Sequential Delivery of Growth Factors from Hydrolytically Degradable Silica-Based Nanoparticles for Cartilage Tissue Engineering

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Abstract

Articular cartilage shows a very limited self-healing capability due to its vascular structure. It has been reported that sequential supplementation of various growth factors such as bone morphogenic protein 7 (BMP-7), transforming growth factor-beta 1 (TGF-β1) and insulin-like growth factor-I (IGF-I) play critical roles in reconstruction of articular cartilage tissue. The objective of this work is to design a drug delivery system which facilitates the controlled sequential delivery growth factors to maximize the extent of chondrogenic differentiation of mesenchymal stem cells and consequently reconstruction of damaged cartilage tissue. Surface-modified silica nanoparticles (SNs) with short segments of lactide (L) and glycolide (G) units were used for grafting and timed-release of growth factors. It was shown that by changing the length of L and G units, the release rate of grafted bovine serum albumin (BSA) on the surface of SNs can be controlled. Nanoparticles with bigger G units showed faster release rate of BSA compared with shorter G units. Additionally, the presence of short LG segments did not significantly change the size distribution of SNs.

Introduction

Articular cartilage can resist a significant amount of mechanical stress and provide a lubricating surface for the gliding joints and a load-bearing matrix attached to the underlying bone [1]. However, due to its avascular nature and low metabolism, it has a very limited self-repair capability upon suffering a trauma [2]. The disease control and prevention center reported that nearly 27 million Americans suffer from joint pain and stiffness, loss of function and disability [3]. While various strategies such as transplantation of autogenous or allogeneic chondrocytes, or the use of mesenchymal stem cells (MSCs) are currently used for cartilage treatment, these strategies always suffer from inherent risks of an immune reaction, lack of suitable donor site, and more importantly they rarely restore the full function to the joint [4,5].

It has been demonstrated that different types of growth factors such as bone morphogenic protein 7 (BMP-7), transforming growth factor-beta 1 (TGF-β1) and insulin-like growth factor-I (IGF-I) play critical roles in tissue engineering of articular cartilage to induce chondrogenic differentiation of Mesenchymal Stem Cells (MSCs) [6-8]. However, the bioavailability and bioactivity of these growth factors are both time and concentration dependent [4,9,10]. Several studies have shown that the sequential supplementation of growth these factors is critical to prevent dedifferentiation of cells by first promoting proliferation with one specific growth factor, and then differentiation and expression of a desired phenotype with another [9,10]. A critical barrier to progress in this way is the lack of suitable delivery systems which work for precise controlled and orchestrated time-dependent delivery of multiple growth factors, although many studies have been conducted to control the delivery of growth factors for different tissue engineering applications. Accordingly, the main challenge of this work is to engineer a programmable delivery system to control the release rate of BMP-7, TGF-β1 and IGF-I growth factors and consequently maximize the chondrogenic differentiation of MSCs.

To address this challenge, the idea is to engineer a silica-based nanoparticle system containing short segments of biodegradable polymers such as polylactic acid and polyglycolic acid to control the release rate of grafted proteins (Figure 1). We have chosen MSNs given their demonstrated biocompatibility, osteogenic potential [11], and efficacy as drug delivery vehicles for sustained release of antibiotics [12] and anti-cancer drugs [13]. We envision that due to the hydrophilic nature of silica nanoparticles, bioactivity of the grafted proteins will be significantly enhanced over solid hydrophobic micro/nanoparticles such as poly lactic-co-glycolic acid (PLGA) which are currently used for drug delivery.
applications. Accordingly, we assume that the protein release rate can be tuned by type or length of the degradable segments. The novelty of this project is to design a low-cost hydrolytically degradable nanocarrier system which facilitates a programmable sequential delivery of multiple growth factors with a controlled timed-release to enhance chondrogenic differentiation of MSCs for cartilage tissue engineering applications.

![Figure 1: Growth factors timed-release by grafting to silica based nanoparticles. The surface of silica nanoparticles are modified by sequential addition of short biodegradable lactide or glycolide for controlling protein release followed by a functionalization with succinimide for protein grafting; Next, the growth factor is grafted to modified silica nanoparticle by the succinimide amine reaction.](image)

Materials and Methods

**Materials**: Lactide (L) and glycolide (G) monomers with >99.5% purity (Ortec, Easley, SC) were dried under vacuum at 40°C for at least 12 h before use. N, N’-disuccinimidyl carbonate (DSC) and bovine serum albumin (BSA) were received from Novabiochem (EMD Biosciences, San Diego, CA) and Jackson Immuno Research (West Grove, PA), respectively. Hydrophilic silica nanoparticles (SN) was kindly donated by Evonik Corporation (New Jersey). All other reagents were purchased from Sigma Aldrich (St. Louis, MO).

