

TOPICAL REVIEW

Biofabrication: a 21st century manufacturing paradigm

V Mironov¹, T Trusk¹, V Kasyanov², S Little³, R Swaja⁴ and R Markwald¹

¹ Medical University of South Carolina, Charleston, SC 29425, USA

² Riga Stradins University, Riga, Latvia

³ South Carolina EPSCoR/IDeA Program, Columbia, SC, USA

⁴ South Carolina Bioengineering Alliance, Charleston, SC 29425, USA

Received 6 February 2009

Accepted for publication 13 May 2009

Published 10 June 2009

Online at stacks.iop.org/BF/1/022001

Abstract

Biofabrication can be defined as the production of complex living and non-living biological products from raw materials such as living cells, molecules, extracellular matrices, and biomaterials. Cell and developmental biology, biomaterials science, and mechanical engineering are the main disciplines contributing to the emergence of biofabrication technology. The industrial potential of biofabrication technology is far beyond the traditional medically oriented tissue engineering and organ printing and, in the short term, it is essential for developing potentially highly predictive human cell- and tissue-based technologies for drug discovery, drug toxicity, environmental toxicology assays, and complex *in vitro* models of human development and diseases. In the long term, biofabrication can also contribute to the development of novel biotechnologies for sustainable energy production in the future biofuel industry and dramatically transform traditional animal-based agriculture by inventing 'animal-free' food, leather, and fur products. Thus, the broad spectrum of potential applications and rapidly growing arsenal of biofabrication methods strongly suggests that biofabrication can become a dominant technological platform and new paradigm for 21st century manufacturing. The main objectives of this review are defining biofabrication, outlining the most essential disciplines critical for emergence of this field, analysis of the evolving arsenal of biofabrication technologies and their potential practical applications, as well as a discussion of the common challenges being faced by biofabrication technologies, and the necessary conditions for the development of a global biofabrication research community and commercially successful biofabrication industry.

Introduction

The launch of the new journal *Biofabrication* was an important milestone which manifested past predictions of the emergence of a new dramatic research field or discipline based on bridging the life, physical, and engineering sciences to address global priorities. Many believe that biofabrication is this anticipated field and that it has the potential to emerge as the leading manufacturing paradigm of the 21st century. The primary objectives of this topical review are to provide a basis for future

activities in the area by presenting (i) a comprehensive survey of the state of the art of biofabrication including objectives, applications, approaches, and enabling technologies; and (ii) a discussion of opportunities and challenges in realising the potential economic and healthcare benefits.

1. Definition and scope of biofabrication

Defining an emerging field of science or technology is essential to provide a uniform framework for the development and

implementation. Biofabrication is a technology, as opposed to a basic science, and is part of the much broader field of biotechnology. Whereas science involves observation, modeling, and explanation of natural phenomena, technology deals with engineering an artificial world. The prefix 'bio' implies that either raw materials, or processes, or final products (or all these) must be biology inspired or biology based. Raw materials for biofabrication can be biological molecules, extracellular matrices, living cells and tissues, and possibly decellularized organs. The term 'fabrication' means making or constructing something from a raw or semi-finished material or creating something that is different from its components. In this context, biofabrication deals with science, engineering and technology or production, based on using living matter as raw materials.

With regard to its relation to other biotechnologies, isolated biological molecules are generally not considered living matter, and the broadly shared and well-established consensus is that only living cells represent elementary units and manifestations of life [1]. Thus, the acellular synthesis of genes or chemical synthesis of biomolecules does not precisely fit the above definition of biofabrication and rather belongs to the domains of synthetic biology or biomaterials science. From another viewpoint, the synthesis and fabrication of protocells or artificial minimal living cells [2] is an overlapping area between biofabrication and synthetic biology. Microfabrication of microfluidic devices and bioreactors also, technically, does not belong to the biofabrication domain, although the situation changes when these devices are used for cell patterning or tissue fabrication. Microfabrication and microfluidics, and nanofabrication and nanotechnology can all contribute to biofabrication when living cells or molecules are used as construction building blocks in combination with micro/nano-featured materials and devices. Although living cells are often employed in industrial biomanufacturing processes, the manufacturing of drugs and molecules using living cells is generally considered to be within the classical biomanufacturing domain which usually does not create more complex living products than the original biological raw materials. However, in some cases (e.g. leather or fur production from living cells and biofuel production from algae), biofabrication and biomanufacturing can overlap. Some areas of biofabrication also encompass manufacturing aspects of tissue engineering. Whereas tissue engineering is a biomedical field focused primarily on replacement, repair, and regeneration of injured or diseased human tissues and organs [3], potential applications of biofabrication are much broader and not limited to biomedical applications or using only human cells.

Based on these considerations, biofabrication can be narrowly defined as the production of complex biological products using living cells, molecules, extracellular matrices, and engineered biomaterials. This definition does limit biofabricated products to living tissues or organs, but it implies that raw materials must include living or bio-inspired matter, component processes must be biology based and intermediate products must be complex living tissue constructs. However, according to a more inclusive definition, biofabrication

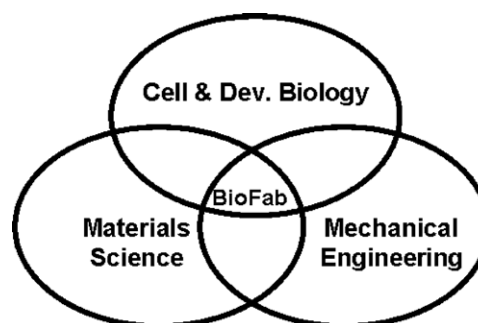


Figure 1. The main disciplines contributing to the emergence of biofabrication: cell and developmental biology, mechanical engineering, and biomaterials science.

encompasses a broad range of physical, chemical, biological, and/or engineering processes with various applications in tissue science and engineering, disease pathogenesis, and drug pharmacokinetic studies, biochips and biosensors, cell printing, patterning and assembly, and emerging organ printing.

2. Origins and components of biofabrication technology

Biology (cell, tissue, developmental, and stem cell), mechanical engineering (CAD/CAM, additive manufacturing), and materials science (primarily biomaterials) constitute the main disciplines and basic technological pillars of biofabrication (figure 1).

The classic 1907 paper [4] of the American developmental biologist Ross Harrison on growing tissue explants *in vitro* was a foundation for modern cell culturing which is an integral component of biofabrication technology. Mainly based on his work in the field, Dr Harrison was nominated several times for the Nobel Prize. The sequential series of 1950s and 1960s pioneering works by developmental biologists such as Malcolm Steinberg led to early attempts to 'reconstruct' (now called 'tissue engineering') 3D tissues *in vitro* from dissociated cells using a self-assembly process [5]. The concepts of tissue fusion and tissue fluidity are fundamental for the modern organ printing technology based on directed tissue self-assembly. The experimental measurements of tissue surface tension using tensiometers performed by Gabor Forgacs validated the concept of tissue fluidity and Steinberg's differential adhesion hypothesis [6–8]. These cell culture techniques, concepts of tissue fluidity and tissue fusion are the important biological bases of biofabrication technology.

Cell survival during the biofabrication process and sequential tissue construct self-assembly, vascularization, and tissue maturation are the important biological problems associated with fabrication. Stem cell biology is a potentially important component for biofabrication because stem cells are the main raw materials for tissue bioassembly. Extensive knowledge of human anatomy and clinical imaging is also critically important, especially in biomedical applications of biofabrication technology. Although in previous centuries, anatomy was predominantly an analytical science employing

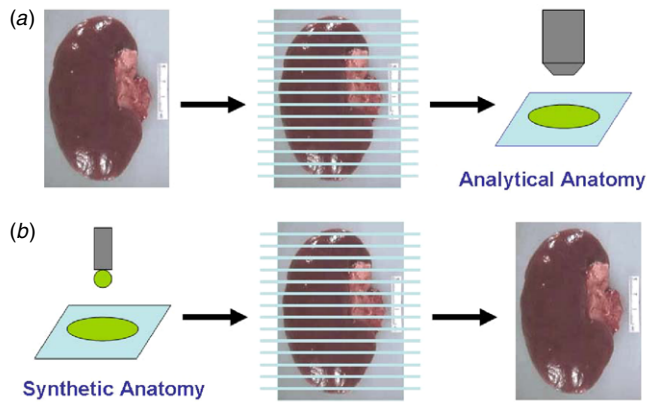


Figure 2. Transformation of analytical anatomy (a) into synthetic anatomy (b).

reductionistic approaches [9], 21st century anatomy is emerging as a synthetic science (figure 2). Current anatomists have the potential to support biofabrication as specialists who can design ‘virtual organs’ [10], provide a ‘blueprint’ for organ and tissue printing, and serve as quality control experts in the biofabrication industry.

Mechanical engineering has always been a fundamental engineering discipline for fabrication and manufacturing technologies. Changing raw materials from non-living to living matter does not change the fundamental role of mechanical engineering in designing fabrication processes and manufacturing technologies. Biomedical applications of rapid prototyping (computer-aided layer-by-layer additive manufacturing) are an important example of how mechanical engineering contributes significantly to the emerging field of biofabrication [11–16]. Mechanical engineering is a second pillar and an integral component of biofabrication, and the contribution of mechanical engineering to additive manufacturing, in particular, and to biofabrication, in general, will undoubtedly grow in the future.

The third integral component of biofabrication is materials science with an emphasis on biomaterials. The design and synthesis of photosensitive functionalized polymers and *in situ* cross-linkable hydrogels are just two examples of areas where materials science and chemical engineering are enabling applications of already existing rapid prototyping technologies (such as stereolithography) and building foundations for novel biofabrication technologies (such as centrifugal casting). Synthesis of precisely tailored, bioprocessible, functional and biomimetic extracellular matrices and stimuli-sensitive or intelligent hydrogels must be based on carefully formulated design specifications for particular biofabrication technologies. The demand for new biomaterials will continue to grow, and the value of materials science in this effort cannot be underestimated.

3. Growing arsenal of biofabrication technologies

3.1. Solid scaffold-based biofabrication

Solid scaffold-based biofabrication is a classic and still the most popular tissue engineering approach (figure 3).

