environment for healthy growth of the overlying cartilage.

Development of a functionally biomimetic OC scaffold which will bridge the gap between small OC defect treatment and joint replacement is still a grand challenge. However, with the advancing of OC scaffold biotechnology, it is hoped that, in the near future, a novel OC scaffold with improved capability for biomechanical and biological fixation would lead to tangible and clinically relevant results in a one-step surgical procedure for the treatment of large OCDs, relieving pain and improving quality of life by keeping people active.

Tables

Table 1 Properties and performance of scaffolds used for in vivo animal studies

Scaffolds in animal studies							
Cartilage Material	Bone Material	Mechanical pro	Other properties - notes	Cell/single vs double step	No samples & duration	Findings	Ref
Collagens I and II (porcine)+hyaluronic acid	Collagen I (bovine)+hydroxyapatite / collagens I and II + hydroxyapatite	0.95kPa (bottom), 0.35kPa (intermediate layer), 0.3kPa (top)	>97% porosity	single	8 rabbits (femoral condyle), 12weeks	ICRS I score of regenerated cartilage: Grade II (nearly normal); bone volume/total volume of 0.4±0.05 compared to 0.35±0.03 in control, evidence of tidemark	102,140
Collagens I/III scaffold (Insoluble bovine tendon collagen type I (4% w/v) with type III)	b-tricalcium phosphate (TCP) MedArtis AG	TCP – compressive stiffness 1MPa	TCP = 65–80% (pore diameter of 600 µm) – size 7mm x 5.45mm	Growth factors: BMP 2, 3, 4, 6, 7, TGF-β1, -2, -3, FGF-1, osteocalcin and osteonectin	27 and 36 minipig	The TCP layer significantly increased new bone formation 29.8±9.68% at 6 weeks and 40.09±4.76% at 12 weeks; at 52 weeks scaffolds almost fully degraded and 31.28±5% defect was restored with trabecular lamellar bone; use of GF increased resorption of TCP but did not increase new bone formation	97,98

Gelatine-chondroitin sulfate- sodium hyaluronate (GCH)	Gelatine- ceramic bovine bone (GCBB)	5.86±0.77 cartilage and 13.44±0.89 MPa bone	In rabbit>3mm defect is large	Two-step -- Group A: with cells, Group B: no cells, Group C: empty	36 rabbit, patella defects 15 x 10 x 15 mm, press-fit	Group A showed hyaline cartilage formation at 6 and 12 weeks while fibrous tissue was observed in Group B and C, by RT-PCR and type-II col staining	99
Chitosan-gelatine + TGF-β1	HAp-chitosan-gelatine +BMP-2		Layers glues with fibrin glue	BMMSCs – 4 groups: gene-activated, DNA-free scaffold, gene-activated monolayers	36 rabbit, 4 x 5mm, at 4, 8 and 12 weeks	In gene activated: At week 4: new trabecular bone formation ; at 8 weeks: stain for Alcian Blue ad col II, at 12 weeks: integration with surrounding tissue/hyaline cartilage/indistinguishable	141
Calcibon granules (200-220um) + chondrocytes	Calcibon: injectable in situ setting bone cement, consists of a powder (α-TCP, CaHPO4, CaCO3, precipitated HA) and a liquid (Na2HPO4)	50MPa compressive strength	Edges were cut before implantation to provide fresh surface	Autologous chondrocytes, culture for 3 weeks before implantation	8 minipigs, femoral condyle, 26 and 52 weeks 2 x 4.5mm	Well integration of cartilage and scaffold No degradation/remodelling of the carrier Absence of subchondral cyst which was seen in TruFit	142

Silk scaffold \pm TGF- β	Silk scaffold \pm BMP-2 \rightarrow two layers	-	Layers attached by fibrin glue	No cells TGF- β and BMP-2	20 rats, 1.8mm x 1 mm, 8 weeks	Formation of neo-OC tissue Good biointegration Mulberry silk favours osseous differentiation (col-I), non-mulberry chondrogenic differentiation (proteoglycan + col-I and col-II)	¹⁰⁰
PolyGraft (PLGA+calcium sulfate) : softer by increasing porosity	PolyGraft (PLGA + calcium sulfate)	+ 95 MPa vs 150 MPa vs 172 MPa for subchondral bone		-	24 sheep, 7.45x10mm, femoral condyle, 3 and 6 months	Scaffold stiffness affected subchondral bone formation but at 6 month mechanical prop of regenerated cartilage were the same in stiff and softer Defects in some animal (rabbit) has more healing potential Stiffness change with porosity but porosity itself affects the behaviour, so not the effect of stiffness alone	¹³⁷

