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Topologically controlled hyaluronan-based gel coatings of hydrophobic grid-like scaffolds to modulate drug delivery

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ABSTRACT

Scaffolds based on poly(ethyl acrylate) having interwoven channels were coated with a hyaluronan (HA) hydrogel to be used in tissue engineering applications. Controlled typologies of coatings evolving from isolated aggregates to continuous layers, which eventually clog the channels, were obtained by using hyaluronan solutions of different concentrations. The efficiency of the HA loading was determined using gravimetric and thermogravimetric methods, and the hydrogel loss during the subsequent crosslinking process was quantified, seeming to depend on the mass fraction of hyaluronan initially incorporated to the pores. The effect of the topologically different coatings on the scaffolds, in terms of mechanical properties and swelling at equilibrium under different conditions was evaluated and correlated with the hyaluronan mass fraction. The potential of these hydrogel coatings as vehicle for controlled drug release from the scaffolds was validated using a protein model.

1. Introduction

Polymer-based materials have been widely investigated for tissue engineering applications for their versatility in terms of chemical, physical and mechanical properties and ease of handling to obtain microporous structures. Among them, poly(ethyl acrylate) (PEA) is a biostable polymer with good biocompatibility with cells such as chondrocytes [1], osteoblasts [2], endothelial cells [3], keratinocytes [4], neural cells [5–8] or dental pulp stem cells [9], and has been proposed as a feeder-free platform for the maintenance and growth of embryonic stem cells [10]. Its scaffolds have been implanted in several tissues with regenerative purposes [5,11–13]. They can be manufactured in a variety of pore architectures including aligned [14] and interwoven [15] microchannels and interconnected microspheres [16] by using porogen leaching techniques. These scaffolds can be further combined with others of dissimilar nature: for example, in [15,16] the authors combined them with a self-assembling peptide gel as filling in their pores. The inclusion of a hydrogel improves cell colonization and maintenance in the pores.

PEA undergoes its vitreous transition around −10 °C, so it shows a rubbery behavior at physiological temperatures [17], characterized by being softer and more elastic than other similar polymers. As grid-like scaffolds with pores diameter of 150 μm and porosity of 76.4 ± 6.1%, its Young's modulus decreases from 0.84 ± 0.08 MPa for bulk PEA to 0.04 ± 0.02 MPa [13]. Its density is 1.13 gcm^{-3} [18]. Additionally, PEA is a hydrophobic polymer and the amorphous networks thereof result in low water uptake (equilibrium water content in PBS of 1.14 ± 0.16% [13]), thus maintaining their size and shape stable in aqueous environments. PEA has been shown to favor the adhesion of proteins such as fibronectin [2,19] and to induce its spontaneous fibrillogenesis, due to the particular mobility and polarity of its side chain, together with its low wettability. These peculiarities encourage its use for soft tissue engineering purposes.

Hyaluronic acid, usually referred to as its sodium salt form, hyaluronan (HA), is a natural polymer of the glycosaminoglycans family that forms part of the extracellular matrix of several tissues. HA contains binding sites for extracellular, intracellular and transmembrane proteins, known as hyaladherins [20]. Additionally, HA molecules possess physical properties and regulate cell signaling pathways in a size-dependent manner [21]: high molecular weight HA at physiological conditions behaves as a viscoelastic material, increases hydration and displays anti-inflammatory properties, whereas low molecular weight HA up-regulates important cell functions implied in the regenerative process including proliferation, migration, angiogenesis and the immune response.

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In spite of its biological interest and degradation by-products, HA has an important drawback: it shows a short residence time in physiological conditions (because of its high solubility) and a fast degradation rate, as well as an excessively low structural stability to be shaped as scaffold for cell support and in growth. For these reasons, HA is usually crosslinked [22,23] or modified [24]. Several HA-based hydrogels have indeed been proposed in the tissue engineering field and, in particular, HA crosslinked with divinyl sulfone (HA-DVS) constitutes one of the most studied, with clinical applications such as intra-cutaneous injections as dermal fillers [25] and intra-articular injections for osteoarthritis [26]. HA-based hydrogels give rise to stable and more easy handling structures with large hydration, exhibiting great diffusion of substances implied in cell survival. They have thus been used alone to develop scaffolds [27] and drug and cell carriers [28–31].

Herein, the HA-DVS crosslinking reaction was performed inside the interwoven channels of hydrophobic PEA scaffolds, resulting in a combined material of peculiar properties and potential applications: the hydrogel as coating provides an artificial and more cell-friendly nanoenvironment, and the good biocompatibility and better mechanical stability of PEA joins the biological benefits and high hydration rates of HA-DVS hydrogel. This work ensues from a previous one [32], which addressed the problems faced to produce different HA coating typologies and to characterize their physical state in the scaffold-coating construct. Firstly, the efficiency of the coating procedure using solutions with different HA concentrations has been assessed, as well as the efficiency of the crosslinking reaction within the microchannels with DVS. Next, the morphology of the coating in the dry and swollen state, its water uptake under diverse conditions and its effect on the mechanical properties of the ensemble have been studied and correlated with the initial HA solution used to produce the coating. Finally, the ability of the HA-DVS hydrogels to absorb and release molecules from the micropores of the hydrophobic scaffolds has been followed with bovine serum protein (BSA) used as protein model.

