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Development of an arginine-based cationic hydrogel platform: Synthesis, characterization and biomedical applications

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ABSTRACT

A series of biodegradable and biocompatible cationic hybrid hydrogels was developed from water-soluble arginine-based unsaturated polymer (Arg-AG) and poly(ethylene glycol) diacrylate (PEG-DA) by a photocrosslinking method. The physicochemical, mechanical and biological properties of these hydrogels were intensively examined. The hydrogels were characterized in terms of equilibrium swelling ratio (Q_{eq}), compression modulus and interior morphology. The effects of the chemical structure of the two Arg-AG precursors and the feed ratio of these precursors on the properties of resulting hybrid hydrogels were investigated. The crosslinking density and mechanical strength of the hybrid hydrogels increased with an increase in allylglycine (AG) content in the Arg-AG precursor, as the gelation efficiency (G_f) increased from 80% to 90%, but the swelling and pore size of the hybrid hydrogels decreased as the equilibrium swelling weight (Q_{eq}) decreased from 1890% to 1330% and the pore size from 28 to 22 μm. The short-term in vitro biodegradation properties of hydrogels were investigated as a function of Arg-AG chemical structures and enzymes. Hybrid hydrogels showed faster biodegradation in an enzyme solution than in a phosphate-buffered saline solution. Bovine serum albumin and insulin release profiles indicated that this cationic hydrogel system could significantly improve the sustained release of the negatively charged proteins. The cellular response of the hybrid hydrogels was preliminarily evaluated by cell attachment, encapsulation and proliferation tests using live–dead and MTT assay. The results showed that the hybrid hydrogels supported cell attachment well and were nontoxic to the cells.

1. Introduction

Hydrogels are materials that can swell and hold a large amount of water in their hydrated state. Due to their high water contents, similar to that of body tissues, and high biocompatibility, hydrogels have attracted significant attention for use as surgical implants, diagnostics, biosensors, bioreactors, bioseparators and matrices for drug delivery and tissue engineering scaffolds [1–4]. Chemically, hydrogels can be broadly divided into two categories: non-biodegradable and biodegradable. Biodegradable hydrogels are more useful in biomedicine because they are fabricated from biodegradable polymer-based precursors and are degraded in the human body and hence do not cause a permanent foreign-body inflammatory response [5–13]. Both synthetic biodegradable polymers, e.g. polylactide, poly(lactide-co-glycolide), poly(ε-caprolactone), and natural biodegradable polymers, e.g. dextran [14], chitosan [15,16] and hyaluronic acid [17], can be used to prepare biodegradable hydrogels for drug delivery and tissue engineering scaffold applications. By controlling the feed ratios of the hydrophilic and hydrophobic precursors, biodegradable hydrogels with a wide range of swelling ratios, mechanical strengths, internal morphologies and degradabilities can be obtained [18–21].

Amino acid-based poly(ester amide) (AA-PEAs), which are prepared from amino acids, fatty alcohols and aliphatic acids, have both ester and amide linkages on their backbones. They have been introduced as a new family of biodegradable materials [22–38] as they combine the good mechanical, thermal and processing properties of polyamides with the degradability of polyesters into a single entity. Our laboratory has very recently developed a new advanced generation of water-soluble and cationic functional AA-PEAs called Arg-AG (where Arg is the positively charged amino acid arginine and AG is DL-2-allylglycine) that have a pendant vinyl functional group for subsequent photo or non-photo reactions [39].

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This new family of water-soluble and cationic functional Arg-AG PEAs was made from two amino acid building blocks: L-Arg (a positively charged and water-soluble α-amino acid) and a derivative of L-Gly (DL-2-allylglycine). The incorporation of AG in the synthesis of this Arg-AG PEA conferred photoreactivity on the resulting AA-PEAs via the pendant unsaturated carbon–carbon double bonds in the AG unit. The pendant double bond content of Arg-AG PEAs is tunable by adjusting the feed ratio of Arg-based to AG-based monomers. This strategy is different to that used for previously reported unsaturated AA-PEAs which were synthesized from unsaturated fatty alcohols/acids, and hence the resulting unsaturated AA-PEAs (UPEAs) have an unsaturated unit in the AA-PEA backbone [22,38,40,41]. As for UPEA polymer, the carbon–carbon double bonds are embedded in the polymer backbone, and the unsaturated content can only be controlled by tuning the molecular weight of the UPEA [22,41]. However, the new approach proposed here allows us to control the unsaturated contents of AA-PEAs by adjusting the feed ratio of two amino acid derivatives (Arg- and Gly-based) during the synthesis of the Arg-AG precursor.