<p>CG= 1%collagen I bovine tendon + 0.044% C6S + crosslink EDC/ NHS → for bone not cartilage</p>	<p>CCP=0.5% col I bovine tendon immersed in biphasic calcium phosphate</p>	<p>1±0.27kPa and 10.3±6kPa</p>	<p>Separate layers implanted, once cell-free and once cell-seeded for 28 days (TE)</p>	<p>Rat BMMSCs</p>	<p>90 rats, 7mm calvarial defect , 4 and 8 weeks</p>	<p>CCP promoted more bone healing compared to CG due to mineral phase TE scaffolds prompted M1 pro-inflammatory macrophage mediated response from the host tissue, while cell-free M2 pro-remodelling macrophage → over-engineering can impair in vivo healing A major barrier to clinical success is capsule formation and avascular necrosis in the centre of scaffold due to barrier to macrophage activity and thus remodelling</p>	<p>¹⁴³</p>
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Table 2 Properties and performance of scaffolds used in clinical trials

Trade name	Materials	Mech. prop	Fixation	clinical	No of samples	Duration	Findings	Ref
Cell-free clinical scaffolds								
MaioRegen	Crosslinked Self-assembled equine atelocollagen-I (30%, 60%) + magnesium-hydroxyapatite (70%, 40%)		Press-fit/ no fixation	clinical	79 patients, 82 defects (41 medial, 26 femoral, 15 trochlea) grade III and IV, age 31±11.3, included previous surgeries and concurrent procedure, size= 3.2±2cm ²	24 months	IKDC: 82.2% improved symptoms at 2 year; 47.4±17.1 to 72.1±18.9 to 76.2±19.6) Tegner: improved from 2.9±2 to 3.8±1.6 to 4.4±1.9 MRI: MOCART score increase significantly Those with OCD better than degenerative lesions	¹¹⁴
			Press-fit/ no fixation/fibrin glue	clinical	27 patients (age 34.9±10.2), 32 defects (7 medial femoral condyles, 5 lateral femoral condyles, 11 patellas, 7 trochleas, and 2 tibial plateaus) defect size 1.5-6 cm ²	2 and 5 years	IKDC : 40.0 ± 15.0 to 76.5 ± 14.5 (2-year) and 77.1 ± 18.0 (5-year) Tegner: 1.6 ± 1.1 to 4.0 ± 1.8 (2-year) and 4.1 ± 1.9 (5-year) MRI: significant improvement in MOCART score and subchondral bone status from 2 to 5 years. At 5 years, complete filling of the cartilage in 78.3%, complete integration of the graft in 69.6, the repair tissue surface was intact in 60.9%, and the structure of the repair tissue was homogeneous in 60.9% of the cases. No control, combined surgeries	^{109,111}

	Bi-layer and tri-layered		Press-fit	Pilot in vivo	2 horses: chondral defects in lateral condyle, OC defects in medial condyle	2 and 6 months	Second look arthroscopy at 2 months: no inflammatory One OC filled with fibrocartilage; Tide mark in one OC region; Cartilage region filled with fibrocartilage → aim is hyaline not fibro	144
		-		Clinical	3 complex cases: 46 years old former athlete, 50 years old woman, and a Olympics level athlete	12 months, 24 months, and 4 years	Due to complexity different approaches were combined; Good results in terms of MRI, level of pain and level of activity	115-117

			Press-fit	clinical	2007: 13 patients (15 defects) 4 medial femoral condyle, 2 lateral femoral condyles, 5 patellas and 4 trochleas. The mean defect size 2.8 cm ² (range: 1.5–5.9 cm ²), grade IV and V according to ICRS	6 months	Attachment: 13 of 15 sites complete graft attachment, 2 partial detachment; Filling & integration: at 6 months, 10 complete filling of lesion, 8 full integration MOCART: 73± 19.7 (6 months) Biopsy: 2 patient required additional surgery. Fibrocartilage with no proteoglycans, mostly type-I collagen	110
			Press-fit & fibrin glue	Human cadaveric knee	Scaffolds in human cadaveric knee subjected to continuous passive motion (CPM)→ significantly improved fixation with fibrin glue compared to only press-fitting			145
				clinical	23 patients with OCD, average size of 3.5 ±1.43 cm ³ , 9mm depth defect created, grade III or IV	12 and 24 months	2 failed cases ; ICRS score:50.9 3± 20.6 to 76.44 ± 18.03 (p<0.0005) to 82.23 ± 17.36 ; EQ-VAS: significant increase at 2 year ; Tegner: significant increase at 2 year from 2.34 ± 0.64 to 5.60 ± 1.72 but didn't reach pre-op; 80% complete filling of lesion, scaffold at bone still detectable after 2 years	112