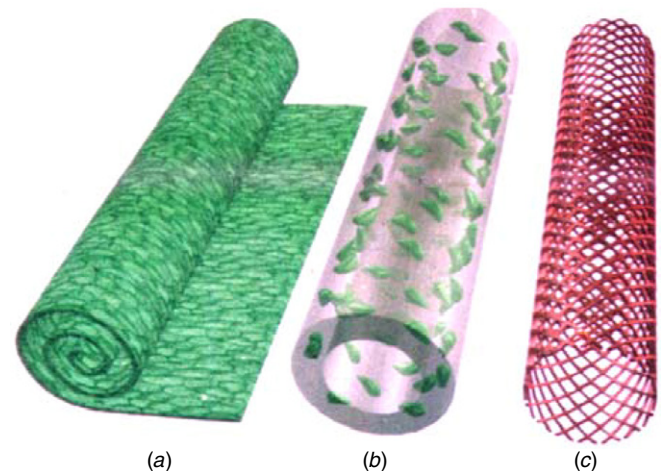


Figure 3. Three most popular biofabrication methods in tissue engineering: (a) cell sheet technology—rolling a cell sheet into a tubular construct; (b) embedding cells into a 3D hydrogel and molding a tubular construct; and (c) cell seeding in a porous solid biodegradable scaffold.

Originally solid scaffold technology was invented by the world famous Robert Langer and his group at MIT. The ground breaking classical science paper written by Robert Langer and Joseph Vacanti is one of the most cited papers in the tissue engineering field [17]. A scaffold is a temporary supporting structure and according to definition it must be biodegradable. There are synthetic and naturally derived solid scaffolds. Synthetic solid scaffold technology allowed us to create the first tissue-engineered products. Innovation in solid scaffold design is continuous. The recent explosion of interest in electrospinning technology (see below) is just one example. Self-assembling matrices and scaffolds are another interesting direction. However, there are certain intrinsic limitations of the ‘top down’ approach which is still predominantly employed in solid scaffold-based biofabrication technology [18].

The recent success of tissue-engineered bladder and bronchus using solid naturally derived scaffolds is another powerful demonstration of the importance of solid scaffold-based technology for tissue engineering of relatively simple organs [19, 20]. However, it is still hard to imagine and probably naive to believe that such complex organs as the human heart, liver, and lung can be engineered by simple seeding or the injection of stem cells into a decellularized matrix of corresponding organs. Artificial human natural solid scaffolds have been recently produced using living human cells *in vitro* [21]. They are allogeneic and thus much better from an immunological point of view than animal-derived xenogeneic natural scaffolds. Moreover, they can be designed and engineered with a high level of control and eventually eliminate the need for cadaveric and animal-derived biomaterials. The biofabrication of decellularized human natural biodegradable solid scaffolds using allogeneic and, maybe eventually, autologous human cells, must be systematically explored. It remains to be seen whether the more controlled and more flexible tissue engineering biofabrication of natural scaffolds allows us to overcome intrinsic limitations of the ‘top down’ solid scaffold-based approach. One potential option is a

‘digitalization’ of scaffolds, which will enable their utilization in a more promising ‘bottom up’ approach such as ‘digital bioprinting’ (see below).

Unfortunately, solid scaffolds fabricated using additive manufacturing are facing the same intrinsic limitations typical for the ‘top down’ solid scaffold-based approach. Thus, besides the obvious possible strong potential for skeletal tissue engineering [11–16], it is difficult to see much advantage in using ‘two step’ biofabrication of solid synthetic biodegradable scaffolds fabricated using a rapid prototyping device (first step) and sequential cell seeding of scaffold in bioreactors (second step) in biofabrication of thick 3D soft tissues and, especially, 3D human organs such as the kidney, liver and heart. However, the ‘one step’ bioprinting approach based on simultaneous deposition of hydrogels mixed with living cells using, for example, stereolithography and a functionalized photosensitive hydrogel with living cells, definitely has much greater appeal, and several groups around the world are already systematically exploring this promising approach [22–26].

3.2. Embedding and molding technology

Embedding or molding biofabrication technologies using a mixture of collagen and other hydrogels with living cells are a very popular approach in tissue engineering (figure 3). There are, however, two intrinsic problems with this technology: undesirable tissue contraction and relatively low initial cell density. It also usually takes a long time to compact the hydrogel and increase cell density and remodel the initial tissue constructs with new extracellular matrix (ECM) synthesized by cells [27–31].

Recent attempts to use hyaluronan and other additive hydrogels to reduce tissue contraction do not solve the problem of low cell density [32–34]. Embedding biofabrication technology demonstrates certain promising results in the cases of biofabrication of tissue-engineered vascular graft and heart valves [35, 36]. New rapidly emerging accelerated tissue maturation technologies can potentially improve biofabrication based on embedding and molding.

3.3. Cell sheet technology

Cell sheet technology is a typical solid scaffold-free self-assembly technology and a serious alternative to solid scaffold-based biofabrication (figures 3(a) and 4). The main advantage of cell sheet technology is initial high cell density. Stacked or rolled layers of engineered tissue can also be fused as a result of the tissue fusion process and form thicker constructs. Professor Teruo Okano from Japan is a world recognized leader in developing, optimization (using thermo-reversible polymer), and clinical translation of this technology [37–40]. Francua Auger’s group in Quebec, Canada, was also instrumental in building the first completely biological tissue-engineered vascular graft using cell sheet technology [41]. The modified variant of this technology is now the commercialized process of Cytograft Tissue Engineering, Novato, CA, USA. The first published results of the ongoing clinical trial look impressive [42–44]. It is logical to

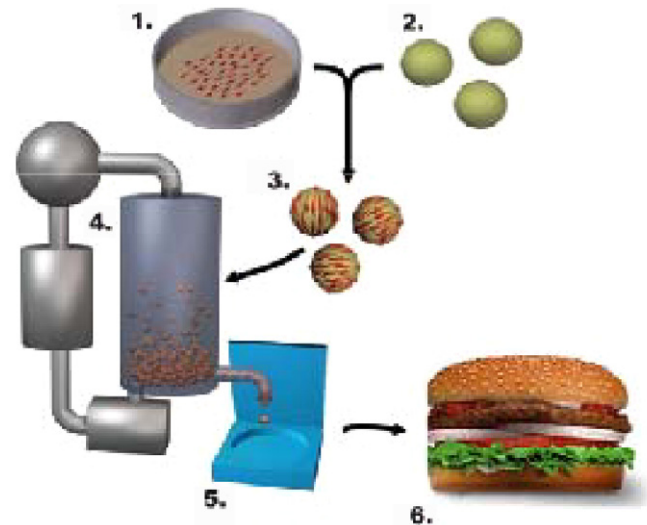


Figure 4. Industrial biofabrication of tissue engineering food: 1—myoblasts—skeletal muscle tissue progenitor cells, 2—porous microspheres from edible polymers, 3—myoblasts seeding on porous microspheres, 4—bioreactor, 5—microwave and 6—hamburger.

assume that the next step will be biofabrication of autologous human heart valves for pediatric patients using cell sheet technology. The development of a ‘robotic cell sheet stacker’ or ‘tissue bioassembler’ will be a logical development of cell sheet technology and an important step toward its commercialization.

3.4. Organ printing: directed tissue self-assembly

We defined organ printing as a biomedical application of rapid prototyping or layered additive biomanufacturing by employing self-assembling tissue spheroids as building blocks [45–48]. The fundamental principle of this technology is a tissue fusion of closely placed tissue spheroids. Tissue fusion is a ubiquitous process during embryonic development [49]; therefore, this biofabrication technology is biomimetic. The recently reported bioprinting of a branched vascular tree using this technology is probably the most exciting accomplishment of this approach [50]. Bioprinting of a functional and perfusable branched intraorgan vascular tree will be an important milestone on the roadmap to organ printing. Robotic computer-aided precise punching of tissue spheroids in sequential layers of sprayed hydrogel with a thickness comparable to the diameter of tissue spheroids is probably the most realistic variant of organ printing technology. Organ printing technology faces a lot of serious challenges [48]. However, fully developed organ bioprinting will allow us to build 3D vascularized functional human organs or living functional organ constructs suitable for surgical implantation. The robotic computer-aided organ printing technology is scalable and can be completely automated, which holds a strong industrial appeal.

3.5. Digital bioprinting

The digital printing work recently introduced by Dr Hod Lipson's group at Cornell University [51, 52] has not yet been systematically applied to the biofabrication of living tissue. However, the striking similarity of the digital materials (plastic spheroids) employed in the Cornell University digital printer with tissue spheroids indicates that the development of a digital bioprinter is just a matter of time and investment. Magnetic interactions instead of electrostatic interactions can be employed in order to control dispensing and the precise placing of tissue spheroids labeled with magnetic nanoparticles. Using 'key and lock' shaped digital living biomaterials or building tissue blocks can also allow fast bioassembly of 3D tissue constructs even without a bioprinter. Ali Khademhosseini from Harvard University is one of the active proponents and emerging leaders of this very promising approach [53, 54]. Professor Michael Sefton's group from University of Toronto, Canada, made impressive advances by putting tissue-engineered modules created from living cells embedded in hydrogels into a confined tubular space [55–57]. However, using confined space and encapsulation methods makes this approach, in certain aspects, similar to traditional molding technology. But as it has been demonstrated, the digitalization of molding technology and the creation of separate tissue modules ('bottom up' approach) can make a big difference and allow us to design tissue constructs suitable for perfusion [57]. This technology has an immediate practical application for extracorporeal tissue-engineered organs such as the liver. The adaptation of this technology for implantable tissue-engineered organ constructs will probably be much more challenging.

