Review TRENDS in Biotechnology Vol.22 No.2 February 2004

The role of bioreactors in tissue engineering

Ivan Martin, David Wendt and Michael Heberer

Departments of Surgery and of Research, University Hospital Basel, Hebelstrasse 20, ZLF, Room 405, 4031 Basel, Switzerland

Ex vivo **engineering of living tissues is a rapidly developing area with the potential to impact significantly on a wide-range of biomedical applications. Major obstacles to the generation of functional tissues and their widespread clinical use are related to a limited understanding of the regulatory role of specific physicochemical culture parameters on tissue development, and the high manufacturing costs of the few commercially available engineered tissue products. By enabling reproducible and controlled changes of specific environmental factors, bioreactor systems provide both the technological means to reveal fundamental mechanisms of cell function in a 3D environment, and the potential to improve the quality of engineered tissues. In addition, by automating and standardizing tissue manufacture in controlled closed systems, bioreactors could reduce production costs, thus facilitating a wider use of engineered tissues.**

Bioreactors are generally defined as devices in which biological and/or biochemical processes develop under closely monitored and tightly controlled environmental and operating conditions (e.g. pH, temperature, pressure, nutrient supply and waste removal). The high degree of reproducibility, control and automation introduced by bioreactors for specific experimental bioprocesses has been key for their transfer to large-scale applications. Bioreactors are classically used in industrial fermentation processing, wastewater treatment, food processing and production of pharmaceuticals and recombinant proteins (e.g. antibodies, growth factors, vaccines and antibiotics).

Tissue engineering has been defined as the application of principles and methods of engineering and life sciences for the development of biological substitutes, to restore, maintain or improve tissue function [1]. In one of the most typical approaches, 3D tissue structures are generated by the association of cells (autologous or allogeneic) with porous scaffolds, which provide the template for tissue development and degrade or are resorbed at defined rates. The *in vitro* culture of 3D cell-scaffold constructs under conditions that support efficient nutrition of cells, possibly combined with the application of mechanical forces to direct cellular activity and phenotype, is an important step towards the development of functional grafts for the treatment of lost or damaged body parts (i.e. functional tissue engineering [2]). The ultimate goal of engineering 3D tissues *in vitro* is not always to generate grafts but could also yield non-implantable structures that can be used as external organ support devices when a compatible donor is not readily available [3]. Furthermore, engineered tissues could provide reliable model systems, facilitating a fundamental understanding of structure–function relationships in normal and pathological conditions, with possible commercial applications in molecular therapeutics (e.g. drug screening) [4]. The generation of 3D tissues *ex vivo* not only requires the development of new biological models (rather than those already established for traditional monolayer or micromass cell cultures [5]) but also poses new technical challenges owing to the physicochemical requirements of large cell-masses.

Here, we review the role of bioreactors in processes that are key for the *ex vivo* engineering of 3D tissues based on cells and scaffolds, including cell seeding of porous scaffolds, nutrition of cells in the resulting constructs, and mechanical stimulation of the developing tissues. Particular focus will be given to the control over environmental conditions and to the automation of bioprocesses that bioreactors can offer. These features are essential not only for controlled fundamental studies of 3D tissue development but also to reduce manufacturing costs of engineered tissues and facilitate their broad clinical use.

Cell seeding on 3D scaffolds

Cell seeding of scaffolds – that is, the dissemination of isolated cells within a scaffold – is the first step in establishing a 3D culture, and might play a crucial role in determining the progression of tissue formation [6]. Seeding cells into scaffolds at high densities has been associated with enhanced tissue formation in 3D constructs, including higher rates of cartilage matrix production [7], increased bone mineralization [8], and enhanced cardiac tissue structure [9]. Thus, engineering autologous grafts for clinical applications using high initial cell densities, while limiting the biopsy size and/or the extent of cell expansion, requires the cells to be seeded with the highest possible efficiency. Furthermore, the initial distribution of cells within the scaffold after seeding has been related to the distribution of tissue subsequently formed within engineered constructs [8,10–12], suggesting that uniform cell-seeding could establish the basis for uniform tissue generation. However, even with a small 3D scaffold (e.g. 5 mm diameter × 2 mm thick), it can be a significant challenge to distribute a high density of cells efficiently and uniformly throughout the scaffold volume.

Corresponding author: Ivan Martin (imartin@uhbs.ch).

 0167-7799/$ - see front matter doi:10.1016/j.tibtech.2003.12.001

Although static loading of cells onto a scaffold is by far the most commonly used seeding method, several studies reported low seeding efficiencies [8,12–15] and non-uniform cell distributions within scaffolds [8,12,14,16–21], owing, in part, to the manual- and operator-dependent nature of the process. Significantly higher efficiencies and uniformities were obtained when poly(glycolic acid) non-woven meshes were seeded in stirred-flask bioreactors [19]. Mixing the dilute cell suspension around stationary scaffolds suspended from the mouth of the flask transports the cells into the scaffolds by convection (Figure 1a). However, owing perhaps to ineffective convection of cells into the interior region of the scaffolds, seeding in stirred-flask bioreactors can also yield low seeding efficiencies [9,12] and non-uniform distributions of cells [16,20], with a higher density of cells lining the scaffold surface [6].

