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Micromolding of shape-controlled, harvestable cell-laden hydrogels

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Abstract

Encapsulation of mammalian cells within hydrogels has great utility for a variety of applications ranging from tissue engineering to cell-based assays. In this work, we present a technique to encapsulate live cells in three-dimensional (3D) microscale hydrogels (microgels) of controlled shapes and sizes in the form of harvestable free standing units. Cells were suspended in methacrylated hyaluronic acid (MeHA) or poly(ethylene glycol) diacrylate (PEGDA) hydrogel precursor solution containing photoinitiator, micromolded using a hydrophilic poly(dimethylsiloxane) (PDMS) stamp, and crosslinked using ultraviolet (UV) radiation. By controlling the features on the PDMS stamp, the size and shape of the molded hydrogels were controlled. Cells within microgels were well distributed and remained viable. These shape-specific microgels could be easily retrieved, cultured and potentially assembled to generate structures with controlled spatial distribution of multiple cell types. Further development of this technique may lead to applications in 3D co-cultures for tissue/organ regeneration and cell-based assays in which it is important to mimic the architectural intricacies of physiological cell–cell interactions.

Keywords: Microgels; Tissue engineering; Micromolding; Biomaterials; Cell encapsulation

1. Introduction

Tightly regulated and controlled *in situ* microenvironments are comprised of cells, soluble factors, and extracellular matrix molecules. [1]. Mimicking the *in vivo* microenvironment can be useful for a variety of applications such as tissue engineering [2], cell-based assays [3], and directed stem cell differentiation [4]. Hydrogels, are three-dimensional (3D) crosslinked networks of hydrophilic polymers that, resemble the physical characteristics of extracellular matrices [5] and are often used to encapsulate cells. They can be tailored to exhibit high permeability to oxygen, nutrients, and other water-soluble metabolites [6]. Cell encapsulating hydrogels can be used for the generation of 3D tissue engineering structures [7] and immunoisolation microcapsules [8,9] as well as for use in scalable bioreactors [10].

Typically, cells are encapsulated within hydrogels through mixing a cell suspension with hydrogel precursors followed by crosslinking of the network. The crosslinking reaction may be controlled by a variety of environmental factors such as temperature, pH and the addition of chelating ions. In addition, hydrogels can be photopolymerized in the presence of photoinitiators via exposure to ultraviolet (UV) light [11]. Both biological hydrogels (e.g. fibrin [12], hyaluronic acid (HA) [13], agarose [8]) and synthetic hydrogels (e.g. poly(ethylene glycol) (PEG) [14,15]) have been used to encapsulate cells. For example,

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photopolymerized PEG diacrylate hydrogels, have been explored for the transplantation of islets of Langerhans for development of a bioartificial endocrine pancreas [16–18]. Similarly, photopolymerized hyaluronic acid hydrogels have been investigated as potential implantable/injectable cell delivery vehicles for cartilage regeneration [19].

The encapsulation of cells within hydrogels has been proposed as a method of enabling the scalable expansion of anchorage dependant cells within stirred bioreactors. However, the immobilization of cells within larger structures decreases the viability of cells in the center of these structures due to the lack of appropriate levels of oxygen and nutrients [16,20]. Spherical microcapsules with high surface area to volume ratios and coated annuli of cells immobilized within polymers have therefore been generated to overcome transport difficulties [8,19]. Currently most approaches to generate such structures have been based on spherical structures because of the available technologies to generate microscale spheres based on emulsification [10] or shear-induced droplet formation from syringes [8]. These approaches have been shown to be capable of forming spherical cell-laden (microscale hydrogels) microgels of controlled sizes; however, they are not amenable for the generation of other well-defined shapes. Thus, the development of approaches to generate cell-laden hydrogels with controlled sizes and shapes in a homogeneous manner may be of benefit.

Recently, photolithography [21–23] and soft lithography [24] have been used to encapsulate live cells within microscale polymeric hydrogels (i.e. microgels) anchored onto two-dimensional (2D) surfaces, which offer great potential for diagnostics and cell screening applications. Alternatively, cell encapsulation within free floating microgels (i.e. in suspension) may be advantageous not only for immunoisolation and bioreactor applications where long term cell culture is imperative, but also for tissue engineering. Such systems allow for the creation of micron sized units of tissue that can be potentially assembled to create tissue engineering constructs of controlled microscale structural and architecture. For such applications, controlling the size and shape of cell-laden microgels is important for minimizing diffusion limitations and for exhibiting control over the macroscopic engineered tissue. Micromolding of hydrogels provides a potentially powerful method for fabricating micro- and nanostructures [25,26]. Micromolding approaches are compatible with soft lithographic technology and therefore greatly minimize the need for costly photolithographic equipment and clean room facilities.

