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Review

Microengineered hydrogels for tissue engineering

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Abstract

Hydrogels have been extensively used in various biomedical applications such as drug delivery and biosensing. More recently the ability to engineer the size and shape of biologically relevant hydrogels has generated new opportunities in addressing challenges in tissue engineering such as vascularization, tissue architecture and cell seeding. Here, we discuss the use of microengineered hydrogels for tissue engineering applications. We will initially provide an overview of the various approaches that can be used to synthesize hydrogels with controlled features and will subsequently discuss the emerging applications of these hydrogels.

Keywords: Microengineered hydrogels; Tissue engineering

Contents

1. Introduction

Tissue engineering is an approach in generating a renewable source of transplantable tissues by using the principles of engineering, physical sciences and medicine [1]. In most cases, cells are seeded on biodegradable materials that have been fabricated in the form of porous scaffolds. As the cells deposit their own extracellular matrix and as the material is degraded a tissue-like structure is formed that can be subsequently transplanted. Despite enormous advances in tissue engineering, which include the clinical approval of tissue engineered skin, a number of scientific barriers still prevent the fabrication of more

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complex organs. These challenges include the difficulties in generating vascularized tissues as well as in mimicking the complex structure and architecture of biological tissues.

In the body, cells reside in close proximity to blood vessels that supply tissues with nutrients and oxygen and remove waste products and carbon dioxide. Therefore, to engineer functional tissues it is important to engineer a vascular network that can perform a similar function. In one approach scaffolds are seeded with endothelial and smooth muscle cells and/or induced to release growth factors that promote angiogenesis into the engineered scaffold. The major challenge in this approach is that during the time required to generate proper vascularization many cells may starve for oxygen and nutrients and lose their viability. Another approach to minimize this challenge is to pre-vascularize the engineered tissues prior to implantation by generating artificial microvasculature within the scaffolds that can integrate with the host's vasculature upon implantation. The major challenges in this approach are the difficulty in fabricating such controlled structures as well as ensuring their proper function after implantation.

Cell–cell contact and tissue architecture are other important parameters that regulate cell behavior. For example, in the bone marrow hematopoietic progenitors and stem cells closely interact with osteoblasts and endothelial cells [2]. Although, cells within tissue engineered scaffolds have a certain capacity to 'self-assemble' to regain important aspects of their cell–cell interactions, many of these interactions are permanently lost during tissue isolation and seeding processes. Furthermore, it is typically difficult to obtain uniform cell seeding throughout the scaffolds, with most cells seeding in the periphery of the scaffolds. Therefore, the ability to control cell–cell interactions and proper tissue architecture as well as uniform cell seeding may aid in generation of functional tissue constructs.

Microengineered hydrogels (i.e. hydrogels with features that are in the order of a few microns in at least one dimension) are potentially powerful engineering tools to overcome a number of tissue engineering challenges [3]. The use of microscale hydrogels in tissue engineering dates back to some of the earliest attempts at generating transplantable tissues. For example hydrogel microcapsules were used in cell microencapsulation, a process in which transplanted cells were protected from the host's immune system by being immobilized within a semi-permeable membrane [4]. However, more recently the ability to engineer the properties of hydrogel materials such as adhesiveness, stiffness, cell signaling potential, size and shape have enabled a wide range of new applications in tissue engineering. Here we will briefly discuss the synthesis and the emerging applications of microscale hydrogels for tissue engineering. We will first discuss the approaches used to fabricate hydrogels with microscale features and then discuss their applications in addressing the challenges of vascularization, cell seeding and tissue complexity within tissue engineered constructs.

2. Synthesis

To generate microengineered hydrogels the crosslinking process must be controlled with a high degree of spatial resolution. A number of techniques have been developed that can be used to generate hydrogels with dimensions as small as a few tens of nanometers. These techniques can be categorized into one of the following categories: emulsification, photolithography, microfluidic synthesis and micromolding.

