Colloids and Surfaces B: Biointerfaces 145 (2016) 347–352

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Janus emulsion mediated porous scaffold bio-fabrication

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ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form 22 April 2016
Accepted 5 May 2016
Available online 10 May 2016

Keywords:
Janus emulsions
Calcium phosphates
Gelatin-chitosan scaffolds,

ABSTRACT

A three dimensional biopolymer network structure with incorporated nano-porous calcium phosphate (CaP) balls was fabricated by using gelatin-chitosan (GC) polymer blend and GC stabilized olive/silicone oil Janus emulsions, respectively. The emulsions were freeze-dried, and the oil droplets were washed out in order to prepare porous scaffolds with larger surface area. The morphology, pore size, chemical composition, thermal and swelling behavior was studied by Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and micro-Differential Scanning Calorimetry (micro-DSC). Microscopic analysis confirmed that the pore size of the GC based sponges after freeze-drying may be drastically reduced by using Janus emulsions. Besides, the incorporation of nanoporous calcium phosphate balls is also lowering the pore size and enhancing thermal stability.

1. Introduction

The purpose of tissue engineering is to fabricate biocompatible and biodegradable scaffolds with similar mechanical properties of the targeted tissue for repair and replacement.

Natural polymers possess biocompatibility, high porosity, biomechanical compatibility, and biodegradability. Consequently, they are considered as biodegradable biomaterials which are used clinically and are still attracting much attention for their application as material for tissue engineering [1].

Gelatin is one of the most popular amphiphilic biopolymer scaffold materials for tissue regeneration. Because gelatin is derived from natural collagen, which forms the extracellular matrix of the bone, it can provide a native like environment for the cells. The main disadvantage of gelatin as a tissue substitute is that gelatin is unstable in aqueous medium. To overcome this weakness gelatin can be associated with other naturally obtained polymers, in particular with chitosan [2]. Structure formation can be realized through electrostatic interactions (polyelectrolyte complex formation), physical binding (blends), or covalent cross-linking (network formation) [3–5]. Studies provide that the dissolution degree of the gelatin-chitosan blends is reduced by increasing the chitosan concentration [5].

As well as gelatin, chitosan has qualified itself for tissue engineering, on account of its outstanding properties, namely high biodegradability, biocompatibility, non-toxicity and anti-microbial properties, osteoconductivity, hemostaticity and last but not least of its low costs [6,7]. Chitosan based scaffolds and gels have been studied extensively for wound healing, tissue engineering and tissue repair of bones, cartilage, liver, or nerve tissues [8,9].

The attention towards biopolymer hard tissue engineering has focused on composites. Since these materials are close to mimic the construction of natural bones, calcium orthophosphate crystals within a collagen fiber have been favored. In composite materials the high mechanical stability of ceramics is advantageously coupled with good elasticity of the polymers [10–12].

Calcium phosphate (CaP) chitosan composites are claimed to have outstanding osteoblast adhesion, migration, differentiation, and proliferation. Furthermore, the bioactivity and cell growth factor are outstanding [13]. Apart from scaffold design chitosan and gelatin are widely applied to influence the mineralization process of calcium orthophosphates, e.g., by constructing diverse hybrid structures [14].

Wang et al. precipitated flower like hybrid chitosan/hydroxyapatite composites under stirring [15]. Li et al. produced calcium phosphate nanoparticles in a matrix mediated synthesis and claimed that the chitosan/pectin network modulates nucleation and growth of the hydroxyapatite crystals [16]. In addition, there are various methods of preparing porous scaffolds using biopolymers, which include fiber bonding, melt molding solvent casting/particulate leaching, gas foaming and phase separation and freeze-drying, among others [17,18].

It is a challenge for tissue engineering to reconstruct certain tissues with appropriate pore size, interconnectivity and porosity. The

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http://dx.doi.org/10.1016/j.colsurfb.2016.05.018
0927-7765/

effect of the pore size on tissue regeneration was demonstrated by several studies, suggesting that the ideal pore size is:

- 5 μm for neovascularization,
- 5–15 μm for fibroblast ingrowth,
- 20 μm for hepatocytes ingrowth,
- 200–350 μm for osteoconduction,
- 20–125 μm for mammalian skin regeneration [2].

With a reference to our latest result, a supramolecular CaP card-house structure (of about 5–15 μm in size) was synthesized in presence of gelatin/chitosan polymer blend. In other words, nano-porous calcium phosphate balls are formed, built up by individual flat CaP platelets [19].

In the present contribution, the freeze-drying method was applied to fabricate porous biopolymer ceramic scaffolds with gelatin-chitosan (GC) networks modified with hybrid calcium phosphate balls [19].

Our approach was to introduce the so called Janus emulsions as a template phase for controlling the surface area and pore size of the three-dimensional structure formed, followed by the freeze-dry technique to fabricate a 3D scaffold.