				Clinical	49 patients (2009-2011), defect size 4.35 ± 1.26 cm ² , 6mm depth, age: 37 ± 14 years	1 and 2 years	IKDC: significant increase ($p < 0.001$) preop to 1 and 2 years, 45.45 ± 19.29 to 70.86 ± 18.08 to 75.42 ± 19.31 ; Significant improvement ($p < 0.005$) in VAS from 6.69 ± 1.88 to 1.96 ± 2.47 ; Significant increase ($p < 0.001$) in Tegner score ; 5 biopsies: 4 from failed grafts Age, type of defect (OCD better outcome), and level of previous activity affected the outcome	¹¹³
TruFit	bilayered, semiporous poly lactide– co-glycolide copolymer (75:25), polyglycolic acid (PGA), and calcium sulfate (licensed for chondral and OC defects in Europe but only for bone void filling in the US)	Modulus of elasticity= 50-80MPa based on diameter (7mm-11mm) ¹²⁶	-	Clinical	26 patients, 2 with OC defect, 15 medial femoral condyle, 4 lateral femoral condyle, 7 trochlea.	12, 18, 24 months	At 12 months: Tegner = 2.4 to 6 ($p = 0.009$), IKDC = 37.7 to 65.1 ($p = 0.004$), KOOS = improved 46 points ($p < 0.001$) At 24 no significant difference	¹⁴⁶
			-	Clinical case	2 cases: 43 year old male three plugs (two 7-mm and one 11-mm) ; 34 year old female two 7-mm plugs	Failure at 9 and 20 months	adverse foreign-body giant cell reaction that was clinically correlated with progressive symptomatic failure at extended follow-up periods of 20 months and 9 months	¹⁴⁷

			-	Clinical	20 patients, 15 follow up	6 and 12 months	3 failed due to foreign body giant cells indicated by histology at 12 months; good filling but fibrous vascularized tissue, disorganized ECM and a lot of fibroblasts KOOS = 214.16 ±92.47 preop to 263.94 ± 139.09 (<i>P</i> < .151) at 6 months to 358.69 ± 112.37 (<i>P</i> < .019) at 12 months Stable MOCART score over time Modest short-term clinical and MRI outcome	124
			-	Clinical	1 case: semi-professional footballer, 3 plugs	6 months and 2 years follow up	Delayed incorporation of plug: at 6 months inflammation and synovitis, at 9 month pain and an effusion, at 12 months improvement, but mild synovitis and lack of subchondral bone filling, at 24 months return to impact sport	148
			-	Clinical	10 patients, grade III and IV, patella defects size mean 2.64cm ²	6, 12, 24 months	Short-time improvement in symptoms specially small lesions, but after 2 years failure in restoring subchondral bone: some degree of plug incorporation failure (replacement of subchn bone with cyst), although predominantly hyaline but lesions (collapse and fissuring) appread → MRI	123

							70% reoperating	
			-	Clinical	13 patients, 1.9±0.7cm ² in femoral chondyle	12±4months	After 1 year the bony part of plug still visible in MRI The plug did not damage the surrounding surface and neo-tissue had cartilage like appearance in MRI	122
			-	Clinical	6 patients, cystic subchondral bone in ankle: medial talar dome and distal tibia	12 months	Fibrous rather than hyaline cartilage Improvement n AOFAS and AOS MRI showed resolution of bony cyst but bony plug remained Authors believe much of the improvement is due to a stable filling of the cystic lesion	126
	Platelet-rich fibrin matrix (PRFM) + cartilage fragments with fibrin glue+ Hyaluronic Acid felt		Press-fit	Pilot in vivo	18 goats (36 defects) unilateral tocklear defects (7mm x 3.5mm)	1, 3 and 6 months		149

ChondraColl	Microfibrillar collagen type-I (bovine)/HAp/collagen-II (porcine)/HyA			commercial	Separate scaffolds in 90 rats for 4 and 8 weeks		150,151	
	Collagen-I on 3D printed PLA, the interface charged with hyaluronic acid and rhBMP-2		Press-fit	animal	70 rabbits, femoral condyle , defect size 3mm x 3mm	6, 12, 24 weeks	Using morphogen led to better integration of the regenerated tissue with the host cartilage. Bone reconstitution and attachment of subchondral bone to cartilage repair tissue were greater with morphogens; with implant only the repair is good but lacks GAGs deposition	152
Agili-C	Hyaluronic acid+aragonite (cartilage) – aragonite (bone)		Press-fit	Clinical	1 case:47 years old, 2cm ² defect , 2 scaffolds	24 months post op	Articular surface appeared to restore (MRI), patient returned to sports IKDC : 50 to 78 Tegner: 5 to 8	96,153

				Animal	14 goats (28 lesions) , 6mm x 8mm defect size	6 months	combined histological evaluation according to the ICRS II-2010 and O'Driscoll et al. 34 ± 4 n = 7 in channeled scaffolds; integration of newly formed hyaline (due to proteoglycans, col II and absence of col I) with surrounding tissue	
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AE=adverse events (in KOOS and VAS evaluation systems)

IKDC= International Knee Documentation Committee

MOCART= Magnetic Resonance Observation of Cartilage Repair Tissue

ICRS=International Cartilage Repair Society

VAS=Visual Analog Scale

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