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ScienceDirect
Acta Biomaterialia 3 (2007) 439–448
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Characterizing multilaminated hydrogels with spatially varying network structure and solute loading using confocal laser scanning microscopy

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Received 1 June 2006; received in revised form 31 October 2006; accepted 7 November 2006

Abstract

Multilaminated controlled release devices were formed through photopolymerization techniques to produce hydrogels with spatially varying solute loadings and network structures composed of poly(hydroxyl ethyl methacrylate) (PHEMA) and poly(ethylene glycol) (PEG). Using low molecular weight fluorescent dyes as model drugs, the distribution profiles were characterized non-invasively in pseudo-real-time with confocal laser scanning microscopy (CLSM) during release studies. For comparison, theoretical modeling based on Fickian diffusion theory was performed in conjunction with experimental work to identify any deviations from expected behavior and to guide in the development of future devices. In multilaminates composed of only PHEMA, the evolution of dye distribution during release and cumulative release profiles agreed well with theoretically predicted data, indicating continuity of diffusion and insignificant interfacial hindrance between layers. However, in devices composed of alternating layers of PHEMA and PEG, differences from predicted behavior were experimentally observed in both concentration profiles and release rates, suggesting interfacial obstruction of diffusion, possibly due to the formation of interpenetrating networks. Finally, the simultaneous release of two dyes at different rates from a PEG/PHEMA multilaminate was monitored to demonstrate the usefulness of CLSM in understanding the complex temporal changes in solute distributions in gel devices.
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Keywords: Confocal microscopy; Controlled drug release; Diffusion; Hydrogel; Photopolymerization

1. Introduction

Diffusion-controlled polymeric drug delivery systems are used extensively in many pharmaceutical applications [1,2]. In monolithic devices with uniform initial drug disbursement, first-order diffusion kinetics are often observed, where the drug release rate is initially high and then tapers off rapidly. In many applications, release rates approaching zero-order behavior, or a near constant rate of release, would be beneficial. To eliminate the burst effect and tailor release profiles in polymeric devices, researchers have investigated a variety of approaches, including alternative geometries [3,4], rate-controlling membranes [5–8] and surface-degrading polymers [9–12].

One alternative method is the construction of multilaminate polymeric devices with spatially varying properties. Several researchers have demonstrated control of release rate profiles both theoretically and experimentally by constructing matrices with initially nonuniform concentration profiles [13–18]. Specifically, Lu and Anseth demonstrated the potential of photopolymerizations to construct poly(2-hydroxyethyl methacrylate) (PHEMA) multilaminates with nonuniform initial solute distributions [15].

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1742-7061/$ - see front matter Published by Elsevier Ltd. on behalf of Acta Materialia Inc.
doi:10.1016/j.actbio.2006.11.006

Photopolymerizations are advantageous in that they proceed very rapidly at room temperature and can be performed in an aqueous environment or in the absence of solvent. These mild reaction conditions enable the safe encapsulation of biological agents, such as living cells [19–23], DNA [24,25] and therapeutic agents [26], with complete spatial and temporal control of the reaction. An additional level of control over release rates can be gained by varying diffusional properties between layers of a multilaminate device. Furthermore, by varying spatial properties within a device, simultaneous release of multiple therapeutics at different rates can be attained.

In crosslinked polymers, such as hydrogels, diffusional properties can be controlled by varying the crosslink density of the network. For example, 2-hydroxyethyl methacrylate (HEMA) requires a crosslinking molecule with functionality greater than 2, such as diethylene glycol dimethacrylate (DEGDMA), to form networks. Here, we are following the convention that vinyl groups in a chain polymerization have a functionality of two, so DEGDMA has a functionality of four. By varying the ratio of HEMA to DEGDMA, one can systematically control the network properties, and thus control the rate of transport of a given solute [15]. For greater variations in diffusional properties, different materials could be utilized with even greater differences in structure.

Multilaminates with combinations of spatially varying diffusion and loading have been explored theoretically using rigorous optimization techniques to design devices with desirable release profiles [18,27]. However, little has been reported with respect to the experimental investigation of such devices, and these studies have been limited to following cumulative release profiles. The objective of this study was to construct PHEMA/poly(ethylene glycol) (PEG) hydrogel multilaminates via photopolymerization and to characterize these devices experimentally and theoretically. These matrix materials were selected, in part, due to their long histories of use in biomedical applications, including drug delivery. Using low molecular weight fluorescent dyes as model drug molecules, devices with spatially varying loading and diffusional properties were constructed with the goal of tuning the overall release rate. Traditionally, release and uptake experiments are characterized by simply quantifying solute concentrations in the release/uptake media. To gain greater insight into the evolving concentration profiles within these devices, confocal laser scanning microscopy (CLSM) was utilized to image model drug distributions within the polymeric matrices during release experiments. Previously, CLSM was demonstrated as an effective tool for non-destructively characterizing molecular transport in monolithic hydrogel networks in pseudo-real-time [28]. Specifically, PHEMA multilaminates with spatially varying initial loading profiles, PHEMA–PEG multilaminates with spatially varying diffusional properties and multicomponent release from multilaminates were investigated with respect to their ability to release low molecular weight (approximately 400–600 Da) drug molecules. In this work, theoretical modeling was performed and compared with experimental work to analyze any divergences from expected behavior as predicted by Fickian theory. Modeling also provided insight into the design and construction of devices to yield desired drug delivery responses.

2. Materials and methods

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) was obtained from Acros Organics and poly(ethylene glycol) 550 dimethacrylate (PEG550DMA) was obtained from Sigma–Aldrich. Diethylene glycol dimethacrylate (DEGDMA) was purchased from Polysciences, Inc. (Warrington, PA). The photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPA) was obtained from Ciba Specialty Chemicals. The model release solutes in this study, Texas Red sulfonyl chloride (TxR, MW = 625 Da) and 2′,7′-difluorofluorescein [Oregon Green 488 (OG488), MW = 368 Da] were obtained from Invitrogen. All chemicals were used as received.

2.2. Equilibrium swelling experiments

Equilibrium swelling experiments were performed by placing poly(PEG550DMA) and PHEMA disks ($n = 3$–5, diameter ~10 mm, thickness ~0.4 mm) in deionized water (DI-H_2O) until constant masses were attained. The disks were then patted dry and the swollen masses were recorded. Dry masses were measured after drying the disks in a vacuum oven for several days. The equilibrium mass swelling ratio, q, was calculated by dividing the swollen mass by the dry mass for each sample.

2.3. Construction of multilaminates

HEMA monomer solutions were created by combining 65 wt.% monomer with 35 wt.% deionized water and 0.1 wt.% DMPA. The HEMA monomer component contained 1–10 wt.% DEGDMA. PEG macromer solutions were created by combining PEG550DMA (65 wt.%) with 0.1 wt.% DMPA in DI-H_2O. Hydrogels of both PHEMA and PEG550DMA exhibit minimal swelling post-polymerization when cured in 35 wt.% water, which is an important property that provides excellent compatibility and adhesion between layers. Furthermore, the minimal swelling in the gels post-polymerization eliminates the confounding effects of water uptake and the complexity of moving boundaries during release experiments. Multilaminates were constructed by photopolymerizing monomer solutions between glass microscopy slides using #2 cover slips (0.19–0.25 mm thick) and squares cut from standard transparencies (~0.11 mm thick) as spacers. All photopolymerizations were performed at 4 °C using a Blak-Ray UV lamp (365 nm, ~20 mW cm^{-2}). After the first layer was suffi-

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