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Bioprinting: A Beginning

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ABSTRACT

An increasing demand for directed assembly of biologically relevant materials, with prescribed three-dimensional hierarchical organizations, is stimulating technology developments with the ultimate goal of re-creating multicellular tissues and organs *de novo*. Existing techniques, mostly adapted from other applications or fields of research, are capable of independently meeting partial requirements for engineering biological or biomimetic structures, but their integration toward organ engineering is proving difficult. Inspired by recent developments in material transfer processes operating at all relevant length scales–from nano to macro–which are amenable to biological elements, a new research field of *bioprinting and biopatterning* has emerged. Here we present a short review regarding the framework, state of the art, and perspectives of this new field, based on the findings presented at a recent international workshop.

INTRODUCTION

The First International Workshop on Bioprinting and Biopatterning was held at the University of Manchester (United Kingdom) in September 2004 and was organized by Prof. Brian Derby (University of Manchester), Dr. Douglas B. Chrisey (Naval Research Laboratory), Dr. Richard K. Everett (ONR Global, London, U.K.), and Dr. Nuno Reis (Universidade de Beira Interior, Covilhã, Portugal). The meeting was held at the Manchester Conference Centre (MCC) and was financially supported by the U.S. Office of Naval Research and the School of Materials, University of Manchester.

The meeting gathered research leaders from around the world (22 speakers from 10 countries) to review the state of the art in bioprinting and biopatterning, establish new collaborations, and conduct discussion concerning the future of the field's research. The workshop was organized into six sessions: Micro and Nanotechnologies for Biological Applications, Bioprinting (two sessions), Photopatterning and Photopolymerization, Biopatterning, and Tissue Engineering. The topics presented covered a wide scope of possible applications of bioprinting technology from the molecular level (protein and DNA patterning), through cell and tissue patterning and tissue scaffold printing, to multi-cellular assemblies ("organ printing"). The list of abstracts is available for online consultation at: http://www.umist.ac.uk/material/bioprint/.

BIOPRINTING FRAMEWORK

For the purpose of the meeting, bioprinting was defined as the use of material transfer processes for patterning and assembling biologically relevant materials–molecules, cells, tissues, and biodegradable biomaterials–with a prescribed organization to accomplish one or more biological functions.

Diversity of approach

Rather than a single approach, bioprinting is defined as a set of techniques that transfer biologically important materials onto a substrate. It is convenient to sub-divide these into two categories in terms of the basic principle

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of the material delivery method: the printing method requires contact between the delivery mechanism and the substrate or delivery occurs without contact and there is a finite standoff between the mechanism and the substrate. Examples of these methods presented at the meeting included:

- Contact bioprinting: dip pen lithography, (micro) extrusion, and soft lithography.
- Contactless bioprinting: laser based forward transfer, ink-jet deposition.
- Other methods: direct photopolymerization.

It would be a mistake to consider these methods as competing technologies. The many applications of bioprinting cover a range of patterning length scales, depending on their biological application. At the finest scale, bioprinting is a molecular delivery mechanism and the important length-scale is that of focal adhesion sites. At its coarsest scale, bioprinting is required to reproduce gross anatomical features, such as a vascular network. In common with the conventional printing industry, bioprinting technologies are likely to be developed and used in a range of different applications, depending on length scale, printing speed, price, single or multiple copies, as well as fundamental aspects of biocompatibility.

Printing resolution

There was a consensus that printing resolution is dependent on the task or goal to be accomplished. The required spatial resolution for molecular, cellular, tissue, and organ printing spans several orders of magnitude, depending on the size of the fundamental building blocks. Printing resolutions of the methods discussed in the meeting were shown to be sufficient for the task in most cases. The resolution of current techniques vary from sub-μm with near-field optical patterning through μ-contact printing to finally extrusion and ink-jet printing, which currently are pressed to achieve better than 100 μm.

One participant introduced a novel concept of *printel,* which he defined as a minimal two-dimensional printing area that could be somehow recognized by living cells. It is obvious that a printel is more biology-inspired and cell-specific than the "pixel" unit familiar from imaging technology.

