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A 'degradable' poly(vinyl alcohol) iron oxide nanoparticle hydrogel

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ABSTRACT

Polymeric materials that contain magnetic nanoparticles are extremely useful in many applications including as multifunctional drug carriers, imaging contrast agents, or scaffold material. There is a need for biomaterials with appropriate chemical, mechanical, and magnetic properties that also have the ability to degrade or dissolve over time so they can be eliminated from the body following use. In this work, we explore the use of iron oxide nanoparticle (IONP) formation in poly(vinyl alcohol) (PVA) as a crosslinking method in conjunction with physical crosslinking achieved using low temperature thermal cycling (LTTC). PVA-IONP hydrogels were fabricated and characterized. IONPs contribute to the crosslinking of the PVA-IONP material, and their subsequent removal reduces crosslinking, and therefore stability, of the material, allowing dissolution to occur. Dissolution studies were performed on PVA-IONP hydrogels and dissolution was compared for films in solutions of varying pH, in the presence of iron chelating agents, and in simulated physiological and tumor conditions in cell culture media. Iron release, mass loss, and mechanical testing data was collected. This work demonstrates the ability of this biomaterial to 'degrade' over time, which may be very advantageous for applications such as drug delivery. This importance of this work extends to other areas such as the use of stimuli-responsive hydrogels.

Statement of Significance

This manuscript explores the stability of an iron oxide nanoparticle (IONP)-containing, physically crosslinked poly(vinyl alcohol) (PVA) hydrogel. The PVA-IONP hydrogel's stability is imparted through crosslinks created through a low temperature thermal cycling process and through the IONPs. Subsequent IONP removal reduces crosslinks so material dissolution can occur, resulting in a 'degradable' and multifunctional biomaterial. PVA-IONP films were fabricated, characterized and evaluated in terms of dissolution in solutions of varying pH and in the presence of chelating agents. Iron release, mass loss, and mechanical testing data demonstrate the ability of the PVA-IONP biomaterial to 'degrade' over time. This degradability has not yet been demonstrated for crosslinked PVA hydrogels. These results are relevant to the development of degradable multifunctional drug carriers, image contrast agents, or magnetic scaffold materials.

1. Introduction

Biocompatible hydrogel is a class of material that finds a broad range of applications in medical devices, drug delivery, tissue engineering and other biomedical areas. However in addition to biocompatibility, additional property requirements have also to be satisfied for a particular application. Stability of a hydrogel serves as an illustration of such a requirement. For medical implants, long-term stability is necessary. For tissue engineering scaffolds, degradability would be required to accommodate the regenerated tissue. In drug delivery applications, and depending on the application environment, degradability may be highly desirable.

Poly(vinyl alcohol) is a hydrophilic and biocompatible, material that possesses desirable properties for many biomedical applications such as hydrophilicity, elasticity, and available pendant alcohol groups. It can be made into a hydrogel via various crosslinking processes including physical crosslinking through a low temperature thermal cycling process. This has the advantages of tunable mechanical and diffusion properties without the use of potentially problematic chemical crosslinking agents [1–4]. In addition, using the LTTC process, mechanical properties, including anisotropy, of

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the resulting hydrogel can be tuned to mimic properties of soft tissues [3]. By adding a reinforcing phase, mechanical properties approaching that of articulating cartilage have been demonstrated [7]. Such a degree of control is possible by controlling the degree of crystallinity and the micro- and nano-pore structure of the hydrogel via the processing parameters. Versions of the LTTC processed PVA hydrogel that are mechanically tuned and surface modified for cell compatibility have also been reported and proposed for PVA-tissue hybrid development [5,6]. Irrespective of the crosslinking method, PVA hydrogel is known to be biostable and non-degradable, due to the inherent stability of the PVA polymer and the crosslinks. There are, however, many applications in which a dissolvable or degradable material is more desirable, including its use as drug delivery vehicles and scaffolds for tissue regeneration. The ability to design a crosslinking system for PVA hydrogel that exhibits degradability would further expand its potential application into these areas that is hitherto not possible.

A plausible approach towards a degradable PVA hydrogel, knowing that PVA is a chemically stable polymer under physiological conditions, is to design a crosslinking system that is 'degradable' either using a physical or a chemical approach, or a combination of both approaches. Efforts have been made to make degradable forms of PVA including coupling PVA with a degradable ester-acrylate molecule [8], and modifying PVA with aldehyde and hydrazide functional groups [9]. These approaches all require the addition of new chemical functionalities to the PVA backbone. In a combination approach, the preferred chemical route would be one that does not impart toxicity to the system. One possible way is to use iron oxide nanoparticles in conjunction with LTTC. Several studies have shown that it is possible to incorporate IONPs into PVA that also undergo the LTTC process to produce stable composite hydrogel both in the bulk and in the form of millimeter diameter beads [10–13]. An appropriate combination of IONP and physical crosslinks by LTTC may result in a degradable PVA hydrogel. In addition to the degradation products being biocompatible, IONPs can also play the role of imaging contrast enhancement for CT and MR imaging, an advantage when using the PVA-IONP system for delivery in applications such as transarterial chemoembolization (TACE) therapy for liver cancer treatment where multifunctionality, such as tracking for the drug location, is required [14]. As the IONPs degrade, the PVA hydrogel will eventually dissolve, thus allowing repeat treatment to the same localized environment.