3.6. Inkjet bioprinting

Inkjet bioprinting is one of the most exciting and elegant biofabrication technologies. The pioneer of this technology, Dr Thomas Boland from Clemson University, South Carolina, and his followers have shown both a high level of control on cell dispensing as well as remarkable viability and certain functionality of printed cell patterns using different cell types [58–63]. There are also certain promising results for bioprinting 3D tissue constructs using inkjet bioprinting [64, 65]. However, the issue of cell density and demonstration of authentic functional and structural 3D tissue organization continue to be an unsolved problem. Several groups around the world are actively working on improvement of inkjet bioprinting technology. Inkjet bioprinting *in vivo* has recently been proposed [66]. The inkjet bioprinting technology can benefit enormously from using the digitalized version of cellularized solid scaffolds and hydrogels. It will allow us to make a new type of 'bioink' which can be both 'inkjettable' and permissive of post-printing tissue self-assembly. One of potential advantages of using pre-assembled and bioprocessible (tolerable to the inkjet printing process) microtissues with a high level of initial cell density instead of single cells in suspension or in a hydrogel drop is a dramatic reduction of printing time. At least one group recently demonstrated the principal feasibility of

such an approach [67]. The 'one cell in drop' approach represents potentially misleading technological specifications by completely ignoring the obvious specific requirements of the tissue bioassembly process, such as initial high cell density. At this stage of the technology development it is very difficult (but not impossible) to imagine how the 'one cell in drop' approach could lead to inkjet bioprinting of thick 3D tissue and organ constructs. However, inkjet printing still has strong immediate short-term applications in molecular and cell patterning and can be effectively employed in genomics, proteomics, as well as in rapid biofabrication of cell-based assays. The most attractive feature of inkjet technology is that inkjet bioprinters are relatively cheap and affordable. Moreover, from the long-term point of view, the existence of profitable and well-established inkjet printer technology with global companies such as Hewlett Packard, Lexmark, and Cannon could be an important advantage especially when the technology becomes mature enough for commercialization. Some industrial research organizations such as Palo Alto Research Center (PARC) [68] are already systematically working on exploring the potential of industrial inkjet bioprinting.

3.7. Centrifugal casting

Centrifugal casting is one of the oldest fabrication technologies, but it has only recently been employed in biofabrication. Centrifugal forces allow us to fabricate a tubular scaffold with high cell density in a porous scaffold. However, the combination of centrifugal casting technology and *in situ* cross-linkable hydrogels synthesized by Glenn Prestwich gave most exciting results and technologies, which have been recently reviewed in [69]. Centrifugal casting in this variant offers a unique opportunity to biofabricate living vascular tubular tissue constructs with high cell density in just 10 min [70, 71]. Another important advantage of centrifugal casting is that it could potentially be a bioreactor-free technology [69]. Centrifugally casted tubular tissue constructs can be made ready for implantation immediately after biofabrication. There are many tubular organs in the human body. However, tubular constructs fabricated by concentric centrifugal casting can be longitudinally cut and, thus, this technology can also be used for production of flat living tissue constructs such as skin. The simplicity of the centrifugal casting technology, its capacity to achieve a high level of cell density and a stratified structure in a very short time and with low cost make it very attractive and promising.

3.8. Biospraying

Although a spraying technique and an associated device (for example the Baxter spraying device) are already commercially available, it has only recently started to be systematically used for biofabrication. The most interesting aspect of biospraying technology is that in certain cases it can be used *in vivo*. Mixing of hydrogels with living cells is very easy in Baxter spraying devices. There were some reports in the popular press on developing a stem cell gun which was probably based on spraying technology. The

fact that the leading medical device company, Baxter, is interested in developing tissue engineering and cell therapy applications of this promising technology and has already developed many clinically proven devices and accumulated certain level of expertise is very encouraging. The rapid commercialization and broad applications of biospraying technology can transform it to one of the leading biofabrication technologies; at least in the biomedical field one can call 'self-directed self-assembly'.

3.9. Dielectrophoresis for biofabrication

Dr Sangeeta Bhatia from MIT first introduced stereolithography as a 'one step' method of robotic biofabrication of tissue-engineered constructs [72], and later developed dielectrophoresis technologies to control the cell pattern in photosensitive hydrogels [73]. The impressive results and elegant simplicity of this innovative approach suggest a strong potential for this unique biofabrication method. Most importantly, the combination of dielectrophoresis and stereolithography opens up opportunities for high-level precision control of cell distribution and cell density and could be an important advancement in biofabrication technology. Another important characteristic of this technology is that rapid prototyping stereolithography machines are already commercially available and just need to be adapted for using new cell-friendly functionalized processible photosensitive polymers.

3.10. Magnetic force-driven biofabrication

The development of magnetic force-driven tissue engineering is one of the most impressive manifestations of nanotechnological impact on biofabrication. Initially developed by a Japanese group [74], this fast evolving technology now has committed followers in the UK [75], and other places [76]. Magnetic nanoparticles and magnetic microbead-labeled endothelial cells have been successfully used for accelerated endothelialization in vascular tissue engineering [77, 78]. The combination of magnetic force-driven tissue engineering and digital bioprinting is potentially one of the most promising areas in biofabrication. The systematic exploration of the magnetic force-driven tissue engineering approach could enable digital bioprinting and lead to the development of powerful biofabrication technology.

3.11. Electrospinning or nanostructuralized scaffold-based biofabrication

Electrospinning is becoming another fast growing area at the interface of nanotechnology and tissue engineering. Both synthetic and natural polymers as well as mixtures have been successfully employed for electrospinning [77, 79]. Nanostructuralized tissue-engineered scaffolds have been explored by Dr Cato Laurensin [80] and some other groups. Electrospinning is a relatively simple and cost-effective electrospinning technology that has certain advantages compared to scaffolds created by traditional methods. Several companies are actively involved in the commercialization of

electrospinning technology in tissue engineering. One of the problems of electrospinning technology is cell seeding. Dense biomimetic nanostructuralized matrices are excellent for cell adhesion, but not often permissive of cell migration. There are several approaches to overcoming this obvious limitation. The first approach is based on using so-called sacrificial biopolymers or cryospinning, which allows us to create holes of the desired size in electrospun matrices [81, 82]. The second, and probably the most innovative idea, is based on simultaneous electrospinning of polymers and cells encapsulated in hydrogels [83]. Finally, it is possible to use rolling technology: after cell seeding electrospun matrices are rolled into tubular constructs [84]. Elegant simplicity, rapid fabrication and cost-effectiveness make this approach very promising. Another problem of electrospun nanostructuralized scaffolds is their inferior biomechanical properties. Although initially encouraging results obtained by using electrospun tissue-engineered vascular graft *in vivo* have been recently reported [84], we must not forget earlier attempts in the 1980s at using electrospun non-biodegradable vascular grafts [85]. Thus, in the case of an effective solution of the above-described technical problems electrospinning could emerge as a biofabrication technology with a strong translational potential.

3.12. Continuous and digital microfluidic-based biofabrication

Microfluidics is still not extensively used in tissue engineering; however, it has a strong potential for large-scale biofabrication technologies [86]. Continuous microfluidics can create solid collagen-based microchannelled scaffolds which can be stacked or rolled and be used for bioassembly of perfusable 3D tissue constructs [87, 88]. Design and fabrication of microfluidic hydrogels is a very recent trend in continuous fluidics [89–91]. It remains to be seen how cellularized and endothelialized microfluidic hydrogels can be effectively perfused in the case of a scalable stacking bioassembly approach. Digital (discrete) microfluidics can be used for large-scale biofabrication of different forms of tissue spheroids. For example, some digital microfluidic devices have a very impressive capacity to generate 10 000 drops s^{-1} . Another possible application of digital microfluidics is designing chaotic micromixers [92, 93], which could enable more effective cell mixing and cell patterning during the dispensing process. The enormous potential of microfluidics, especially in large-scale biofabrication, remains to be systematically explored. The large-scale generation of standardized living building blocks offered by digital microfluidics, as well as microfabrication of perfused microchannelled scaffolds and microfluidic hydrogels, are the two most promising areas of potential application of microfluidics in biofabrication. Microfluidic devices are a promising technological platform for bioreporters and biosensors developed by NASA and can also be used for maintaining viability of *in vitro* cell- and tissue-based drug discovery and drug toxicity assays [94].

4. Common challenges in biofabrication technologies

The fast evolving arsenal of biofabrication technologies and biofabrication technological platforms is very diverse. However, there are certain identifiable common challenges which are shared by all biofabrication approaches. First, fabrication is a multidisciplinary field and technology and the assumption that one person can somehow equally successfully comprehend and effectively implement all aspects of biofabrication technology is rather naive. Thus, creating a well-managed multidisciplinary team and even a multidisciplinary research center is not just wishful thinking, it is a must, or necessary precondition, for sustainable technological progress and advances. Such multidisciplinary centers must have a critical mass of well-integrated and well-managed experts with diverse disciplinary backgrounds and have access to expensive shared equipment and infrastructure. Second, access to biofabrication raw materials such as a broad spectrum of stem cells, hydrogels and biomaterials is also essential. Third, biofabrication research centers must have sophisticated hardware and software which include biofabrication tools such as cell sorters, bioprinters, rapid prototyping machines, and CAD software. Affordable biofabrication tools are essential for small biofabrication groups, whereas for a large research center the effective utilization of expensive equipment is the main challenge. Fourth, research centers and groups must be equipped with, or at least have unlimited access to, broad spectrum destructive and non-destructive living and non-living material characterization tools for structural and functional characterization, and bioreactor-based monitoring of biofabricated constructs. Fifth, research centers or groups must have experts in mathematical modeling and computer simulation of all the steps of the biofabrication process. *In silico* biofabrication can enhance and ensure effective optimization of all the steps of the biofabrication process. Sixth, the viability and sterility of biofabricated constructs are extremely important due to the living nature of biofabricated products. The development of a new generation of sophisticated perfusion bioreactors is a critical component of biofabrication technology. The viability for at least some types of tissue constructs is closely related to solving the problem of vascularization. Seventh, in order to ensure smooth industrial translation and commercialization, the often ignored issues of scalability and cost-effectiveness of biofabrication technology must be an integral part of intellectual challenges in biofabrication already at the ideation stage. Finally, the effective functioning of, and the systematically integrated technology development in, a complex multidisciplinary team of fabrication research centers are only possible in the case of long-term sustainable financial support. The switching of research direction without systematic analysis and solving of fundamental biofabrication problems, and failure to accumulate the necessary expertise in technology development and optimization is a recipe for designing inferior and non-translatable biofabrication technology. Forceful accelerated technology development for premature delivery

usually does not produce a desirable outcome. The biofabrication technology has to be 'ripe'.