In this paper, we present a micromolding approach for generating cell-encapsulating 3D hydrogels of controlled shapes and sizes in the form of harvestable free units. Cells were suspended in a hydrogel precursor solution containing photoinitiator, deposited onto hydrophilic poly(dimethylsiloxane) (PDMS) patterns, crosslinked under UV radiation, and retrieved upon hydration. Two common photocrosslinkable hydrogel materials, methacrylated hyaluronic acid (MeHA) and poly(ethylene glycol) diacrylate (PEGDA), were tested using this technique, yielding shape-controlled microgels with homogeneous cell distribution at various viable cell densities. Hyaluronic acid is a natural component of the extracellular matrix known for its biodegradable, bioresistant properties [27] and its role in facilitating cellular functions such as adhesion, proliferation, and migration [28], while PEG is an inert, non-biofouling synthetic material often used as templates for immobilizing cells on 2D surfaces [25,26] or within microfluidic channels [25]. These two diverse materials were both shown to be compatible with this micromolding approach, suggesting the versatility of this technique and the feasibility of developing it further for tissue engineering applications in mimicking the architectural intricacies of physiological cell–cell interactions.

2. Materials and methods

2.1. Cell culture

All cells were manipulated under sterile tissue culture hoods and maintained in a 95% air/5% CO_2 humidified incubator at 37 °C. NIH-3T3 mouse embryonic fibroblast cells were maintained in Dulbecco's modified Eagle media (DMEM) supplemented with 10% FBS. Confluent dishes of NIH-3T3 cells were passaged and fed every 3–4 days. Murine embryonic stem (ES) cells (R1 strain) were maintained on gelatin treated dishes with media comprised of 15% ES qualified FBS in DMEM knockout medium. ES cells were fed daily and passaged every 3 days at a subculture ratio of 1:4.

2.2. Prepolymer solution

Two macromers were used: poly(ethylene glycol) and hyaluronic acid. The synthesis of MeHA was previously described [29]. In brief, the synthesis was performed by the addition of 1 wt% methacrylic anhydride (Sigma) to a solution of 1 wt% HA (Lifecore, MW = 67 kDa) in deionized water. The reaction was performed for 24 h on ice and maintained at a pH of 8–9 through the addition of 5 N NaOH. The macromer solution was then purified by dialyzing (Pierce Biotechnology, MW cutoff 7 kDa) for 48 h in deionized water and lyophilized for 3 days, resulting in a final dry form which was frozen for storage. The prepolymer form of MeHA was created by dissolving dry MeHA in PBS (Gibco) at 37 °C for 24 h to facilitate full dissolution. Immediately prior to UV photopolymerization, varying concentrations of photoinitiator solution was added to the prepared prepolymer solution. The photoinitiator solution used was 33 wt% 2-hydroxy-1-(4-(hydroxyethoxy)phenyl)-2-methyl-1-propanone (Irgacure 2959, CIBA Chemicals) in methanol.

To generate PEG hydrogels, a solution containing 10% (w/w) poly(ethylene glycol)-diacrylate polymer, PEGDA, (MW 575, Sigma) in phosphate-buffered saline (PBS, Gibco) was prepared prior to experiments in order to allow the PEGDA to adequately dissolve into solution. Immediately prior to UV photopolymerization, photoinitiator solution was added to the prepolymer solution at 1 wt%. The photoinitiator solution used was also 33% (w/w) 2-hydroxy-1-(4-(hydroxyethoxy)phenyl)-2-methyl-1-propanone (Irgacure 2959, CIBA Chemicals) in methanol.

2.3. PDMS mold fabrication

PDMS micropatterns of various shapes were fabricated by curing prepolymer (Sylgard 184, Essex Chemical) on silicon masters patterned with SU-8 photoresist. The patterns on the masters had protruding shapes (squares, circles, long rectangles) of various sizes (ranging from 50 to