Exploiting the principle of convective transport for scaffold seeding, the flow of a cell suspension directly through the pores of 3D scaffolds using a multi-pass filtration seeding technique produced more-uniformly seeded scaffolds compared with static seeding [14]. When direct perfusion was incorporated into an automated bioreactor for 3D-scaffold seeding, higher seeding efficiencies and more-uniform cell distributions were achieved compared with either static seeding or the stirred-flask bioreactor [20]. A variety of scaffolds can be effectively and reproducibly seeded in an automated and controlled process using this straightforward concept and a simply designed bioreactor. Additionally, perfusion seeding can be readily integrated into a perfusion bioreactor system capable of performing both seeding of the scaffold and subsequent culturing of the construct. These seeding and culturing bioreactors have been designed for engineering vascular grafts [22] and have recently been used in engineering cartilage [23] and cardiac [24] tissues, and in maintaining hepatocyte function within 3D scaffolds [25]. These systems not only streamline the engineering process but also reduce the safety risks associated with the handling and transferring of constructs between separate bioreactors.

Increase of mass transport

It has long been known that the supply of oxygen and soluble nutrients becomes critically limiting for the *in vitro* culture of 3D tissues. The consequence of such a limitation is exemplified by early studies showing that cellular spheroids larger than 1 mm in diameter generally contain a hypoxic, necrotic center, surrounded by a rim of viable cells [26]. Similar observations were reported for different cell types cultured on 3D scaffolds under static conditions. For example, glycosaminoglycan (GAG) deposition by chondrocytes cultured on poly(glycolic acid) meshes was poor in the central part of the constructs (~400 μm from the outer surface) [27] (Figure 2a), and deposition of mineralized matrix by stromal osteoblasts cultured into poly(DL-lactic-*co*-glycolic acid) foams reached a maximum penetration depth of 240 μm from the top surface [28]. Because engineered constructs should be at least a few mm in size to serve as grafts for tissue replacement, mass-transfer limitations represent one of the greatest challenges to be addressed.

