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Bioreactors for Tissue Engineering: An Update

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Highlights

- Summary of the different up-to-date bioreactor designs being used for different cell types and special design scaffolds.
- The advantages and special characteristics of each bioreactor systems
- Current challenges in designing bioreactor systems
- The future trends in bioreactor system development

Abstract
One of the greatest puzzles in wound healing is how to substitute or replace the defect caused by loss and damaged tissue or organs. In regenerative medicine, Tissue Engineering has been proposed to supply this demand by generating tissues *in vitro*. Bioreactors are the key to translate these cells and tissue-based constructs into large-scale biological products that are clinically effective, safe and financially pliable. In this review, we summarise the different up-to-date bioreactor designs being used for different cell types and special design scaffolds, and highlight advantages of different bioreactors, current challenges and the future trends. It is our belief that with efforts combined from multiple disciplinary participants, a novel bioreactor system that is capable of fast, large scale tissue culture would come about in near future.

Abbreviations
AAA: Abdominal Aorta Aneurysm
ASC: Adipose Stem Cells
ALP: Alkaline Phosphatise
BMSC : Bone Marrow Stem Cells
DNA: Deoxyribonucleic Acid
DO: Dissolved Oxygen
EC: Endothelial Cell
ECM: Extra-Cellular Matrix
EPC: Endothelium Progenitor Cell
EpSC: Epidermal Stem Cell
ESS: Engineered Skin Substitute
FEP : Fluorinated Ethylene Propylene
GAG : Glycosaminogylcan
HP: Hydrostatic Pressure
MDP: Markov Decision Process
mRNA: Messenger Ribonucleic Acid
MSC: Mesenchymal Stem
NASA: National Aeronautics and Space Administration
PCR: Polymerase Chain Reaction
PGA: Polyglycolic Acid
PLGA: Poly Lactic-Glycolic Acid
PMMA: Polymethlmethacrylate
POSS-PCU: Polyhedral Oligomeric Silsesquioxane Poly (caprolactone-urea) Urethane
PTFE: Polytetrafluoroethylene
PU: Polyurethane
RCCS: Rotating Cell Culture System
SEM: Scanning Electron Microscope
SMC: Smooth Muscle Cell
STSG: Split Thickness Skin Graft
TE: Tissue Engineering
TEHV: Tissue Engineered Heart Valve
VTE: Vascular Tissue Engineering

Keywords: Bioreactors; Dynamic Stiumation; Growth Kinetic; Immobilised Cells; Spinner flask; Rotating vessel

1. Introduction

The loss and damage of organs cause a huge social and economic burden. With a limited capacity for self-repair and surgeon-dependent techniques, restoration of the original form and structure are impossible, and new methods for organ regeneration are required [1]. Currently, implantable medical devices are being used to substitute a number of damaged tissues and organs. For instance, hip and knee replacements have been utilised for a number of years, yet still have a time-span limitation and possible implant failure due to poor bone infiltration. These commercially available products also fall short of the requirements for active individuals. Articular cartilages are often damaged from degenerative joint diseases and congenital abnormalities, for which there are no suitable substitutes for surgical reconstruction. Surgeons currently restore peripheral nerve injuries using autologous tissue, however there are limitations in harvesting adequate substitutes depending on the amount required by the autoplastic technique for restoration. Overall, there is a large demand for providing a medical alternative to restore severely damaged tissues when required [2]. One way to meet this demand has been proposed by TE via generating tissues *in vitro*. One of the aims of TE is to develop viable substitutes to restore, maintain or improve tissue function *in vivo* using materials capable of mimicking natural ECM. The major challenge is to translate these cells and tissue-based constructs into large-scale biological products that are clinically effective, safe and financially plausible [3].

Bioreactors have played a vital role in TE as they are capable of cultivating mammalian cells under a controlled environment up to an industrial scale [3]. Several operational conditions can be modified and controlled including pH, temperature, oxygen tension and perfusion of the cells as well as external stimuli such as mechanical forces etc. [4]. Bioreactors can be used to aid in the *in vitro* development of new tissues. They provide the biochemical and physical regulatory signals required for cells to proliferate, differentiate and to produce ECM [5]. For example *in vivo* cells will respond to mechanical stimulation which bioreactors can provide, aiding cells to produce ECM within shorter a time-span under the optimal mechanical stiffness for the construct [6]. Mechanical stimulation has also been shown to encourage stem cells down different lineages, enabling different cell types to be obtained [5].

The designs of bioreactors are both tissue and application specific. Several common principles can be employed when designing bioreactors. First of all, they should be quick and easily assembled and efficient in tissue forming in a short time span and most importantly keep products sterile. Secondly, the materials being used to create these bioreactors must be non-toxic to most or specific tissue types. This has ruled out most metals as they have the potential of releasing ions into the media which could be highly toxic to cells in most cases. The bioreactors must also capable of being sterilised if they are to be used again. Furthermore sensors need to be built into them for accurately monitoring culturing conditions [7]. At least, the pumps must be accurate in creating mechanical stimuli to the smallest degree as most cells will respond to extremely small forces [8].

Besides the aforementioned conventional bioreactors, there are also *in vivo* bioreactors. The principle of an *in vivo* bioreactor is to implant a well-designed TE scaffold with or without cell seeding onto a well vascularized site of a living organism and harvest when matured. There are two vectors of choice, xenogenic or autogenic [9]. Liu *et al* in 2014 implanted growth factors loaded scaffolds onto the latissimus dorsi muscles of pigs and successfully induced in-situ MSCs to differentiate into osteoblasts and generated well vascularized bone tissue products [10]. Kokemueller *et al* in 2010 implanted a β-tricalcium phosphate scaffold loaded with morcellized autologous bone graft from the iliac crest to the latissimus dorsi of a patient suffered from chronic mandibular osteomyelitis requiring resection. After 6 months, a well vascularized construct was harvested and used for reconstructing his mandible, and at 12 months of following up the reconstructed mandible was still viable [11]. The future trends of *in*

vivo bioreactors lie with implantable devices which are able to monitor and control the *in vivo* culturing condition and scaffolds with higher biocompatibility that is suitable for long term implantation with minimal rejections [12]. This type of bioreactor does not fit the focus of bioreactors that are suitable for large industrial scale TE constructs, and have been mentioned to provide context and will be explored no further in this paper.

This review aims to provide a concise discussion of the different types of bioreactors used for generating cartilage, bone, vascular tissue, skin and nerve. We will highlight the success in engineering tissues using bioreactors, describing novel designs and explain the future work that is required to expand the use of bioreactors in regenerative medicine.

2. Bioreactors for Cartilage Tissue Expansion

Upon the natural development of human cartilage, forces are constantly applied on them during movements of the body joints. It is been well documented that mechanic forces promotes proliferation and differentiation of chrondrocytes [2]. For the very same reason, the key principle for cartilage bioreactors is to mimic the *in vivo* mechanical loading [13]. There are different types of bioreactors systems designed to induce shear stress, perfusion, HP and compression for stimulating cartilage formation.

2.1. Bioreactors for Inducing Shear Force

Bioreactors for inducing shear force have been used widely for cartilage tissue engineering for decades. They are commonly made up of rotating vessels, spinner flasks and stirred double chambers. Human cartilage progenitor cells have shown to differentiate into mature chrondrocytes on a hydroxyapatite, collagen and chondroitin sulfate scaffold after 6 weeks using a rotating wall vessel bioreactor with chondrogenic induction media [3]. BMSCs have also been shown to differentiate into chrondrocytes after 3 weeks of culture in rotating bioreactors [2]. Marlovits *et al* showed that with the rotating bioreactor, large cylindrical cartilaginous tissues can be formed in dimensions of 1.25 ± 0.06 X 0.60 ± 0.08 cm, with confirmed mRNA expression of aggrecan, collagen type I and II, GAG/DNA ratio within 12 weeks [14]. To improve the design of rotating vessels Chang *et al* formulated a double chamber bioreactor that is capable of forming biphasic osteochondral grafts in a single apparatus (Figure 1A). This device consists of two tubular glass chambers with a magnetic bar stirrer separated by a silicon rubber septum with holes, attached to a tube coated with biphasic composite scaffolds. The scaffolds were coated with gelatin before 10 million porcine chrondrocytes were injected, the graft can be harvested after cultivating for 4 weeks [15].