2. Materials and methods

2.1. Scaffolds preparation

Scaffolds were prepared as described in [33]. Briefly, a solution of ethyl acrylate monomer (99%, Aldrich), 0.1 wt.% azobisbutyronitrile (99%, Fluka), and 2 wt.% ethylene glycol dimethacrylate (98%, Aldrich) was injected in a nylon fabrics template. The monomeric mixture was allowed to polymerize for 24 h at 60 °C and post-polymerize for 24 h at 90 °C. The materials were next washed to eliminate the porogen template. The resulting 1.6 mm thick scaffolds were thoroughly dried and punched into 1 cm-diameter discs.

2.2. Preparation of HA solutions and coating procedure

Aqueous solutions of hyaluronic acid sodium salt (99%, Sigma, 1.63 MDa) in 0.2 M sodium hydroxide (NaOH, extrapure, Scharlau) were prepared at 0.5, 1, 2 and 5 wt.% and stirred for 24 h. These solutions were forced into the scaffolds pores by applying vacuum with a syringe and next thoroughly dried as described in [32]. After preparation, the hybrids were stored under vacuum until use. Coated scaffolds will be designed as *y*HA*x*, where *y* is the concentration of the coating solution, and an *x* is added to the series of homologous crosslinked samples.

2.3. Crosslinking of HA coating

The HA coating of PEA scaffolds was crosslinked with divinyl sulfone (97%, Aldrich, 118.15 Da) in a series of samples. Firstly, 6 replicates of coated samples were immersed during 20 min in 5 mL of a 80/20 vol. mixture of acetone (synthesis grade, Scharlau)/0.2 M (aq) NaOH with a pH of 11–12 adjusted with HCl (≥99.8%, Sigma–Aldrich). DVS was next added in a 1:0.9 molar ratio of DVS to HA monomeric units, dissolved in 1 mL of the acetone/NaOH solution. Samples were left in the solution for 24 h to ensure that the crosslinking reaction was completed. Next, samples were rinsed in a 20/80 vol. acetone/distilled water mixture for 30 min, followed by a washing with distilled water for 30 min, and dried as described.

2.4. Preparation of HA discs

In order to obtain HA discs to be used as control, hyaluronic acid sodium salt was dissolved in a NaOH (aq) 0.2 M solution to a concentration of 5 wt.% by gently shaking for 24 h and stirring at 200 rpm for 2 extra hours. Next, DVS was added and stirred at 300 rpm for 1 minute. 10 mL of the DVS/HA solution were poured into a 8.5 cm diameter Petri dish and the crosslinking reaction was let to occur for 24 h. The obtained gel was punched into 5 mm-diameter discs. The discs were washed 5 times for 24 h in a 50/50 vol. acetone/distilled water mixture, and dried in an oven at 40 °C for 5 h followed by 24 h under vacuum at room temperature. Samples were kept under vacuum and darkness until use.

2.5. Determination of the amount of HA entrapped in the scaffolds

The amount of HA entrapped in the scaffolds was quantified before and after its crosslinking by comparing the weight of bare samples with that after coating and after crosslinking, in all cases followed by drying. A Mettler AE 240 balance (Mettler-Toledo Inc., Columbus, OH, USA) was used for this purpose. These data allowed the estimation of the filling efficiency and the mass loss on account of crosslinking.

The amount of HA adsorbed on the scaffolds surface during the coating process and the HA remaining after crosslinking was quantified by the HA mass fraction in the scaffold, ω_{HA}, defined as:

$$\omega_{HA} = \frac{m_{HA}}{(m_{HA} + m_{\text{bare}})},$$

where m_{bare} is the mass of bare scaffold and m_{HA} is the mass of dry HA coating. The relative loss during the crosslinking ($HA_{\text{loss}}(\%)$) was determined as:

$$HA_{loss} = \frac{m_{H\,\text{Ainitial}} - m_{HA\,\text{final}}}{m_{HA\text{initial}}} \times 100,$$

where $m_{HA\text{initial}}$ is the mass of HA incorporated during the coating process and $m_{HA\text{final}}$ is the mass of HA remaining after the crosslinking step. Measurements were performed 3 times for each HA concentration.

2.6. Morphological observation

A JSM-5410 scanning electron microscope (SEM; JEOL Ltd., Tokyo, Japan) was used to characterize the obtained materials. Surfaces and inner sections of the samples (exposed by fracturing the samples previously frozen by immersion in liquid nitrogen) were observed after a sputter-coating with gold. The working distance was fixed at 15 mm and the acceleration voltage at 15 kV.

Samples swollen for 4 days (by immersion in phosphate buffer saline (PBS), by immersion in distilled water, or maintained in an atmosphere of PBS, PBS(RH)), were mounted on a specimen holder and immersed in nitrogen slush. Once frozen, these samples were transferred to a JSM 6300 microscope (JEOL Ltd., Tokyo, Japan) in the cryoSEM device. An inner section was then exposed by fracturing the sample, and ice sublimation started at −80 °C. After 40 min, samples were sputter-coated with gold and examined at 20 kV of acceleration voltage.