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Review

Interplay of biomaterials and micro-scale technologies for advancing biomedical applications

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Abstract—Micro-scale technologies have already dramatically changed our society through their use in the microelectronics and telecommunications industries. Today these engineering tools are also useful for many biological applications ranging from drug delivery to DNA sequencing, since they can be used to fabricate small features at a low cost and in a reproducible manner. The discovery and development of new biomaterials aid in the advancement of these micro-scale technologies, which in turn contribute to the engineering and generation of new, custom-designed biomaterials with desired properties. This review aims to present an overview of the merger of micro-scale technologies and biomaterials in two-dimensional (2D) surface patterning, device fabrication and three-dimensional (3D) tissue-engineering applications.

Key words: BioMEMS; biomaterials; surface patterning; micro-fluidics; tissue engineering; review.

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INTRODUCTION

Microfabrication technology was first developed for the semiconductor/microelectronics industry and was then adapted to the field of Micro-Electro-Mechanical Systems (MEMS) for fabrication of microsensors and other microdevices in the 1980s and 1990s. MEMS technology can be used to generate features at length scales ranging from a few tens of nanometers to hundreds of micrometers in a reproducible manner. In the past few years there has been much interest in the use of MEMS for biomedical applications in order to miniaturize diagnostic devices and to facilitate high-throughput experimentation. As a result of this widespread interest in the biomedical and biological applications of MEMS, the field of BioMicro-Electro-Mechanical Systems (BioMEMS) has emerged [1]. BioMEMS is defined as 'devices or systems, constructed using techniques inspired from micro/nanoscale fabrication, that are used for processing, delivery, manipulation, analysis, or construction of biological and chemical entities' [2]. The devices and integrated systems using BioMEMS are also known as lab-on-a-chip and micro-total analysis systems (μTAS). In addition, micro-scale technologies allow for the unprecedented abilities to control the cellular microenvironment in culture and to miniaturize assays. Thus, they can potentially be used as powerful tools for addressing challenges in tissue engineering and *in vitro* cell culture studies.

The use of microfabricated systems has been increasingly widespread in the past few years, and this has been made possible by the emergence of soft lithography [1, 3], among other techniques that can be used to fabricate microdevices without the use of expensive 'clean rooms' and photolithographic equipment. Soft lithography is a set of microfabrication techniques that use elastomeric stamps to print or mold materials at resolutions as low as a few tens of nanometers [4–7]. In soft lithography, micro- and nanostructures are made by curing the pre-polymer on previously fabricated masters. This master is typically a photoresist pattern, which is microfabricated by photolithography. However, after the initial step of photolithographic patterning, the subsequent fabrication steps can be performed in 'wet labs'. Therefore, soft lithographic approaches minimize the amount of clean room time and equipment that are required. Soft lithography can be used to control the topography and the spatial distribution of molecules on a surface and the subsequent deposition of cells [8, 9], as well as to fabricate microfluidic channels and scaffolds for tissue engineering in a convenient, rapid and inexpensive manner [3, 10]. Another techique that facilitates the use of microfabricated systems is photolithography, which can be used to fabricate micro-scale features based on selective exposure and cross-linking of a material to light. The simplification of photolithographic techniques makes it more easily accessible for many research laboratories.

The emergence of BioMEMS devices that interact with cells and biomolecules has generated a need to integrate biocompatible materials within these devices. Traditional materials, such as silicon, which have been extensively used for microelectronics applications, are not optimized for biological samples. Hence, the development of synthetic and natural materials that can be used to fabricate micro-

scale devices and structures or to modify the surface of existing devices to increase their biocompatibility is an active area of research.

BioMEMS devices can be fabricated using three classes of materials [2]: (1) microelectronics-related materials such as silicon and glass; (2) synthetic polymers such poly(dimethylsiloxane) (PDMS) and poly(ethylene glycol) (PEG); and (3) biological materials such as hyaluronan and collagen, which are components of the native extracellular matrix. Microelectronics-related materials have been well-characterized as components in MEMS devices, and synthetic polymers have been widely used because of their strengths in ease of fabrication. In comparison, biological materials are relatively unexplored, and the use of micro-scale technology may be the key to realizing their potential. Micro-scale devices, for example, can be used to generate biomaterials with controlled and unique features, such as photo-cross-linkable materials with spatial distributions of functional units or cross-linking densities as well as homogeneous nanomaterials.

In this review, we discuss the interplay of materials technologies with BioMEMS technologies. Specifically we will discuss examples of both the use of novel or existing biomaterials in device fabrication, as well as the use of micro-scale tools to generate novel biomaterials either in the form of controlled structures or biomaterials that exhibit unique spatial properties.

