Microgel-based Delivery of Soluble Factors for Articular Cartilage Engineering

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Introduction

Chondrocytes encapsulated in a three-dimensional hydrogel scaffold elaborates extracellular matrix with time in culture. However, the deposited matrix decreases the ability of nutrients to diffuse into the construct, causing spatially inhomogeneous mechanical properties with weaker tissue in the center of the construct [1]. Uniform matrix deposition and mechanical integrity is critical for successful implantation of engineered cartilage, as the tissue will experience large compressive forces in vivo. Creating macrochannels in the scaffold has demonstrated improved homogeneous matrix deposition and mechanical properties [1], but may be limited by the number of channels that can be created without compromising the integrity of the constructs. An alternative solution is to embed nutrient-encapsulated polymeric microgels which can release the contents in a desired timeframe controlled by the polymer degradation rate and also has the advantage of a uniform localized delivery of the nutrients or growth factors. Such carriers have several advantages including the controlled release rates through variation in molecular weights and hydrophobicity of the polymers. Polymers with anhydride linkages (e.g. poly(sebacic anhydride), PSA) offer one of the best ways to tailor the degradation rate because their hydrophobicity can be manipulated by changing the molecular weight. The PSA and its hydrolysis products are known to be biocompatible. There are several studies that have used PSA and its copolymers as an encapsulating agent [2-4]. Thus, the goal of our study is to investigate the use of PSA microgels embedded in a chondrocyte-seeded agarose scaffold as a delivery vehicle for essential nutrients and other bioactive agents to overcome problem of decreased convection and diffusion can be overcome. Towards this aim, we tested the biocompatibility of PSA microgels using tissue culture studies and determined the release rate of amitriptyline molecules.

Methods

Synthesis of poly(sebacic anhydride) and encapsulated microgel: The poly(sebacic anhydride) was synthesized from sebacic acid and methacrylic anhydride (or acetic anhydride) by melt polymerization method at 80-120 ^{0}C. Microgels were synthesized using a double emulsion method (W/O/W) of water/methylene chloride/water system. The poly(vinyl) alcohol (PVA) was used as an emulsifier in the external water phase and the encapsulating polymer, PSA, was dissolved in oil phase. The solvent evaporation method was used to remove oil phase and leave a coating of the polymer encapsulating the internal water phase (containing bioactive molecules).

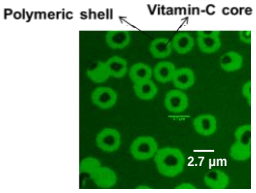

Figure 1. Polyanhydride-microgels embedded in agarose scaffold. Green color indicates the polymeric shell (containing fluorescein acrylate label). Black core is the encapsulated Vitamin-C.

Release kinetics: The release kinetics from the microgels was studied using amitriptyline. The amitriptyline was encapsulated as discussed above and the release was studied using UV-Vis absorption at the wavelength, 239 nm. 150 µL of 100 mg/mL amitriptyline solution in PBS-1x buffer was encapsulated using 200 mg of PSA as described above and freeze dried. The dry microgels were suspended in 10 mL of PBS-1x buffer and release of amitriptyline was quantified every few days using UV absorption of extracted buffer (after centrifugation) after which a fresh volume of PBS-1X was added.

Cell Culture: To study the biocompatibility and effect of microgels on the viability of chondrocytes and tissue growth, the microgels were embedded in our hydrogel scaffold. Microgels were embedded in a slab 2% wv agarose with a chondrocytes concentration of 30M/mL. The microgels were added at a concentration of 0.1% wv and were encapsulated with ~10 mg of vitamin-C (Figure 1). A control set without the embedded microgel was prepared for comparison. The culture medium, CM, was used throughout the culture period of 15 days. The Young's (E_Y) was measured under unconfined compression at 10% strain. Raman spectroscopic measurements were performed on the constructs as well as on a section of juvenile-bovine articular cartilage.

Results

Release kinetics: A linear release of amitriptyline was observed until 12 days (Figure 2A). Within these days, ~ 28 % of the drug was released.

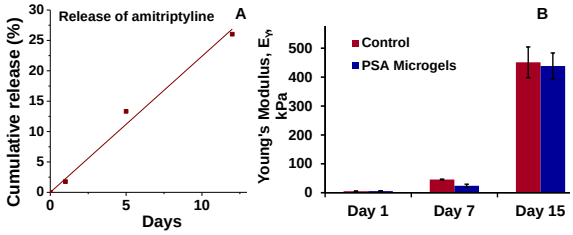

Figure 2. A) Release of amitriptyline drug from poly(sebacic anhydride)-based microgels. B) Young's modulus (E_Y), of both control and microgel containing constructs up to day-15.

Cell Culture: The Young's modulus of both the control and microgel constructs (Figure 2B) as well as the cell count (not shown) did not reveal any significant variation between these two sets. These results indicate that the microgels did not affect cell viability or tissue growth. Interestingly, a comparison of the Raman spectra of both the control and microgel constructs with that of a juvenile-bovine articular cartilage clearly indicates that collagen content (collagen peak at 936 cm^{-1} relative to GAG peaks) and the helical conformation (peak at 1665 cm^{-1}) was still low in the engineered constructs (Figure 3).

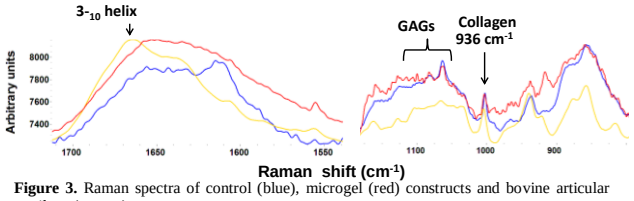

Figure 3. Raman spectra of control (blue), microgel (red) constructs and bovine articular cartilage (orange).

Discussion

In this study, we demonstrate that polymeric microgels can be incorporated into a clinically-relevant hydrogel scaffold (agarose [5]) without inhibiting tissue growth or decreasing cell viability. A sustained linear delivery of the drug via microgel delivery with no initial burst effect was observed and is promising. Additionally, the degradation of these embedded polymeric microgels can act as a delayed porogen that can foster greater nutrient exchange in later stages of the culture period. Although the mechanical properties of microgel-loaded constructs were similar to the control and were approaching native levels, the collagen conformation and content of both remain low relative to native bovine cartilage. We anticipate that the latter can be improved by encapsulating chondroitinaseABC (chABC) in the microgels, a future aim. The chABC is used in tissue engineering to increase the relative content of collagen by controlled digestion of glycosaminoglycans (GAG) [6,7].

Significance

Long-term controlled delivery of nutrients using encapsulated polymeric microgels embedded in a hydrogel scaffold can overcome the problems of suppressed convective and diffusive transport in engineered cartilage constructs associated with increasing levels of tissue formation with culture time.

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