Spinner flasks bioreactors have also been showed high efficiency in cartilage regeneration confirmed by PCR and histological observation [4], they have also been proven to support *in vitro* chrondrogenic differentiation of ASCs, attributed to the formation of a spheroid culture allowing for cell-cell interactions [4]. Rabbit MSCs have also showed to macroaggregate on a PLGA scaffold after being cultivated in 4 weeks in spinner flasks in the hope of making trachea grafts [5].

2.2. Bioreactors for Inducing Perfusion

Bioreactors applying perfusion have also shown to be extremely successful for cartilage tissue formation. Cylindrical shaped glass vessels filled with culture medium are generally used for this application. (Figure 1B) Perfusion bioreactors have found to be increasing cartilage quality as they improve ECM retention. For instance, increasing flow rates of 0.075–0.2 mL min^{-1} to human chrondrocytes seeded on PLGA scaffolds for up to 5 weeks increased the percentage of GAG retained in the ECM [7]. Furthermore, cartilage cultivated on perfusion bioreactors was found to be viable and homogeneously cartilaginous, with biomechanical properties resembling most of native cartilage [8]. Most perfusion bioreactors are unidirectional, the culture medium is perfused from a medium reservoir then throughout the cylindrical bioreactor. Wendt *et al* illustrated a bi-directional bioreactor which was more effective than spinner flasks and static seeding of chrondrocytes (Figure 1C) [16]. Scaffolds

were placed in polysulfone/Teflon chambers and positioned at the bottoms of two glass columns and connected through a U-shaped glass tube at their base. Flow of cell suspension was induced with the use of a vacuum pump whilst the flow rate regulated by a flow meter. The direction of flow reversed when the cell suspension reached the level of the sensors placed near the top of each glass column. Perfusion seeding of chondrocytes into poly-active foams using the perfusion system resulted in "viable cell seeding efficiencies," defined as the percentages of initially loaded cells that were seeded and remained viable. It was significantly higher (75 ± 6%) than those by static (57% ± 5%) and spinner flask seeding (55% ± 8%) [16].

2.3. Bioreactors for Inducing Hydrostatic Pressure

Bioreactors that induce HP also showed to have significantly enhanced cartilage formation. HP can be applied to a monolayer of cells seeded on a petri-dish by covering them with culture medium and placing them in a pressure chamber where a gas phase acts on both sides of the dish. HP bioreactors consist of fluid filled chambers attached to a water pump. The vessel is completely filled with water and a syringe sealed at the top. The syringe contains the culture volume with the sample. The water pump compresses the water inside the vessel, resulting in syringe moving and transmitting HP to the culture volume. The optimal magnitude, frequency, and duration of application of HP have not been identified yet. It was suggested that dynamic HP has a beneficial effect on chrondrocytes grew in a monolayer compared with static HP [17]. Interestingly, recent studies found static HP to be more efficient on chondrocytes in 3D culture. This is further verified in Toyoda's study as immature bovine chondrocytes on 3D collagen sponges exposed to static HP at 2.8MPa for up to 15 days showed greater GAG production at 5 and 15 days of culture [18]. On the other hand, dynamic HP loading of chrondrocytes in 3D culture yielded better tissue formation over time and the possibility of promoting differention. Correia *et al* illustrated that application of 0.4 MPa pulsatile HP on human nasal chrondrocytes for 3 weeks encapsulated in gellan gum hydrogels encouraged enhanced tissue formation compared to continuous 0.4 MPa HP and static loading [19]. Candiani *et al* applied 10 MPa HP at the frequency of 0.33 Hz for 4 hours/day resulted in enhanced differentiation of bovine chrondrocytes toward a mature chondrocytic phenotype on synthetic 3D porous scaffolds (DegraPol) [20].

2.4. Bioreactors for Inducing Compression

Compression bioreactors are becoming more widely used in cartilage tissue engineering nowadays. They apply dynamic compression loading which is similar to the natural physiological loading of cartilage. Compression bioreactors can improve mass formation and increase the elastic modules of the cartilage formed to approach that of native cartilage [21, 22]. Mauck *et al* in 2000 seeded cells on agarose disks in dynamic compressions with a peak-to-peak compressive strain amplitude of 10% at a frequency of 1 Hz, 3 times (1 hour on, 1 hour off)/day, 5 days per week for 4 weeks resulted in a six-fold increase in the equilibrium aggregate modulus over free swelling controls after 28 days of loading (100 ± 16 kPa versus 15 ± 8 kPa, $p < 0.0001$). Sulfated GAG content and hydroxyproline content was also found to be greater in dynamically loaded disks compared to free swelling controls at day 21 ($p < 0.0001$ and $p = 0.002$, respectively) [23].

2.5. Bioreactors for Inducing Combined Mechanical Force

Bioreactors applying combined mechanical force are being developed. Shahin *et al* illustrated that human chrondrocytes benefit from combined application of intermittent unconfined shear and compressive loading at a frequency of 0.05 Hz using a peak-to-peak compressive strain amplitude of 2.2% superimposed on a static axial compressive strain of 6.5% for 2.5 weeks. GAG and collagen type II productions were enhanced between 5.3- and 10-fold after simultaneous stimulation [8]. We foresee that future research would be centered in a more complex mechanic system that mimics the *in vivo* mechanical loading condition of natural cartilage.

3. Bioreactors for Bone Tissue Expansion

Bioreactors for bone are similar to the ones for cartilage. In TE studies, one of bone's characteristics resembles vascular tissue in as much as it allows the perfusions of nutrients and oxygen. *In vivo*, loadings that are applied to bone cause fluid to flow through the bone, while shear stress influence osteoblasts and osteocytes to differentiate and mineralize [24]. Therefore in bioreactors designed for bone formation, shear stress and perfusion are constantly highlighted.

Much like bioreactors for cartilage, spinner flasks are used to induce shear force. Research suggests that their use enhance both early osteoblastic marker ALP and late osteoblastic markers osteocalcin. Mygind *et al* in 2007 cultured human MSCs on a coralline porous scaffold presenting with enhanced proliferation and differentiation on spinner flasks than static controls [25]. Stiehler *et al* in 2008 repeated the experiment on PLGA scaffolds for up to 3 weeks and demonstrated a 20% increase in DNA content (21 days), enhanced ALP specific activity (7 days and 21 days), a more than tenfold higher Ca^{2+} content (21 days), and significantly increased transcript levels of early osteogenesis markers (e.g., COL1A1, BMP2, RUNX-2) as compared with static culture [26].

Rotating wall vessel bioreactors are also commonly used in bone TE. TE constructs from these type of bioreactors have demonstrated enhanced osteoblast cell phenotypic expression and mineralized matrix synthesis comparing with static conditioning bioreactors [27]. It provided better-controlled oxygen supply, caused fewer turbulence while inducing shear stress in comparison to spinner flasks [28]. Song *et al* in 2007 demonstrated that rat osteoblasts cultured on rotating wall vessel biorectors expanded by more than ten times than spinner flasks and static controls, and they presented better morphology, viability and stronger ability to form bone tissue [29]. Concerns had been raised on the collision of scaffolds with the walls of the rotating vessel chamber as the scaffolds were usually denser than the medium [30]. However, several groups succeeded in developing scaffolds with densities lower than water. Studies with these scaffolds in a rotating wall vessel bioreactor showed greater expression of ALP and calcium deoposition in osteoblasts compared to static controls [31].

Another type of bioreactor being used in bone TE is the perfusion bioreactor. They are able to effectively pump media directly through the scaffold subsequently allowing a better exchange of oxygen and nutrients. This is useful in large tissue mass constructs as it allows for more precise control of the culturing environment. Bancroft *et al* described a commonly used perfusion bioreactor system as shown in Figure 1B. The result showed that mineralized matrix production dramatically increased when compared to statically cultured constructs. The total calcium content of the cultured scaffolds were also elevated as flow rate increased [32]. Flow rate is one of the most essential parameters for bone TE. The optimal range varies according to the design of the bioreactor and the cell type used. Grayson *et al* illustrated in a novel bioreactor design that osteoblasts proliferation and mineral content of the final construct were significantly increased as flow rate increased within the range of 0.3~3ml/min [33]. In addition to flow rate, further development was made to optimize perfusion bioreactors for bone tissue in the hope of finding a more cost effective and less complex alternative. For instance, scaffolds with a central channel can enhance bone formation [34]. Jansen *et al* showed that with a central channel perfused β-tricalcium phosphate scaffold, the proliferation of MSCs seeded was enhanced and osteoblast differentiation was observed after 19 days [35]. Frohlich *et al* in 2010 cultured ASCs in a similar bioreactor system resulting in formation of compact and viable bone tissue after 5 weeks [36]. It is clear that perfusion bioreactors promote osteogenic differentiation and bone formation [37].