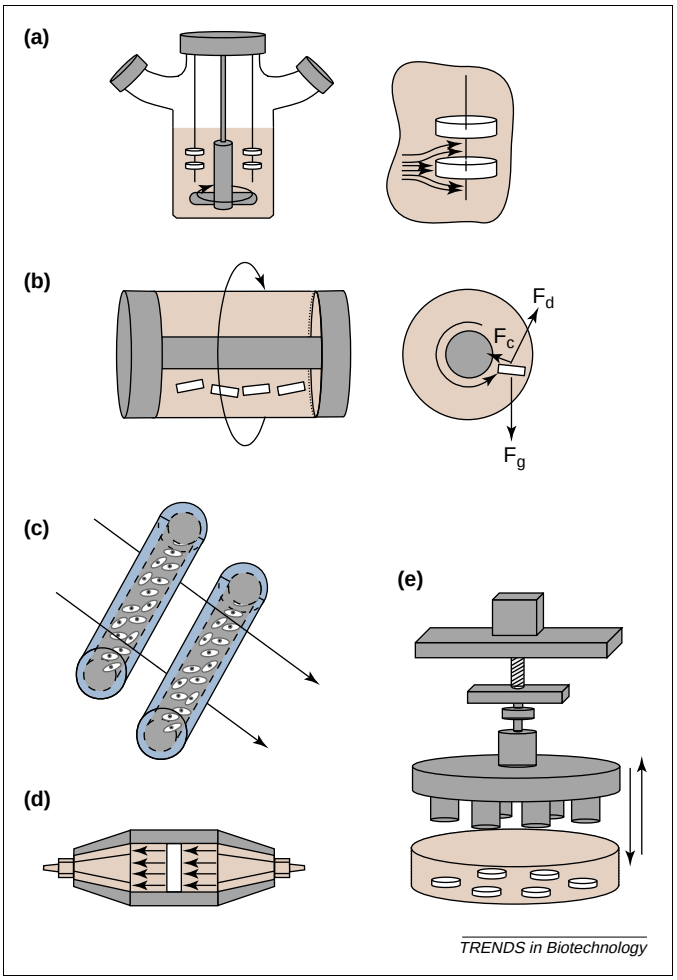

Figure 1. Representative bioreactors for tissue engineering applications. **(a)** Spinner-flask bioreactors have been used for the seeding of cells into 3D scaffolds and for subsequent culture of the constructs [19]. During seeding, cells are transported to and into the scaffold by convection. During culture, medium stirring enhances external mass-transfer but also generates turbulent eddies, which could be detrimental for the development of the tissue. **(b)** Rotating-wall vessels provide a dynamic culture environment to the constructs, with low shear stresses and high mass-transfer rates. The vessel walls are rotated at a rate that enables the drag force (F_d), centrifugal force (F_c) and net gravitational force (F_g) on the construct to be balanced; the construct thus remains in a state of free-fall through the culture medium [34]. **(c)** Hollow-fiber bioreactors can be used to enhance mass transfer during the culture of highly metabolic and sensitive cell types such as hepatocytes. In one configuration, cells are embedded within a gel inside the lumen of permeable hollow fibers and medium is perfused over the exterior surface of the fibers [64]. **(d)** Direct perfusion bioreactors in which medium flows directly through the pores of the scaffold can be used for seeding [20] and/or culturing 3D constructs. During seeding, cells are transported directly into the scaffold pores, yielding a highly uniform cell distribution. During culture, medium flowing through the construct enhances mass transfer not only at the periphery but also within its internal pores. **(e)** Bioreactors that apply controlled mechanical forces, such as dynamic compression, to engineered constructs can be used as model systems of tissue development under physiological loading conditions, and to generate functional tissue grafts. Compressive deformation can be applied by a computer-controlled micro-stepper motor, and the stress on the constructs can be measured using a load cell [58,59].

External mass-transfer limitations can be reduced by culturing constructs in a stirred flask (Figure 1a). As one of the most basic bioreactors, the stirred flask induces mixing of oxygen and nutrients throughout the medium and reduces the concentration boundary layer at the construct surface. Culture of bovine chondrocytes on poly(glycolic acid) non-woven meshes in a stirred flask induced an increase in both the synthesis of GAG [29] and the fractions of GAG accumulated within the central construct

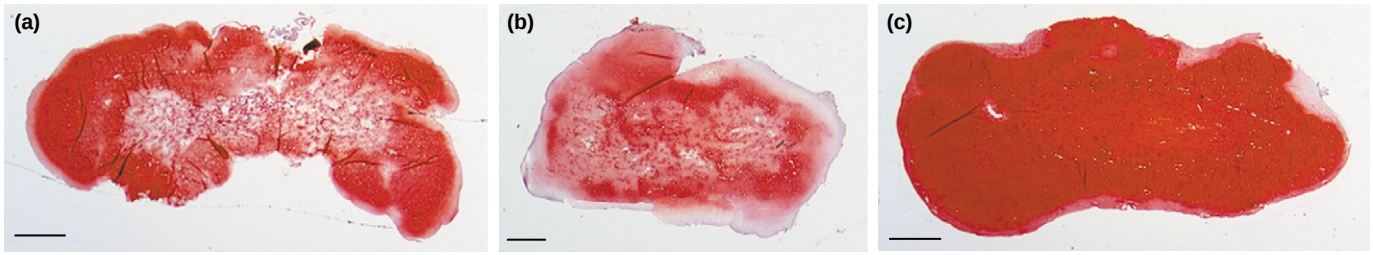

Figure 2. Safranin-O-stained cross-sections of engineered cartilage tissues after six weeks of culture under different hydrodynamic conditions [glycosaminoglycan (GAG) is stained red]. **(a)** Statically cultured constructs contain central regions with negligible GAG deposition. **(b)** Spinner-flask cultured constructs stain more intensely for GAG in their central regions but are encapsulated by fibrous tissue at their periphery. **(c)** Rotating-wall vessel (RWV) cultured constructs stain intensely and homogeneously for GAG throughout their cross-sectional area. The improved structural features following RWV culture (observed histologically and confirmed biochemically) were shown to be related to increased equilibrium modulus, dynamic stiffness and reduced tissue permeability [27,30]. Adapted with permission from [65]. Scale bars, 1 mm.

regions [27]. However, the net average GAG fractions accumulated throughout the constructs was reduced [29], and a 400-μm-thick fibrous capsule was formed at the construct surface [27] (Figure 2b). This was probably caused by the turbulent eddies generated within the stirred-flask bioreactor, which increased the rate of GAG released into the culture medium and were associated with chondrocyte dedifferentiation.

A dynamic laminar flow generated by a rotating fluid environment is an alternative and efficient way to reduce diffusional limitations of nutrients and wastes while producing low levels of shear. The efficacy of rotating-wall vessel (RWV) bioreactors (Figure 1b) for the generation of tissue equivalents has been demonstrated using chondrocytes [30], cardiac cells [9] and various tumor cells [31,32]. After a few weeks of cultivation in the RWVs, cartilaginous constructs had biochemical and biomechanical properties superior to those of static or stirred-flask cultures and approaching those of native cartilage [30] (Figure 2c) [33], whereas cardiac tissue constructs consisted of elongated cells that contracted spontaneously and synchronously [9]. Prostate [31] and melanoma [32] cancer-derived cells cultured in RWV bioreactors had 3D structures that reflected the cellular architecture and heterogeneous composition of the tumor site *in vivo*. On the basis of these studies, it was proposed that the RWV bioreactor would support the engineering of tissues and organoids as *in vitro* model systems of tissue development and function [34]. In particular, 3D cell cultures in the RWV bioreactor were used to identify and control various biochemical factors that influence cell function, tissue growth and integration capacity [35], as well as to study phenotypic instability, radiation resistance, and the response of cancer cells to drugs [34].