MATERIALS FOR SURFACE PATTERNING

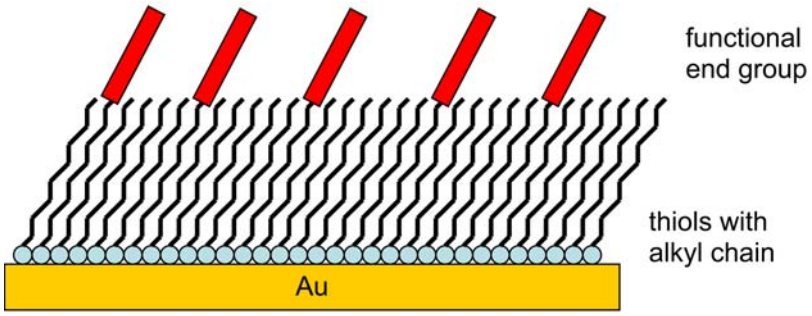
Microfabrication techniques have been widely utilized for generating patterns of living cells on surfaces with potential applications in fundamental cell biology, tissue engineering and cell-screening studies [1]. In addition, proteins, polysaccharides, DNA, RNA and peptides have also been micropatterned for diagnostic and screening applications. Micropatterns of biological entities have typically been made using microfabrication technologies such as photolithography or soft lithography. Photolithography has been widely used for patterning cells and materials on hard materials [11–19]. Alternatively, soft lithography can be used to fabricate functional structures with dimensions in the range of tens of nanometers to hundreds of micrometers [6, 7, 17]. Soft lithographic approaches commonly utilize a microstructured surface made with an elastomeric material, PDMS, to generate patterns on surfaces. Most micropatterning approaches use materials to modify surface properties. These materials are either synthetic or natural and use surface charge, hydrophobicity, and hydrogen and covalent bonding to interact with the substrates and biological entities such as cells and proteins.

Synthetic materials

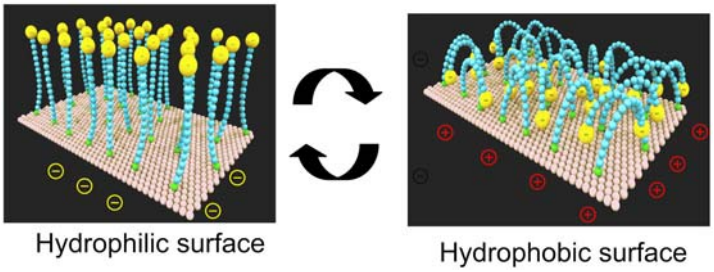
Synthetic polymers and alkanethiols are commonly used materials to pattern surfaces. These materials are desirable since their properties like chain length and functional units can be engineered to control their macroscopic properties.

Alkanethiol self-assembled monolayers. Alkanethiols are used to micropattern metal surfaces such as gold by forming densely packed self-assembled monolayers (SAMs) (Fig. 1). The sulfur end-groups strongly bind to metal surfaces, and the rest of the molecule can then be used to control surface chemistry, wetting, and protein and cells resistance. For example, alkanethiols can be conjugated to poly(ethylene glycol) (PEG) molecules to render surfaces protein and cell resistant. To micropattern surfaces using alkanethiols, a variety of methods have been employed such as microcontact printing (μCP) [6, 20–23]. In this approach, PDMS molds are inked with the alkanethiol solution and transferred to the gold surface by conformal contact between the relief pattern and the substrate. Using μCP, micropattens of SAMs terminated with PEG chains have been generated (Fig. 1a). These micropatterns have been used to immobilize proteins and cells on specific regions of a surface by selectively modifying surfaces with the non-biofouling PEG patterns [24, 25].

To generate surfaces that can change their properties dynamically and switchably, SAMs have been generated that can change surface hydrophobicity in a dynamic manner [26]. In this approach, SAMs were generated with ionic head groups that could be attracted to the substrate using surface charge. The interaction of the alkanethiol head group with the surface could result in 'bending of the molecule' and the exposure of the hydrophobic alkane group (Fig. 1b). The exposure of



(a)



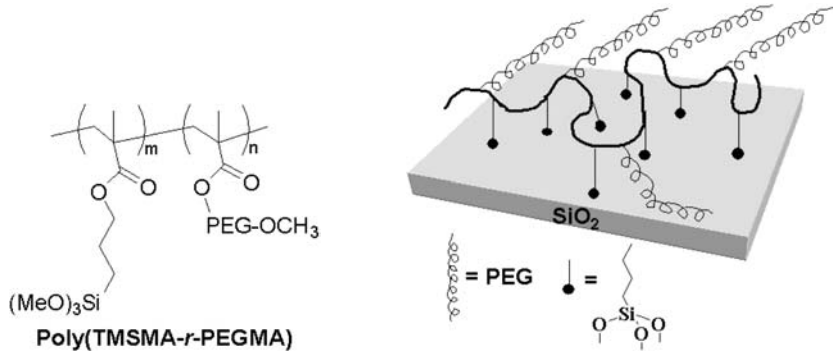
(b)

Figure 1. (a) Schematic diagram of alkanethiols on a gold substrate. (b) Schematic diagram of the reversibly switchable surfaces [26]. This figure is published in colour on http://www.ingenta.com

the alkanes could greatly enhance the hydrophobicity of the surface and could be potentially used for applications such as sensors and valves. Thus, SAMs represent a powerful approach for controlling surface properties in a simple, reproducible and versatile manner.