2.1. Emulsification

Emulsification is the most widely used method for fabricating microgels. In this process a multi-phase mixture is stirred to generate small aqueous droplets of the hydrogel precursors within an organic phase. The size of the droplets can be controlled by the degree of mechanical agitation, viscosity of each phase, as well as the presence of surfactants that can modify the surface tension between the two phases. The resulting droplets can be gelled using a variety of crosslinking mechanisms to generate spherical microgels (Fig. 1A). This process can be used to fabricate microgels made from a variety of materials including agarose, alginate and collagen. By adding cells to the aqueous phase, cell-laden microgels can be fabricated for applications such as immunoisolation, as carriers within bioreactors or for analyzing stem cell biology [5].

The major advantage of emulsification is the ease with which it can be used to generate microgels. Depending on the process conditions the size distribution in the gels can be minimized, however, the process typically has a larger size distribution than other synthesis approaches. Furthermore, there is little control of the resulting shapes since the emulsification process typically produces spherical droplets.

2.2. Photolithography

Photolithography is a technique that has been widely developed for the microelectronics industry. More recently, photolithographic techniques have been used for a variety of biomedical applications to engineer microengineered hydrogels. This has been partly enabled by the development of synthetic and natural photocrosslinkable prepolymers that can crosslink to form hydrogels [3]. In photolithographic processes a thin film of a polymer is exposed to UV light through a mask. As the light reaches the photosensitive polymer through the transparent regions of a mask it causes a photoreaction that crosslinks the polymer (Fig. 1B).

Photolithography can be used to create microstructured hydrogel scaffolds or to immobilize cells within microengineered hydrogels. A number of studies have demonstrated

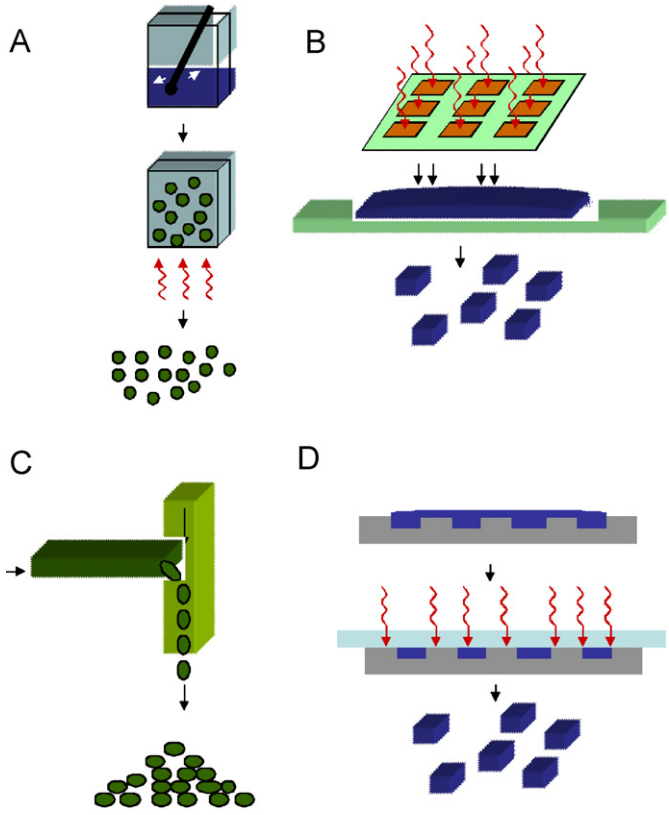

Fig. 1. Schematic diagram of the techniques to microengineer the size and shape of hydrogels: emulsification (A), photolithography (B), microfluidics (C) and micromolding (D).

that encapsulated cells within photocrosslinkable microgels can be made from various types of synthetic polymers such as poly(ethylene glycol) (PEG) [6,7]. In addition, natural photocrosslinkable pre-polymers have been developed to enable the formation of microengineered hydrogels from natural materials [8]. Photolithography can also be used to conjugate chemical entities to hydrogels with controlled spatial resolution [9,10].