4. Bioreactors for Vascular Tissue

The incidence of peripheral vascular diseases is gradually increasing in both developed and undeveloped countries. The primary treatment strategy for peripheral vascular diseases is

surgical bypass operations. However, due to the shortage of usable vessels, suitable vascular substitutes are in great demand [38, 39]. Currently, there are two major trends in developing vascular substitute. One is looking at new synthetic biomaterial such as Dacron and PTFE. They are the only commercially used materials in the development of cardiovascular implants. Dacron is used mostly in large diameter bypass graft such as AAA etc. and PTFE is used in other bypass grafts with diameters over 6 mm. When comes to bypass grafts with a smaller diameter however, neither show an ideal biocompatibility and patency, that is to say that the patency of PTFE bypass grafts for lower limbs are only 25% in 5 years [40]. These materials are over 25 years old and still being used currently for cardiovascular implants, and still do not meet all clinical needs [41]. Alternatively, VTE aims to construct vascular bypass using ECs and SMCs with a biodegradable or non-biodegradable scaffold [42].

4.1. Bioreactors for Vascular Bypass Grafts

Bioreactors that could closely monitor and control physical, biochemical and mechanical conditions are essential for VTE [43]. There are four major components in a vascular bioreactor which are: culturing chambers, motor-driven pumps, medium reservoirs and a temperature controller. All components are assembled into a double-enclosure circuit loop. The culturing circuit consists of two separated circulating loop: an incubating loop and a medium-replenishing loop. With the incubating loop, the pump gives a pulsatile flow which drives the culturing medium to supply oxygen and nutrients. In the medium-replenishing loop, a one-direction pump (not necessarily pulsatile load) drives fresh medium into the incubating chamber and old medium into the gas/liquid and liquid/liquid exchanger to replenish oxgyen, nutrients and to remove waste products (Figure 2) [44]. Song *et al* reported an integrated vascular bioreactor system which added a PH sensor, a buffer-driven PH controller, a DO sensor, a pump-driven DO controller, an ozonizer, a position detector and a force monitor on the basic layout. The system was designed for complete computer-control to maintain a stasis-balanced condition and to mimic *in vitro*, the physiological mechanical stimulation and fluid flow effect that presented in arteries in vivo. Engineered bypass grafts were put into the computer-controlled integrated vascular bioreactor system that could provide a dynamic stasis conditioning and a static conditioned bioreactor for a two-week culture. Results showed that the proliferation rate of cells in dynamic conditioning was significantly higher than the one in static conditioning [44].

Another group of researchers are looking at MDP in developing the next generation of VTE bioreactors. MDP is a system designed to find out the growth model in any VTE bioreactors using symbolic regression [45]. Symbolic regression is a method based on evolutionary computation and genetic programming [46]. It is designed for the purpose of searching mathematical expressions and minimizing possible error matrices. With MDP, a mathematical framework is generated for optimizing problems under stochastic environment and forecasting successive action interference [47]. It performs calculations to explore whether the system is likely to work prior to assembly. It can also be used to explore the combination of optimal parameters for greatest efficiency such as optimal flow rate, oxygen in-flow, nutrients top up frequency etc. While the MDP system being used to exploit models in VTE bioreactors is still theoretical, it is likely to be a future trend.

Different cell sources could result differently even under the same VTE bioreactor system. ECs and SMCs are the conventional choices of cell sources for VTE. Lei Song *et al* successfully developed a small-diameter vascular construct with exact histological mimicking of natural artery using rat aortic SMCs and ECs on the external and internal surface of a de-cellularised rabbit aorta scaffold in a similar structured pulsatile pump bioreactor system [44]. Various stem cells are also being investigated as cell sources in VTE such as ASCs, MSCs and EPCs [48]. Recent research has suggested adding EPCs into the incubating loop along with a surface functionalised scaffold that process EPC-capturing feature resulted in EPCs

immobilization onto the scaffold's inner surface and differentiate into monolayer of EC coverage [49, 50].

4.2. Bioreactors for Stents

In addition to bypass grafts, VTE bioreactors are increasingly used as stent testing and evaluating platforms for their biocompatibility, thrombogenicity and ability of in-situ endothelialization *in vitro*. ECs or EPCs are usually added into the incubating loop to test the cell capturing efficiency of the tested stent. Stent bioreactors resemble the design of VTE bioreactors, with the outer medium-replenishing loop absent and with a roller pump instead of a pulsatile one [51]. Weinandy *et al* used a stent bioreactor system to test the endothelialization of BioStent (a nitinol stent covered with fibrin gel) with ECs added into the loop resulting is successful cell anchoring, growth and generation of ECMs in-situ (Figure 3) [52].

4.3. Bioreactors for Heart Valves

TEHVs is a potential route to substitute for heart valve prostheses that are essential for treating valvular diseases [53]. To create TEHVs *in vitro* requires yet another integrated bioreactor system. The general layout of the TEHV bioreactors includes only two major components, a motor-driven pulsatile pump and an incubating chamber. The incubating chamber is designed to have two separated compartments isolated by a septum. In the middle of the septum is the position for the fixation of the TEHV scaffold, on the side are one-direction valves that open opposite to the opening of TEHV (Figure 4). When the pulsatile pump starts working, it drives the culture medium to burst through the TEHV into the distal compartment. This also pushes the culture medium in the outer compartment to flow through those one-direction valves back to the proximal compartment, thus forming an intra-chamber, inter-compartmental flow circuit [54, 55].

Driven by insufficient supply of vascular bypass and heart valve substitutes and the high up in demand, VTE bioreactors are progressing swiftly in smart culturing. By combining research models with mathematical frameworks, we now have the means to select and isolate optimal parameters that achieve the best results. Based on literature, we believe that the next generation of bioreactors for vascular tissue will be an integrate system which have "smart" control over multiple parameters in the manner of "monitoring-feedback-control". It is also our belief that in the absence of ideal testing models and while the financial and ethical cost of using animal as testing subjects are growing, the role of VTE bioreactor be used as a testing and evaluating *in vitro* tool will become prominent in the near future [56].

5. Bioreactors for Skin Expansion

Skin is the largest organ of our body. It provides physical protection, prevents fluid loss, as well as thermal regulation, sensation and immunological functions. Loss of integrity of skin area is usually caused by trauma, burn and surface tumour/carcinoma. Restoration of skin is of essence in treating those conditions. It is also a primary treatment strategy for post-traumatic conditions, such as scars (especially hypertrophic scars) and regional cosmetic changes. However, insufficiency of donor skin supply has led a huge demand for a suitable skin substitute. There are already several commercial available skin substitute, such as: Integra (collagen based regeneration template), Oasis (cellular intestinal mucosa), Dermagraft (tissue engineered product) [57-61]. Yet there are limitations in these products like low clinical success rate – the success rate of Integra in large wounds is only 60% (reported by Machens, HG *et al* in 2001), many products failed commercially such as Dermagraft due to its high cost.

5.1. The Kerator

ESSs are epidermal substitutes of autologous keratinocytes, attaching to a dermal analogue with populated autologous fibroblasts. It showed great clinical efficiency in related studies [62]. An integrated skin bioreactor system with dynamic conditioning is required for culturing ESSs. The Kerator was originally designed to produce an autologous wound dressing made up of gas-permeable FEP film containing keratinocytes [63]. It is a closed loop system with a "Smart"

computer-controlled function. It has multiple monitoring built in and allows automatic feedback controlling. The basic layout of the Kerator and the image of actual system are illustrated in (Figure 5) [64].

5.2. Expanding Bioreactors for skin grafts

It is a very common procedure for surgeons to expand full skin grafts via incising patterns or to mesh STSGs to obtain larger segments of autoglogous skin [65]. Ladd *et al* in 2009 designed a bioreactor system to apply continuous physio-mechanical stress load in culture with the hope of harvesting a larger area of products. It contained an actuator mounted on the tissue container which secures the culturing skin or scaffold, a linear motor-driven device and an incubator. It is programmed to uniaxially expand the fixed tissue or scaffold to 20% of its initial length per day, so as it will double the surface area in 5 days approximately. Human foreskin cultured in this system for 6 days showed a 110.7% ± 12.2% increase in surface area, while maintenance of cell viability proliferative potential of cells (Figure 6). Histomorphological and ultrastructural analyses of the expended tissue confirmed that the integrity of dermal structure was well preserved [66].