Photo-cross-linkable and chemisorbed PEG. Synthetic polymers that can covalently bind to the substrate can be used to micropattern surfaces with control over the chemical composition as well as the topographical features of a substrate. PEG based polymers can potentially be generated to conjugate to surfaces and form monolayers with the ability to control surface topography. For example, we have synthesized a PEG-based random co-polymer, that can spontaneously immobilize to silicon oxide or glass substrates [27]. This polymer is composed of a backbone with brush-like 'anchoring' (trialkoxysilane) or 'functional' (PEG) extensions. The chemical structure of the co-polymer and its proposed monolayer formation onto Si/SiO_2 surfaces are shown in Fig. 2. Incorporation of the surface-reactive trimethoxysilyl group in the monomer allows the co-polymer to form multiple covalent bonds with the surface. Consequently, non-specific protein adsorption and cell adhesion can be significantly reduced due to the PEG functional group. Since monolayers can be spontaneously formed on materials such as silicon and PDMS, this material can be used to fabricate microdevices that are resistant to protein adsorption and cell adhesion [27]. In addition, the polymer could be merged with existing micropatterning approaches such as capillary force lithography [28] to generate micropatterns with controlled surface chemistry and topography [8].

Photo-cross-linked PEG has also been used to pattern surfaces [15] and microchannels [29] using techniques such as photolithography and soft lithography. These techniques can be used to pattern cells or proteins for diagnostic and screening applications [30–32]. In addition, the ability to photo-cross-link



(a) (b)

Figure 2. Chemical structure of poly(TMSMA-r-PEGMA) and its monolayer structure. (a) A chemical structure of (trimethoxysilyl)propyl methacrylate and PEG methacrylate random co-polymer, poly(TMSMA-r-PEGMA). (b) A schematic diagram of monolayers of the co-polymer on a SiO_2 surface.

environmentally-responsive photo-cross-linkable PEG has been used to fabricate valves and actuators within microdevices. For example, pH-sensitive photo-cross-linkable PEG-based hydrogels, that actuate in response to changes in the pH of the solution, were used as functional valves within microfluidic channels [33]. Other methods of actuating valves using light, temperature, electrical and chemical stimuli that induce change in the stimuli-responsive polymers is now an active area of investigation.

Physisorbed PEG. Physisorption of synthetic polymers can also be used to surface modify various substrates. Physisorption does not require chemical synthesis and conjugation steps, making this approach simpler and more widely applicable. However, physisorbed surfaces may not be as robust or stable as covalently-bonded micropatterns. PEG molecules, being hydrophilic, tend not to adsorb onto the surfaces of many materials; therefore, to immobilize these PEG molecules, block co-polymers can be used, as their hydrophobic blocks can be used to induce physisorption of the polymer onto surfaces while PEG groups still repel proteins and cells. Using this approach, block co-polymers have been used to generate patterns on a variety of hydrophobic substrates to generate micropatterns of proteins and cells [34, 35]. Interestingly, patterns that are stable for a few weeks have been observed demonstrating that non-covalent interactions are sufficient for long-term patterning.

Natural materials

Whereas synthetic materials have the advantage of greater control over material properties such as degradation rate, porosity, strength and chemistry, natural materials more closely mimic the native cellular environment and may present natural cues for biorecognition. Such materials include proteins and polysaccharides. The main disadvantage of natural materials is that they exhibit structural complexity and can be difficult to manufacture, purify and modify in a reproducible manner [36]. In the following section we will review some of the most common natural materials used for cell and protein patterning.

Direct protein patterning. Proteins can be directly patterned on surfaces. Arrays of proteins can be used to carry out protein-protein, protein-DNA, protein-drug, or enzyme substrate screening assays in a sensitive, parallel and automated manner. Direct patterning of proteins on surfaces can be performed using a variety of approaches such as drop dispensing arrays, microfluidic patterning and microcontact printing. Various proteins have been patterned on surfaces to facilitate cell adhesion. For example, collagen has been used in a variety of patterning approaches to improve cell attachment of myocytes [37] and hepatocytes [38] to micropatterns, to alter cell shape and alignment of myocytes [37] and of vascular smooth muscle cells [39], and to create a step gradient to examine haptotaxis of endothelial

cells [40]. Micropatterns of collagen have also been used to affect the proliferation and spreading of vascular smooth muscle cells [39], to quantify the traction of cells that are constrained within μm-sized islands [41], and to create patterned co-cultures [42]. In addition to collagen, micropatterns of other proteins or peptides such as fibronectin and laminin, or combinations thereof, have been produced for a variety of applications [37, 43–48].

Polysaccharide patterning. Polysaccharides are polymers made from monosaccharide residues joined together by glycosidic linkages. Polysaccharides can potentially produce hydrogels through hydrogen bonding or ionic interactions. Agarose which undergoes thermal gelation and chitosan which undergoes pH-dependent gelation are two examples of polysaccharides which typically assemble through hydrogen-bonding events. Alternatively, alginate may bond through ionic interactions. Many of these materials may also be chemically modified to render them photo-cross-linkable [49–53]. Polysaccharides that have been used for micropatterning applications include agarose, chitosan and hyaluronan.

Agarose is a thermally reversible hydrogel that is extracted from a family of polysaccharides called agars that are obtained from algae such as seaweed. Agarose is a linear galactose polymer (galactan) consisting of alternating D-galactose and 3,6-anhydro-L-galactose units. This chemical structure gives agarose the capacity to form strong gels even at low temperatures. The gels form an open mesh which can be adjusted simply by varying the concentration of the agarose, and the absence of ionic groups makes the gel a neutral structure allowing macromolecules to migrate through the gel without interaction. Agarose has been used in a variety of micropatterning approaches to create microfeatures that serve as scaffolds for chondrocyte culture [54], to stamp arrays and gradients of proteins [55], and to stamp arrays of cells [56]. Agarose has also been used to obtain multicolor micropatterns of thin films of dry gels [57] and to create adhesive biochemical channels for guided 3D cell growth and migration [53].

