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Transparent, elastomeric and tough hydrogels from poly(ethylene glycol) and silicate nanoparticles

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ABSTRACT

The structures and mechanical properties of both physically and covalently cross-linked nanocomposite hydrogels made from poly(ethylene glycol) (PEG) and silicate nanoparticles (Laponite RD) are investigated. Injectable nanocomposite precursor solutions can be covalently cross-linked via photopolymerization. The resulting hydrogels are transparent and have interconnected pores, high elongation and toughness. These properties depend on the hydrogel composition, polymer–nanoparticle interactions and degree of cross-linking (both physical and covalent). Covalent cross-linking of polymer chains leads to the formation of an elastic network, whereas physical cross-linking between nanoparticles and polymer chains induces viscoelastic properties. At high deformations covalent bonds may be broken but physical bonds rebuild and to some extent self-heal the overall network structure. Addition of silicate also enhances the bioactivity and adhesiveness of the hydrogel as these materials stick to soft tissue as well as to hard surfaces. In addition, MC3T3-E1 mouse preosteoblast cells readily adhere and spread on nanocomposite hydrogel surfaces. Collectively, the combinations of properties such as elasticity, stiffness, interconnected network, adhesiveness to surfaces and bio-adhesion to cells provide inspiration and opportunities to engineer mechanically strong and elastic tissue matrixes for orthopedic, craniofacial and dental applications.

1. Introduction

Hydrogels are of great interest for biotechnology, tissue engineering and drug delivery applications due to their hydrophilic character, porous structure and often biocompatible nature [1–5]. Poly(ethylene glycol) (PEG)-based hydrogels have been extensively used in these applications and a variety of formulations are available [5–9]. Despite the many advantages of PEG-based hydrogels, they often lack appropriate mechanical properties which limit their application to space-filling scaffolds, soft tissue repair, or matrixes used for delivery of bioactive molecules and cells [10,11]. To overcome some of these limitations, nanoparticle-containing polymer hydrogels have been developed with properties that can be tailored towards mechanical strength, bioactivity and biocompatibility [12–14]. Among a variety of nanoparticles, silicates such as Laponite have been used to design and develop mechanically strong nanocomposite polymer hydrogels [12–14]. Laponite consists of synthetic and charged silicate nanodiscs that may dissolve in aqueous environments under pH-dependent conditions [15]. The chemical composition of these nanoparticles is $(Na^{+}_{0.7}[(Mg_{5.5}Li_{0.3})Si_8O_{20}(OH)_4]^{-}_{0.7})$ and the dissolution products include Na^{+}, $Si(OH)_4$, Mg^{2+}, Li^{+}. Similar to bioactive glasses [15–18], Laponite dissolution products such as $Mg^{2+}$and $Si(OH)_4$ have been found to enhance osteoblast proliferation and cell differentiation [18–21].

We are especially interested in evaluating the use of Laponite in polymer hydrogels as compared to other bioactive glass nanoparticles, because the dissolution of Laponite into orthosilicic acid can be accelerated at local lower pH. We found that the presence of H^{+} from acidic polymer degradation products (e.g. added by formulation with polylactic acid) accelerates the dissolution of the Laponite within the polymer hydrogels. Thus Laponite can be used to establish an internal buffer system within a degradable polymer hydrogel formulation by preventing acidic local environments during polyacid degradation. As a result the in vivo degradation of the Laponite-containing hydrogels should also be faster than the in vitro degradation due to local changes in pH during natural inflammation around an implant. Our group is currently working on evaluating the above-mentioned concepts, and work presented in this paper is the first step towards mapping out the influence of Laponite on some biologically relevant hydrogel properties. In this paper the experiments were performed in a time frame in which the dissolution of Laponite is negligible.

Fundamental research done previously by us and other groups has shown that in aqueous solutions PEG chains readily adsorb

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to the charged Laponite surfaces via physical interactions [22–26]. At low concentrations viscoelastic solutions and "shake gels" form [27,28], and at higher concentrations a variety of physically cross-linked and permanent hydrogels can be generated [13,24,29,30]. More recently, moldable hydrogels were reported, for example, by Wang et al. [31] who used a variety of non-covalent approaches to fabricate nanocomposite hydrogels with high water content from Laponite and dendritic PEG-based macromolecules. These moldable hydrogels have robust mechanical properties and preserved the biological activity of entrapped proteins while maintaining self-healing properties [31].

Although research on physically cross-linked PEG–Laponite solutions and hydrogels goes back more than a decade [22,32], biomedical relevance has been suggested only recently when cell growth studies showed that cells cultured on the surfaces of poly(ethylene oxide) (PEO)–Laponite gels attach and proliferate easily [19,33–37]. The addition of silicate nanoparticles to the bio-inert PEO induced cellular adhesion and proliferation [19], and increased the mechanical strength of the nanocomposite substrate [19,33]. In a similar study, Pek et al. proposed the use of thixotropic nanocomposite hydrogels made from PEG and silica nanoparticles for three-dimensional cell culture [38]. This group took advantage of the reversible gelation potential of these hydrogels to entrap cells and other biological macromolecules within the network. They showed that differentiation of cells can be controlled by changing the matrix stiffness, which, in turn, can be tuned by varying the concentration of nanoparticles. Collectively, these studies suggest that addition of silicate to PEO hydrogel networks improves some physical as well as biological properties.

