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Cytocompatible and spontaneously forming phospholipid polymer hydrogels

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ABSTRACT

Given the importance of regulating living cell functions in three-dimensional (3D) environments, we have designed a cytocompatible and spontaneously forming polymer hydrogel matrix. Water-soluble phospholipid polymers bearing a phenylboronic acid unit, poly(2-methacryloyloxyethyl phosphorylcholine-*co*-*n*-butyl methacrylate-*co*-*p*-vinylphenylboronic acid) (PMBV), and poly(vinyl alcohol) (PVA), are candidate systems for preparing the hydrogel matrices. Aqueous solutions of PMBV and PVA can be used to form hydrogels based on reversible complexation between the phenylboronic acid groups in PMBV and the diol groups in PVA, even under cell culture conditions. Uniform cell encapsulation is easily achieved with hydrogel formation, and cells survived well in the hydrogel. By applying a spinning-assisted layer-by-layer (LbL) process, multilayered PMBV/PVA hydrogels containing living cells can be assembled. This multilayered hydrogel, which mimics the stratified structure of *in vivo* tissues, allows the layer-specific encapsulation of cells and temporary storage of bioactive molecules. Distance-dependent cell–cell interactions are investigated using the multilayered hydrogel where two cell-laden layers are separated by a finely controlled multilayered hydrogel. In addition, dual-crosslinked multilayered hydrogels are also assembled by alternative depositions of PMBV and photoreactive-PVA solutions, followed by photoirradiation. The dual-crosslinked hydrogel has a lower diffusivity of bioactive molecules than that of single-crosslinked hydrogel and therefore acts as a diffusion-controlling barrier. We demonstrate the utility of this dual-crosslinked hydrogel by examining ways to regulate the diffusion of bioactive molecules in the hydrogel and investigating the diffusion-dependent effects on cell behavior. In conclusion, these hydrogel matrices can provide insights into the regulation of cell behavior in 3D matrices. In turn, our results may contribute to the future design of 3D cell culture systems and tissue regenerated medicine based on cell engineering.

1. Introduction

The past few decades have witnessed gradual progress in the field of biomaterials, which has split into two directions: (i) clinical therapies, *e.g.*, encapsulation of bioactive molecules or even cells in constructs to replace human skin, cartilage, bone, and other tissues, and (ii) basic research in tissue physiology and pathophysiology *in vitro*, *e.g.* experimental tissue models for drug development, toxicology, pharmacokinetics, and developmental biology [1]. In particular, the use of polymer hydrogels has led to progress in *in vivo* tissue repair and the design of tightly controlled *in vitro* models that can be used to study stem cell fate, tissue morphogenesis, and disease pathogenesis [2–4].

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Hydrogels are constructed using hydrophilic polymer networks and absorb large amounts of water (typically comprising 90–99% of an aqueous medium) while remaining insoluble in aqueous solutions because of the chemical or physical crosslinking of individual polymer chains. They are a type of soft materials that mimic some of the physicochemical aspects of natural tissues. As we know, some biodegradable polymers, such as poly(lactic acid) (PLA) and poly(lactide-*co*-glycolide) (PLGA), have been applied in the medical fields as matrices for tissue regeneration. They have limited water-absorption capabilities (<5–10 wt%) based on their hydrophobic nature. On the contrary, hydrophilic hydrogels exhibit many unique physicochemical properties that render them suitable for biomedical applications [2,3]. For example, hydrogels can be used in three-dimensional (3D) cell culture and encapsulations of biomolecules, such as proteins and DNA, which are fragile species and can easily be denatured in hydrophobic environments. Hydrogel matrices contain high amount of water, therefore, they can provide viscoelastic mechanical properties similar to many soft tissues and permit diffusion of water as well as nutrients, proteins and signaling molecules [1–3]. All together mimicked some key features of the native extracelluar matrix (ECM). 3D cell culture in hydrogel matrices is better than conventional two-dimensional (2D) cultures, because 3D cell culture provides another dimension for mechanical stimulation and for cell–cell interaction and cell–ECM interaction, which dramatically affects expression of integrin, cell contraction and associated intracellular signaling [1,4]. The 3D matrix can also affect diffusion of soluble factors, such as some growth factors, thereby establishing tissue-scale solute concentration gradients, which involve in regulation of cell behaviors [5,6]. Mimicry of these concentration gradients in hydrogel could be important in functional tissue engineering, which aim to create natural tissue structure and function [7].