5.3. The Rotating Cell Culture System

The RCCS is a bioreactor system developed by the NASA with the purpose of mimicking microgravity condition during culture [67]. Study by Yuge *et al* suggested RCCS promotes the viability and proliferation of MSCs [68]. Lei *et al* cultured human EpSCs on Cytodex 3 (a micro-carrier) scaffold coated by collagen type IV in a RCCS (Figure 7). Results showed RCCS have positive effects on the differentiation and proliferation of EpSCs when compared with plain culturing. It also promoted a three dimentional epidermis growth *in vitro* [69, 70]. At this point, only data on MSCs and EpSCs with RCCS is available, more cell types need to be tested to explore this system.

6. Bioreactors for Nerve Tissue Expansion

In peripheral nerve injuries, the most common repairing technique is direct end-to-end suturing. In the circumstance that the two nerves ends cannot be aligned tensionless, gap bridging with and nerve autograft materials are required. The re-innervation rate for this kind of procedure is 80% [71]. However, the harvest of donor graft means loss of sensory or motor function of the donor site and so alternative approaches of obtaining nerve graft are being investigated. Nervous TE is one of the prospective approaches for producing nerve graft substitutes [72]. Nervous tissue requires strict culturing conditions and it is even harder in inducing differentiation and integration. Currently, there are only few ongoing studies and even fewer encouraging results generated for this matter. Tao *et al* in 2008 designed a bioreactor system for nerve conduit. It consisted of a 9 cm circular tissue culturing polystyrene Petri dish, a peristaltic pump and a medium reservoir. All components were connected together using silicone tubes as shown in Figure 8. Schwann cells cultured in this bioreactor system showed cellular adhesion and alignment on the longitudinal axis under fluorescence microscopy [73].

7. Summary and Future Trends

Bioreactors have been utilised for all aspects of TE and research in this area has brought us a step closer to develop numerous tissues *in vitro* for large-scale medical application. A summary of bioreactors used for a variety of different tissue types as presented in Table 1.

In cartilage tissue engineering, mechanical stimulation is vitally important to form tissue that has similar elastic modulus to native tissue and for cells to produce an effective ECM [21]. Rotating bioreactors have shown to upregulate gene expression in osteoblasts effective for bone tissue engineering [74]. Perfusion bioreactors have been useful in bone engineering due to their capability for stimulating bone cells to increase levels of bone formation markers and mineralisation of the scaffold [32]. Despite these encouraging studies, there is a greater need to elucidate the specific biochemical and biomechanical factors need for optimal bone and cartilage engineering *in vitro*. For vascular tissue engineering, bioreactor technology is still

under-explored but bioreactors that provide adequate perfusion and fluid flow has shown to provide an optimal environment for adequate tissue formation [43]. Future work will need to specify the optimal cell source used to create an optimal tissue engineered vascular construct [75]. With the rapid expansion in skin tissue engineering, multiple bioreactors have been utilised for *in vitro* skin generation. The kerator has emerged to decrease labour costs and material requirements but further studies are required to understand its capability for large-scale production for clinical use [76, 77]. Bioreactors for nerve engineering are still in their infancy with few studies showing further potential for effective tissue formation *in vitro*.

In this review we have summarised most of the success in tissue regeneration using bioreactors in recent years and highlight the progress that has been made in large-scale three-dimensional tissue constructs or substitutes that are suitable for clinical uses. However, most bioreactors to date still have a low volume output, and are time consuming and labour extensive. They require considerable time for tissue formation and therefore still await optimization to meet the need of clinical practices.

In addition to the mass-production of tissue substitutes, another future trend for bioreactor design would be assisting developing artificial organs that are tailored to each individuals. In our lab in the centre for nanotechnology in the Royal Free Campus of UCL, we have developed and patented a family of nanocomposite polymer namely POSS-PCU. It is an anti-thrombogenic [78], biocompatible [79] and non-toxic [80, 81] biomaterial that is suitable to be used as the next generation of biomaterial for surgical implants. Our studies have also revealed that it is an inert biomaterial that it does not cause inflammatory reactions in the surrounding host tissue and show calcification resistance [82-85]. In 2011, we used this novel biomaterial and produced an synthetic trachea seeded with patient's own stem cells for 36 hours before being implanted to replace the damaged trachea and the following-up showed implant survival and patient currently live without complication with the artificial trachea [86]. We have also developed lacrimal duct conduits and lower limb bypass grafts with this technology and are planning to use them in the treatments for patients. We are currently designing new generations of bioreactors to manufacture these artificial organs on a larger scale and faster time span to meet the needs of the market.

The future success of bioreactors for TE to achieve effective replacements for clinical applications will depend on the collaboration between engineers, clinicians and biologists. With multi-disciplinary collaboration, we are soon to create a novel complex bioreactor system that is capable of large scale of tissue culture under short time span *in vitro*.

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Junjie Zhao and Michelle Griffin conceived and planned the structure and wrote the manuscript. The rests of the author proof read the manuscript and gave valuable feedback. All authors read and approved the final manuscript.

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Figure Captions

Figure 1. A: The double chamber bioreactor, consists of two tubular shape glass chambers, each containing medium with outflow and inflow and oxygen ventilation. The two chambers, separated by silicon rubber has multiple holes to hold the biphasic scaffold. The bioreactor forms two independent medium circuits. **B**: Flow perfusion Chamber. Each flow chamber contains a cassette sealed by two neoprene O-rings heed in place by a screw top. Media flow from top to bottom though the scaffolds. Media drawn from the first media reservoirs through the flow chamber and then back to the second reservoir to complete the circuit. **C:** Bidirectional bioreactor. The cell suspension oscillates between the two glass columns, flowing into the sample chamber containing the scaffold. The direction of the media flow reverses when it reaches the sensors levels. (Adapted and modified from Bancroft *et al*)

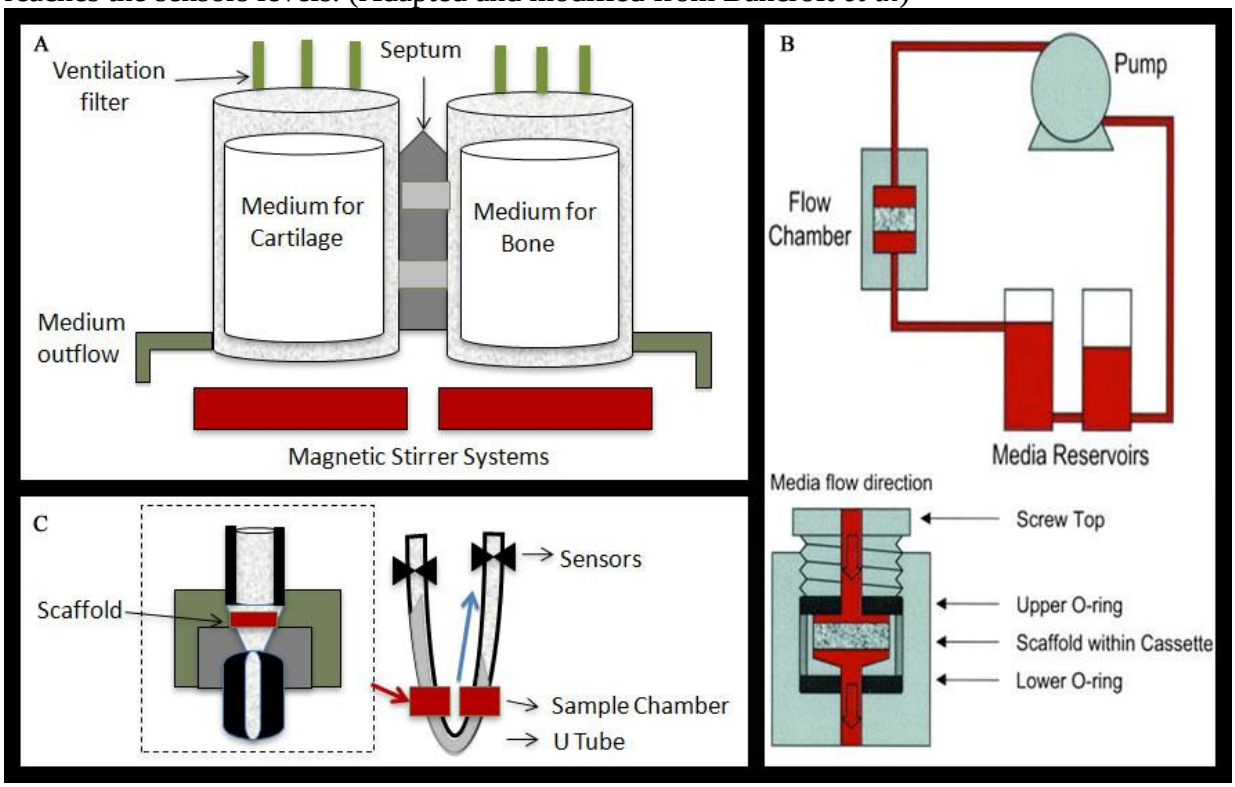


Figure 2. The schematic diagram of a vascular tissue bioreactor. The yellow circle indicates the incubating loop, the red circle indicates the median-replanish loop, and the blue circle indicates the computer-controlled integrated system. (Adapted and modified from Song *et al*)