Janus emulsions are dual emulsions of commonly known multiple emulsions, where two non-mixable oil components, e.g., silicone and vegetable oil, are combined in one drop. Introduced by Nisisako et al. [20], Janus emulsions immediately attracted extensive attention, because of the compelling correlation between interfacial tension and the drop topology [21–23]. Droplet topology can be controlled, e.g., in a one-step high energy mixing procedure [24].

Immiscible liquids control the morphology of patchy emulsions, but for the present contribution these emulsions were prepared by traditional vibration emulsification, according to Hasinovic et al. [25,26]. One criterion for the spontaneous Janus droplet formation, numerically evaluated [27] and experimentally determined [28], is that the interfacial tension of the more hydrophobic oil towards the aqueous phase is less than the sum of the two remaining interfacial tensions [27]. However, most relevant for the present publication are a new type of Janus emulsions, introduced by Kovach et al. [29], where the oil droplets are stabilized by means of biopolymers, namely gelatin (G) and chitosan (C). The authors claimed that in a first step the GC complexes were strongly adsorbed at the olive oil/water interface, before a rigid skin-like polymer composite layer is formed [29]. GC polymer blends can build up three dimensional networks and stabilize completely engulfed Janus emulsions, and can control the formation of supramolecular ordered CaP nano-porous card-house structures [29,19]. Taking this into account, the aim of the present research was to utilize CaP balls for constructing porous composite scaffolds and applying Janus emulsions as a template phase to affect the pore size of the GC scaffold.

2. Experimental

2.1. Materials

The low molecular weight chitosan with a degree of deacetylation of 81.2%, and a moisture content ≤12 wt% was obtained from Sigma-Aldrich®, and used without further treatment. Gelatin powder (Type A, isoelectric point ≈7, Bloom number 140) with a moisture content ≤11 wt% was purchased from Carl Roth®. The polymers were used without any further treatment. Silicone oil (SiO) (viscosity: 10–mPa s), olive oil (OO) and ethanol ≥99.5% were obtained from Sigma-Aldrich®.

Recently, we were able to show that well defined supramolecular structured, spherical CaP card-house structures are formed in the presence of gelatin-chitosan blends in a wet chemical procedure at 90 °C [19]. The resulting CaP powder (without calcification) was used here as given. All reagents were dissolved in Millipore Milli-Q deionized water.

2.2. Preparation of the gelatin-chitosan (GC) blend

Chitosan powder (2.5 wt%) was dissolved in 0.1 mol acetic acid, and the solution was homogenized by stirring overnight. Gelatin (2.5 wt%) was dissolved in 0.1 mol acetic acid, under continuous stirring and heating up to 40 °C for 5 min.

The 2.5 wt% GC blend was prepared by mixing the 2.5 wt% chitosan solution with the 2.5 wt% gelatin solution, in the ratio of 1:1, under constant stirring at room temperature for 72 h.

2.3. Preparation of GC-CaP suspensions

The nano-porous, ball-like calcium phosphate particles (with diameter between 5 and 15 μm) synthesized according to Ref. [19], were added to the 2.5 wt% GC blend by stirring for 2 min, in order to obtain the GC-CaP suspension. The resulting GC-CaP suspension contains 0.5 wt% calcium phosphate.

2.4. Preparation of Janus GC and Janus GC-CaP emulsions

The GC blend solution and GC-CaP suspension were used as the aqueous phase for preparing Janus emulsions. Each mixture consists of 0.15 g silicone oil, 0.15 g olive oil and 0.7 g of the GC solution or GC-CaP suspension. The emulsification with the oil components was made in a 2 ml Eppendorf tube by mixing with Minishaker IKA (Roth®) at 2500 rpm in order to produce completely engulfed

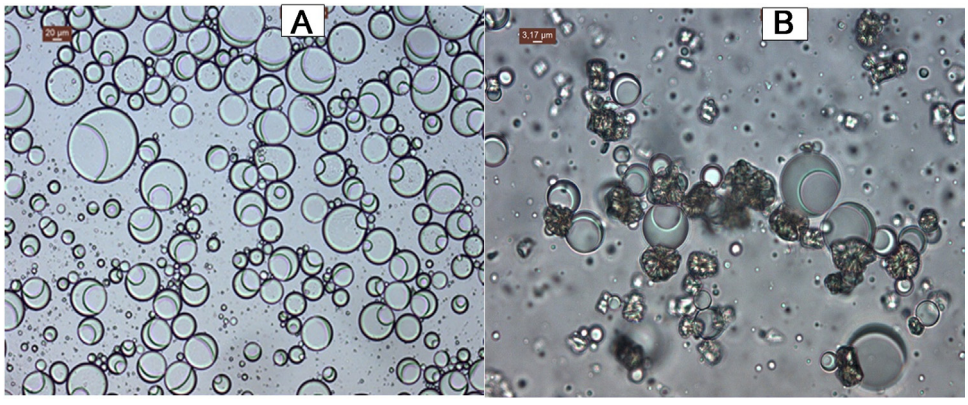

Fig. 1. Micrograph of the GC blend Janus emulsion (A) and the corresponding GC-CaP Janus emulsion (B).