While physically cross-linked silicate polymer hydrogels have attractive biological properties, their application as scaffold structures for tissue engineering are limited due to insufficient mechanical properties. However, by covalent cross-linking of polymeric chains in the presence of silicate, mechanically robust and dissolution-resistant hydrogels can be obtained [14,39]. For example, Fukasawa et al. [40] fabricated such robust hydrogels from tetra-PEG and silicate nanoparticles (Laponite XLG). Uniform dispersion of the silicate nanoparticles and formation of covalently cross-linked networks resulted in mechanically robust hydrogels with high elongations (600–1000%) [40].

In this work, we report on the synthesis and formulation of injectable silicate–PEG nanocomposite precursor solutions that can be covalently cross-linked to form transparent, highly elastic (~2000% strain) and tough hydrogels. We show that the addition of silicate nanoparticles to the covalently cross-linkable PEG network improves the elongation properties, induces adhesion (stickiness) to soft and hard surfaces, and mammalian cell adhesion. The unique property combinations of the hydrogels reported in this article suggest new approaches to engineering complex viscoelastic biomaterials.

2. Experimental

2.1. Materials

PEG with a molecular weight M_w of 35000 g mol^{-1} was purchased from Fluka Analytical (Sigma–Aldrich). The hydroxyl groups of PEG were acrylated by a 5-fold molar excess of acryloyl chloride and a 5-fold molar excess of triethylamine in 100 ml dichloromethane under nitrogen. The reaction was stirred at room temperature for 24 h. The resulting solution was then filtered to remove any precipitates. Afterwards PEG–diacrylate (PEGDA) was precipitated out from the filtrate by pouring into cold diethyl ether. The white PEGDA precipitate was isolated and subsequently dried under vacuum for 1 day. The acrylation degree of PEGDA was over 80%, as determined by 400 Hz ^{1}H nuclear magnetic resonance on a BrukerARX400 spectrometer and calculated by the ratio of acryl protons of PEGDA (=CH_2, δ = 5.8–6.4) to –CH_2O– (δ = 3.63) of PEG.

Synthetic silicate (Laponite RD (LRD)) nanoparticles were used as physical cross-linker to PEG. LRD, a hectorite-type synthetic silicate with the chemical composition of $Na^+_{0.7}[(Mg_{5.5}Li_{0.3})Si_8O_{20}(OH)_4]^-_{0.7}$, was purchased from Southern Clay Products Inc. The disk-like nanoparticles are approximately 25–30 nm in diameter and 1 nm thick. The pyrophosphate anhydrous ($Na_4P_2O_7$) was purchased from Alfa Aesar, and the initiator, IRGACURE 2959, is a photosynthetic activator purchased from Ciba AG (Basel, Switzerland).

2.2. Preparation of PEG–silicate nanocomposite hydrogel

The initiator solution was prepared by mixing 0.5% pyrophosphate and 0.2% initiator in 10 ml deionized water. Pyrophosphate was added to enhance the solubility of the silicate nanoparticles and to avoid formation of agglomerates. Silicate nanoparticles (0%, 1%, 2.55% and 5%) were dissolved in the initiator solution with the help of a vortexer and a sonicator. Then appropriate amounts of PEGDA (15% or 20%) were added and mixed vigorously. The mixtures were then subjected to ultracentrifuging (3000 rpm for 10 min) to remove any entrapped air bubbles. The transparent precursor solutions were injected into a mold using a syringe. Then the precursor solutions were photo cross-linked using a high-intensity UV light (B-100AP, Ultra-Violet Products Ltd., Upland, CA; power = 100 W; 365 nm wavelength UV) for 10 min. The intensity of the UV lamp at 2 in/10 in is 21,700/8900 μW cm^{-2}. The distance between the lamp and sample was kept constant at 15 cm. Different types of sample shapes were prepared for the material characterizations. For tensile testing, a dumb-bell-shaped specimen was prepared (5 mm in length, 3 mm wide and 1 mm thick). For the rheological studies, a cylindrical sample with a diameter of 20 mm and height of 1 mm was used. For compression testing, a cylindrical sample 5 mm in diameter and 10 mm high was prepared.

2.3. Hydration kinetics

The saturated hydration degree of as-prepared hydrogels was determined in phosphate-buffered saline solution (PBS) at 37 °C. After removing the gel from the solution and blotting off excess water with kim wipes, the hydrogel weight was recorded. The hydration degree of the gels was then calculated by taking the ratio of the percentage of absorbed water to the original weight of the hydrogel:

$$\text{Hydration Degree} = \frac{m_t - m_0}{m_0} \times 100\%$$

2.4. Mechanical properties

Mechanical properties of the as-prepared nanocomposite hydrogels were tested using an AR2000 stress-controlled rheometer and an ARES strain-controlled rheometer (TA Instruments Ltd.) (n = 3). Flow experiments were carried out at ambient temperatures (25 °C) with a 40 mm parallel plate. Precursor solution was placed at the center of the machine and pressed to a gap of 400 μm. The viscosity of the precursor solution was determined as a function of shear rate (from 1 to 100 s^{-1}). The rheological properties of covalently cross-linked hydrogels were determined by oscillatory sweep experiments. Dynamic stress sweeps (0.1–1000 Pa, 1 Hz) and frequency sweeps (0.01–100 Hz, 10 Pa) were carried out at physiological temperatures (37 °C) with a 20 mm parallel plate at a gap of 800 μm. In both tests the storage