Bioreactors that perfuse medium either through or around semi-permeable hollow fibers have been used successfully to maintain the function of highly metabolic cells (e.g. hepatocytes) by increasing the mass transport of nutrients and oxygen (Figure 1c). This concept has been extended to engineered tissues by perfusing culture medium directly through the pores of the cell-seeded 3D scaffold, thereby reducing mass transfer limitations both at the construct periphery and within its internal pores (Figure 1d). Direct perfusion bioreactors have been shown to enhance: (i) growth, differentiation and mineralized matrix deposition by bone cells [36,37], (ii) proliferation of human oral keratinocytes [38], (iii) albumin synthesis rates by hepatocytes [25], (iv) expression of cardiac-specific markers by cardiomyocytes [39], and (v) GAG synthesis and accumulation by chondrocytes [23,40]. When incorporated into a bioreactor design, direct perfusion can thus be used as a valuable tool for enhancing cell survival, growth and function. However, the effects of direct perfusion can be highly dependent on the medium flow-rate and the maturation stage of the constructs, as recently demonstrated for 3D cultures of chondrocytes [23]. Therefore, optimizing a perfusion bioreactor for the engineering of a 3D tissue must address a careful balance between the mass transfer of nutrients and waste products to and from cells, the retention of newly synthesized extracellular matrix components within the construct, and the fluid-induced shear stresses within the scaffold pores.

The optimal flow conditions of a bioreactor should not be determined through a trial-and-error approach, but rather should be supported by simulation methods. Computational fluid dynamic (CFD) software packages (e.g. FLUENT, Fluent; http://www.fluent.com) have been increasingly developed during the past decade and are a powerful tool to calculate flow fields, shear stresses and mass transport within and around 3D constructs. Computational fluid dynamic models have been used to calculate the momentum and oxygen transport within a concentric cylinder bioreactor for cartilage tissue engineering and to design and characterize a scaled-up version [41]. A CFD model of direct perfusion through a 3D mesh demonstrated that the random fiber architecture of the scaffold would yield highly variable shear stresses; a scaffold with a homogeneous distribution of pores would therefore enable more precise control over the shear stresses [42]. In this regard, it is becoming increasingly clear that design requirements for scaffolds used in tissue engineering should not be limited to considerations on biocompatibility and mechanical properties [43], but should also include a critical evaluation of pore structure and interconnectivity [44,45], which must be tuned to the specific flow conditions used [46].

Mathematical models of the flow within a perfused RWV bioreactor have been developed and validated against experimental laser-Doppler velocimetry measurements to determine the effects of rotational speed and

perfusion flow rates [47]. Similarly, the flow field and shear stresses around a 3D construct within a spinner-flask bioreactor were characterized through CFD models used in conjunction with particle-image velocimetry measurements [48]. These simulations and measurements could be subsequently combined with histological data of engineered tissues, relating the local fluid-dynamic environment around the construct to the growth process and final properties of the generated tissue, as an extension of a previously developed diffusive model for GAG deposition in cartilaginous constructs grown in a RWV bioreactor [49].

Mechanical conditioning

Increasing evidence suggests that mechanical forces, which are known to be important modulators of cell physiology, might increase the biosynthetic activity of cells in bioartificial matrices and, thus, possibly improve or accelerate tissue regeneration *in vitro* [2]. Various studies have demonstrated the validity of this principle, particularly in the context of musculoskeletal tissue engineering. For example, cyclical mechanical stretch was found to: (i) enhance proliferation and matrix organization by human heart cells seeded on gelatin-matrix scaffolds [50], (ii) improve the mechanical properties of tissues generated by skeletal muscle cells suspended in collagen or Matrigel [51], and (iii) increase tissue organization and expression of elastin by smooth muscle cells seeded in polymeric scaffolds [52]. Pulsatile radial stress of tubular scaffolds seeded with smooth muscle cells improved structural organization and suture retention of the resulting engineered blood vessels, and enabled the vessels to remain open for four weeks following *in vivo* grafting [53]. Dynamic deformational loading or shear of chondrocytes embedded in a 3D environment stimulated GAG synthesis [54,55] and increased the mechanical properties of the resulting tissues [55,56]. Translational and rotational strain of mesenchymal progenitor cells embedded in a collagen gel induced cell alignment, formation of orientated collagen fibers, and upregulation of ligament-specific genes [57]. This study provided evidence that specific mechanical forces applied to 3D cellular constructs might not only enhance the development of an engineered tissue but also direct the differentiation of multi-potent cells along specific lineages.

Despite numerous proof-of-principle studies showing that mechanical conditioning can improve the structural and functional properties of engineered tissues, little is known about the specific mechanical forces or regimes of application (i.e. magnitude, frequency, continuous or intermittent, duty cycle) that are stimulatory for a particular tissue. In addition, engineered tissues at different stages of development might require different regimes of mechanical conditioning owing to the increasing accumulation of extracellular matrix and developing structural organization. In this highly complex field, a comprehensive understanding can only be achieved through hypothesis-driven experiments aimed at elucidating the mechanisms of downstream processes of cellular responses to well-defined and specific mechanical stimuli. In this context, bioreactors can have an important role, providing controlled environments for reproducible and accurate application of specific regimes of mechanical forces to 3D constructs [58] (Figure 1e). This must be coupled with quantitative analysis and computational modeling of the physical forces experienced by cells within the engineered tissues, including mechanically induced fluid flows and changes in mass transport.