The resolutions that can be achieved by using photolithography ranges from sub micron scale to millimeters. Considering that a cell is about 10 microns in diameter, photolithography can be used to generate structures that are much smaller or much larger than a cell making it suitable for biological applications such as tissue engineering. Potential disadvantages with photolithography include the need for photocrosslinkable materials, as well as the effects of UV light on cell function. Furthermore, since photolithography is inherently a 2D process it forms structures that may require further assembly to generate 3D tissues.

2.3. Microfluidics

Microfluidics has emerged as a potentially powerful method for generating microengineering hydrogels. The unique flow properties within microfluidic channels and the precision with which these devices can be fabricated present a unique opportunity in generating hydrogels with controlled features. A common method of using microfluidic systems to generate microparticles is by using multiphase systems. In these approaches the viscous and surface tension forces are used to create homogeneous particles that can be crosslinked to form microscale hydrogels (Fig. 1C). A range of particle sizes and shapes can be created by generating the properly designed microfluidic channels. For example, by changing the dimensions of the microchannels, the flow rates and the droplet shapes it is possible to create hydrogels in the form of spheres and rods [11].

An interesting aspect of microfluidic fabrication of microgels is that the spatial properties of hydrogels can be controlled. For example, by using a microfluidic device that can create concentration gradients of two or more inlets it is possible to create hydrogels with controlled gradients of signaling molecules or material properties embedded in the hydrogel [12]. These hydrogels can be used for various tissue-engineering applications in which concentration gradients are desired in the scaffolds. In addition, it is possible to generate Janus particles (i.e. particles in two or more distinct sides) by flowing multiple streams and generating droplets of the two or more regions [13]. Finally, it is possible to merge microfluidic techniques with other approaches such as photolithography [14–16]. For example, it is possible to flow hydrogels containing cells within microfluidic channels and then capture the cells within particular regions by crosslinking the surrounding hydrogels by using a photomask [17]. Also, by using this approach hydrogels can be fabricated with engineered bar-codes and analysis regions for biosensing and high-throughput applications [15].

2.4. Micromolding

Micromolding is another technique that is capable of generating hydrogels with controlled features. Micromolding has become particularly appealing due to soft lithography which has enabled easy fabrication of poly (dimethyl siloxane) (PDMS) molds from prefabricated silicon wafers. Thus by using micromolding approaches it is possible to generate a great variety of micromolded structures in a simple manner. To generate micromolded hydrogels, precursor polymers are initially molded and subsequently gelled to generate structures of a variety of shapes and sizes [18–20] (Fig. 1D). In addition to PDMS, other materials could also be used for micromolding. For example, by using a novel fluoro-based material with higher surface energies and enhanced fabrication resolution it was possible to micromold nanoscale particles of controlled shapes and sizes [21]. Although the immediate application of such high-resolution nanoparticles is in drug delivery, it is envisioned that the ability to engineer the nanoscale topography of hydrogels will be important in

generating improved tissue engineering scaffolds. Micromolding has also been used to generate microengineered hydrogels from a variety of materials including hyaluronic acid (HA) [8,19], chitosan [18] and PEG [22].

Until recently micromolding techniques were unable to fabricate microengineered hydrogels of controlled shapes and sizes from a class of materials that require the addition of gelling agents such as divalent cations. These materials encompass a large class of hydrogels including alginate and fibrin. To alleviate this limitation hydrogel micromolds have been developed that deliver the crosslinking agent in a controlled manner. Therefore, these molds enable molding the hydrogel precursors and subsequently delivering the curing agent in a desired manner [23].

Finally it is possible to use micromolding approaches to generate 3D hydrogel microstructures. This can be accomplished by first using a sacrificial template around which the hydrogel can be formed. This approach can be used to generate 3D interconnected macroporous hydrogels in which the macromers were formed around a packed bed of polymeric beads that were subsequently dissolved [24]. Also, to generate microfluidic channels within hydrogels a dissolvable gelatin-based template was used [25].

