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Simultaneous formation and mineralization of star-P(EO-*stat*-PO) hydrogels

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ABSTRACT

Natural bone is an organic-inorganic composite of highly ordered collagen fibrils and ~60–70% nanocrystalline hydroxyapatite (HA) crystals resulting in a high fracture resistance for various mechanical loading situations. This study aimed to synthesize highly mineralized hydrogels to mimic the mechanical properties of cancellous bone. A six armed star molecule functionalized with isocyanate groups as reactive termini (NCO–sP(EO-stat-PO)) was used to build up a hydrogel matrix, which was then subsequently mineralized with hydroxyapatite nanocrystals following the hydrolysis of incorporated α-tricalcium phosphate particles. The advantage of this dual setting approach in comparison to simply adding unreactive filler particles to the hydrogel was demonstrated to be a strength improvement by the factor of 30. After 1–28 d setting, the mechanical properties of a composite with 30 wt% NCO–sP(EO-stat-PO) such as elasticity (5.3–1.4%), compression strength (11–23 MPa) and E-modulus (211–811 MPa) were found to be similar to the properties of cancellous bone.

1. Introduction

Hydrogels are commonly used to mimic the extracellular matrix of soft tissues in tissue engineering applications. Due to their highly porous network structure and hydrophilicity, hydrogels provide a high water uptake capacity, whereby adequate diffusion rates of nutrients and biological agents can be realized resulting in the possibility to encapsulate bioactive drugs or even living cells [1]. Poly(ethylene oxide) (PEO) is a common basis polymer to build up three dimensional hydrogel matrices due to its cytocompatibility and the availability in a wide range of molecular weights [2,3]. PEO is a linear polyether chain which can be functionalized by hydroxy, amine, carboxylic acid, thiol and others functional groups at the distal endings of the chain to adapt the properties to a specific application [4]. In comparison to the commonly used linear PEO molecules, branched PEOs (e.g. star shaped molecular architectures) enable a higher amount of functional groups per molecule with the possibility for three dimensional network formation [5]. Star shaped molecules possess unique properties caused by the defined structure of the arm's number and length [6,7]. Based on the stereoscopic arrangement of the arms, the higher functional group density results in crosslinked gels with a comparable higher strength compared to gels from bifunctional linear PEO molecules [8].

The current study used a six armed star shaped molecule functionalized with isocyanate groups as reactive termini (NCO–sP(EO-*stat*-PO)) [9]. Both the PO content and the isophorone-diisocyanate (IPDI)-derived NCO-groups are hydrophobic, so that the reactive prepolymers are amphiphilic in nature and not crystalline in bulk. The hydrolysis of NCO–groups in aqueous solutions forms amines, which leads to an autocatalytic crosslinking of the star molecules to dense and well defined hydrogel structures by forming biocompatible urea groups with unreacted isocyanates. In previous studies this system was used to produce ultrathin coatings on polymeric substrates to avoid unspecific protein adsorption [10–12]. This concept was extended to surface functional three dimensional fiber substrates [13–15] and also bulk hydrogels can be prepared from these prepolymers, either as hybrid systems with biopolymers [16] or solely based on NCO-sP(EO-stat-PO) molecules [17]. Furthermore, NCO–sP(EO-*stat*-PO) hydrogels are cytocompatible, show no negative side effects in vivo [18] and were shown to be promising drug delivery systems in an animal model concerning cochlear implants [19].

This study aimed to transfer the NCO–sP(EO-*stat*-PO) hydrogel system from soft tissue applications into hard tissue bone regeneration by applying an in situ mineralization step occurring simultaneously to hydrogel formation. Natural bone is an organic-inorganic composite of highly ordered collagen I fibrils (~95% of the organic phase) and ~60–70% nanocrystalline hydroxyapatite (HA) crystals resulting in a high fracture resistance for various mechanical loading situations [20,21]. It is hence obvious that mimicking the mechanical properties of bone tissue requires the defined mineralization of a polymeric template

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matrix [22]. Since many hydrogels commonly show only a low intrinsic mineralization capacity [23], nucleation sites for the mineralization have to be introduced. Gkioni et al. summarized the strategies for hydrogel mineralization in three groups: I) a biomimetic mineralization approach, II) chemical modification of the hydrogel with ion binding motifs and III) mineralization by adding inorganic particles [24]. The latter strategy includes a high diversity of nanocomposite hydrogels based on the addition of carbon [25], metal [26,27] silica [28–30], HA [31,32] or bioglass [33,34] particles to the hydrogel prior to gelation, commonly accompanied by a reinforcement effect caused by the embedded particles. However, the preparation of nanocomposites with approx. 60–70% mineral load similar to natural bone [35,36] is challenging due to insufficient mixing properties of two dissimilar phases with potential inhomogeneity caused by agglomeration and sedimentation of the nanoparticles within the gel [37].