The role of bioreactors in applying mechanical forces to 3D constructs could be broadened beyond the conventional approach of enhancing cell differentiation and/or extracellular matrix deposition in engineered tissues. For example, they could also serve as valuable *in vitro* models to study the pathophysiological effects of physical forces on developing tissues, and to predict the responses of an engineered tissue to physiological forces on surgical implantation. Together with biomechanical characterization, bioreactors could thus help in defining when engineered tissues have a sufficient mechanical integrity and biological responsiveness to be implanted [59]. Moreover, quantitative analysis and computational modeling of stresses and strains experienced both by normal tissues *in vivo* for a variety of activities and by engineered tissues in bioreactors, could lead to more precise comparisons of *in vivo* and *in vitro* mechanical conditioning, and help to determine potential regimes of physical rehabilitation that are most appropriate for the patient receiving the tissue [2].

Manufacture of engineered grafts

Ex vivo engineering of cell-based grafts to restore damaged or diseased tissues has the potential to revolutionize current therapeutic strategies for severe physical disabilities and to improve the quality of life. One of the major challenges in reaching this ambitious goal is to translate research-scale production models into clinically applicable manufacturing designs that are reproducible, clinically effective and economically acceptable, while complying with Good Manufacturing Practice (GMP) requirements [60]. Similar to pharmaceuticals, tissue-engineered products have multimillion-dollar expenditures associated with winning regulatory approval, but, unlike many pharmaceuticals, they have not been able to recoup the regulatory costs and enjoy the same high profit margins. Given that any profits that can be recovered over the costs of R and D and clinical trials are highly dependent on the efficiency of the manufacturing system, problems with automation and scale-up have been suggested to be major factors in the failure to commercialize tissue-engineered products successfully (see http://www.spectrum.ieee.org/webonly/wonews/dec02/tissue.html). Therefore, the application of cell-based tissue engineering approaches in routine clinical practice depends crucially on the development of innovative bioreactor systems.

The manufacture of Dermagraft® by Advanced Tissue Sciences (ATS) was a pioneering example of the use of bioreactors in the large-scale production of tissue-engineered products. ATS developed a robotic system to automate culture medium exchange during the cell-expansion phase [61], and a closed bioreactor system to seed and culture 96 tissue-engineered skin grafts with automated medium perfusion [62]. Despite efforts to streamline and automate the tissue engineering process, the bioreactor system was not highly controlled and produced many

batches that were defective, making overall production costs high (see http://www.spectrum.ieee.org/webonly/wonews/dec02/tissue.html).

As envisioned recently for the expansion of hematopoietic stem cells [63], and for the reproducible generation of cell therapeutic products (e.g. AastromReplicell™ System, Zellera; http://www.zellera.de); for the automated manufacturing of tissues bioreactors will need to include state-of-the-art systems for monitoring and controlling the physicochemical culture parameters. Typical environmental factors (e.g. temperature, pH and oxygen) will have to be maintained at defined levels to ensure reproducibility and standardization, as is routinely achieved in classical bioreactors for the production of recombinant proteins. Because the development of engineered tissues might progress at varying rates for different cell batches, additional parameters to be monitored would be cell number, phenotype and metabolism, or specific tissue mechanical properties. Development of the tissue could be further monitored through the incorporation of advanced technical tools for online micro- or macro-scopic observation of the structural properties of the tissue (e.g. video microscopy, magnetic resonance imaging and micro-computerized tomography). All collected inputs could be analyzed by a microprocessor unit and fed back to the bioreactor system to optimize the control of culture parameters at pre-defined levels. Additionally, computational modeling of the parameters acquired online could potentially be integrated to predict the development of the tissue with time, enabling timely planning of the surgery. Although it is unlikely that a bioreactor system capable of monitoring, controlling and modeling all of these parameters is realistic or economically viable in the near-future, the current commercial availability of analysis systems such as the Stat Profile® Critical Care Xpress, or BioProfile® 400 (Nova Biomedical; http://www.novabiomedical.com) demonstrate the feasibility of designing a highly compact, automated and sophisticated system capable of analyzing and managing a large number of physicochemical inputs.

Advanced control systems would facilitate streamlining and automation of the numerous labor-intensive steps involved in the *in vitro* engineering of 3D tissues within a closed bioreactor unit. Owing to similar requirements and constraints, the development of such devices could be drawn from the vast and sophisticated technology used in spaceflight applications, in which compact, automated and controlled systems are essential. Such an approach has been taken by Millenium Biologix (http://www.millenium-biologix.com) where aspects of the space-flown OSTEO™ bioreactor system have been incorporated into the design of ACTES™, an ongoing effort to generate an on-site hospital based Advanced Clinical Tissue Engineering System. Starting with a patient's tissue biopsy, a bioreactor system could isolate, expand and seed specific cell types on a scaffold, and culture the resulting construct until a suitably developed graft is generated, thereby performing the different processing phases within a single closed and automated system (Figure 3). This bioreactor would enable competent hospitals and clinics to carry out