Physical aspects of bioprinting

A key aspect in all bioprinting methods is the effect the transfer process has on the biological material used. For example, it was stated that in contactless methods, transfer typically involves accelerations of 100–1000 times that of gravity and a large impact force on the substrate. Preliminary studies of both ink-jet and laser forward transfer indicated that, in some regimes of the deposition conditions, cells can survive these forces. However, concerns regarding molecular folding, cell viability, and expression of the appropriate markers are justified. Modified substrate surfaces in laser-assisted forward transfer have shown to be a helpful strategy in retaining biological functionality. Problems associated with ink-jet delivery of cell suspensions may also come about from the high shear stresses observed during ejection and impact of a fluid drop. Additionally, ink design must account for flow requirements, with viscosity, density, surface tension, and acoustic velocity all being important parameters.

While it is desirable to design and optimize a bioprinting process in such a way that associated potentially damaging effects (physical and chemical) are reduced or eliminated, it is also possible to develop genetically modified cell lines that can temporally tolerate (e.g., by blocking signal transduction pathways) printing-associated physical effects without damage. In many cases, the bioprinting process requires that before and during printing, cells and molecules must be carried in a fluid vehicle that shortly after printing requires consolidation and should consequently behave as a viscoelastic solid. This phase change must occur without damage to the biochemicals, cells, or more complex units within the fluid, which presents a considerable challenge for future development of biomaterials.

Biological aspects of bioprinting

Demonstration of cell viability during and immediately after printing is a priority task for bioprinting. Some participants tried to state that biological engineering is not ready for bioprinting technology, because there is not even any satisfactory control for printed tissue constructs, whereas others argued that developmental biology or adult tissue histology, biochemistry, biomechanics, and physiology is the best possible control. In the short term, bioprinting must deal with two biological problems. First, it must be proven that the selected printing method is neither toxic nor irreversibly damaging for cells and their DNA. Second, printed constructs must be assembled and be able to rapidly evolve into a cohesive and mechanically stable tissue. Finally, printed constructs must be suitable for perfusion and be able to survive *in vitro*, demonstrating organ-typical or tissue-specific functions and spatial organizations. Demonstration of bioprinted tissue and organ integration *in vivo* is a final step.

Mechanisms of printed tissue assembly

It was argued that viscous flow is the mechanism of tissue self-assembly and that tissue fluidity is necessary for post-printing self-assembly. Alternative approaches are based on using fast-solidifying, stimuli-sensitive hydrogels (photo-, thermal-, or chemically-sensitive). However, the ability of these hydrogels to allow the development of functional tissues and organs from directed

molecular and cell assembly remains to be demonstrated. It is clear that existing hydrogels must be optimized to enable a new generation of biomaterials allowing for biological assembly functions, which simultaneously meet the requirements and specifications of the appropriate bioprinting process.

TECHNOLOGY STATE OF THE ART

Printing scaffolds for tissue engineering

Biodegradable scaffolding is a fundamental concept of classical tissue engineering. The design and manufacture of 3-D scaffolds with controlled (and prescribed) functionality can be readily achieved using bioprinting techniques inspired by rapid prototyping methods. Methods based on micro-extrusion, 3-D printing, and lost mold casting using synthetic and natural biomaterials, such as extracellular matrix proteins, were demonstrated. The presentations covered a number of topics, including biocompatibility of the biomaterials and the possibility of designing scaffolds exhibiting vascular-like internal channels. A limiting factor remains the difficulty of seeding these complicated constructs with cells.

Two-dimensional vs. three-dimensional bioprinted cell assays

Two-dimensional, printed cell assays and arrays are immediate applications of emerging bioprinting technology. Some participants expressed the opinion that 2-D bioprinted cell assays and arrays could provide enough valid information for basic and applied research, as, for example, human brain could be considered a 2-D folded structure. Not all participants agreed with such a radical point of view. Opponents of this point reasonably argued that native tissues are three-dimensional and that 2-D cell-based bioprinted man-made arrays and assays will have low or very limited predictive power because of an absence of complexity. It is a well-established fact that cells show different biological activity in 2-D and 3-D environments. Finally, printing living tissues and organs clearly requires a 3-D approach. Manual assembly and packaging of 2-D cell sheets into 3-D tissue-like structures have been demonstrated, but scalability, automation, and the low speed of this assembling technology are limited compared to the potential of fully automated 3-D bioprinting. Finally, some participants argued that printing so-called 2-D tissues consisting of three to four cell layers is a realistic short-term goal.