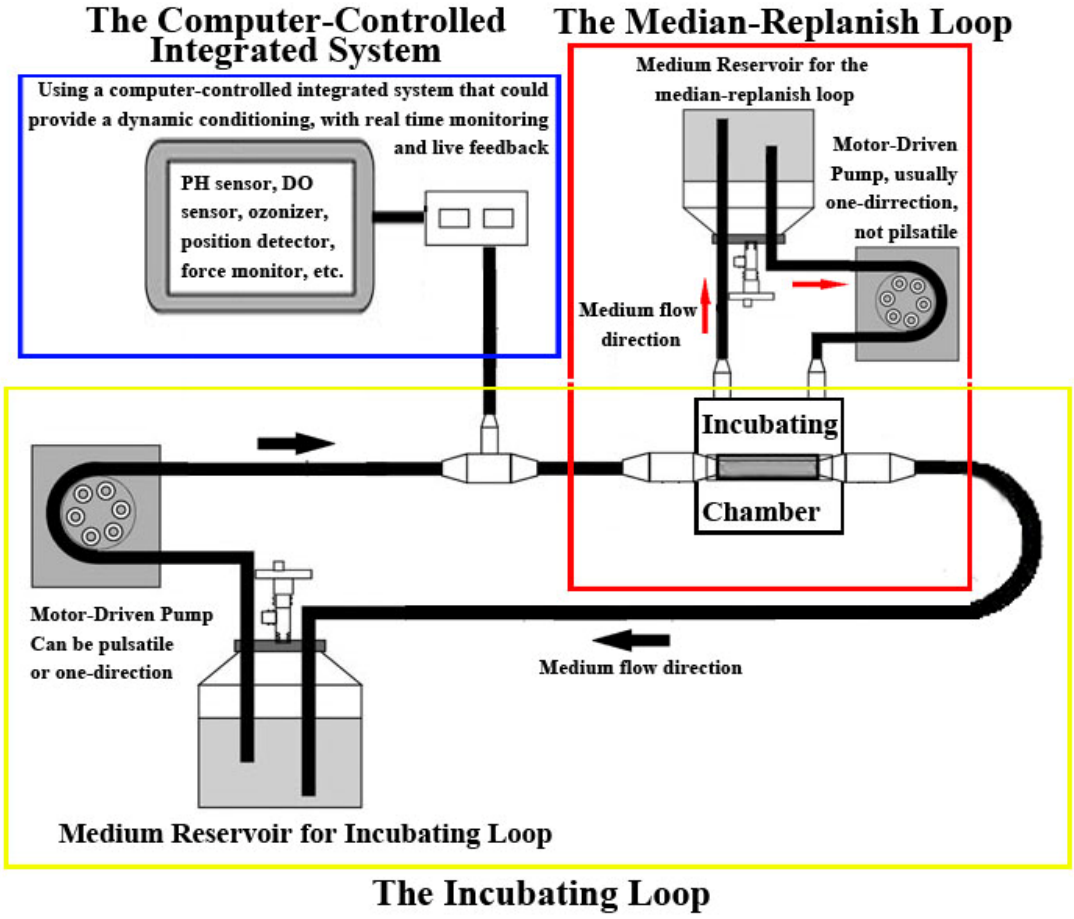


Figure 3. Biostent after 2 weeks culture in the stent bioreactor system: A. The pre-culture BioStent, B and C. HE staining of PMMA section of BioStnet. (Adapted from Stefan *et al*)

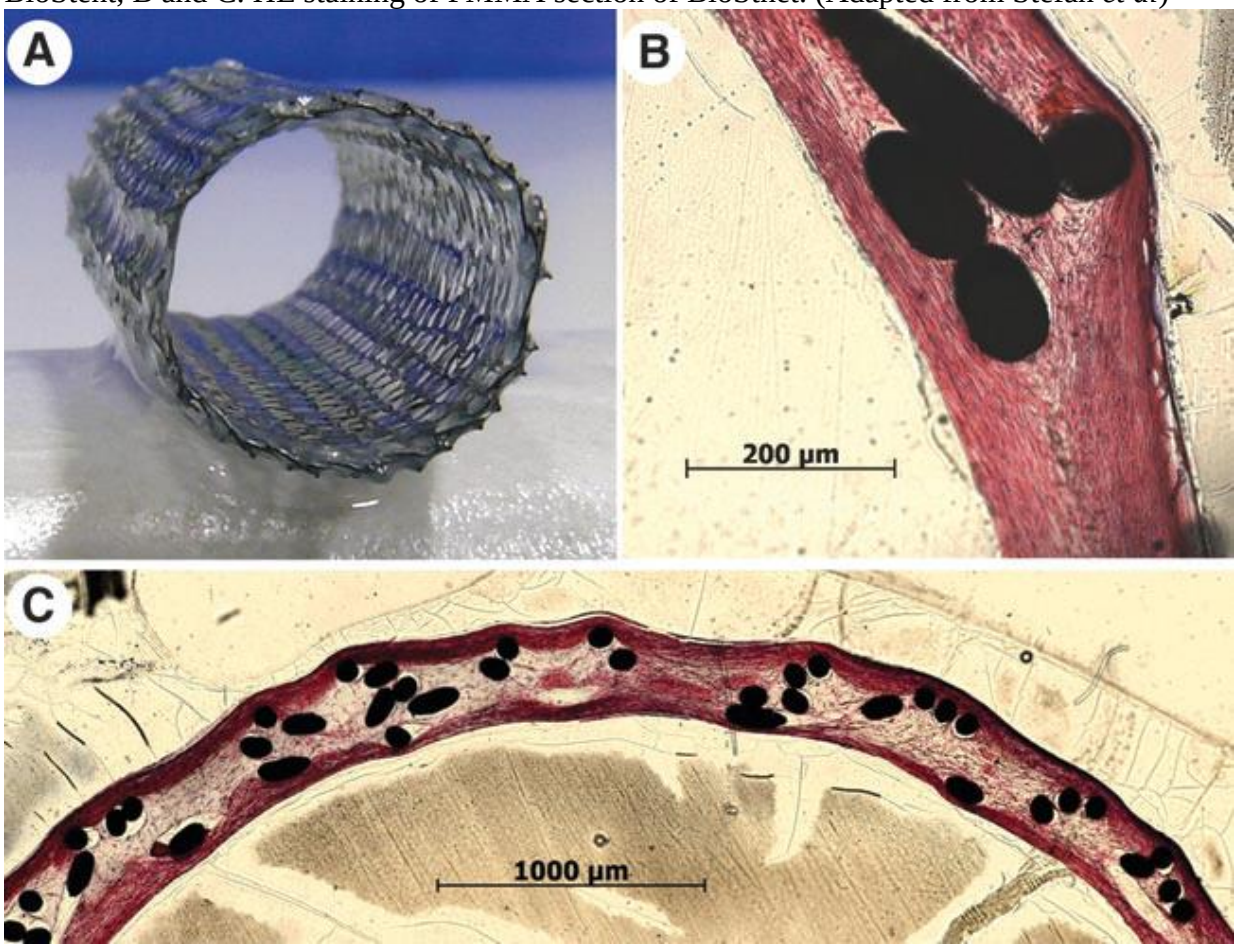


Figure 4. A pulsatile bioreactor for TEHVs with an endoscopic monitoring unit and a flow velocity monitoring and control system. A: Schematic diagram of the flow circulation inside the incubating chamber: the pulsatile pump drives the culture medium (shown in red arrow) to burst through the TEHV set in the middle of the septum while also pushing valve (1) is pushed upwards and seals the tubular connection (2) between the proximal and distal chambers. As the culture medium flows upwards and through the small holes, it flows downwards (shown in blue arrow) through the disc (3). It flows back to the proximal chamber through (2) and continue in the circulation. B: The over view of the pulsatile bioreactor for TEHVs. (Adapted from Konig *et al* and Aleksieva *et al*)

