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Polyethylene glycol-albumin/fibrin interpenetrating polymer networks with adaptable enzymatic degradation for tissue engineering applications

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ABSTRACT

A new series of polyethylene glycol-albumin/Fibrin interpenetrating polymer networks (PEG-BSA/Fb IPNs) combining a fibrin gel with a co-network of polyethylene glycol and serum albumin has been developed in order to study the enzymatic degradation of such architectures combining protein and synthetic polymer partners. BSA provides biodegradability thank to its many segments cleavable by enzymes while PEG ensures material resistance (shape preservation, mechanical moduli, …). Previous the degradation study of these easily manipulable PEG-BSA/Fb IPNs, it was shown that each partner (fibrin, PEG and BSA) contributes to the elastic modulus value which is only possible in an IPN architecture where the different polymer partners form a continuous phase in the whole material. The homogeneous protein distribution in the materials was also confirmed by confocal microscopy.

Then, their biodegradability was studied by combining three complementary characterizations: on the one hand, absorbance of the hydrolytic enzyme solution in which they were immersed to quantify the proportion of protein fragment extracted and, on the other hand, viscoelastic moduli of the hydrogels after immersion in the same enzyme to evaluate their mechanical resistance, and finally, MEB imaging to check the hydrolysis homogeneity. The proteolytic enzyme degrades quickly IPNs containing less than 3 wt% PEG while other IPNs remain resistant to proteolysis over long periods, although their viscoelastic properties are reduced by 70% and morphology at the microscopic level changed in a few hours. The degradation rate of these materials is thus easily tunable by composition adjustment.

1. Introduction

For efficient applications in tissue engineering, biomaterials must offer specific properties. For instance, to replace soft tissues, the developed biomaterials must present suitable mechanical properties, i.e. an elastic modulus between 1 and 4 kPa, controlled degradation kinetics and the retention of their volume upon time. However, these properties are not enough and materials have primarily to be biocompatible. It is thus difficult to combine all these properties to one single material.

Fibrin-based hydrogels present high potentialities for clinical applications due to their capacity to promote cell adhesion, migration and proliferation [1,2]. In addition, they have a key role as a provisional matrix in physiological processes such as hemostasis and wound healing. However, at the physiological concentration (~5 mg/mL), their mechanical moduli are very weak (between 13 and 100 Pa) [3,4] they are rapidly degraded in the presence of proteases and also contract when embedded with cells. To make up for these shortcomings, fibrin network has been combined with other polymer networks in interpenetrating polymer network (IPN) architecture defined as a combination of two polymer networks that are cross-linked in the presence of each other [5,6].

On the one hand, fibrin network has been associated with other natural polymers. While adding collagen to a fibrin gel in IPN increases to a 2–3 fold of its elastic modulus, the obtained biomaterial strongly retracts (25% for the first day) when cells are encapsulated [7]. Contrarily, the degradation of a fibrin gel/modified hyaluronic acid IPN in the presence of nattokinase is strongly

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slowed (total degradation observed after 5 days instead of 2 days for single fibrin gel) [8] but their elastic moduli are not improved. Similar results were obtained when hyaluronic acid was modified with other functions [9]. The structural integrity of fibrin-based IPNs can be increased up to several weeks of cell culture when fibrin is associated with an alginate gel [10,11]. In these materials, fibrin network is rapidly hydrolyzed by plasmin and metalloproteases produced by the encapsulated cells, while the slow alginate degradation insures the mechanical support of the material. However, the elastic moduli of these IPNs even without cells remain low (300 Pa). Thus, association of natural polymers to fibrin gel to improve its mechanical properties on long term is systematically limited by their sensitivity to enzymes, and the mechanical improvement is very moderate.

On the other hand, the mechanical modulus of fibrin gel can be significantly improved when it is associated with a synthetic polymer. Thus, the elastic modulus of a fibrin gel at physiological concentration ($G' = 80$ Pa [12]) can be increased up to about 3000 Pa when it is combined with either a poly (ethylene glycol) network (PEG) [13], or polyvinyl alcohol one (PVA) [14]. However, these materials are not biodegradable. To render PVA/Fibrin IPNs degradable, albumin chemically modified with methacrylate groups was included inside the materials by copolymerization with PVA modified with the same groups. The partner network then contains protein cross-linking nodes with several segments recognized by the metalloproteases active *in vivo*. Degradability of these materials by enzymes can thus be modulated according to the protein amount [15]. However, this degradation has not been studied in detail. As the IPN's properties highly depend on their morphology, special attention should be granted to it.