5. Engineering principles in biofabrication

In Drew Endy's influential paper 'Foundations for engineering biology' in *Nature* several important basic engineering principles have been identified [95]. They include standardization, decoupling, and abstraction. Although the cited paper focuses on a new field of synthetic biology, it also provides an interesting insight into general engineering principles, which can be effectively employed in the biofabrication field. System engineering also means continuous optimization of the process based on feedback or statistical analysis of the measurable and quantitative parameters of the process performance and outcome is essential for the development of reproducible biofabrication technology.

5.1. Standardization

Standardization is a keystone of modern engineering, fabrication, and manufacturing. Without generally accepted and enforced standards and standardization, it will not be possible to assemble any complex machine in a global world with increasing labor division and outsourcing practice. Standardization means employing the modular principle of assembly and fabrication. For example, using tissue spheroids as a building block is a classic example of implementation of the modular approach in biofabrication. Standardization also means that the design parameters and measurable product specifications and boundary conditions of the biofabrication process must be carefully formulated from the beginning of the project, as early as at the product and process ideation stage. Only precise specifications will allow us to find an optimal compromise between biologists and mechanical engineers in designing the biofabrication process. Finally, standardization and the associated modular approach are necessary preconditions for large-scale automated industrial biofabrication.

5.2. Decoupling

The second engineering principle is decoupling. Decoupling is the idea that it is useful to divide a complicated problem into many simpler problems that can be worked on independently, such that the resulting work can eventually be combined to produce a functioning whole. The decoupling of design and fabrication is also essential. The art of system engineering is in its capacity to reduce or decouple engineering problems of a complex project, such as organ printing, into a series of simple, doable but separated immediate research tasks without compromising the long-term compelling conceptual vision which ensures that all the separated developed technology components are not only integrable but also scalable.

5.3. Abstraction

Abstraction is the third engineering principle. Abstraction is a powerful technology for managing biofabrication project complexity. The essential information specifying desirable biological functions of tissue constructs must be organized across levels of complexity using abstraction hierarchies. The abstraction hierarchies must allow us to work at any one level of complexity without regard for the details that define the other levels. Abstraction is an important tool for managing a multidisciplinary group of specialists in a biofabrication project. Modern implementation of the abstraction principle of system engineering needs is based on employing and effective utilization of sophisticated mathematical modeling and computer simulation approaches (see more below).

6. Why affordable biofabrication tools are essential?

The selection of research topics and following scientific trends is a very complicated multifaceted process. It is based on the perception and reception of certain signals. One potential signal is a situation where suddenly well-known, respected and world-recognized research leaders in the scientific community start moving aggressively in a new area, or when top journals start publishing papers related to this new research direction, or when funding agencies and bodies start promoting certain specific research areas, or when a new journal is launched. The potential followers must be sure that the research topic is fundable, that research in this direction is in high demand and publishable and that there is plenty of room for their creative contribution. There is no doubt that biofabrication provides such opportunities. Unfortunately, biofabrication research tools such as rapid prototyping machines and bioprinters are still very expensive and not accessible to the majority of potential followers. In this context, it is extremely important to provide access to affordable biofabrication tools to a maximum number of possible followers or scientists and engineers who want to join the evolving field of biofabrication. One innovative strategy, which has been perfectly executed by a group at Cornell University, is enabling access to an affordable printer which they call 'fabber' to a maximal number of interested people by putting detailed information about the design and assembly of the printer on the website, and by providing all possible technical assistance including creating an analogy of an international social network (see [fab@home](#)). The second possible strategy is to work at the small scale by focusing on small and narrow but critically important questions using, for example, manual biofabrication. This approach works well in the case of manual bioprinting at small scale using self-assembling tissue spheroids. Finally, the third strategy is an *in silico* approach or performing all research including design, biofabrication and testing of biofabricated products using mathematical modeling and computer simulation. It is obvious that the more the biofabrication tools are accessible the faster this field and associated research community will grow.

7. Role of mathematical modeling and computer simulation

Modern fabrication is unthinkable without employing sophisticated mathematical modeling and computer simulation. Moreover, not only the designing process, but also the assembly process, including designing the assembly line and final product testing, is now performed *in silico*. We strongly believe that biofabrication from the start must be a predictable technology and built on predictable models and measurable parameters. Practically, all essential steps of the biofabrication process could be and must be the subject of rigorous mathematical modeling and computer simulations. Our experience demonstrates that even the biological processes associated and explored during the biofabrication process can be the subject of predictable mathematical modeling and computer simulation [96]. The open access CAD software packages posted on the [fab@home](#) website allow us to create a blueprint of tissue-engineered scaffolds and biofabricated organs and tissues. Finite element analysis software packages allow us to predict permeability and the mechanical properties of fabricated scaffolds and tissue constructs based on their porosity and employed biomaterial mechanical properties. For example, the extrusion process can be effectively modeled with MoldFlow software [97]. Tissue assembly can be modeled with the molecular dynamics and Surface Evolver software (<http://www.susqu.edu/brakke/evolver/evolver.html>) [98]. Perfusion in bioprinted constructs created from partially fused tissue spheroids can be modeled and simulated using the lattice Boltzman approach (LBflow software) and computational fluid dynamics software such as Fluent [99]. Thus, there is plenty of room for mathematicians, computer engineers, and mechanical engineers to use their mathematical modeling and computer simulation skills in biofabrication.

8. Emerging biofabrication research community

8.1. Essential steps in building a biofabrication research community

The organization of a vibrant global biofabrication research community is the first and probably the most essential step in ensuring the emergence of future biofabrication technology and industry. Such a process usually takes several years and is driven by recruitment to the emerging field of a critical mass of experts from different disciplines. In the biofabrication field, this process has already been initiated but not completely formalized. The indicators of the evolution of a new research community around a new research field include the emergence of research groups and specialized research centers; organization of special sessions, symposiums, conferences, and congresses; publication of new journals and textbooks; development of training courses; and, finally, organization of professional societies.

8.2. Emerging biofabrication research groups and centers

There are already several research groups in the biofabrication field around the world and some of them are in the process of transforming into specialized research centers. The incomplete and fast growing list includes Wei Sun's group at Drexel University, Philadelphia, PA, USA; Sangeeta Bhatia's group at MIT; Gabor Forgacs's group at the University of Missouri, Columbia, USA; Scott Hollister's group at the University of Michigan, Ann Arbor, USA; Thomas Boland's group at Clemson University, SC, USA; Dr Lee Weiss and Dr Phil Campbell's group in Pittsburg, PA, USA; Dr Morgan's group at Brown University, Providence, RI, USA; Bioprinting Research Center at Medical University of South Carolina, Charleston, USA; Professor Mulhaupt's group at University of Freiburg, Germany; CESAR Institute in Germany, Dr Brian Derby's group at the University of Manchester, UK; Paolo Bartolo's group at Leiria Technological University, Portugal; Giovanni Vozzi's group in Pisa, Italy; Professor Yan's group at Tsinghua University, Beijing, China; several active groups at the National University of Singapore and Nanyang Technological University, Singapore; Dietmar Hutmacher's group in Brisbane, Australia; Makoto Nakamura's group in Japan, and many others. The development of a global network of collaborating and competing national biofabrication centers is the next logical step and probably the best possible recipe for the advancing biofabrication field around the world.

8.3. Biofabrication meetings and new journals

The members of biofabrication research groups tried to organize special sessions at meetings of Tissue Engineering, Biomaterials, Rapid Prototyping, Artificial Organs, American Association of Anatomist, and other societies. The support of already existing societies is extremely valuable and must be greatly appreciated. However, it is not surprising that there is an increasing demand for specialized meetings, conferences, and workshops which are essential for the consolidation and growth of the biofabrication research community. A series of successful international bioprinting and biofabrication workshops have been organized in Freiburg, Germany; Manchester, UK [100]; Charleston, SC, USA [101, 102]; Tokyo, Japan; and Singapore. The next international biofabrication conference is planned on 6–8 July 2009 in Bordeaux, France (<http://www.u577.u-bordeaux2.fr/3B09/>). The important milestones were bi-national USA–China [103] and USA–India workshops organized by Dr Wei Sun. The organization of the new journal *Biofabrication* with an impressive Editorial Board, Editor, and enthusiastic support from Dr Jane Roscoe, the IOP Publisher, is an extremely important step toward further growth and consolidation of the international biofabrication community.

9. Practical applications of biofabrication technologies

9.1. Biofuel production from algae

Energy consumption has a strong linear correlation with the economic growth of a nation, which makes sustainable energy

production a top priority for any country. Recent emphasis on global warming is another motivation for searching for an alternative source of sustainable energy production. Recently, several excellent reviews [104] have been published which provide strong evidence that algae growing at first in a perfused closed bioreactor and then in open ponds can be a most effective method for biofuel production.

According to some estimates it will be 300 times more effective than a leading plant source for biofuel production such as palm oil [104]. Because the growth of algae in bioreactors is associated with the problem of algae aggregation (biofilm formation) and algae flocculation is an integral step of biofuel production technology, it can be considered in certain ways to be a biofabrication problem at least according to the broad definition of biofabrication.

9.2. Animal-free meat biofabrication

Extremely popular in the media, the concept of animal-free production of tissue-engineered meat is another possible application of biofabrication for solving global problems (for more information, see <http://www.new-harvest.org/resources.htm>). The concept of large-scale industrial production of animal-free tissue-engineered meat includes five essential elements: (a) cell source (stem cells or myoblast cell line from edible domestic animals); (b) economically effective cell culture media; (c) porous scaffold spheres from edible stimuli-sensitive polymers such as collagen or chitosan; (d) perfusion bioreactor and (e) optimal regime of mechanical and chemical conditioning and directed cell and tissue differentiation [105]. These technological elements are achievable and technologically tissue-engineered meat looks like a feasible concept, but economically it is still not very sound. As compared with tissue engineering of skeletal muscle for biomedical applications, tissue-engineered skeletal muscle biofabricated for food consumption does not need to be vascularized. The elimination of the necessity for vascularization makes this technology scalable. The goal of a concept of tissue-engineered meat is not to create natural looking steak (which somehow many observers mistakenly assumed) but rather to develop scalable production of 'hydroponic' skeletal muscle tissue, which can be used in the production of sausages, hamburgers, nuggets, and food bars (figure 4).