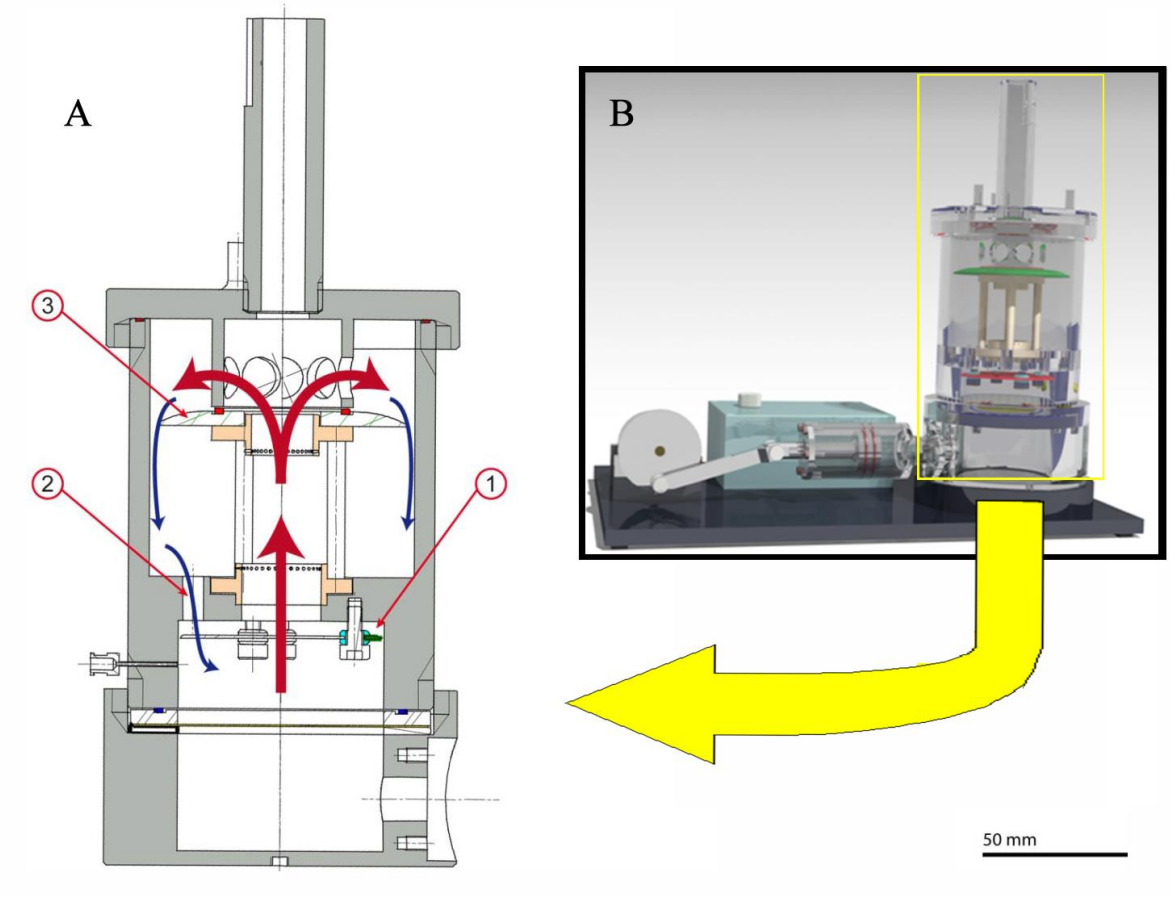


Figure 5. The basic layout of the Kerator and the image of actual system. Upper: A. The growth chamber (five-layered), B. Monitoring camera, C. Tilting acuator, D. Rocking platform, E. Pneumatic pinch valves. Lower: 1. Cold medium reservoir, 2. Warm medium reservoir, 3. Cell seeding and collection reservoir, 4. Waste product 5.Carbondioxide supply, 6. Air supply, 7. Flow controller, 8. Humidifier bottle, 9. Carbondioxide detector, 10. Peltier cooler, 11. Compressed air supply. (Adapted from Balaji *et al*)

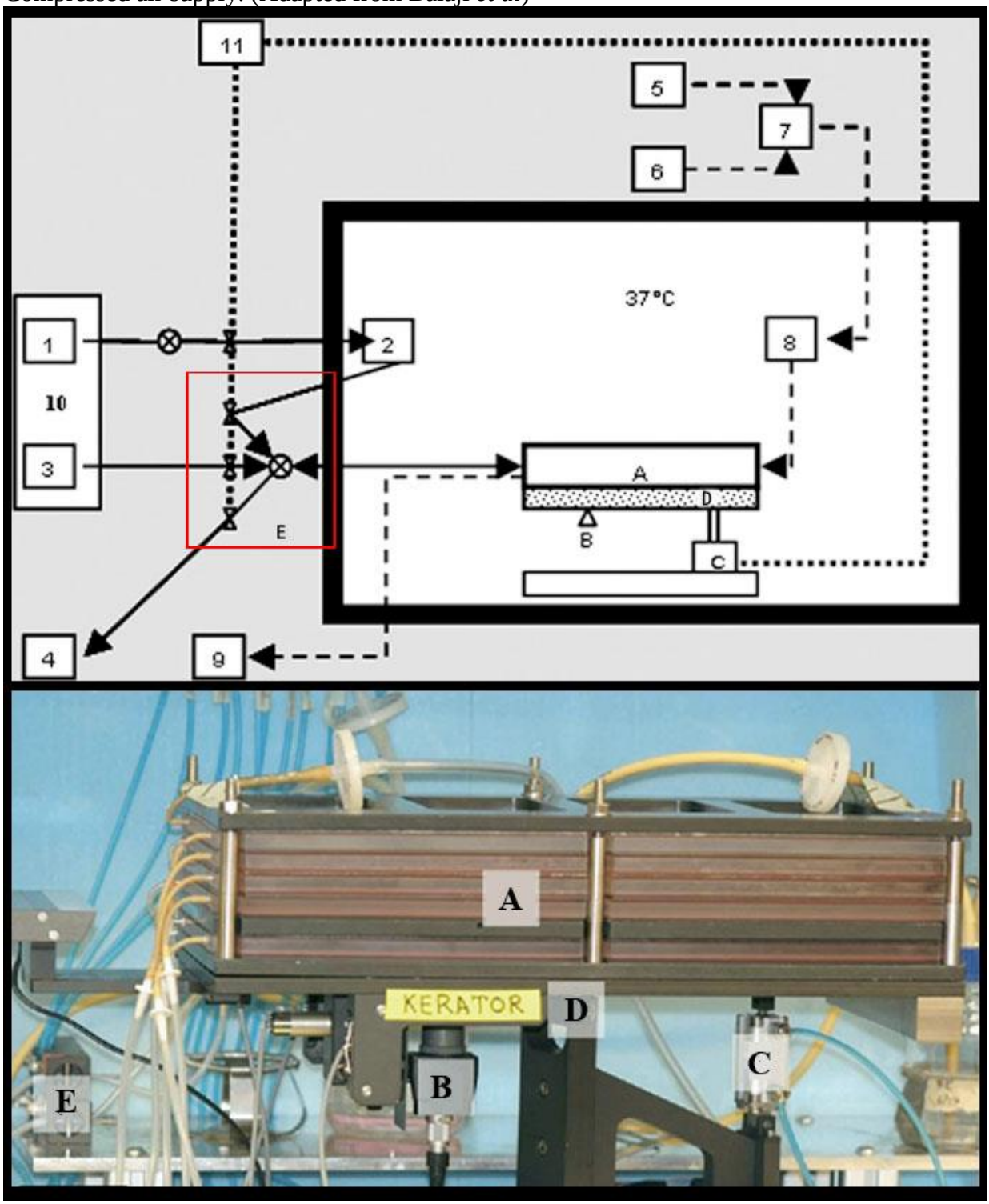


Figure 6. Skin expanding bioreactor: A. Unexpanded skin loaded in the bioreactor, B. skin in the bioreactor after expansion. (Adapted from Mitchell *et al*)