To carry out this study, a series of new PEG-BSA/Fb IPNs combining a fibrin gel with a co-network of polyethylene glycol and serum albumin had to be developed. Indeed, in order to obtain a homogeneous degradation of IPNs over time, the distribution of synthetic compounds which do not degrade, and proteins which degrade, must be homogeneous in the whole material. PEG was thus chosen by respect to PVA because it can be synthesized from polyethyleneglycol dimethacrylate (PEGDM) oligomer with defined molar weight between methacrylate functions ($Mn = 750$ g in this case). The cross-linking density of the partner network should be thus more homogenous which is important for its controlled morphology and then its degradation. As in previously studies, BSA thanks to its many segments cleavable by enzymes should lead to biodegradable materials.

After checking of their synthesis, morphology and elastic moduli of the materials were characterized according to their composition. Then, their biodegradability was studied as a function of the immersion time of the materials in an enzymatic solution by combining three complementary methods of characterization, which is unusual. On the one hand, absorbance of the immersion solution has been measured in order to quantify the proportion of protein fragment extracted and, on the other hand, viscoelastic moduli of the hydrogels have been quantified under the same conditions. Finally, the hydrolysis homogeneity was checked by MEB imaging of the material surfaces.

2. Materials and methods

2.1. Materials

Thrombin (BP 25432) and sodium chloride was purchased from Fisher Reagents, and bovine fibrinogen (Fg - 341573) from Calbiochem. Tris(hydroxymethyl)aminomethane (Tris), and calcium chloride were purchased from VWR and Riedel-deHaën, respectively. Albumin from bovine serum (BSA - purity > 98%, A7906), phalloidin-fluorescein isothiocyanate (phalloidin-FITC, P-5282), and thermolysin (Th) from *Geobacillus stearothermophilus* (P1512) were obtained from Sigma. The oligomer α,ω-poly(ethylene glycol) dimethacrylate (PEGDM - $Mn = 750$ g/mol, density = 1.11) was purchased from Aldrich, and 2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone (Irgacure, I_{2959}) from Ciba. Alexa Fluor 568-labeled anti-rabbit antibody was obtained from Invitrogen.

2.2. Material synthesis

Serum albumin was modified with methacrylate groups (BSAm), as previously described [15].

PEG-BSA/Fb IPNs with variable compositions were synthetized as follows: All reactants were solubilized in a 50 mM Tris-HCl buffer at pH 7,4 and incubated at 37 °C prior to mixing. In the reaction medium, the sum of BSAm and PEGDM concentrations was fixed to 100 mg/mL and the PEGDM concentration varies between 20 and 50 mg/mL, while the concentration of BSAm varies simultaneously from 80 to 50 mg/mL. The I_{2959} photoinitiator was introduced at a molar ratio of 1 for 16 methacrylate groups. For all the compositions, the fibrinogen concentration was fixed at 5 mg/mL. The thrombin was used at 0.2 U/mL and was the last component to be introduced in the mixture. All materials were synthetized in a 50 mM Tris-HCl buffer containing 0.15 mol/L NaCl and 0.02 mol/L $CaCl_2$. The solution of precursors prepared in a microvial was then placed in a previously described mold [14]. The mold was put at 37 °C under a 365 nm UV lamp (VL-6, Bioblock, 1.16 mW/cm^2) for 1 h. The materials thus obtained were then unmolded with a thin plate of Teflon®.

For most analyses, the materials were fully hydrated (swelling in 50 mM Tris-HCl buffer for at least 24 h).

BSA(10)/Fb (without PEGDM) and PEG(10)/Fb IPNs (without BSAm) were also synthesized according the same protocol as IPN synthesis and used as reference materials. They were prepared from a 100 mg/mL of either BSAm or PEGDM solutions in 50 mM Tris-HCl buffer. I_{2959} was introduced at a molar ratio of 1 mol for 16 mol and for 155 mol of methacrylate function for BSA(10)/Fb IPN and PEG(10)/Fb IPN, respectively.

2.3. Soluble fractions

The soluble fraction of the synthetic polymer networks was extracted for 48 h in Soxhlet with dichloromethane. The samples were dried under vacuum and weighed before (w_i) and after (w_f) the extraction. The soluble fractions were determined as:

$$SF\ (\%) = \frac{w_i - w_f}{w_i} \times 100$$

Unreacted proteins contained in the IPNs were extracted by immersing for 24 h in a 10-fold volume of 50 mM Tris-HCl buffer containing 0.15 mol/L NaCl and 0.02 mol/L $CaCl_2$. The concentration of the extracted proteins was assessed by a Bradford assay. The absorbance at 595 nm was measured using a spectrophotometer (Uvikon).

The buffer used for protein degradation in material was composed of 160 mM Tris, 8 M urea (VWR), 2% SDS (Fisher), and 2% β-mercaptoethanol (Sigma-Aldrich).

The average value of the water contact angle was determined from at least 3 measurements and the uncertainty corresponds to the standard deviation.