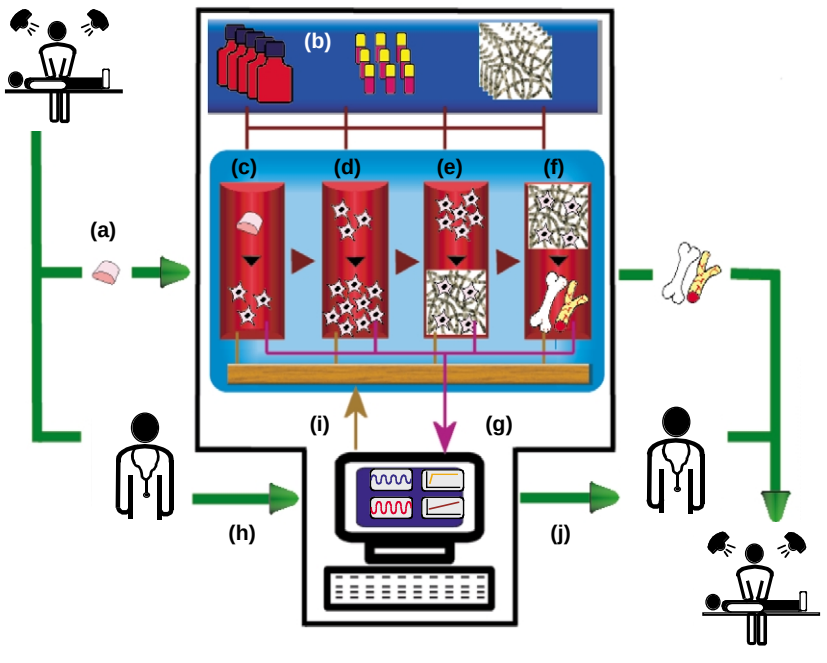

Figure 3. Vision for a closed-system bioreactor for the automated production of tissue-engineered grafts. **(a)** The surgeon would take a biopsy from the patient and introduce it into the bioreactor located on-site at the hospital. **(b)** All reagents (e.g. culture medium, medium supplements, and scaffolds) would be stored in compartments under appropriate conditions (i.e. temperature, humidity). The bioreactor system could then **(c)** automatically isolate the cells, **(d)** expand the cells, **(e)** seed the cells onto a scaffold, and **(f)** culture the construct until a suitably developed graft is produced. **(g)** Environmental culture parameters and tissue development would be monitored and inputs fed into a microprocessor unit for analysis. In conjunction with data derived from clinical records of the patient **(h)**, the inputs would be used to control culture parameters at pre-defined optimum levels automatically **(i)** and provide the surgical team with data on the development of the tissue, enabling timely planning of the implantation **(j)**. Figure generated by M. Moretti.

autologous tissue engineering for their own patients, eliminating logistical issues of transferring specimens between locations. This would also eliminate the need for large and expensive GMP tissue engineering facilities and minimize operator handling, with the final result of reducing the cost of tissue engineered products for the health system and for the community. Altogether, when efficiently designed for low-cost operation, novel bioreactor systems could thus facilitate the distribution of novel and powerful therapeutic approaches that would otherwise remain confined within the context of academic studies or restricted to elite social classes or systems.

Concluding remarks

Progress made in the *in vitro* generation of 3D tissues starting from isolated cells has been hindered by the complexity of the process and by the interplay between different culture parameters. By providing a comprehensive level of monitoring and control over specific environmental factors in 3D cultures, bioreactors can provide the technological means to perform controlled studies aimed at understanding which specific biological, chemical or physical parameter plays which function in engineering a defined tissue. This fundamental interdisciplinary research will provide the basis for identifying environmental and operating conditions required for the generation of specific tissues. At this stage, the transition from laboratory- to industrial-scale will require a switch from highly flexible bioreactors to specialized bioreactors, implementing the defined bioprocesses in a standardized way. The resulting devices will provide an economically viable approach to the automated manufacture of functional grafts, possibly bringing cell-based tissue engineering approaches down from the ivory tower and making them clinically accessible at a larger scale.