3. Tissue engineering applications

Microfabrication techniques are potentially powerful tools in tissue engineering since they can be used to replicate structures that are in the order of 0.1–10 μm, to control the microenvironment of individual cells, 10-~400 μm to control the structure of clusters of cells as well as >400 μm to control the interactions between multiple cell clusters. Thus the merger of microengineered hydrogels and microfabrication techniques has significant potential to generate tissue constructs that can overcome the limitations associated with the current tissue engineered constructs. Recently, two different approaches have emerged in using microengineered hydrogels for tissue engineering that can be classified as either "top-down" or "bottom-up".

3.1. Top-down tissue engineering

Top-down tissue engineering approach utilizes microengineering approaches to control the microscale features of relatively large pieces of hydrogels. Early examples of the top-down tissue engineering approaches have aimed to generate controlled featured within existing tissue engineering scaffolds. For example, to engineer microvasculature within tissue engineering scaffolds a number of approaches have been used to design the shapes of the scaffolds and then to micromold these structures from biomaterials [26,27]. More recently the ability to engineer the shapes and sizes of hydrogels has enabled the engineering of hydrogel-based microfluidic channels that can be potentially used to engineer tissue microvasculature [28]. It has been demonstrated that cells that are seeded in close proximity to the engineered hydrogel microchannels maintain their viability while cells that are further from these channels lose their viability over time [20]. Microengineered hydrogel microchannels can be fabricated from various hydrogels including collagen, HA or PEG and therefore show great potential in tissue engineering applications [29].

Microengineered hydrogels can also be used to control the interactions of cells relative to each other in 3D scaffolds. For example to control the size of cell aggregates in 3D, dielectrophoretic methods have been used to localize cells within specific regions of hydrogels that can be subsequently gelled [30]. Using these approaches it is possible to probe the effects of cell–cell interactions in 3D and to generate tissue engineered structures with control of the location of the cells relative to each other. Another approach is to generate 3D microengineered scaffolds with appropriate tissue architecture. These scaffolds can be fabricated using either standard printing technologies or by using some of the techniques that were previously described. For example, it is possible to fabricate layers of hydrogels (either alone or containing cells) with desired porosity and structure. These layers can be stacked on top of each other or used as is to generate 3D scaffolds with desired architectures. For example, a layer-by-layer microfluidic technique was used to immobilize cell-matrix assemblies to build multilayer constructs that mimicked the arterial structure by using three types of vascular cells [31].

3.2. Bottom-up tissue engineering

Tissue engineered constructs can also be fabricated by the assembly of smaller building blocks. This approach mimics much of the native biology that is often made from repeating functional units. For example, in the liver, the sinusoid is the repeating functional unit. Bottom-up approaches can be used to generate functional units that can be assembled in a modular approach to generate larger scaffolds. An interesting example of the use of modular components for generating tissues was recently demonstrated [32]. In this approach rod-shaped collagen microgels that were seeded with HepG2 hepatocytes on the inside and endothelial cells on their surfaces were 'packed' together within a bioreactor and perfused with medium or whole blood (Fig. 2A and B). It was demonstrated that the spaces between the modules formed interconnected channels that exhibited delayed clotting times. In another approach, shape controlled cell-laden microgels were created by micromolding photocrosslinkable hydrogels such as HA. The rectangular microgels generated using this approach could be seeded with different cell types and assembled to generate 3D tissue structures made from various cell types with controlled architecture and cell–cell interactions. The gels could be subsequently further crosslinked into each other to stabilize their interactions.

Tissue printing is an emerging approach that can also be used to form tissues from smaller building blocks [33]. By