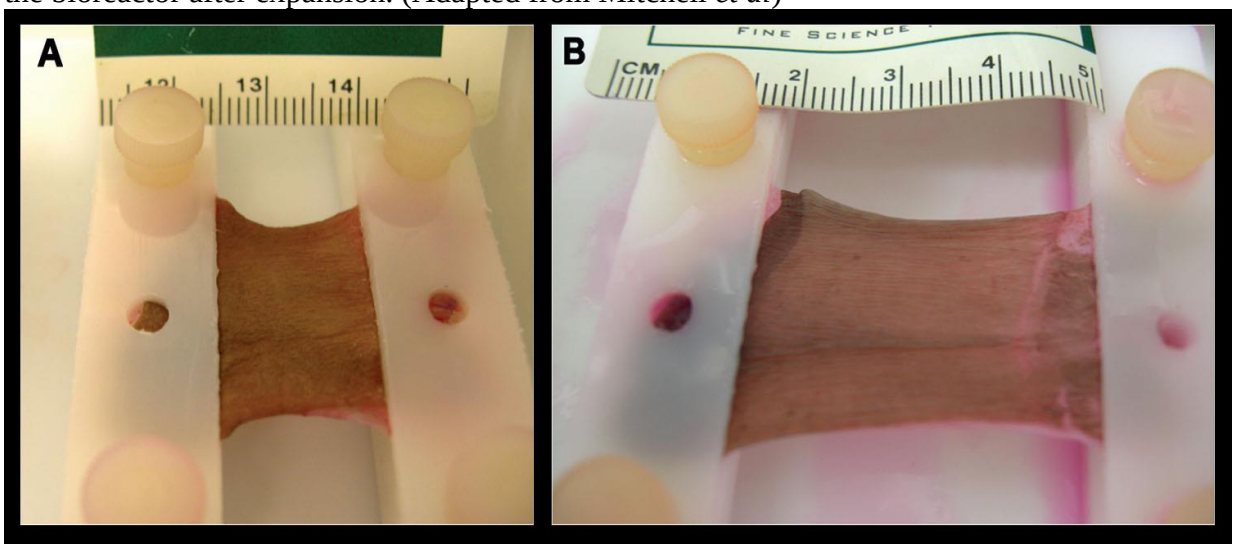


Figure 7. A RCCS used to culture human EpSCs. A: The RCCS in culture. B: The EpSCs and Cytodex 3 was loaded into the RCCS (the red round vessels), and the vessels were rotating in a clockwise direction. C: T over view of the RCCS. (Adapted from Antoni *et al*)

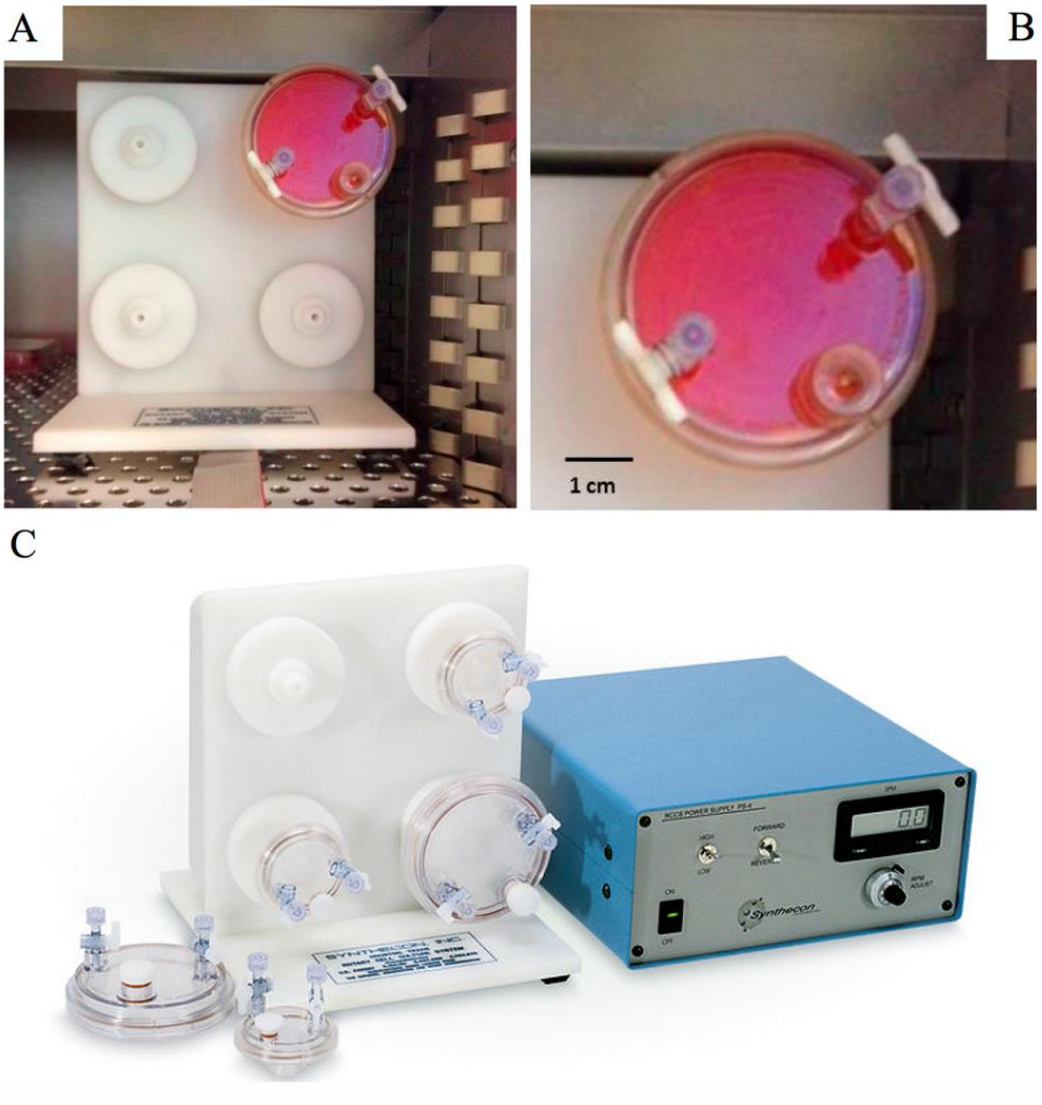
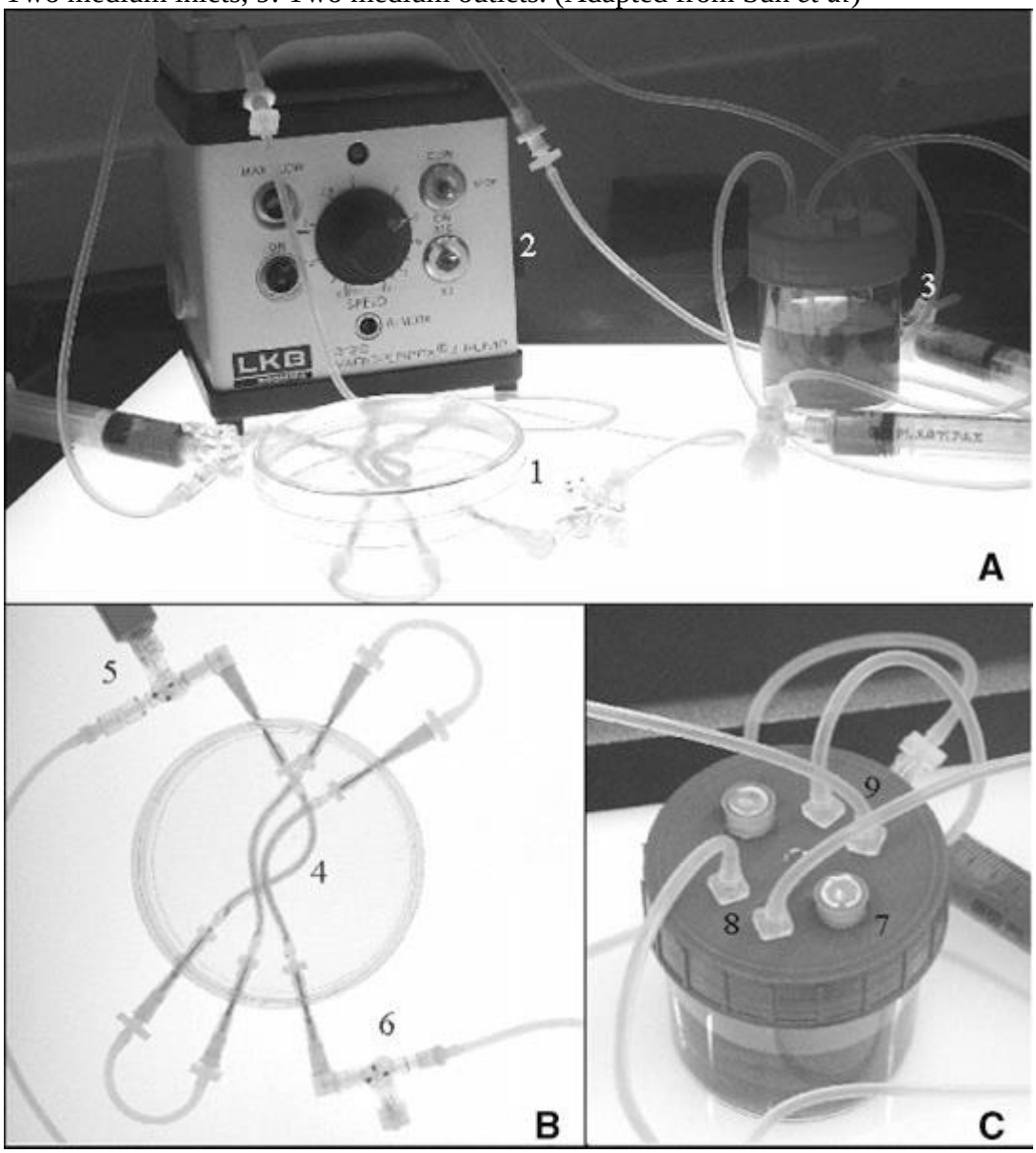