Is organ printing feasible?

The ultimate goal of tissue engineering is the manufacture of living functional tissues and organs suitable for transplantation in reasonable time scales. Organ printing could be defined as computer-aided 3-D tissue engineering of living organs based on the simultaneous deposition of cells and hydrogels with the principles of self-assembly. This can be divided into three essential technological steps: pre-processing or development of design-files for organs, actual printing, and finally, post-processing or organ conditioning and accelerated tissue and organ maturation. Thus, the proposed organ printing approach includes 1) precise computer-aided and controlled placement or deposition of cells or cell aggregates; 2) simultaneous deposition of cells and hydrogels; and 3) utilization of the principles of biological self-assembly or fusion of cell aggregates.

It was argued that using self-assembled cell aggregates in permissive stimuli-sensitive hydrogels will dramatically accelerate tissue and organ assembly. It was also argued that ultimately organ printing could be done not only *in vitro* but also *in vivo* with the eventual design of clinical bioprinting devices or even some form of novel bioprinting-based surgical instruments. The concept of organ printing based on the principles of directed layer-by-layer deposition and sequential biological tissue self-assembly opens a new window of opportunities and challenges. The significant advantage of the technology is the possibility of being able to organize cells and molecules in three dimension with the desired local density, functionality, and anatomical shape mimicking their distribution in organs.

Can cells and rigid scaffolds be printed simultaneously?

Reports indicate that thus far printed cell/hydrogel tissue constructs are not able to maintain their shape over a wide range of external conditions and hence are subject to melting and/or distortion post-printing. Simultaneous printing of cells (or cell aggregates) and biodegradable materials, which can be consolidated to the desirable level in a controlled fashion, would overcome this problem. Techniques, such as two-photon polymerization (2PP), could be explored for this purpose. It was demonstrated that 2PP of inorganic-organic hybrid polymers is a promising approach for the fabrication of complicated 3-D micro- and nano-structures directly from computer files. Thus, simultaneous printing of viscoelastic, fluidic, self-assembling tissue in a photosensitive material is probably feasible. The bottleneck is the development of the material (or material combination) that can be co-deposited and modified *in situ* without affecting the biological activity of the living elements.

CONCLUSIONS AND FUTURE OUTLOOK

Participants at the First International Workshop on Bioprinting and Biopatterning agreed that this was a successful and useful meeting although it was unclear at the

time if bioprinting had achieved a critical mass of scientists and engineers warranting the formation of a more structure organization. However, there was a consensus that workshops such as this one should be held on a regular basis, either as independent events or as a section of already well-established conferences or meetings. The eventual creation of a specialized society, with regular meetings and even a specialty journal on bioprinting and biopatterning, seemed premature but should not be excluded from consideration in the long term. Time will determine whether a relatively cohesive group of specialists will emerge.

In order to consolidate this new direction in bioengineering at least three steps must be accomplished. First, a conceptual and theoretical basic science and engineering framework (theory, methodology, specialized terminology–e.g., printel, bioink, printing resolution, organ printing, and so on) must be established. Realistic short-term (printing 2-D molecular and cell-based assays) and long-term goals (printing 3-D functional tissues and organs) must be identified. Secondly, practical and reliable bioprinting methods and other deposition devices must be developed; more sophisticated, intelligent hydrogels, bioinks, and other biomaterials suitable for bioprinting technologies are also required. Finally, funding agencies should consider the field a priority. There is a need for bioprinting technologies, products, and know-how to be acquired from related disciplines. Applications of successfully implemented bioprinting technology are unlimited. We believe that 2-D printed cell assays and printing of 3-D scaffolds for tissue engineering are realistic goals in the short term. Bioprinting of 3-D tissues and organs is a challenging but worthwhile pursuit in the medium and long term.

Future workshops on bioprinting are already being planned and will take place in the USA and Japan. This indicates how realistic these expectations and predictions are.

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