In this work, we explored the use of IONPs formed *in situ* in PVA as a crosslinking agent in conjunction with physical crosslinking achieved using the LTTC process. Dissolution studies were performed on the IONPs containing hydrogel material as a function of pH and in the presence of iron chelating agents. Iron release, mass loss and changes in mechanical properties were parameters used as indicators of sample degradation. The importance of this work in TACE therapy and other areas of biomedical applications will be discussed.

2. Materials and methods

All chemicals used were ACS reagent grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Distilled water was used for all experiments.

2.1. Solution preparation

A PVA solution was made by dissolving 5.88 wt% PVA, MW 146,000–186,000, 99+% hydrolyzed, into water. This was heated to 90 °C for approximately 3 h, or until complete dissolution. Separately, iron solution was made by dissolving 20.66 wt% iron (III) chloride ($FeCl_3$) and 12.66 wt% iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_20$) (2:1 M ratio of Fe^{3+} to Fe^{2+}) in water. This was mixed with a magnetic stirring for several hours. Once cooled, 85 wt% PVA solution was added to 15 wt% iron solution, and mixed with magnetic stirring. The final solution composition is 5 wt% PVA, 3.1 wt% $FeCl_3$ and 1.9 wt% $FeCl_2{\cdot}4H_2O$ in water. This solution was filtered through a 5 μm filter (Acrodisc syringe filter with Versapor Membranes) to ensure any suspended impurities were removed. A 5 wt % PVA solution, prepared in the same way as the PVA solution described, was used as a control.

2.2. Hydrogel film fabrication

The final solution was poured into moulds (Buna-N rubber) with rectangular openings (10 cm by 5 cm by 0.2 cm) sandwiched between sheets of Teflon. The Teflon sheets were held between aluminum sheets and screwed together to ensure the moulds were tightly sealed. The moulds were placed in a water bath for 6 freeze-thaw cycles (FTCs) from 20 °C to −20 °C to 20 °C at 0.1 °C/min, with one holding hour at the temperature limits. Subsequently, films were removed from the moulds and submerged in a 0.5 M sodium hydroxide (NaOH) solution for 24 h. Films were removed and then submerged in water for one day with constant water replacement until the pH reached neutral. Films were then cut into 5 mm by 5 mm strips and wrapped in plastic wrap and sealed.

2.3. Scanning electron microscopy and energy dispersive X-ray spectroscopy

Films were dried at 60 °C overnight and freeze fractured in liquid nitrogen to expose the inside of the film. Samples were coated with osmium (OPC-60A Osmium Plasma Coater) and images of the cross-section were taken with a scanning electron microscope (LEO (Zeiss) 1540XB FIB/SEM). Energy dispersive X-ray spectroscopy (EDX) (LEO (Zeiss) 1540XB FIB/SEM) was used for elemental analysis of the sample.

2.4. X-ray diffraction

Films were dried at 60 °C overnight and crushed using mortar and pestle with liquid nitrogen. X-ray diffraction (XRD) was performed using a Rigaku-Rotaflex Diffractometer (RU-200BH) with a Co-kα radiation (λ = 1.79 Å) at 30 kV and 44 mA. Spectra with a 2θ diffraction angle were scanned from 0° to 82° with a 0.2° step size. The PVA-IONP film was loaded onto a glass slide and the PVA film was loaded onto a glass slide with double-sided tape. A background scan was performed on the blank slide and blank slide with tape, and the relative peaks were subtracted from the sample peaks of the applicable sample. Spectra were plotted from a 2θ of 10° to 82°.

2.5. Vibrating sample magnetometry measurement

A model 74035 vibrating sample magnetometer (LakeShoreCryotronics Inc.) was used to test the magnetic properties of a dried PVA-IONP film. The sample was exposed to a magnetic field range of ±10 000G at 25 °C.

2.6. Iron content quantification

Wet PVA-IONP films were dried at 60 °C overnight. Dry samples were dissolved in 5 mL concentrated hydrochloric acid and 5 mL water. Samples were placed in an ultrasonic bath for 30 min and then left to stir for 24 h. Iron content was measured on diluted dissolved samples using atomic absorption spectroscopy (AAS)