Figure 8. The bioreactor system for culturing nerve conduits: 1. 9 cm Polystyrene Petri dish, 2. A peristaltic pump, 3. A medium reservoir, 4. Three silicone tubes containing aligned microfiber scaffold inside the Petri dish (length: 40mm, diameter: 1.2mm, connected in series), 5. Two closable inlets, 6. Two closable outlets, 7. Vent windows with an air permeable film, 8. Two medium inlets, 9. Two medium outlets. (Adapted from Sun *et al*)



Tables

Table 1: Summary overview of the different bioreactors used for tissue engineering of various of tissue types.

Author	Year	Tissue Type	Bioreactor Characteristics	Scaffold Used	Cell Type Used	Outcome
Takebe *et al*	2012	Cartilage	Shear Force, Rotating Vessel	Collagen, hydroxyapatite and chondroitinsulfate	Bovine and human articular chondrocytes	Enhanced type II collagen production.
Akmal *et al*	2006	Cartilage	Shear Force, Rotating Vessel	2% alginate hydrogel	Human cartilage progenitor cells	Cells formed elastic cartilage-like tissue.
Yoon *et al*	2012	Cartilage	Shear Force, Spinner Flask	Fibrin Gel	ASCs	Chrondrogenic differentiation of ASCs.
Liu *et al*	2010	Cartilage	Shear Force, Spinner Flask	PLGA	Rabbit MSCs	Formation of tubular tracheal grafts.
Vunjak-Novakovic *et al*	1998	Cartilage	Shear Force, Spinner Flask	PGA	Chondrocytes from femur	Cartilaginous tissue formation.
García *et al*	2012	Cartilage	Shear Force, Stirred	Chitosan	Human chondrocytes	Enhanced aggrecan and collagen type II production.
Shangkai *et al*	2007	Cartilage	Stirred	Fibroin sponge	Chondrocytes	Enhanced DNA content and GAG.

Shahin *et al*	2011	Cartilage	Perfusion	PGA	Human chondrocytes	Enhanced ECM on scaffold.
Santoro *et al*	2010	Cartilage	Perfusion	Hyaff-11	Human chondrocytes	Form cartilage construct over two weeks.
Wendt *et al*	2011	Cartilage	Perfusion	Polyactive foams and Hyaff-11	Human articular chondrocytes	Bi-directional chamber shows enhanced cartilage formation.
Mauck *et al*	2000	Cartilage	Compression Dynamic	Agarose gel	Human chondrocytes	Enhanced aggregan and hydroproline content of cells on scaffolds.
Wang *et al*	2013	Cartilage	Compression Dynamic	Chitosan/collagen	Rabbit chondrocytes	Rabbit chondrocytes showed enhanced total GAG deposition on the scaffold.
Wang *et al*	2009	Cartilage	Compression Dynamic	Chitosan/collagen	Rabbit chondrocytes	The expression of type II collagen and aggrecan was upregulated after 3 hours of compression when compared with the free-swelling samples of rabbit chondrocytes.

Correia *et al*	2012	Cartilage	Hydrostatic Dynamic	Gellan Gum Hydrogel	Human nasal chondrocytes	Enhanced cartilage formation of human nasal chondrocytes.
Candiani *et al*	2008	Cartilage	Hydrostatic Dynamic	Degrapol	Bovine Chondrocytes	Enhanced differentiation on 3D scaffold.
Lammi *et al*	1994	Cartilage	Hydrostatic Static	None	Bovine chondrocytes	37% decrease in GAG synthesis, decreased aggrecan mRNA levels.
Mizuno *et al*	2002	Cartilage	Hydrostatic Static	Collagen sponges	Bovine chondrocytes	Greater GAG production at 5 and 15 days of culture.
Toyoda *et al*	2003	Cartilage	Hydrostatic Static	Agarose gels	Human chondrocytes	Enhanced aggrecan and collagen II mRNA expression in chondrocytes
Mygind *et al*	2007	Bone	Shear Force, Spinner Flask	Coralline hydroxyapatite	Human MSCs	Enhance osteoblastic markers – alkaline phosphastase and osteocalcin
Yu *et al*	2004	Bone	Shear Force, Rotating vessel	PLGA	Human MSCs	Enhance osteoblast cell phenotype.
Song *et al*	1999	Bone	Shear Force, Rotating vessel	None	Rat osteoblast	Produce greater bone

						formation in bioreactors than static controls.
Grayson *et al*	2008	Bone	Perfusion	Decellularized bovine trabecular bone	Human MSCs	Increased flow rate increased mineralised matrix formation by osteoblast
Chen *et al*	2013	Vascular tissue	An integrated bioreactor that mimic the physiological pulsatile stimuli	3D PGA scaffold	ASCs	An elastic small diameter vessel with certain functional and mechanical properties similar to natural arteries
Song *et al*	2012	Vascular tissue	1). A computer-controlled integrated vascular bioreactor system. 2). Consists of two separated circulation loop: The incuating loop and the medium-replenishing loop	Decellularized rabbit aorta tissue	Rat aortic ECs and SMCs	Higher cell proliferation rate than in normal bioreactor systems. And a successful small-diameter vascular constructs harvest in end point.
Stefan *et al*	2012	Stents	1). Single loop system: Culture medium circuit. 2). ECs or EPCs are often added into the circling culture medium to test the cell capturing property of the stent	BioStent (a nitinol stent covered with fibrin gel)	ECs	Cell growth and generation of ECMs

Genoveva *et al*	2012	Heart Valves	1). Once direction flow circuit 2). Pulsatile flow model	PU scaffolds	Human vascular ECs and fibroblasts	SEM results showed a high density of adherent cells on the surface valves from both groups
Li *et al*	2011	Skin	A Rotary Bioreactor: Rotating Cell Culture System mimicking microgravity condition	Collagen type IV coated Cytodex 3 (a micro-carrier)	Human EpSCs	Show promotion of proliferation and inhibition of differentiation in 3D cell culture. A 3D epidermis tissue harvest in end point.
Mitchell *et al*	2009	Skin	The Skin "expander": 1). Conventional incubator 2). Programmed linear motor-drive device: Uniaxially expand fix tissue or seeded scaffold in a slow and gradual manner	None	Human foreskin	A greater in surface area while maintenance of cell viability and proliferative potential, and the integrity of dermal structure.
Prenosil *et al*	1999	Skin	The Kerator: 1). Has a specific liquid and gas exchange surface 2). "Smart" computer-controlled system: Multiple monitoring and feedback controlling, Automatically changing culturing medium	FEP film	Keratinocytes	An autologous wound dressing
Tao *et al*	2008	Nerve Conduit	1). Aligned microfiber inserted into the lumen of silicone tubes as conduits	Acrylic Acid Plasma Polymerized Viscose Rayon Fiber and	Schwann cells	Fluorescence microscopy examination after culture showed cellular adhesion

			2). The conduit can provide a locally enriched environment of growth factors and prevent the infiltration of fibroblast and inflammatory cells 3). Combined the above conduit approach with Schwann cell delivery and a conduit filling material	Polystyrene Microfiber as Scaffolds		and alignment on the longitudinal axis.