In the long term, tissue-engineered food is the inescapable future of humanity. However, in the short term the extremely high prohibitive cost of the biofabrication of tissue-engineered meat is the main potential obstacle, although large-scale production and market penetration are usually associated with a dramatic price reduction. Issuing of the first patent and organization of the first international symposium on tissue-engineered food in Norway in 2008 (see <http://invitromeat.org/content/view/14/29/>) strongly indicates that this exciting application of biofabrication technology will eventually take off. Another interesting potential application of biofabrication technology in the food industry is using robotic dispensing for food decorations and even for food fabrication [106].

9.3. *Animal-free leather and fur production*

The world trade in leather, which is one of the most widely traded commodities, stands at over US\$60 billion a year, and is growing. There are several patents issued on *in vitro* synthesis of collagen by cultured fibroblasts. Tissue-engineered skin for medical applications and drug toxicity is already a clinically proven reality. The world fur market is around \$12 billion and is fast growing due to an increase in disposable income in China and Russia. The epidermis stem cells have been isolated and growth of hair *in vitro* in cell culture has been demonstrated [107]. What is missing—a cost-effective scalable biofabrication technology. Fur farming is the main practice of fur production and it was banned in England, Wales, and Scotland by the Fur Farming (Prohibition) Act and was justified principally on grounds of public morality. Biofabrication can open up new opportunities for developing large-scale animal-free technology for natural leather and fur production [108]. It can also enable the creation of unique new tools for designing and patterning more sophisticated fur, non-existent in nature. Maintaining viability of skin tissue constructs in large-scale biofabrication is another main technological challenge. Using microfluidic hydrogels and microchannelled collagen or synthetic microchannelled scaffolds can be a potential solution of this problem.

9.4. *Biofabrication of human tissues and organs for implantation*

One of the most obvious and highly desirable practical applications of biofabrication technology is bioengineering of living human tissues and organs suitable for implantation. Advanced biofabrication technology can help to design scalable cost-effective industrial production of living human organs or living and implantable organ constructs. Recently published medical economic data have strongly suggested that a tissue-engineered vascular graft could be sold for \$25–30K and may even under certain conditions sell for \$50K [109]. According to our calculation, a \$250K price for a tissue-engineered kidney is economically justifiable [109]. There are 100 000 patients in the USA alone who are waiting for kidney transplantation. The shortage of human organs for transplantation is an urgent, serious and still unsolved medical problem. Kidney biofabrication alone can potentially create a \$25 billion market [109]. Biofabrication of living organs can save thousands of human lives and dramatically reduce the cost of health care. Thus, investment in artificial human organ biofabrication technologies is both economically and socially justifiable and morally sound.

9.5. *Biofabrication of extracorporeal living tissue including devices*

Living tissue spheroids are the integral part of a certain extracorporeal artificial organ-mimicking device. Most advanced extracorporeal tissue-based devices are extracorporeal liver and extracorporeal kidney devices [110, 111]. Scalable production of living tissue spheroids from isolated human cells and their integration into extracorporeal

devices is a biofabrication problem. These devices either have already been clinically tested or are very close to clinical translation. Maintaining the viability and functionality of living tissue spheroids in such devices is one of the main technological challenges. It is not surprising that a perfused hollow fiber bioreactor is a popular strategy for designing an extracorporeal artificial organ device. Using extracorporeal liver and kidney devices for the treatment of some acute diseases and toxicological conditions could be life-saving procedures. However, they are not very well suited for long-term treatment of chronic liver and kidney diseases. Thus, even commercially and clinically successful tissue-based extracorporeal artificial organ devices could not undermine the urgent need in developing organ printing and organ biofabrication technologies.

9.6. *Biofabrication of in vitro 3D tissue models of human diseases*

Biofabricated 3D *in vitro* models of human diseases could be superior compared with traditional 2D *in vitro* cell culture on Petri dishes as well as with animal *in vivo* studies. The main challenge here however is an identification and availability of a proper human cell source. It is generally assumed, but not yet satisfactory proven, that induced human stem cells could solve the problem of a proper human cell source for biofabrication of an *in vitro* 3D model of human disease. However, recent progress in this direction is very encouraging. It is also becoming increasingly recognized that *in vitro* 3D tissue models biofabricated from animal cells have a relatively limited value as compared with human cells.

9.7. *Drug toxicity and drug discovery assays*

Drug discovery tissue-based assays must help to identify and validate potential therapeutic targets, whereas drug toxicity tissue-based assays are dealing primarily with toxicological aspects of already selected therapeutic targets or compounds. The superiority and higher level of authenticity of 3D tissue assays as compared with 2D assays are already proven [112]. Unfortunately, most of published biofabricated *in vitro* 3D tissue models were not systematically validated nor was their predictive power tested. Until the systematic validation and retrospective checking of the prediction power of the proposed tissue-based assays becomes a standard requirement for publication of novel *in vitro* toxicology assays, their potential value will remain uncertain. Providing limited viability and functional data is certainly not enough to validate an assay and prove its predictive power. Overcoming these limitations and the development of automated non-destructive real-time biomonitoring methods can turn robotically biofabricated *in vitro* 3D tissue models into a powerful high content and high throughput tool for drug discovery and drug toxicity.

9.8. *Biosensors and bioreports in space research*

Although the USA, China and Russia are still actively pursuing rather expensive and risky human space exploration and

manned space trips, the European Union is more focused on an alternative approach—unmanned automatic space exploration. However, even in the case of planned Moon and Mars manned missions, the development of sophisticated cell- and tissue-based bioreporters and biosensors is essential to check radiation safety. Thus, future applications of biofabrication in space research will probably be related to designing, fabricating, and testing sophisticated miniaturized tissue-based radiation biosensors and bioreporters. One such sophisticated microfluidic bioreporter has already been developed for NASA. The most challenging goal is to create an *in vitro* tissue analog of human organisms or organs which mimic the essential aspects of complexity of human organisms, including the radiation sensitive immune system with circulating lymphocytes and stem cells.

9.9. Biofabrication and bioart

The constant search for new art materials, techniques, and media is an integral part of the evolution of art. Growing semi-living tissue-engineered sculptures has already been employed in an art project by provocative bioartist Oron Catts (<http://www.symbiotica.uwa.edu.au/>) who considers tissue engineering as a new art medium [113]. He was trained in tissue engineering technology in the world famous Dr Joseph Vacanti Lab at Harvard University and he is one of the leading bioartists. Another emerging bioartist, Julia Reodica, is also using tissue engineering (<http://www.vivolabs.org/>). Although we are not sure that creating beauty is an ultimate goal of modern art, the living biosculptures created from fluorescent-labeled living tissue spheroids can indeed be very beautiful. From a commercial point of view, it is important to mention that the most expensive bioart object, which has ever been sold, is a huge shark, which was fixed in formaline and placed in a transparent glass box. Rapid prototyping is already broadly used in art and jewelry and it is logical to assume that biofabrication technology will also be applied in bioart.

9.10. Biogames and bioentertainment

Using biofabrication technology for creating biogames sounds like a really strange idea. However, an attempt to create a self-assembled moving microdevice driven by tissue-engineered muscle has already been reported [114]. One can imagine the organization of bioolympic games in the future when 'biofabbists' from different countries will compete in running, jumping, swimming, and may even be with their flying biofabricated biocreations. It cannot also escape our notice that using biofabricated living devices for personal entertainment is also technologically feasible [115], but this extremely controversial and culturally sensitive issue is beyond the scope of this review.

10. Toward biofabrication industry

10.1. Concept of biofabrication industry cluster

The concept of industry cluster is probably the most popular and broadly adopted concept of industrial development. In

essence, the concept of industry cluster is about putting industry on the map or the selection of the most favorable geographic locations for industry growth by systematic implementation, enhancing and enforcing industry cluster promotion and enhancing factors. One of the most interesting and still unpredictable aspects of industry cluster formation is so-called path dependence, when a chain of small events lock the location and initiate growth of an industry cluster. There are already several start-up companies which are trying to explore the biofabrication concept. Thus, the question about the future location of a biofabrication industry cluster is no longer entirely abstract.

10.2. Biofabrication industry cluster formation

What are the necessary and sufficient conditions for promoting biofabrication industry cluster formation? First of all, it is safe to predict that a biofabrication industry cluster will evolve in an area where there is already strong industrial expertise in fabrication, manufacturing, and biotechnology. Close proximity of research universities with corresponding strong research and technological expertise as well as access to educational and training institutions providing well-trained specialists and technologists is equally essential. The access to raw materials (stem cells, cell culture media, biomaterials, hydrogels) and biofabrication tools (bioprinters, dispensers, bioreactors, software) and the proximity of potential markets (biofabrication product consumers) are other very important factors. The strong transportational infrastructure including a proximity of large seaports, efficient international airports and ground transportation such as highways is also a great plus point. Industry cluster growth enhancing institutions, such as the presence of research and training biofabrication centers, as well as state and governmental targeted sustainable financial support, is another important factor. For example, South Carolina already has some world recognized experts and research centers in biofabrication at local research universities, and the headquarters of the world's leading rapid prototyping company 3D Systems Inc. and its educational branch 3D System University are both located in Rock Hill, SC, USA (figure 5). What is most essential in the forthcoming competition for a biofabrication industry cluster is capitalization on initial advantage and exploring path dependence.