References

1 Langer, R. and Vacanti, J.P. (1993) Tissue engineering. *Science* 260, 920–926
2 Butler, D.L. *et al.* (2000) Functional tissue engineering: the role of biomechanics. *J. Biomech. Eng.* 122, 570–575
3 Mazariegos, G.V. *et al.* (2002) First clinical use of a novel bioartificial liver support system (BLSS). *Am. J. Transplant.* 2, 260–266
4 Griffith, L.G. and Naughton, G. (2002) Tissue engineering – current challenges and expanding opportunities. *Science* 295, 1009–1014
5 Cukierman, E. *et al.* (2001) Taking cell-matrix adhesions to the third dimension. *Science* 294, 1708–1712
6 Vunjak-Novakovic, G. *et al.* (1998) Dynamic cell seeding of polymer scaffolds for cartilage tissue engineering. *Biotechnol. Prog.* 14, 193–202
7 Freed, L.E. *et al.* (1997) Tissue engineering of cartilage in space. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13885–13890
8 Holy, C.E. *et al.* (2000) Engineering three-dimensional bone tissue *in vitro* using biodegradable scaffolds: investigating initial cell-seeding density and culture period. *J. Biomed. Mater. Res.* 51, 376–382
9 Carrier, R.L. *et al.* (1999) Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. *Biotechnol. Bioeng.* 64, 580–589
10 Freed, L.E. *et al.* (1998) Chondrogenesis in a cell-polymer bioreactor system. *Exp. Cell Res.* 240, 58–65
11 Ishaug-Riley, S.L. *et al.* (1998) Three-dimensional culture of rat calvarial osteoblasts in porous biodegradable polymers. *Biomaterials* 19, 1405–1412
12 Kim, B.S. *et al.* (1998) Optimizing seeding and culture methods to engineer smooth muscle tissue on biodegradable polymer matrices. *Biotechnol. Bioeng.* 57, 46–54
13 Bruinink, A. *et al.* (2001) The stiffness of bone marrow cell-knit composites is increased during mechanical load. *Biomaterials* 22, 3169–3178
14 Li, Y. *et al.* (2001) Effects of filtration seeding on cell density, spatial distribution, and proliferation in nonwoven fibrous matrices. *Biotechnol. Prog.* 17, 935–944
15 Xiao, Y.L. *et al.* (1999) Static and dynamic fibroblast seeding and cultivation in porous PEO/PBT scaffolds. *J. Mater. Sci. Mater. Med.* 10, 773–777
16 Burg, K.J. *et al.* (2002) Application of magnetic resonance microscopy to tissue engineering: a polylactide model. *J. Biomed. Mater. Res.* 61, 380–390
17 Glicklis, R. *et al.* (2000) Hepatocyte behavior within three-dimensional porous alginate scaffolds. *Biotechnol. Bioeng.* 67, 344–353
18 Nehrer, S. *et al.* (1997) Matrix collagen type and pore size influence behaviour of seeded canine chondrocytes. *Biomaterials* 18, 769–776
19 Vunjak-Novakovic, G. *et al.* (1996) Effects of mixing on the composition and morphology of tissue-engineered cartilage. *AIChE J.* 42, 850–860
20 Wendt, D. *et al.* (2003) Oscillating perfusion of cell suspensions through three-dimensional scaffolds enhances cell seeding efficiency and uniformity. *Biotechnol. Bioeng.* 84, 205–214
21 Xie, Y. *et al.* (2001) Three-dimensional cell-scaffold constructs promote efficient gene transfection: implications for cell-based gene therapy. *Tissue Eng.* 7, 585–598
22 Sodian, R. *et al.* (2002) Tissue-engineering bioreactors: a new combined cell-seeding and perfusion system for vascular tissue engineering. *Tissue Eng.* 8, 863–870
23 Davisson, T. *et al.* (2002) Perfusion increases cell content and matrix synthesis in chondrocyte three-dimensional cultures. *Tissue Eng.* 8, 807–816
24 Radisic, M. *et al.* (2003) High-density seeding of myocyte cells for cardiac tissue engineering. *Biotechnol. Bioeng.* 82, 403–414
25 Kim, S.S. *et al.* (2000) Dynamic seeding and *in vitro* culture of hepatocytes in a flow perfusion system. *Tissue Eng.* 6, 39–44
26 Sutherland, R.M. *et al.* (1986) Oxygenation and differentiation in multicellular spheroids of human colon carcinoma. *Cancer Res.* 46, 5320–5329
27 Martin, I. *et al.* (1999) Method for quantitative analysis of glycosaminoglycan distribution in cultured natural and engineered cartilage. *Ann. Biomed. Eng.* 27, 656–662
28 Ishaug, S.L. *et al.* (1997) Bone formation by three-dimensional stromal osteoblast culture in biodegradable polymer scaffolds. *J. Biomed. Mater. Res.* 36, 17–28
29 Gooch, K.J. *et al.* (2001) Effects of mixing intensity on tissue-engineered cartilage. *Biotechnol. Bioeng.* 72, 402–407
30 Vunjak-Novalovic, G. *et al.* (1999) Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. *J. Orthop. Res.* 17, 130–138
31 Rhee, H.W. *et al.* (2001) Permanent phenotypic and genotypic changes of prostate cancer cells cultured in a three-dimensional rotating-wall vessel. *In vitro Cell Dev. Biol. Anim.* 37, 127–140
32 Licato, L.L. *et al.* (2001) A novel preclinical model of human malignant melanoma utilizing bioreactor rotating-wall vessels. *In vitro Cell Dev. Biol. Anim.* 37, 121–126
33 Pei, M. *et al.* (2002) Bioreactors mediate the effectiveness of tissue engineering scaffolds. *FASEB J.* 16, 1691–1694
34 Unsworth, B.R. and Lelkes, P.I. (1998) Growing tissues in microgravity. *Nat. Med.* 4, 901–907
35 Obradovic, B. *et al.* (2001) Integration of engineered cartilage. *J. Orthop. Res.* 19, 1089–1097
36 Bancroft, G.N. *et al.* (2002) Fluid flow increases mineralized matrix deposition in 3D perfusion culture of marrow stromal osteoblasts in a dose-dependent manner. *Proc. Natl. Acad. Sci. U. S. A.* 99, 12600–12605
37 Goldstein, A.S. *et al.* (2001) Effect of convection on osteoblastic cell growth and function in biodegradable polymer foam scaffolds. *Biomaterials* 22, 1279–1288
38 Navarro, F.A. *et al.* (2001) Perfusion of medium improves growth of human oral neomucosal tissue constructs. *Wound Repair Regen.* 9, 507–512
39 Carrier, R.L. *et al.* (2002) Perfusion improves tissue architecture of engineered cardiac muscle. *Tissue Eng.* 8, 175–188