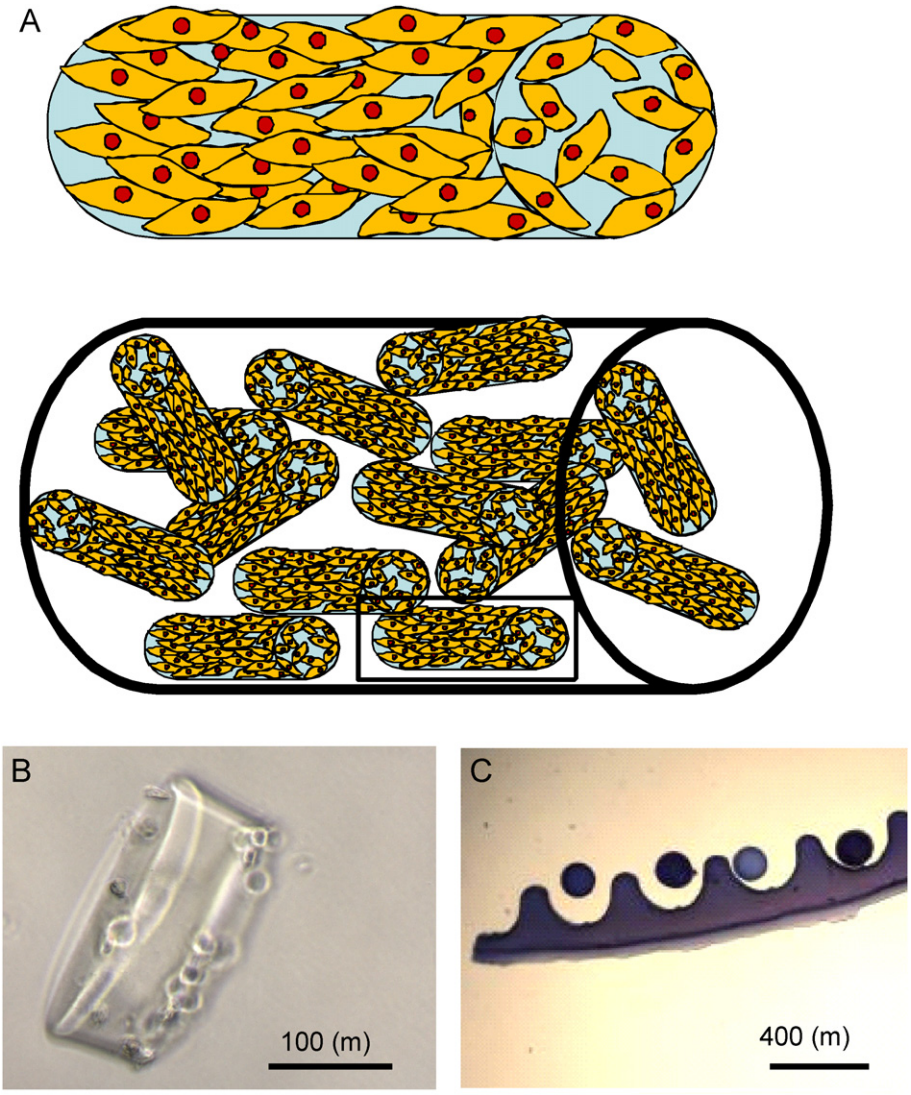

Fig. 2. Bottom-up tissue engineering by using microengineered hydrogels. A schematic of a cell-laden microengineered hydrogels (top) and a packed bed of hydrogels (bottom) (A). Cells can be encapsulated within microgels of controlled shapes (B). The modules can be 'assembled' by a number of mechanisms. For example, spherical microgels can be fit in larger microgels using a lock-and-key process (C).

using tissue printing it is possible to generate microvasculature and desired architecture in tissues. Although a number of challenges such as ejector clogging and poor tissue mechanics have limited the current applications of this technology, it is anticipated that through further research tissue printing will become a powerful 'bottom-up' approach to engineer complex 3D tissues.

4. Conclusion

We have described the use of microengineered hydrogels in tissue engineering. Over the past few years the ability to engineer the shapes and sizes of biologically relevant hydrogels has led to new approaches in generating tissues. These approaches can be used to engineer microvasculature and tissue architecture inside cell-containing hydrogels. Also, it is envisioned that the ability to engineer functional tissue units can be used to generate a 'modular approach' to tissue engineering in which smaller building blocks can be assembled to generate larger tissues with appropriate function and structure. Overall, it appears that our ability to engineer microgels and microscale hydrogels has significant potential in overcoming many of the challenges that have plagued the field of tissue engineering.

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