Chitosan is a natural glycosaminoglycan (GAG) that is derived from chitin, a molecule found in great abundance in the exoskeleton of shellfish like shrimp, lobsters and crabs. Chitin is a cellulose-like polymer consisting mainly of unbranched chains of N-acetyl-D-glucosamine. Chitosan is a polycationic co-polymer that is generally produced industrially through the deacetylation of chitin. Unlike chitin, chitosan is soluble in dilute acids and can have a degree of acetylation between approximately 0% and 60%; the upper limit depends on parameters such as processing conditions, molar mass and solvent characteristics [58]. In addition, chitosan exhibits a minimal foreign body reaction and is considered biodegradable and nontoxic [59]. Chitosan has been used to create micropatterns to exhibit precise spatial control over cell spreading and orientation [60], to achieve microfluidic patterning of cells in extracellular matrix bio-polymers [61] and to develop an agarose gel 'biomask' for the sequential assembly of single-stranded DNA [62]. Chitosan has also been used to create techniques for patterning nucleic acids and proteins [63]

and for creating spatially controlled co-cultures of neurons and glial cells to investigate their interactions for potential applications in the repair or regeneration of the nervous system [64].

Hyaluronan (HA), also known as hyaluronic acid or sodium hyaluronate, is closely related to chitin, insofar as half of its sugars are N-acetyl-glucosamines. However, HA is found within the extracellular matrix of higher animals, especially in soft connective tissues such as cartilage, vitreous, umbilical cord, Wharton's jelly and skin. It is composed of linear, unbranching, polyanionic disaccharide units consisting of glucuronic acid and N-acetyl glucosamine joined alternately by beta 1-3 and beta 1-4 glycosidic bonds. Since one of the sugars in HA is modified with an amino group (NH_2) like chitin, HA is considered as a subset of GAGs. Interestingly, the water binding ability of HA is directly related to its molecular weight and can be as high as 6 l/g [65]. The widespread occurrence of receptors for HA indicates that HA recognition is an important biological function [66]. Non-biofouling HA has been used for protein and cell patterning, and subsequent ionic absorption of poly-L-lysine or complexing with collagen was used to switch the HA surfaces from cell repulsive to adherent, facilitating the adhesion of a second cell type (Fig. 3) [67, 68].

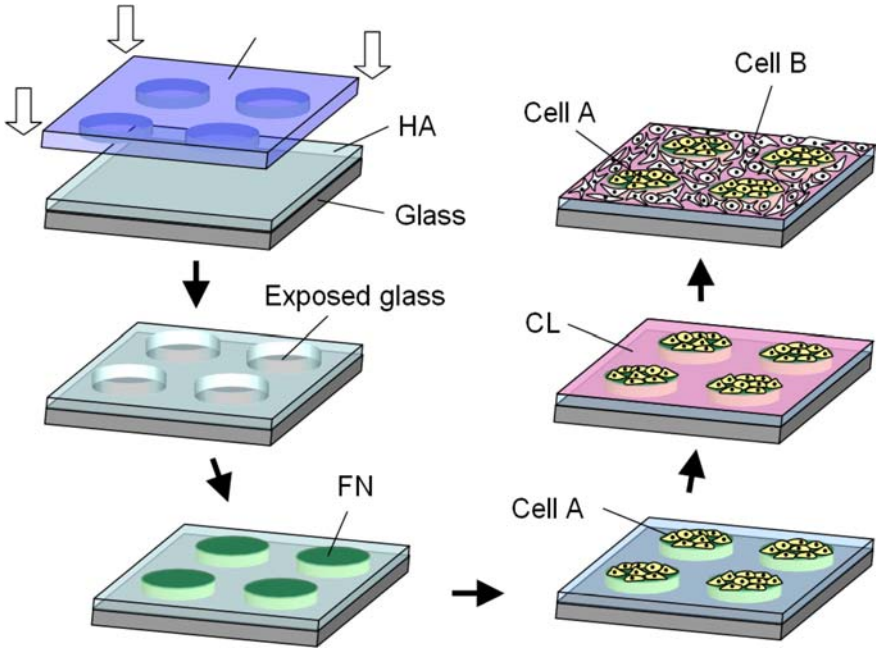


Figure 3. The scheme for fabrication of the co-culture system using capillary force lithography and layer-by-layer deposition. A few drops of hyaluronic acid (HA) solution were spin-coated onto a glass slide, and a PDMS mold was immediately placed on the thin layer of HA. HA under the void space of the PDMS mold receded until the glass surface became exposed. The exposed region of a glass substrate was coated with FN, where primary cells could be selectively adhered. Subsequently, the HA surface was complexed with collagen, allowing for the subsequent adhesion of secondary cells. This figure is published in colour on http://www.ingenta.com

Micropatterns of HA have also been used to study the adhesion, migration and alignment of chondrocytes [69] and melanocytes [70] and to study the adhesion of platelets [71]. In addition, HA has been used with microfluidics technology to create gradients of immobilized molecules and cross-linking densities [72].