However, the access to capital and the proximity to venture capital firms are other extremely important, and may even be key, factors, which make South Carolina's hope and ambitions a little bit more modest. Carefully orchestrated assembly of all the critical and essential industry cluster promoting factors is probably the most secure economic strategy, but it needs well-integrated efforts, superb management and, of course, a significant amount of money. In order to make investment effective, the state and federal funding agencies must carefully consider the complexity of all industrial cluster promoting factors. The important 'nucleation and catalytic factor' for the emergence of a biofabrication industry cluster is the organization of a biofabrication engineering research and training center.

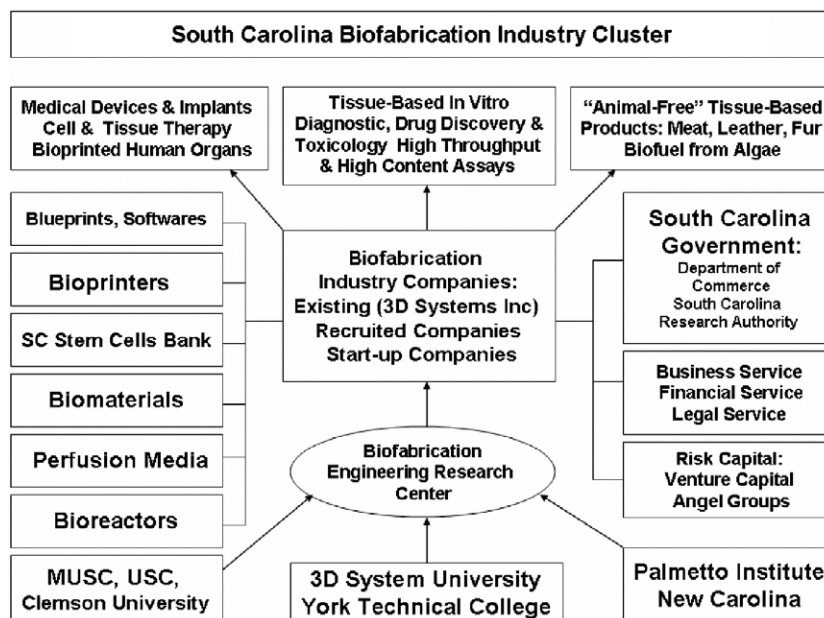


Figure 5. Scheme of emerging South Carolina biofabrication industry cluster.

10.3. International competition in biofabrication

It is still difficult to predict which country will take a leading role in building the future biofabrication industry, but the potential main players are obviously economically developed scientific and technological giants such as the USA, Japan, European Union (especially Germany with strong expertise in precision engineering and manufacturing), increasingly China, South Korea, Taiwan and relatively small but research-intensive and technologically superior nations such as Switzerland, Israel, and especially Singapore (which according to the <http://www.gpubmed.com> search engine is a world leader in the application of rapid prototyping and nanotechnology in tissue engineering). Canada (Toronto), Australia (Brisbane), Brazil (Campinas), and India (Bangalore) also have a chance to compete. However, the existing geographical location of rapid prototyping industry clusters and biotechnology clusters can provide powerful insight into the future economic geography of the emerging biofabrication industry. Path dependence does matter and it is extremely difficult to build competing new industry clusters from scratch. It will take a lot of time, money, and systematic organizational efforts. The huge multibillion market for emerging biofabrication industries is a good justification for these efforts.

10.4. Historic lessons from the development of other industries

In his influential book *Innovation and Entrepreneurship*, Peter Drucker provided a series of interesting, concrete, historic examples about conditions for the emergence of new industries which we believe can give a powerful insight into the future of the biofabrication industry. For concrete examples, due to the limit of space, we will refer interested readers to Peter

Drucker’s book [116]. Here, we will try to extract the essence and present these historic lessons in a laconic way.

Lesson number 1. ‘The lead time for knowledge to become applicable technology and begin to be accepted on the market is between twenty-five and thirty-five years’. It is interesting that Thomas Kuhn in his influential, and very popular book among scientists, *The Structure of Scientific Revolution* [117], also showed that it takes about 30 years before new scientific theory becomes a new paradigm.

Lesson number 2. ‘The second characteristic of knowledge-based innovation—and a truly unique one—is that they are almost never based on one factor but on the convergence of several different kinds of knowledge’. In the case of biofabrication as we have discussed above, it is a convergence of cell and developmental biology, mechanical engineering, and biomaterials science.

Lesson number 3. ‘Knowledge-based innovation requires careful analysis of all the necessary factors, whether knowledge itself, or social, economic or perceptual factors’. Putting biofabrication technology in a broader social and economic context is essential for perceptivity into it. Economic and social considerations must be an integral part of the intellectual challenge starting from an ideation phase of technology development.

Lesson number 4. ‘The important “requirement of knowledge-based innovation is a clear focus on the strategic position’. Biofabrication is a multidisciplinary field and interested players, for proper development and strategic positioning, need to invest in the most essential and critical components of this technology.

Lesson number 5. ‘To be successful, a knowledge-based innovation has to be “ripe”; there has to be receptivity to

it. ... If we want knowledge-based innovation we must gamble on receptivity to it'. Negative opinion leaders can destroy perception of the biofabrication field and delay its development whereas enthusiastic support from influential leaders and experts and media can dramatically enhance the rapid development of biofabrication. In short, perception does matter.

Lesson number 6. 'Precisely because the inherent risk of knowledge-based innovation is so high, entrepreneurial management is both particularly necessary and particularly effective'. Nothing, besides substantial and sustainable funding, can enhance the development of the biofabrication field more than a superb management of corresponding organizations and institutions.

Lesson number 7. 'The risk in high-tech innovation can be substantially reduced by integrating new knowledge as a source of innovation...[it] requires a great deal of system and self-discipline, and has to be organized and purposeful' [117]. Thus, in order to develop a new biofabrication industry, the creation of long-term, well-funded multidisciplinary technological research and training biofabrication centers with 'program research' or a mission-oriented approach is a must.

Any state or country which really wants to be a world leader in 21st century manufacturing must create a network of well-funded and, most importantly, well-managed national biofabrication engineering research and technology centers and/or national biofabrication technology development programs. Unfortunately, only economically developed and rich countries can afford such investment and sooner or later they will invest in biofabrication technology (no doubt about that), because it will guarantee their future technological superiority and increasing level of economic prosperity.

10.5. Defining the biofabrication industry

According to Peter Drucker [118], there are declining, mature, and growing industries. A growing industry is one in which the demand for its products, whether goods or services, grows faster than the national income and/or population. Investment in a declining industry such as the automobile industry is usually economically counterproductive. Investment in a growing industry is usually most profitable, but it needs to take the lead in innovation and needs to be willing to take risks of substantial up-front investment in uncertain market conditions. Four growing economic sectors during the 20th century were government, health care, education, and leisure [118]. Leisure and entertainment is now becoming a mature industry. Some influential economists predicted that health care will continue to grow, up to 20–30% of USA economy, in the 21st century [119]. Thus, biofabrication is a growing industry, because it addresses the growing demand for more sophisticated health care products and novel therapeutic modalities in fast growing health care and other sectors of developed countries' economy.

Anita McGahan in her book *How Industries Evolve* [120] identified progressive, creative, intermediate, and radical industries. Biofabrication, as well as pharmaceutical industries, the motion-picture industry, and oil and gas industries, is a creative industry. The rules of change in such an industry include committing to high-potential projects which need substantial up-front investment without reliable market information; developing a system for bringing a successful project to the market; and constant abandoning of failing projects. Opportunities for innovation in a creative industry are based on the development of an efficient system for delivering projects to the market. The goal is to build core assets and core activities based on an emerging dominant industry model and dictate the terms and rules. Competitive advantage will occur when companies both achieve operational effectiveness on the dominant model and go further to offer a distinctive value in some way [120]. Thus, in a creative industry performance depends on several primary capabilities: (i) project management skills that allow a firm to develop a new asset efficiently and effectively; (ii) risk assessment capabilities for managing across a portfolio of projects; and (iii) the development of a network of complementary upstream and downstream relationships for commercialization of a new product efficiently. Thus, an emerging biofabrication industry in order to be successful must learn how to operate from Hollywood, Big Pharma, as well as from oil and gas companies.

Whether biofabrication will be a vertically or horizontally integrated industry remains to be seen, but from an economic perspective horizontal integration of an evolving industry at a certain stage guarantees technological superiority and economic effectiveness. It means that a biofabrication company must not have expertise, competitive assets and core activities equally spread in all components of the biofabrication technology but rather focus on and specialize in one or two carefully selected key components in which it can reach unparallel expertise and superiority.

Thus, biofabrication can be defined as a growing, creative, and potentially horizontally integrated industry. It needs huge up-front investment and superb management which in the long term will lead to a prohibitive entrance barrier for potential competitors. As a creative industry, biofabrication is inherently risky but also potentially extremely profitable. The state or country which does not hesitate to invest in the development of the biofabrication industry will benefit enormously by creating new products, highly paid high-tech jobs and ensuring long-term technological superiority and associated economic prosperity.

11. Conclusion

Biofabrication represents a potentially powerful technological platform to support sustainable manufacturing by creating new and transforming existing industries. Biofabrication could be the dominant paradigm for 21st century manufacturing. To help advance the field and turn this vision into reality by fostering communication and information dissemination among the global scientific and engineering community is the noble mission of the new journal *Biofabrication*.

Acknowledgments

This research was supported by NSF FIBR grant (EF-0526854), NSF EPSCOR grant and MUSC Bioprinting Research Center Grant.