40 Pazzano, D. *et al.* (2000) Comparison of chondrogenesis in static and perfused bioreactor culture. *Biotechnol. Prog.* 16, 893–896
41 Williams, K.A. *et al.* (2002) Computational fluid dynamics modeling of steady-state momentum and mass transport in a bioreactor for cartilage tissue engineering. *Biotechnol. Prog.* 18, 951–963
42 Raimondi, M.T. *et al.* (2002) Mechanobiology of engineered cartilage cultured under a quantified fluid-dynamic environment. *Biomech. Model. Mechanobiol.* 1, 69–82
43 Risbud, M.V. and Sittinger, M. (2002) Tissue engineering: advances in *in vitro* cartilage generation. *Trends Biotechnol.* 20, 351–356
44 Gomes, M.E. *et al.* (2003) Effect of flow perfusion on the osteogenic differentiation of bone marrow stromal cells cultured on starch-based three-dimensional scaffolds. *J. Biomed. Mater. Res.* 67A, 87–95
45 Zein, I. *et al.* (2002) Fused deposition modeling of novel scaffold architectures for tissue engineering applications. *Biomaterials* 23, 1169–1185
46 Boland, T. *et al.* (2003) Cell and organ printing 2: fusion of cell aggregates in three-dimensional gels. *Anat. Rec.* 272A, 497–502
47 Begley, C.M. and Kleis, S.J. (2000) The fluid dynamic and shear environment in the NASA/JSC rotating-wall perfused-vessel bioreactor. *Biotechnol. Bioeng.* 70, 32–40
48 Sucosky, P. *et al.* Fluid mechanics of a spinner-flask bioreactor. *Biotechnol. Bioeng.* (in press).
49 Obradovic, B. *et al.* (2000) Glycosaminoglycan deposition in engineered cartilage: experiments and mathematical model. *AIChE J.* 46, 1860–1871
50 Akhyari, P. *et al.* (2002) Mechanical stretch regimen enhances the formation of bioengineered autologous cardiac muscle grafts. *Circulation* 106, I137–I142
51 Powell, C.A. *et al.* (2002) Mechanical stimulation improves tissue-engineered human skeletal muscle. *Am. J. Physiol. Cell Physiol.* 283, C1557–C1565
52 Kim, B.S. *et al.* (1999) Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. *Nat. Biotechnol.* 17, 979–983
53 Niklason, L.E. *et al.* (1999) Functional arteries grown *in vitro*. *Science* 284, 489–493
54 Davisson, T. *et al.* (2002) Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. *J. Orthop. Res.* 20, 842–848
55 Waldman, S.D. *et al.* (2003) Effect of biomechanical conditioning on cartilaginous tissue formation *in vitro*. *J. Bone Jt Surg. Am.* 85A (Suppl. 2), 101–105
56 Mauck, R.L. *et al.* (2000) Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. *J. Biomech. Eng.* 122, 252–260
57 Altman, G.H. *et al.* (2002) Cell differentiation by mechanical stress. *FASEB J.* 16, 270–272
58 Démarteau, O. *et al.* (2003) Development and validation of a bioreactor for physical stimulation of engineered cartilage. *Biorheology* 40, 331–336
59 Démarteau, O. *et al.* (2003) Dynamic compression of cartilage constructs engineered from expanded human articular chondrocytes. *Biochem. Biophys. Res. Commun.* 310, 580–588
60 Ratcliffe, A. and Niklason, L.E. (2002) Bioreactors and bioprocessing for tissue engineering. *Ann. New York Acad. Sci.* 961, 210–215
61 Leventon, W. (2002) Synthetic skin. *IEEE Spectr.* 39, 28–33
62 Naughton, G.K. (2002) From lab bench to market: critical issues in tissue engineering. *Ann. New York Acad. Sci.* 961, 372–385
63 Cabrita, G.J. *et al.* (2003) Hematopoietic stem cells: from the bone to the bioreactor. *Trends Biotechnol.* 21, 233–240
64 Jasmund, I. and Bader, A. (2002) Bioreactor developments for tissue engineering applications by the example of the bioartificial liver. *Adv. Biochem. Eng. Biotechnol.* 74, 99–109
65 Martin, I. *et al.* (2000) Modulation of the mechanical properties of tissue-engineered cartilage. *Biorheology* 37, 141–147