MATERIALS FOR MICROFLUIDICS

The development of bioMEMS devices comprised of microfluidic channels may revolutionize how biological analyses and assays are performed [1], based on their unprecedented advantages in minimal reagent consumption and capacity for high-throughput analysis. While significant progress has been made in the advancement of the technology, efforts remain to be pursued at the interface of such microdevices with compatible biomaterials suitable for cellular investigations.

Microfluidic devices developed in the early 1990s were fabricated from silicon and glass using photolithography and etching techniques. These processes were costly, labor intensive and disadvantageous from a materials standpoint. Soft materials, such as elastomeric polymers, have emerged as alternatives for microfluidic device fabrication since they can be molded into microstructures using soft lithography. In addition, soft materials make possible the easy manufacture and actuation of devices containing valves, pumps and mixers [74].

PDMS has rapidly become the material of choice for many microfluidic device applications. PDMS is optically transparent, permeable to gases, elastomeric and durable, which also makes it suitable for cell applications. Upon cross-linking, PDMS becomes an elastomeric material with a low Young's modulus of approx. 750 kPa, which enables it to conform to surfaces and form reversible seals [132] with a low surface energy around 20 erg/cm^2, PDMS can easily be released from molds after patterning [133]. In addition, PDMS has sufficient gas permeability to sustain cells seeded inside microchannels. Despite these advantages, however, PDMS swells in most organic solvents, such as hexanes, ethyl ether, toluene, dichloromethane, acetone and acetonitrile [73]. The swelling of PDMS-based devices makes it impossible for organic solvents to flow inside the channels. Thus, there is a need for easily fabricated microfluidic channels made from materials that can be used with organic solvents.

Photocurable perfluoropolyethers (PFPEs), which are chemically non-adhesive (similar to Teflon) and resistant to swelling in organic solvents, were recently developed as an alternative to PDMS [74]. The addition of 2,2-dimethoxy-2-phenylacetophenone to commercially available PFPE diol functionalized with isocyanatoethyl methacrylate facilitates photo-cross-linking of the material with exposure to UV radiation. In addition, living radical photo-polymerization (LRPP) has been used to generate microfluidic channels [75]. LRPP involves photo-polymerization of a monomer formulation that includes a photoinitiator and a photoiniferter precursor [134]. The photoinitiator enables the bulk polymerization to take place, and the photoiniferter facilitates the formation of subsequent layers

by providing a source of reinitiatable radicals on fully cured polymer surfaces. Therefore, LRPP fabrication can be used to form covalently bonded, multilayer devices and to graft a range of functional materials to surfaces. Within a microfluidic channel, for example, a macroporous polymer plug formed *in situ* could be used as a static mixer or as a fast-acting hydrogel valve. Compared to thermal curing of PDMS, photo-cross-linkable materials are advantageous since they can greatly minimize the time required to cure the polymer.

In addition, biodegradable materials could also be used to fabricate microchannels. These microchannels could have specific applications that range from environmentally friendly devices to tissue engineering. Towards that end, biodegradable microfluidic channels have been generated from poly(DL-lactic-*co*-glycolide) (PLGA) using melt-processing techniques that circumvent the problems presented by solvent-based processing of biodegradable films [76].

Finally, to form microfluidics within hydrogels, alginate hydrogels containing microchannels have been formed by diffusion at the interface of two laminar flows of alginate and calcium ions [77]. The growth or shrinkage of 'gel bars' at the flow interface can be controlled by varying ratios of calcium ions and EDTA and these gel bars may be used to control the immobilization or release cells.

MATERIALS FOR MICRO-SCALE TISSUE ENGINEERING

Tissue engineering and 3D *in vitro* cell culture systems could revolutionize therapeutics, diagnostics and drug discovery. Micro-scale approaches could potentially provide additional complexity and control to the porous 3D scaffolds that are commonly used in tissue engineering. Thus, micro-scale tissue engineering may be used to generate tissues that mimic the architecture and cellular organization of native tissues. Despite such promise, many of the materials that have traditionally been used for micro-scale device fabrication are not suitable for tissue engineering. Addressing issues of biomaterial selection is a critical factor in the development of micro-scale tissue-engineering systems. Materials traditionally used in microfabricated devices, such as silicon [78], silicon oxide and PDMS [79], are not biodegradable and have limited biocompatibility for tissue-engineering applications.

Recent focus has been redirected towards modifying microfabrication processes to accommodate biomaterials that are more suitable for drug-delivery and tissue-engineering systems, such as poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA) and PLGA. PGA and PLLA are common biocompatible polymers that are used for many biomedical applications. PGA is hydrophilic and, therefore, susceptible to hydrolysis and degradation. Alternatively, PLLA is hydrophobic and relatively stable in the body [80]. Through these unique properties polymers such as PLGA have been derived that are made from both glycolic acid and lactic acid components. The ability to change the ratio of these two components of the polymer has been used to change the degradation rate of PLGA *in vivo*. Due to the ability to control

its properties and, since it is inexpensive and biocompatible, PLGA is widely used in drug delivery [81–83] and tissue engineering [84, 85].