Among various hydrogels, functional hydrogels that are injectable into living organisms have attracted significant interest in biomedical applications [8], as summarized in Fig. 1. Normally, the gelation mechanisms of these hydrogels are chemical crosslinking or sol–gel phase transition of the polymer chains. These hydrogels are available to be injected anywhere, and their shapes can be adjusted to conform to the administered area because these hydrogel systems are flexible aqueous solutions before administration. However, once injected, rapid gelation occurs in response to the physiological condition. These hydrogel systems allow the simple incorporation of bioactive molecules and cells, via mixing before injection. After gelation, these matrices serve as sources of drug release or as scaffolds for cell growth and differentiation for tissue regeneration. Injectable hydrogels can be simply classified into two categories, *in situ* forming hydrogels and spontaneously forming hydrogels, based on the different crosslinking mechanisms.

Sol–gel phase transitions are caused by changes in environmental conditions, such as changes in temperature and pH, thus resulting in *in situ* hydrogel formation. Hydrogels formed *in situ* have been well summarized in several reviews [9,10]. For instance, Bae reviewed thermosensitive sol–gel reversible hydrogels. Lee specifically commented on *in situ* gelling stimuli-sensitive block copolymer hydrogels for drug delivery. As *in situ* forming hydrogel systems that undergo sol–gel transitions have been adequately summarized, they will not be emphasized in this review.

We briefly introduced a typical *in situ* forming hydrogel based on poly(*N*-isopropylacrylamide) (PNIPAM). It is a well-known thermosensitive polymer that acts as a model for physical and chemical studies and is applicable in biomedical applications, such as drug delivery and tissue engineering [10]. Its aqueous solution precipitates above a low critical solution temperature (LCST) of about 32 °C. Below the LCST, the enthalpy term, which is mostly contributed by the hydrogen bonding between the polar polymer groups and the water molecules, results in the dissolution of the polymer. Above the LCST, the entropy term (hydrophobic interactions) dominates, leading to the precipitation of the polymer in water [10]. However, this polymer is non-biodegradable and was found to exhibit nerve toxicity caused by residual acrylamide-like monomers, making it difficult to be used *in vivo* [8].

Another method of preparing injectable hydrogel systems is to form hydrogels spontaneously after mixing at

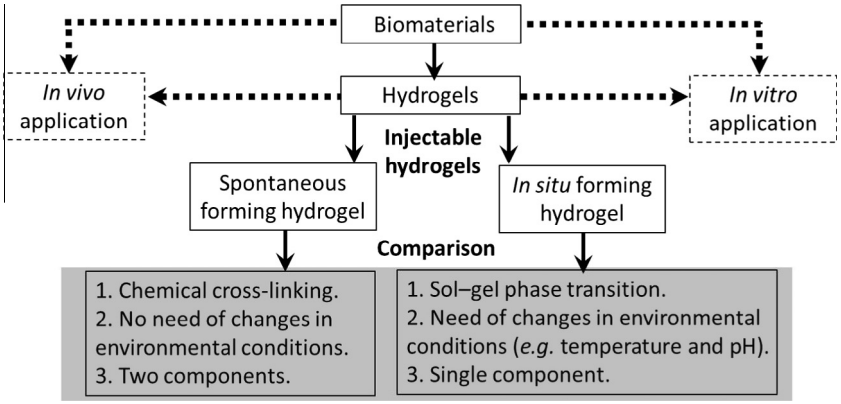

Fig. 1. Injectable polymer hydrogel system — a new biomaterial.