The aim of this work is to demonstrate the feasibility of forming a series of new biodegradable cationic hybrid hydrogels through photocrosslinking the pendant carbon–carbon double bonds in the Arg-AGs with other non-AA-PEA co-precursors such as poly(ethylene glycol) diacrylate (PEG-DA). Compared with our previously reported phenylalanine-based hybrid hydrogel systems [42,43], the Arg-based hydrogels have the ability to carry positive charge due to the strong basic guanidino group, which provides the potential to condense negatively charged DNA. The electrostatic interaction between the positive charge on the arginine of the hydrogels and the negative charge on DNA should result in better interactions with cells and achieve sustained release of ionic drugs [25]. The wide variety of hydrogel components and parameters should allow engineering of hydrogels with a wide range of cytokine release kinetics. In our very recent arginine-based hydrogel research [38], double bonds of Arg-PEA were introduced into the PEA backbone via the Arg-UPEA precursor, instead of the pendant double bond groups investigated in this current work. The embedded backbone double bond contents could only be controlled by the molecular weight of the Arg-UPEAs, and the equilibrium swelling ratio (Q_{eq}) of the resulting hydrogels decreased with an increase in the Arg-UPEA precursor. The incorporation of Arg-UPEA precursor into the F127 hybrid hydrogels lowered their overall compressive moduli dramatically. In this work, the Arg-AG/PEG-DA hybrid hydrogels were characterized by their gel fraction (G_f), equilibrium swelling ratio (Q_{eq}), compressive modulus and internal morphology. The effects of the precursor feed ratio on the property of the cationic hybrid hydrogels were studied and compared in detail with our very recent research [38]. Negatively charged proteins were loaded into the hydrogels to evaluate the sustained release performance of this hydrogel system. A preliminary assessment of the cell interaction with these cationic hybrid hydrogels was conducted using a bovine aortical endothelial cell proliferation assay.

2. Experimental

2.1. Materials

DL-2-Allylglycine (AG), L-Arginine (Arg), *p*-toluenesulfonic acid monohydrate (TosOH·H_2O), diethylene glycol, tetraethylene glycol, hydroquinone, sebacoyl chloride, succinyl chloride, 1,4-butanediol, 1,6-hexanediol (Alfa Aesar, Ward Hill, MA) and *p*-nitrophenol (J.T. Baker, Phillipsburg, NJ) were used without further purification. Bovine serum albumin (BSA) and insulin were purchased from Sigma–Aldrich (St. Louis, MO) and used directly. PEG-DA (PEG M_n = 4000) was synthesized as described previously [42].

2.2. Synthesis of Arg-AGs polymer

The functional Arg-AGs with pendant double bonds were synthesized through the solution polycondensation of di-*p*-nitrophenyl diester (monomer **II**) with a mixture of di-*p*-toluenesulfonic acid salts of bis-L-Arginine (Arg-y) (monomer **I**) and bis-DL-2-allylglycine diesters (AG-nEG) (monomer **III**) in a predetermined feed ratio [39]. The combinations used in this work were listed in Table 1 and illustrated in Fig. 1.

2.3. Preparation of Arg-AG-based hybrid hydrogels Arg-AG/PEG-DA

Arg-AG/PEG-DA biodegradable hydrogels were prepared by the photopolymerization of both Arg-AG and PEG-DA precursors. The PEG-DA precursor (PEG M_n = 4000) was synthesized as described previously [42]. Irgacure 2959® was added as a photoinitiator. The Arg-AG itself, however, could not form hydrogels via photocrosslinking.

An example of the synthetic method for such a hydrogel from 8-Arg-4-AG-4EG and PEG-DA (designated as 8-Arg-4-AG-4EG-G) is given below. A weight ratio of 1:3 of 8-Arg-4-AG-4EG to PEG-DA (0.08 g 8-Arg-4-AG-4EG, 0.24 g PEG-DA) was added to a vial and dissolved in 2 ml of dimethylacetamide to form a clear, homogeneous solution with a light yellow color. The photoinitiator Irgacure 2959® (0.016 g, 5 wt.% of the total amount of the precursors) was added to the solution of the precursors and dissolved completely at room temperature. The mixed solution was poured into a Teflon mold and irradiated by a long-wavelength UV lamp (365 nm, 100 W) for 15 min at room temperature, resulting in gel formation.

The resultant hydrogel (diameter 25 mm, thickness 4 mm) was carefully removed from the mold and washed with distilled water to remove any residual chemicals. The distilled water was replaced periodically. After this purification process, the hydrogels were soaked in distilled water until swelling equilibrium and then removed and dried in vacuo at room temperature for 48 h before subsequent characterization. The gel fraction, G_f, was used to describe the extent of the hydrogel formation through the following equation:

$$G_f = \frac{W_d}{W_p} \times 100\%, \quad (1)$$

where W_d is the weight of the dry hydrogel and W_p is the total feed weight of the two macromer precursors and the photoinitiator.

2.4. Internal morphology of hydrogels

Scanning electron microscopy (SEM) was used to analyze the internal morphology of the Arg-AG/PEG-DA hydrogels as a function of the precursor feed ratio. A cryofixation technique was used to observe the swollen hydrogel structure with minimal artifacts. Typically, an individual hydrogel was immersed in distilled water at room temperature for 3 days to reach its equilibrium swelling. The hydrogel was then gently removed and immediately transferred into liquid nitrogen to freeze and retain its swollen structure. The sample was subsequently freeze-dried for 72 h in a

Table 1
List of monomers and polymers synthesized by different monomer combinations.

Monomer I	Monomer II	Monomer III	Polymer	
Arg-4	N-2	AG-2EG	2-Arg-4-AG-2EG	2-Arg-6-AG-2EG
Arg-6	N-8		8-Arg-4-AG-2EG	8-Arg-6-AG-2EG
Arg-4	N-2	AG-4EG	2-Arg-4-AG-4EG	2-Arg-6-AG-4EG
Arg-6	N-8		8-Arg-4-AG-4EG	8-Arg-6-AG-4EG