Biodegradable materials have been combined with microfabrication technology for drug delivery applications. For example, PLLA has also been microfabricated for use as a resorbable drug-delivery chip in which drugs are loaded into microwells and sealed with PLGA membranes of various compositions [86]. The release of the drugs from the wells can be pre-determined by simply varying the composition of the PLGA membranes that seal each well. In addition, microfabricated biodegradable microneedles have been created from biodegradable polymers for the delivery of therapeutic molecules [87–89]. These devices can be used for transdermal drug delivery or other applications such as intestinal delivery.

Biomaterials and microfabrication have also been merged for generating tissue engineering scaffolds with improved spatial resolutions. Traditional routes of fabricating biodegradable scaffolds using materials such as PLGA for tissue engineering systems include casting/porogen leaching [90], gas foaming [91] and three-dimensional printing [92]. To generate microfabricated scaffolds, PLGA has been micro-molded on PDMS substrates by melting and compressing raw polymer pellets onto the flexible microfabricated substrates [93, 94]. Three-dimensional biodegradable microfluidic networks have been fabricated through thermal lamination of replica-molded micropatterned PLGA sheets [76]. More sophisticated methods of producing biodegradable scaffolds using solvent-casting methods adapted for PLGA in combination with spin-coating, microsyringe deposition and micropatterning have also been reported [95]. PLGA surfaces with micro-scale topography have been used as contact guidance systems to promote and organize regeneration of implants [96].

PLGA, while biodegradable and useful for a variety of medical applications, exhibits sub-optimal properties for numerous applications because of its rigidity [97], poor bulk-degradation kinetics [98] and limited biocompatibility in some cases [99]. High concentrations of PLGA by-products have also been shown to be cytotoxic [100], which is a major limitation in the prospect of fabricating large, organ-size scaffolds. Recent focus has been directed towards the development of improved biomaterials with improved biocompatibility and mechanical properties. Poly(glycerol-sebacate) (PGS), a biocompatible and biodegradable elastomer with superior mechanical properties [98], has emerged as a promising alternative material for tissue-engineering scaffolds, nerve guide materials [102] and other micro-scale tissue-engineering systems. PGS is a tough, biodegradable elastomer that is biocompatible, inexpensive and easy to synthesize from glycerol and sebacic acid. Glycerol and polymers containing sebacic acid have already been approved for use in medical applications. Biocompatibility studies [98, 99] suggest improved cellular response and morphology of PGS when compared to PLGA. PGS is also a suitable material for microfabricated scaffolds from a processing perspective. PGS pre-polymer can be replica molded and cured on silicon masters [103] to form layers as thin as 100 μm in a process that is analogous to replica molding of PDMS [79].

Others have reported similar synthetic biomaterials with improved substrate–tissue interactions and desirable mechanical properties [103].

In addition to making scaffolds for cell adhesion, micro-scale technologies such as microfluidics have also been used to embed microvasculature directly into engineered tissues. For example, microfabricated capillary networks have been fabricated out of biodegradable elastomers, such as PLGA and PGS [76, 102]. These artificial capillary networks could be coated with fibronectin and seeded with endothelial cells, which grow to confluence within a few days. In addition, it is envisioned that the individual layers could be superpositioned and stacked on top of each other to generate structures (Fig. 4) [76].

While PGS and PLGA can potentially be used to fabricate 3D scaffolds on which cells can be seeded, hydrogels can provide a more 3D environment, since they can surround individual cells, providing a more biomimetic setting. PEG hydrogels represent the most broadly-used class of materials for tissue engineering [104–107]. PEG is biocompatible, hydrophilic and resists cell and protein adhesion. It is highly customizable in terms of chain length and can be functionalized with a number of molecules [108]. Also, photo-cross-linkable PEG hydrogels can be easily synthesized and functionalized. Thus, PEG hydrogels can be engineered that can have specific functionality such as addition of RGD and laminin peptides [109] or

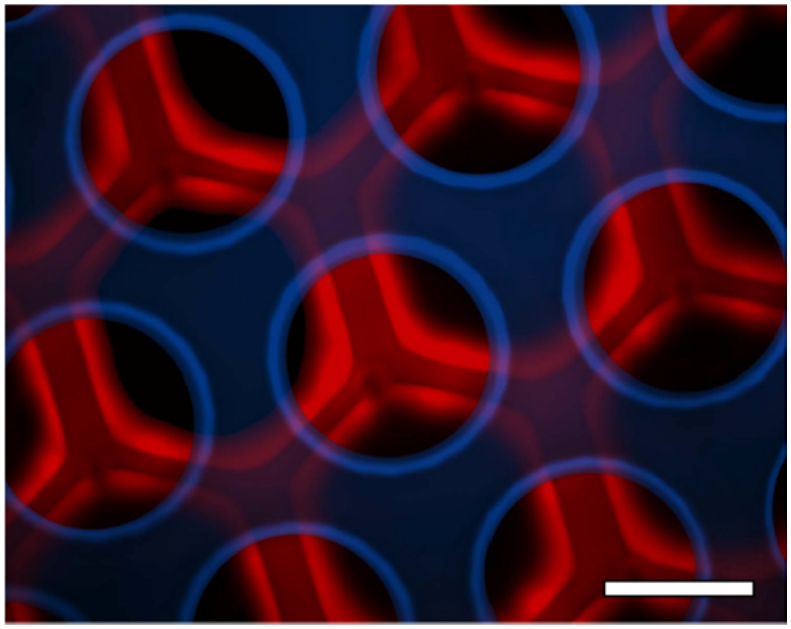


Figure 4. Three-dimensional biodegradable microfluidics. Composite image of fluorescent dyes flowing through a microfabricated three-dimensional network. Microfluidic systems with up to five layers have been fabricated using poly(glycerol-co-sebacate), a novel flexible biodegradable elastomer. Model hepatocarcinoma cells have been seeded into these networks and perfused for up to one week (scale bar is 200 μm) [131]. This figure is published in colour on http://www.ingenta.com

proteins [110, 111], and can be degradable [112, 113]. Photo-cross-linkable PEG systems are easily incorporated into various micro-scale technologies and have been used to encapsulate cells within microgels [18, 114, 115].

