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Processing a polyelectrolyete complex hydrogel at the micro-scale

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KEYWORDS

Methacrylated Gellan Gum, Chitosan, Polyelectrolyte complex, Microfabrication.

ABSTRACT

Natural tissues are structured at the micro- and nano-scale. Thus, a lot of effort has been devoted to develop biomaterial systems enabling replicating those natural structures *in vitro*. Microfabrication techniques have been applied in the development of these biomaterials structures with tailored architectural details. Moreover, by combining in a single device both the architectural and the biochemical cues, one can design a biomaterial device with improved biological affinity, that more closely mimics that extracellular micro-environment.

INTRODUCTION

Hydrogels have been selected for the development of engineered tissues, mainly due to their similarity with the extracellular matrix (ECM) of many tissues (Peppas NA 2006). Several mechanisms for the development of hydrogels have been reported, namely the use of two oppositely charged polyelectrolytes that complex when mixed together, forming a physical hydrogel (Bhatia SR 2005). This hydrogel is held together by molecular entanglements. These interactions are reversible and can be disrupted by changes in physical conditions (such as pH). To overcome this limitation, we used a previously developed a photocrosslinkable polymer (methacrylated gellan gum, MeGG) that enabled the stabilization and microengineering of the polyelectrolyte complex (PEC) hydrogel. Herein, MeGG was combined with chitosan (CHT). A key feature of hydrogels is their ability to be micro-processed into devices with specific sizes and shapes (Khademhosseini A 2007a). Micromolding can be employed to micro-fabricate hydrogels that are crossliked by physical mechanisms. However, photolithographic methods allow for a better control of the features developed and to obtain reproducible shapes. Thus, herein we have microfabricated a photocrosslinked PEC hydrogel using two mechanisms in order to obtain different relevant micro-features: photolithography and microfluidics.

MATERIALS AND METHODS

Materials and hydrogel fabrication

MeGG was synthezised as described elsewhere (Coutinho DF 2010). MeGG (1%, w/v) was dissolved in dionized water. CHT was dissolved at at 1% (w/v) in an acetic acid solution (1%, v/v). Anionic MeGG was added to cationic CHT and exposed to UV light to form PEC hydrogels.

Polyelectrolyte hydrogel characterization

The morphology of the hydrogel was evaluated by scanning electron microscopy (SEM). The chemistry of the surface and bulk of the hydrogel was analyzed by Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). Transmission electron (TEM) and confocal microscopy of the

hydrogel using fluorescein-CHT showed the distribution of both polymers within the hydrogel.

Cell encapsulation and Microfabrication

Rat cardiac fibroblasts were isolated as described elsewhere (Khademhosseini A 2007b). Cells were suspended in the MeGG solution before PEC formation and microfabrication. Microfabricated structures were produced either by placing a photomask on top of the PEC or by flowing both solutions into a microfluidic channel.

RESULTS AND DISCUSSION

The anionic MeGG was added to the cationic CHT, forming an hydrogel capsule. Experimental results showed the presence of fibrous structures over the pores of the hydrogel. The chemical structure of the PEC hydrogel was evaluated by FTIR, revealing the presence of the absorption peaks characteristic of both polymers. Further analysis of the bulk of the hydrogel revealed that the initial electrostatic interactions that occurred upon contact between the two polymers did not allow for an instantaneous mixing. Instead, upon hydrogel formation, the mixing of both polymers proceeded slowly. TEM and confocal of fluorescein-labeled CHT suggested the migration of CHT to the interior of the apparent MeGG capsule. FTIR and XPS chemically validated these findings. The photocrosslinkable feature of MeGG further enabled the formation of a number of micro-scaled units. Encapsulated rat cardiac fibroblasts kept viable after being exposed to the processing methodologies used. Micrometer-size building blocks with different shapes and sizes were fabricated simply by changing the pattern used in the photomask. These micro-units could potentially be used for replicating the micro-environment features of tissues. On the other hand, the microfluidic channel used to direct the formation of the PEC hydrogel resulted on the engineering of a fibrous hydrogel that replicates at a micro-scale the architecture of fiber bundles found in the extracellular matrix of a variety of tissues.

CONCLUSIONS

We successfully fabricated a stable photocrosslinkable PEC hydrogel using cationic CHT and photocrosslinkable anionic MeGG. This biomaterial system is potentially useful for a variety of biomedical applications simply by adjusting the geometry of the microfabrication tools used.

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