**Methods**: To activate the silanol groups of SNs were activated by adding 1.5×10⁻⁴ mol triethyl amine (TEA) to 250 mg of SN containing 3×10⁻⁴ mol SiOH groups in 70 ml toluene as the solvent. In the next step, 6×10⁻⁴ mol of isopropyl alcohol was added to the mixture to continue the reaction for 2 h at 50 °C. Then, the desired amount of L was added to the mixture along with 1 ml Sn (II) 2-ethylhexanoate as the reaction catalyst and the reaction was run for 6 h. The lactide chain-extended silica was used as an initiator for chain extension with G monomer with a predetermined L to G ratio. The reaction was allowed to proceed for 6 h at 50°C and the product was precipitated in ice-cold hexane to remove the unreacted monomers. In separate reactions, the mole ratio of L and G was changed from 100% (L100) to 75% (L75/G25) and 50% (L50/G50) while the total amount of L and G was kept fixed at 3×10⁻³ mol. The synthesized copolymer on SNs surface was functionalized with succinimide groups by reacting hydroxyl end-groups of the copolymer with DSC as we described previously [14]. The product was purified by dialysis against DI water and lyophilized.

To attach BSA on the surface of modified SNs, 10 mg SNs was suspended in 0.5 mL PBS by sonication for 1 min. Next, 0.5 mL of the protein in PBS (20 mg/mL for BSA) was added to the SNs suspension. The amine group of the protein was allowed to react with succinimide end-groups of LG in the surface of SNs under ambient conditions for 12 h as we previously described (Figure 1) [15]. The protein grafted SNs were freeze-dried to obtain a free-flowing powder. To determine grafting efficiency, the protein grafted SNs were resuspended in PBS and centrifuged at 18,000 rcf for 10 min and the supernatant was analyzed for total protein content with the ninhydrin reagent as we described previously [16]. Grafting efficiency was determined by dividing the amount of attached protein (total - free protein) by the initial amount in the grafting reaction.

Size distribution of the SNs was measured by dynamic light scattering with a Submicron Particle Sizer (Model 370, NICOMP, Santa Barbara, CA) as described previously [16,17]. For measurement of release kinetic, 1 mg protein grafted SNs were incubated in 1 mL PBS at 37°C as we previously described [15]. At each time point, the suspension was centrifuged at 18,350 rcf for 10 min, the supernatant was removed, the SNs were resuspended in 1 mL fresh PBS and incubated until the next time point. The amount of BSA in the supernatant was measured with the ninhydrin reagent as described [16,18].

Results and Discussion

The calculated grafting efficiency of BSA to SNs based on the procedure explained in the method section was 52±9%. The effect of L and G segments on average diameter and size distribution of nanoparticles are shown in (Figure 2a). It can

be observed the addition of short L and G parts in SNs does not significantly change the average diameter of particles. The particle sizes are between 120±12 and 165±15 for SN and SN-L100, respectively. Figure 2b reveals the effect of the length of L and G segments on release rate of BSA from nanoparticles. The results demonstrated that by increasing the length of lactide the release rate of BSA will decrease significantly. The cumulative release percentage of BSA is 51±5, 65±8 and 91±4 for L50/G50, L75/G25 and L100, respectively, after 24 days. The average release rate of BSA from L50/G50, L75/G25 and L100 is 3.2, 2.4 and 2.1 wt%, respectively, during the first 24 days.

It has been reported that the release kinetic of BSA grafted to the PLG copolymer follows the degradation rate of the copolymer [19]. Additionally, it has been previously shown that the degradation kinetic of LG based micelles depends on the proximity of water molecules to L and G ester groups [20,21], which is dependent on hydrophobicity of the degradable units [19]. Therefore, the fraction of less hydrophobic G in LG segments has a profound effect on LG unit degradation and consequently the release rate of protein. As a result, by increasing the fraction of G, which is less hydrophobic than L, from 0 to 50%, the the average release rate of BSA increased from 2.1 wt% to 3.2 wt%.

**Conclusion**

It was shown that by surface modification of silica nanoparticles with short segments of lactide and glycolide, the controlled release of growth factors can be achieved. Based on the release profile of BSA from surface modified silica nanoparticles, by increasing the ratio of glycolide segment from 0% to 50% with respect to lactide segment, the average release rate of BSA will increase from 2.1 wt% to 3.2 wt% per day. Some of the potential challenges of using nanoparticulate systems for drug delivery applications are the stability of the nanoparticles in aqueous environment, bioactivity of growth factors and controlling release mechanism of growth factors from nanoparticles. Therefore, further studies need to be done on the properties of surface modified nanoparticles as well as controlled release of target growth factors like of BMP-7, TGF-β1 and IGF-Iand their effect on chondrogenic differentiation of MSCs.

**References**


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