Naturally-occurring hydrogels can often be advantageous in tissue engineering applications since they are typically biocompatible and non-toxic. Indeed, a number of natural polymers, such as HA and collagen, have already been used for tissue engineering applications. The merger of microfabrication approaches and natural hydrogels promises to deliver a new generation of tissue engineered constructs that provide true 3D microenvironments for cells. Examples of this merger include micromolding of cells in collagen [116] and photo-cross-linkable HA [117] microgels. Such cell-laden microgels may be stacked on top of each other to generate 3D tissues comprised of heterogeneous layers [118] (Fig. 5). Controlled hydrogels and microfluidics have been used to generate 3D tissues through use of layer-by-layer microfluidic patterning; cells and matrix bio-polymers were flowed through channels with controlled flow rates [67]. By sequential deposition of cells

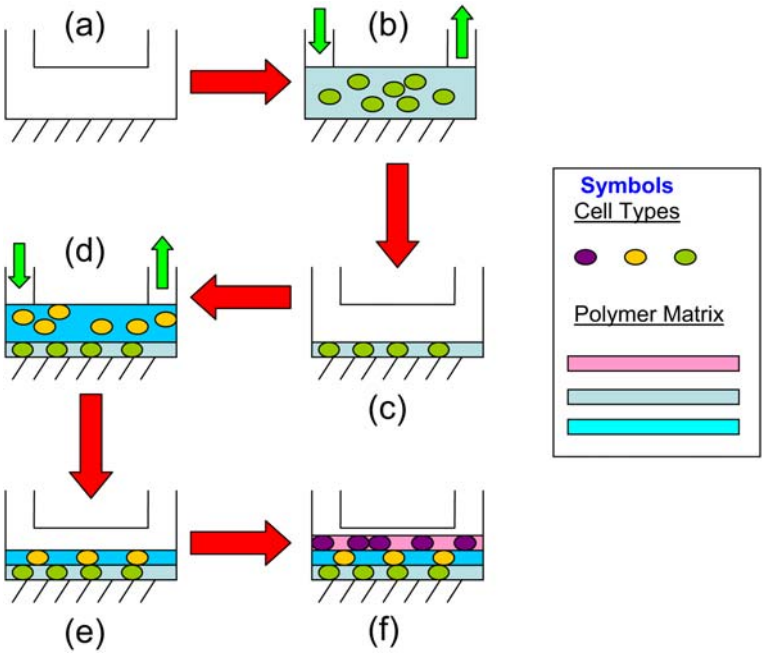


Figure 5. Schematic of the layer-by-layer microfluidics approach for generating micro-scale 3D tissues. Pre-polymer solutions containing different cell types are sequentially deposited within microfluidic channels: (a) the microfluidics channel, (b) a pre-polymer solution containing one cell type is flowed through the channel, (c) the pre-polymer flow is stopped, a layer of pre-polymer with cells is deposited, (d) another pre-polymer solution containing another cell type is flowed through the channel, (e) the pre-polymer flow is stopped and a layer pre-polymer is deposited on top of the original layer and (f) the process is repeated to deposit additional layers. This figure is published in colour on http://www.ingenta.com

and matrix on particular regions within the microchannels, 3D structures were generated with cells deposited in specific locations in a controlled manner.

In addition, calcium alginate [119] has also been molded to form microfluidics channels that could potentially be used to generate hydrogel microchannels [120]. These channels can be fabricated to generate the microvasculature of the scaffold. Such approaches, although at their infancy, provide hope for the fabrication of controllable hydrogel scaffolds made from natural materials.

USE OF MICRO-SCALE TECHNOLOGIES TO MAKE MATERIALS

Micro-scale technologies can also be used to control the homogeneity and spatial properties of materials, as well as to facilitate high-throughput experimentation of materials for biomedical applications.

One application of micro-scale fabrication technologies is to create materials of controlled shapes and sizes. Previously, suspension polymerization, emulsion, precipitation and dispersion techniques had been utilized to generate micro- and nanoparticles. These nanoparticles had a relatively large distribution of sizes which required further separation methods. Recently, a number of groups have demonstrated the use of microfluidic and micromolding approaches for generating monodisperse particles. Using microchannels, droplets containing pre-polymer solutions were made within non-aqueous mineral oil and perfluorocarbon phases and photo-polymerized at controlled flow rates to produce beads of different sizes [121]. Although original methods were unable to control the shapes of the resulting microspheres, more recent approaches have yielded the ability to control the shape of microparticles generated within microchannels [122, 123]. Furthermore, micromolding approaches have been used to generate monodisperse, shape-specific particles [124]. The merger of these tools with biomaterials and cells for the tissue engineering and drug-delivery applications appears as a promising area of research.