References

- [1] Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P 2002 *Molecular Biology of the Cell* 4th edn (NY, USA: Garland)
- [2] Rasmussen S *et al* 2008 *Protocells: Bridging Nonliving and Living Matter* (Cambridge, MA: MIT Press)
- [3] Lanza R, Langer R and Vacanti J 2007 *Principles of Tissue Engineering* 3rd edn (New York: Academic)
- [4] Harrison R G 1907 Observations on the living developing nerve fiber *Anat. Rec.* **1** 116–8
- [5] Steinberg M S 1963 Reconstruction of tissues by dissociated cells. Some morphogenetic tissue movements and the sorting out of embryonic cells may have a common explanation *Science* **141** 401–8
- [6] Forgacs G, Foty R A, Shafir Y and Steinberg M S 1998 Viscoelastic properties of living embryonic tissues: a quantitative study *Biophys. J.* **74** 2227–34
- [7] Foty R A, Forgacs G, Pflieger C M and Steinberg M S 1994 Liquid properties of embryonic tissues: measurement of interfacial tensions *Phys. Rev. Lett.* **72** 2298–301
- [8] Foty R A, Pflieger C M, Forgacs G and Steinberg M S 1996 Surface tensions of embryonic tissues predict their mutual envelopment behavior *Development* **122** 1611–20
- [9] Oppenheimer J M 1967 *Essays in the History of Embryology and Biology* (Cambridge, MA: MIT Press)
- [10] Hunter P, Smith N, Fernandez J and Tawhai M 2005 Integration from proteins to organs: the IUPS Physiome Project *Mech. Ageing Dev.* **126** 187–92
- [11] Hollister S J 2005 Porous scaffold design for tissue engineering *Nat. Mater.* **4** 518–24
- [12] Huttmacher D W, Sittinger M and Risbud M V 2004 Scaffold-based tissue engineering: rationale for computer-aided design and solid free-form fabrication systems *Trends Biotechnol.* **22** 354–62
- [13] Peltola S M, Melchels F P, Grijpma D W and Kellomaki M 2008 A review of rapid prototyping techniques for tissue engineering purposes *Ann. Med.* **40** 268–80
- [14] Sun W and Lal P 2002 Recent development on computer aided tissue engineering—a review *Comput. Methods Programs Biomed.* **67** 85–103
- [15] Weigel T, Schinkel G and Lendlein A 2006 Design and preparation of polymeric scaffolds for tissue engineering *Expert Rev. Med. Devices* **3** 835–51
- [16] Yeong W Y, Chua C K, Leong K F and Chandrasekaran M 2004 Rapid prototyping in tissue engineering: challenges and potential *Trends Biotechnol.* **22** 643–52
- [17] Langer R and Vacanti J P 1993 Tissue engineering *Science* **260** 920–6
- [18] Mironov V, Viconti R P, Kasyanov V, Forgacs G, Drake C J and Markwald R R 2009 Organ printing: tissue spheroids as building blocks *Biomaterials* **30** 2164–74
- [19] Atala A, Bauer S B, Soker S, Yoo J J and Retik A B 2006 Tissue-engineered autologous bladders for patients needing cystoplasty *Lancet* **367** 1241–6
- [20] Macchiarini P *et al* 2008 Clinical transplantation of a tissue-engineered airway *Lancet* **372** 2023–30
- [21] Dahl SL, Koh J, Prabhakar V and Niklason L E 2003 Decellularized native and engineered arterial scaffolds for transplantation *Cell Transplant.* **12** 659–66
- [22] Cohen D L, Malone E, Lipson H and Bonassar L J 2006 Direct freeform fabrication of seeded hydrogels in arbitrary geometries *Tissue Eng.* **12** 1325–35
- [23] Dhariwala B, Hunt E and Boland T 2004 Rapid prototyping of tissue-engineering constructs, using photopolymerizable hydrogels and stereolithography *Tissue Eng.* **10** 1316–22
- [24] Landers R, Hubner U, Schmelzeisen R and Mulhaupt R 2002 Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering *Biomaterials* **23** 4437–47
- [25] Liu Tsang V *et al* 2007 Fabrication of 3D hepatic tissues by additive photopatterning of cellular hydrogels *Faseb J.* **21** 790–801
- [26] Wang X, Yan Y and Zhang R 2007 Rapid prototyping as a tool for manufacturing bioartificial livers *Trends Biotechnol.* **25** 505–13
- [27] L'Heureux N, Germain L, Labbe R and Auger F A 1993 *In vitro* construction of a human blood vessel from cultured vascular cells: a morphologic study *J. Vasc. Surg.* **17** 499–509
- [28] Ross J J *et al* 2006 Cytokine-induced differentiation of multipotent adult progenitor cells into functional smooth muscle cells *J. Clin. Invest.* **116** 3139–49
- [29] Seliktar D, Nerem R M and Galis Z S 2003 Mechanical strain-stimulated remodeling of tissue-engineered blood vessel constructs *Tissue Eng.* **9** 657–66
- [30] Syedain Z H, Weinberg J S and Tranquillo R T 2008 Cyclic distension of fibrin-based tissue constructs: evidence of adaptation during growth of engineered connective tissue *Proc. Natl Acad. Sci. USA* **105** 6537–42
- [31] Weinberg C B and Bell E 1986 A blood vessel model constructed from collagen and cultured vascular cells *Science* **231** 397–400
- [32] Allison D D, Braun K R, Wight T N and Grande-Allen K J 2009 Differential effects of exogenous and endogenous hyaluronan on contraction and strength of collagen gels *Acta Biomater.* **5** 2019–26
- [33] Gillette B M *et al* 2008 *In situ* collagen assembly for integrating microfabricated three-dimensional cell-seeded matrices *Nat. Mater.* **7** 636–40
- [34] Tang S and Spector M 2007 Incorporation of hyaluronic acid into collagen scaffolds for the control of chondrocyte-mediated contraction and chondrogenesis *Biomed. Mater.* **2** S135–41
- [35] Isenberg B C, Williams C and Tranquillo R T 2006 Small-diameter artificial arteries engineered *in vitro* *Circ. Res.* **98** 25–35
- [36] Robinson P S, Johnson S L, Evans M C, Barocas V H and Tranquillo R T 2008 Functional tissue-engineered valves from cell-remodeled fibrin with commissural alignment of cell-produced collagen *Tissue Eng A* **14** 83–95
- [37] Masuda S, Shimizu T, Yamato M and Okano T 2008 Cell sheet engineering for heart tissue repair *Adv. Drug Deliv. Rev.* **60** 277–85
- [38] Nishida K *et al* 2004 Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium *N. Engl. J. Med.* **351** 1187–96
- [39] Yang J *et al* 2006 Cell delivery in regenerative medicine: the cell sheet engineering approach *J. Control. Release* **116** 193–203
- [40] Yang J *et al* 2007 Reconstruction of functional tissues with cell sheet engineering *Biomaterials* **28** 5033–43
- [41] L'Heureux N, Paquet S, Labbe R, Germain L and Auger F A 1998 A completely biological tissue-engineered human blood vessel *Faseb J.* **12** 47–56
- [42] L'Heureux N, Dusserre N, Marini A, Garrido S, de la Fuente L and McAllister T 2007 Technology insight:

- the evolution of tissue-engineered vascular grafts—from research to clinical practice *Nat. Clin. Pract. Cardiovasc. Med.* **4** 389–95
- [43] L'Heureux N, McAllister T N and de la Fuente L M 2007 Tissue-engineered blood vessel for adult arterial revascularization *N. Engl. J. Med.* **357** 1451–3
- [44] König G *et al* 2009 Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery *Biomaterials* **30** 1542–50
- [45] Jakab K, Neagu A, Mironov V and Forgacs G 2004 Organ printing: fiction or science *Biorheology* **41** 371–5
- [46] Mironov V 2003 Printing technology to produce living tissue *Expert Opin. Biol. Ther.* **3** 701–4
- [47] Mironov V, Boland T, Trusk T, Forgacs G and Markwald R R 2003 Organ printing: computer-aided jet-based 3D tissue engineering *Trends Biotechnol.* **21** 157–61
- [48] Mironov V, Kasyanov V, Drake C and Markwald R R 2008 Organ printing: promises and challenges *Regen. Med.* **3** 93–103
- [49] Perez-Pomares J M and Foty R A 2006 Tissue fusion and cell sorting in embryonic development and disease: biomedical implications *Bioessays* **28** 809–21
- [50] Jakab K *et al* 2008 Tissue engineering by self-assembly of cells printed into topologically defined structures *Tissue Eng. A* **14** 413–21
- [51] Hiller J and Lipson H 2007 Methods of parallel voxel manipulation for 3D digital printing *18th Solid Freeform Fabrication Symp. 2007* pp 200–11
- [52] Hiller J and Lipson H 2009 Design and analysis of digital materials for physical 3D voxel printing *Rapid Prototyping J.* **15** 137–49
- [53] Du Y, Lo E, Ali S and Khademhosseini A 2008 Directed assembly of cell-laden microgels for fabrication of 3D tissue constructs *Proc. Natl Acad. Sci. USA* **105** 9522–7
- [54] Khademhosseini A, Langer R, Borenstein J and Vacanti J P 2006 Microscale technologies for tissue engineering and biology *Proc. Natl Acad. Sci. USA* **103** 2480–7
- [55] McGuigan A P, Leung B and Sefton M V 2006 Fabrication of cell-containing gel modules to assemble modular tissue-engineered constructs [corrected] *Nat. Protoc.* **1** 2963–9
- [56] McGuigan A P and Sefton M V 2006 Vascularized organoid engineered by modular assembly enables blood perfusion *Proc. Natl Acad. Sci. USA* **103** 11461–6
- [57] McGuigan A P and Sefton M V 2008 The thrombogenicity of human umbilical vein endothelial cell seeded collagen modules *Biomaterials* **29** 2453–63
- [58] Boland T, Xu T, Damon B and Cui X 2006 Application of inkjet printing to tissue engineering *Biotechnol. J.* **1** 910–7
- [59] Phillippi J A, Miller E, Weiss L, Huard J, Waggoner A and Campbell P 2008 Microenvironments engineered by inkjet bioprinting spatially direct adult stem cells toward muscle- and bone-like subpopulations *Stem Cells* **26** 127–34
- [60] Saunders R E, Gough J E and Derby B 2008 Delivery of human fibroblast cells by piezoelectric drop-on-demand inkjet printing *Biomaterials* **29** 193–203
- [61] Xu T *et al* 2006 Viability and electrophysiology of neural cell structures generated by the inkjet printing method *Biomaterials* **27** 3580–8
- [62] Xu T, Jin J, Gregory C, Hickman J J and Boland T 2005 Inkjet printing of viable mammalian cells *Biomaterials* **26** 93–9
- [63] Yamazoe H and Tanabe T 2009 Cell micropatterning on an albumin-based substrate using an inkjet printing technique *J. Biomed. Mater. Res. A* DOI:10.1002/jbm.a.32312
- [64] Nakamura M *et al* 2005 Biocompatible inkjet printing technique for designed seeding of individual living cells *Tissue Eng.* **11** 1658–66
- [65] Nishiyama Y *et al* 2009 Development of a three-dimensional bioprinter: construction of cell supporting structures using hydrogel and state-of-the-art inkjet technology *J. Biomech. Eng.* **131** 035001
- [66] Campbell P G and Weiss L E 2007 Tissue engineering with the aid of inkjet printers *Expert Opin. Biol. Ther.* **7** 1123–7
- [67] Tang Q, Zhou Y, Chen F and Tan W 2008 Preparing engineering tissue *in vitro* by macroporous microcarriers *China J. Biotechnol.* **24** 74–82
- [68] <http://www.parc.com/research/projects/dropletdispensing/printheads.html>
- [69] Mironov V, Kasyanov V, Markwald R R and Prestwich G D 2008 Bioreactor-free tissue engineering: directed tissue assembly by centrifugal casting *Expert Opin. Biol. Ther.* **8** 143–52
- [70] Kasyanov V A *et al* 2009 Rapid biofabrication of tubular tissue constructs by centrifugal casting in a decellularized natural scaffold with laser-machined micropores *J. Mater. Sci. Mater. Med.* **20** 329–37
- [71] Mironov V *et al* 2005 Fabrication of tubular tissue constructs by centrifugal casting of cells suspended in an *in situ* crosslinkable hyaluronan-gelatin hydrogel *Biomaterials* **26** 7628–35
- [72] Tsang V L and Bhatia S N 2007 Fabrication of three-dimensional tissues *Adv. Biochem. Eng. Biotechnol.* **103** 189–205
- [73] Albrecht D R, Sah R L and Bhatia S N 2004 Geometric and material determinants of patterning efficiency by dielectrophoresis *Biophys. J.* **87** 2131–47
- [74] Ito A *et al* 2005 Novel methodology for fabrication of tissue-engineered tubular constructs using magnetite nanoparticles and magnetic force *Tissue Eng.* **11** 1553–61
- [75] Dobson J 2008 Remote control of cellular behaviour with magnetic nanoparticles *Nat. Nanotechnol.* **3** 139–43
- [76] Lin R Z, Chu W C, Chiang C C, Lai C H and Chang H Y 2008 Magnetic reconstruction of three-dimensional tissues from multicellular spheroids *Tissue Eng. C* **14** 197–205
- [77] Mironov V, Kasyanov V and Markwald R R 2008 Nanotechnology in vascular tissue engineering: from nanoscaffolding towards rapid vessel biofabrication *Trends Biotechnol.* **26** 338–44
- [78] Perea H, Aigner J, Heverhagen J T, Hopfner U and Wintermantel E 2007 Vascular tissue engineering with magnetic nanoparticles: seeing deeper *J. Tissue Eng. Regen. Med.* **1** 318–21
- [79] Sill T J and von Recum H A 2008 Electrospinning: applications in drug delivery and tissue engineering *Biomaterials* **29** 1989–2006
- [80] Li W J, Laurencin C T, Catterton E J, Tuan R S and Ko F K 2002 Electrospun nanofibrous structure: a novel scaffold for tissue engineering *J. Biomed. Mater. Res.* **60** 613–21
- [81] Baker B M *et al* 2008 The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers *Biomaterials* **29** 2348–58
- [82] Leong M F, Rasheed M Z, Lim T C and Chian K S 2008 *In vitro* cell infiltration and *in vivo* cell infiltration and vascularization in a fibrous, highly porous poly(D,L-lactide) scaffold fabricated by cryogenic electrospinning technique *J. Biomed. Mater. Res. A* DOI:10.1002/jbm.a.32208
- [83] Stankus J J, Guan J, Fujimoto K and Wagner W R 2006 Microintegrating smooth muscle cells into a

- biodegradable, elastomeric fiber matrix *Biomaterials* **27** 735–44
- [84] Hashi C K *et al* 2007 Antithrombogenic property of bone marrow mesenchymal stem cells in nanofibrous vascular grafts *Proc. Natl Acad. Sci. USA* **104** 11915–20
- [85] de Cossart L, How T V and Annis D 1989 A two year study of the performance of a small diameter polyurethane (Biomer) arterial prosthesis *J. Cardiovasc. Surg. (Torino)* **30** 388–94
- [86] Andersson H and Van Den Berg A 2004 Microfabrication and microfluidics for tissue engineering: state of the art and future opportunities *Lab Chip* **4** 98–103
- [87] Nazhat S N *et al* 2007 Controlled microchannelling in dense collagen scaffolds by soluble phosphate glass fibers *Biomacromolecules* **8** 543–51
- [88] Vernon R B, Gooden M D, Lara S L and Wight T N 2005 Native fibrillar collagen membranes of micron-scale and submicron thicknesses for cell support and perfusion *Biomaterials* **26** 1109–17
- [89] Choi N W, Cabodi M, Held B, Gleghorn J P, Bonassar L J and Stroock A D 2007 Microfluidic scaffolds for tissue engineering *Nat. Mater.* **6** 908–15
- [90] Khademhosseini A and Langer R 2007 Microengineered hydrogels for tissue engineering *Biomaterials* **28** 5087–92
- [91] Ling Y *et al* 2007 A cell-laden microfluidic hydrogel *Lab Chip* **7** 756–62
- [92] Stremmer M A, Haselton F R and Aref H 2004 Designing for chaos: applications of chaotic advection at the microscale *Phil. Trans. R. Soc. A* **362** 1019–36
- [93] Xia H M, Wan S Y, Shu C and Chew Y T 2005 Chaotic micromixers using two-layer crossing channels to exhibit fast mixing at low Reynolds numbers *Lab Chip* **5** 748–55
- [94] Chang R, Nam J and Sun W 2008 Direct cell writing of 3D microorgan for *in vitro* pharmacokinetic model *Tissue Eng. C* **14** 157–66
- [95] Endy D 2005 Foundations for engineering biology *Nature* **438** 449–53
- [96] Jakab K, Neagu A, Mironov V, Markwald RR and Forgacs G 2004 Engineering biological structures of prescribed shape using self-assembling multicellular systems *Proc. Natl Acad. Sci. USA* **101** 2864–9
- [97] <http://www.moldflow.com/stp/>
- [98] Brakke K A 1992 The surface evolver *Exp. Math.* **1** 141–65
- [99] Huttmacher D W and Singh H 2008 Computational fluid dynamics for improved bioreactor design and 3D culture *Trends Biotechnol.* **26** 166–72
- [100] Mironov V, Reis N and Derby B 2006 Review: bioprinting: a beginning *Tissue Eng.* **12** 631–4
- [101] Mironov V 2005 The second international workshop on bioprinting, biopatterning and bioassembly *Expert Opin. Biol. Ther.* **5** 1111–5
- [102] Mironov V 2006 Toward human organ printing: Charleston Bioprinting Symposium *Am. Soc. Artif. Inter. Org. J.* **52** e27–30
- [103] Sun W, Yan Y, Lin F and Spector M 2006 Biomanufacturing: a US–China National Science Foundation—sponsored workshop *Tissue Eng.* **12** 1169–81
- [104] Chisti Y 2008 Biodiesel from microalgae beats bioethanol *Trends Biotechnol.* **26** 126–31
- [105] Edelman P D, McFarland D C, Mironov V A and Matheny J G 2005 Commentary: *in vitro*-cultured meat production *Tissue Eng.* **11** 659–62
- [106] Periard D, Schaal N, Schaal M, Malone E and Lipson H 2007 Printing food *18th Solid Freeform Fabrication Symp. 2007 (Austin, TX)* pp 564–74
- [107] Sterodimas A, De Faria J, Correa W E and Pitanguy I 2009 Tissue engineering in plastic surgery: an up-to-date review of the current literature *Ann. Plast. Surg.* **62** 97–103
- [108] Lee W *et al* 2009 Multi-layered culture of human skin fibroblasts and keratinocytes through three-dimensional freeform fabrication *Biomaterials* **30** 1587–95
- [109] Mironov V, Drake C and Wen X 2006 Research project: Charleston Bioengineered Kidney Project *Biotechnol. J.* **1** 903–5
- [110] Allen J W and Bhatia S N 2002 Improving the next generation of bioartificial liver devices *Semin. Cell Dev. Biol.* **13** 447–54
- [111] Ding F and Humes H D 2008 The bioartificial kidney and bioengineered membranes in acute kidney injury *Nephron. Exp. Nephrol.* **109** e118–22
- [112] Lee G Y, Kenny P A, Lee E H and Bissell M J 2007 Three-dimensional culture models of normal and malignant breast epithelial cells *Nat. Methods* **4** 359–65
- [113] Catts O and Zurr I 2002 Growing semi-living sculptures: the tissue culture & art project *Leonardo* **35** 365–70
- [114] Xi J, Schmidt J J and Montemagno C D 2005 Self-assembled microdevices driven by muscle *Nat. Mater.* **4** 180–4
- [115] De Filippo R E, Bishop C E, Filho L F, Yoo J J and Atala A 2008 Tissue engineering a complete vaginal replacement from a small biopsy of autologous tissue *Transplantation* **86** 208–14
- [116] Drucker P F 1986 *Innovation and Entrepreneurship. Practice and Principles* (New York: Harper Business)
- [117] Kuhn T S 1962 *The Structure of Scientific Revolutions* (Chicago: University of Chicago Press)
- [118] Drucker P F 1999 *Management Challenges for the XXIst Century* (New York: Harper Business)
- [119] Krugman P 1998 *The Accidental Theorist and Other Dispatches from the Dismal Science* (New York: W.W. Norton & Company)
- [120] McGahan A M 2004 *How Industries Evolve. Principles for Achieving and Sustaining Superior Performance* (Boston: Harvard Business School Press)