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Characterization of protein release from photocrosslinkable hyaluronic acid-polyethylene glycol hydrogel tissue engineering scaffolds

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Abstract

The goal of this work was to utilize the naturally derived bioactive polymer hyaluronic acid (HA) to create a combination tissue engineering scaffold and protein delivery device. HA is a non-immunogenic, non-adhesive glycosaminoglycan that plays significant roles in several cellular processes, including angiogenesis and the regulation of inflammation. In previous work, we created photopolymerizable glycidyl methacrylate-hyaluronic acid (GMHA) hydrogels that had controlled degradation rates, were cytocompatible, and were able to be modified with peptide moieties. In the present studies, we characterized the release of a model protein, bovine serum albumin (BSA), from GMHA and GMHA-polyethylene glycol (PEG) hydrogels. Although BSA could be released rapidly (>60% within 6 h) from 1% GMHA hydrogels, we found that increasing either the GMHA or the PEG concentrations could lengthen the duration of protein delivery. Preliminary size exclusion chromatography studies indicated that the released BSA was almost entirely in its native monomeric form. Lastly, protein release was extended to several weeks by suspending BSA-poly(lactic-co-glycolic acid) microspheres within the hydrogel bulk. These initial studies indicate that the naturally derived biopolymer HA can be employed to design novel photopolymerizable composites that are suitable for delivering stable proteins from scaffolding in tissue engineering applications.

Keywords: Hyaluronic acid; Polyethylene glycol; Protein release; Hydrogel; Tissue engineering

1. Introduction

The fields of tissue engineering and biopharmaceutics provide a number of complex opportunities for the design of new implant materials [1–5]. Each individual application relies on rationally designed scaffolds and devices that interact with living systems in a highly controlled manner. For these reasons, it is likely that no one biomaterial will satisfy all of the design parameters in all applications.

The focus of our work has been soft-tissue engineering applications, such as peripheral nerve repair. Ideal implants for soft tissues are degradable, porous and highly permeable, able to maintain a desired shape, and capable of specifically modulating biological responses. In previous work, we made the first steps towards meeting these aims by creating new hydrogel scaffolds from crosslinked hyaluronic acid (HA; Fig. 1) [6,7]. HA presents a unique combination of advantages for biomaterial formulations: it is a naturally derived, non-immunogenic, non-adhesive, bioactive glycosaminoglycan that has been associated with several cellular processes, including angiogenesis and the regulation of inflammation [8]. We developed glycidyl methacrylate-modified HA (GMHA), which can be photocrosslinked to form a highly hydrated and degradable tissue engineering scaffold [6]. Further enhancements of these

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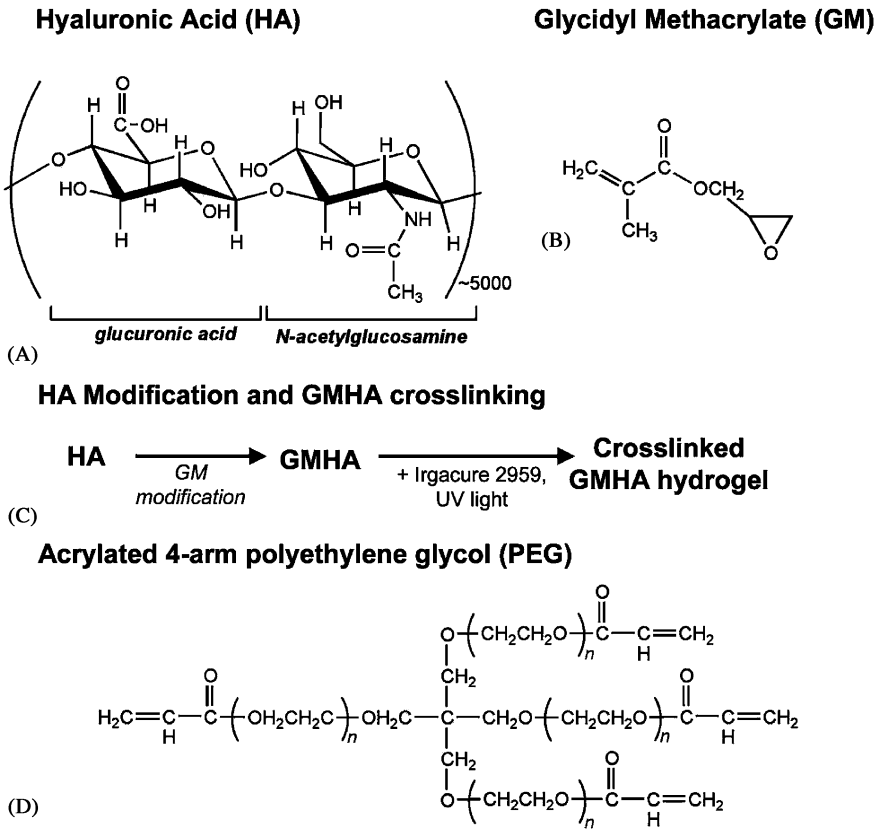

Fig. 1. Schematic of HA modification and crosslinking. The biopolymer HA (A), composed of repeated disaccharides of glucuronic acid and N-acetylglucosamine, was modified with glycidyl methacrylate (B) to yield the photocrosslinkable conjugate, GMHA. In the presence of the photoinitiator Irgacure 2959 and ultraviolet light, GMHA was crosslinked (C) to form an insoluble GMHA hydrogel. To increase the hydrogel crosslink density, acrylated 4-arm PEG (D) was added to the gelation solution, resulting in crosslinked composite GMHA–PEG hydrogels.

GMHA hydrogels focused on methods to attach peptides [7]. Such sequences could allow control over several adhesion-related cellular processes, including migration and proliferation [9–11].

In this study, we built upon these HA-based hydrogels to create composite materials that not only are bioactive, degradable, hydrated and pliable, but also allow the controlled release of a model protein. Other researchers have reported controlled protein delivery using a variety of hydrogel materials, including degradable and non-degradable forms of synthetic and naturally derived materials [12–19]. Many of these materials are able to deliver proteins over extended periods of time (days to weeks) because their structure greatly restricts the diffusion of macromolecules [20]. This design makes sense for the controlled delivery of therapeutic proteins such as insulin [21] or antigenic proteins for vaccination [22]. However, in tissue engineering applications, materials with limited permeability pose a key drawback: it is likely that if the release of bioactive proteins is diffusion limited, that the diffusion of other soluble factors from the implant surroundings is also restricted. A number of regenerative processes, such as the guided growth of axons during peripheral nerve regeneration, rely on the diffusion of soluble growth factors and cytokines from the surrounding tissue [23]. Furthermore, regeneration is a highly demanding metabolic process, and the unhindered diffusion of nutrients and metabolic waste is critical for optimal cellular growth.

Aside from hydrogels, most HA-based materials designed for controlled release devices (e.g., microspheres, soluble gels, and films) have structural or degradation properties that are not suitable for soft-tissue engineering scaffolds (e.g., two-dimensional or particulate structure, long degradation rates, or very quick dissolution times) [24–31]. Despite these challenging criteria, we were encouraged by the promising results from such HA-based strategies; thus, our aim was to investigate protein release from our GMHA hydrogels that were previously designed to serve as scaffolds for soft-tissue applications.

Given that the mesh size of GMHA hydrogels is relatively large [6], we expected that protein release from these materials would be rapid. However, some tissue engineering systems, such as peripheral nerve repair, can