Microchannels can also be used to synthesize hydrogels with unique properties [135]. One recently illustrated example is in controlling the spatial properties of materials. Controlling the spatial properties of materials could be useful for a variety of biomedical applications such as tissue engineering and drug delivery. Previously, to synthesize gels with spatially distinct properties, cumbersome methods were required. Recently, microfluidic systems have been used to control the spatial properties of materials. By generating a concentration gradient of photocross-linkable monomers within a microfluidic channel it is possible to fabricate gels with controlled spatial properties [125]. In this method, two sets of monomer solutions with initiator are placed within separate inlets of a gradient generator [126] and flowed through branching winding microchannels to form a gradient (Fig. 6). The two sets of monomer solutions have varying properties, and gradients of these properties (such as functionalization of a monomer or photoinitiator concentration) are generated. Gels can be synthesized with gradients of signaling or adhesive molecules or

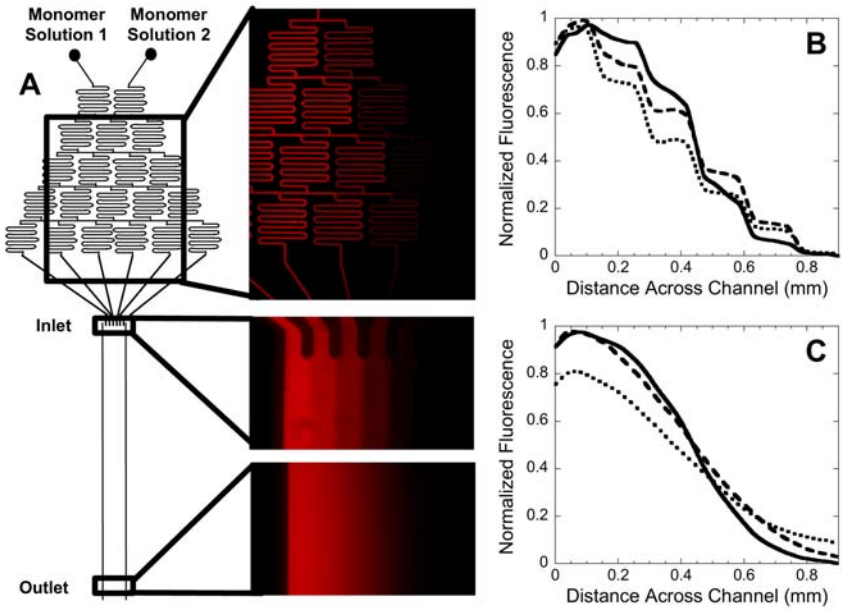


Figure 6. Schematic of the micro-scale channel used in the microfluidics/photo-cross-linking process (A) along with fluorescent images of the gradient maker and channel gradients at the inlet and outlet (approx. 20 mm downstream of the inlet), where rhodamine is incorporated into monomer solution 1 and the monomer solutions are flowed at a rate of 0.3 μl/min. Gradient quantification at the inlet (B) and outlet (C) for monomer solution flow rates of 1.0 μl/min (solid), 0.3 μl/min (dashed) and 0.05 μl/min (dotted).

with varying cross-linking density across the material. In addition, this technique has been applied to generate gradient-compliance substrates [127], upon which the elastic modulus and other mechanical properties varied. Such gels can be used to release drugs in a spatially-dependent manner, to induce directed cell migration and adhesion within the gel, or to study biological systems.

Micro-scale technologies can miniaturize assays and facilitate high-throughput experimentation and, therefore, provide a promising tool for screening libraries. Robotic spotters capable of dispensing and immobilizing nanoliters of material have been used to fabricate microarrays, where cell–matrix interactions can be tested and optimized in a high-throughput manner. For example, synthetic biomaterial arrays have been fabricated to test the interaction of stem cells with various extracellular signals [128]. In this approach, thousands of polymeric materials were synthesized and their effects on differentiation of human ES cells [128] and hMSC [129] were evaluated. These interactions have led to unexpected and novel cell–material interactions. In addition, using a similar approach the effect of combinatorial matrices of various natural ECM molecules was evaluated for the ability to maintain the function of differentiated hepatocytes and induce hepatic differentiation from murine ES cells [130]. Although the molecular mechanisms associated with the biological responses have yet to be clarified, the ability to use micro-scale

technologies to test cell–microenvironment interaction in a high-throughput manner could be important for the identification of cues that induce desired cell responses or novel biomaterials for tissue engineering.

CONCLUSIONS

The widespread use and availability of lithographic approaches have made micro-scale technologies a powerful tool for a number of biomedical applications. Microfabrication techniques and engineered biomaterials are being integrated to develop novel materials and functional microdevices for biomedical applications. Researchers are currently developing a number of micro-scale-enabling technologies, including bioreactors, valves, switching mechanisms, scaffolds, high-throughput libraries and channel architectures that require desired material properties. Also, microdevices have been used to make homogeneous and controlled biomaterials that can control cell behavior and generate functional tissues. Future integration of materials and micro-scale devices promises to lead to biomedical breakthroughs in both therapeutic and diagnostic applications.

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