An approach to fabricate organic/inorganic composites with adequate mineral concentrations is to work with a dual setting system by adding relatively large (~10 μm) and reactive particles to the gel, which simultaneously convert into nanoparticles during gelation by a dissolution/precipitation reaction. This creates a composite with high mineral loads of up to 75 wt% and two entangled polymer and ceramic matrices. As a suitable ceramic particle systems, α-tricalcium phosphate (α-TCP) was applied in this study, which is known to form calcium deficient hydroxyapatite in a cementious reaction within a couple of hours [38,39]. Here, it is hypothesized that the combination of NCO–sP(EO-*stat*-PO) prepolymer in water with α-TCP particles results in a simultaneous cross-linking of the hydrogel phase together with subsequent mineralization with HA nanoparticles by α-TCP hydrolysis. This is thought to lead to mechanical material properties equal to natural bone and a mutual reinforcing effect of the elastic hydrogel by the brittle inorganic mineral phase. The NCO–sP(EO-stat-PO) prepolymer was chosen since the cross-linking reaction is initiated by water without the need of adding further initiators. In addition, due to the absence of Ca^{2+} complexing functional groups, no chemical interaction between the formed hydrogel and the mineral is expected. This helps to study the effect of the in situ mineralization process on the mechanical performance of the composite without any other influencing parameters.

2. Materials and methods

2.1. Hydrogel preparation and mineralization

Hydrogels were produced by mixing NCO–sP(EO-*stat*-PO) (DWI Leibniz-Institute for Interactive Materials, Aachen, Germany) in 2.5 wt% $(NH_4)_2HPO_4$ solution at a concentration of 10, 30 and 50 wt% casted in cylindrical molds (Ø = 5 mm; h = 7 mm) and sealed for 24 h. The polymer backbone consists of a statistical copolymer of 80% ethylene oxide and 20% propylene oxide resulting in star molecules with a molecular weight of 12 kDa. For the mineralization 50, 67 or 75 wt% α-tricalcium phosphate (α-TCP) powder was mixed with the NCO–sP(EO-*stat*-PO) hydrogel precursor and treated the same way. α-TCP was prepared by sintering $CaHPO_4$ (Mallinckrodt-Baker, Germany) and $CaCO_3$ (Merck, Germany) in a molar ratio of 2:1 for 5 h at 1400 °C following quenching to room temperature. The sintered cakes were crushed and passed through a 125 μm pore size-sieve followed by ball milling at 200 rpm for 4 h.

2.2. Characterization of non-mineralized hydrogel

Gelation kinetics were analyzed by Fourier Transform Infrared Spectroscopy (FT-IR; Nicolet is10, Thermo Scientific, Waltham, MA) in a range from 400 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}. Compression test was performed using a dynamical mechanical testing machine (BOSE 5500 system, ElectroForce, Eden Prairie, MN, USA) and a 200 N load cell. The samples were loaded parallel to their long axis and tested at a constant cross head displacement rate of 0.01 $mm \cdot s^{-1}$. For the swelling stress measurement the samples were fixed in the mechanical testing machine, phosphate buffered saline (PBS) was added and the emerging force due to swelling was recorded over time. To analyse the swelling behavior the hydrogels were stored in 1 ml PBS, which was changed every 24 h whereby the hydrogel's mass and dimension were measured. The equilibrium water content EWC (1) was determined by

$$EWC = \frac{w_s - w_d}{w_d} \cdot 100\ \mathrm{wt\%} \quad (1)$$

and the swelling ratio based on the weight Q_w (2) by

$$Q_w = \frac{w_s}{w_d} \quad (2)$$

where w_s is the weight of the swelled hydrogel and w_d the weight of the dried hydrogel.

2.3. Mineralized hydrogel characterization

Mechanical testing of mineralized NCO–sP(EO-*stat*-PO) hydrogels was performed using a static mechanical testing machine (440, Zwick, Ulm, Germany) and a 2.5 kN load cell. For compression tests the samples were loaded parallel to their long axis and tested at a constant cross head displacement rate of 1 $mm \cdot min^{-1}$ under water. X-ray diffraction (XRD) patterns of samples were recorded using monochromatic CuK_α radiation (D5005, Siemens, Karlsruhe, Germany) in a 2Theta range from 2θ = 20–40° with a step size of 0.02° and a normalized count time of 3 $s \cdot step^{-1}$. The phase composition was checked by means of JCPDS reference patterns for α-TCP (PDF Ref. 29-0359) and HA (PDF Ref. 09-0432). A crossbeam scanning electron microscope CB 340 (Zeiss, Oberkochen, Germany) was used to analyse the surfaces of the composite monoliths. Samples were imaged using an accelerating voltage of 1 kV.

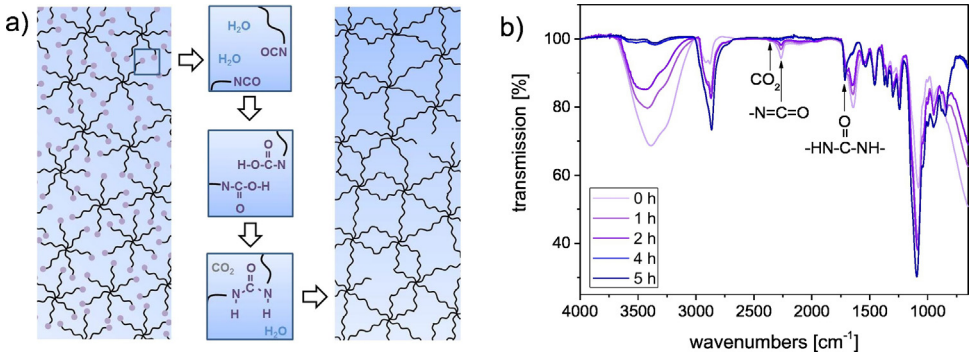

Fig. 1. a) reaction scheme and b) FT-IR spectra of 30 wt% NCO-sP(EO-*stat*-PO) solution during gelation.