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Design and characterization of microporous hyaluronic acid hydrogels for in vitro gene transfer to mMSCs

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ABSTRACT

The effective and sustained delivery of DNA locally could increase the applicability of gene therapy in tissue regeneration and therapeutic angiogenesis. One promising approach is to use porous hydrogel scaffolds to encapsulate and deliver nucleotides in the form of nanoparticles to the affected sites. We have designed and characterized microporous (μ-pore) hyaluronic acid hydrogels which allow for effective cell seeding in vitro post-scaffold fabrication and allow for cell spreading and proliferation without requiring high levels of degradation. These factors, coupled with high loading efficiency of DNA polyplexes using a previously developed caged nanoparticle encapsulation (CnE) technique, then allowed for long-term sustained transfection and transgene expression of incorporated mMSCs. In this study, we examined the effect of pore size on gene transfer efficiency and the kinetics of transgene expression. For all investigated pore sizes (30, 60, and 100 μm), encapsulated DNA polyplexes were released steadily, starting by day 4 for up to 10 days. Likewise, transgene expression was sustained over this period, although significant differences between different pore sizes were not observed. Cell viability was also shown to remain high over time, even in the presence of high concentrations of DNA polyplexes. The knowledge acquired through this in vitro model can be utilized to design and better predict scaffold-mediated gene delivery for local gene therapy in an in vivo model where host cells infiltrate the scaffold over time.

1. Introduction

Vascularization of tissue engineering constructs remains the primary reason for construct failure in vivo [1]. Without the rapid infiltration of blood vessels, diffusion alone is insufficient to sustain migrating endogenous or exogenously implanted cells far from the construct surface. Researchers have recently been focusing on macroscopic biomaterial design to help promote branching from existing blood vessels into the biomaterial. Microscale interconnected pores produced through salt-leaching [2,3], gas foaming [4–6], lyophilization [7–10], and sphere templating [11–14] have shown to be effective in allowing for cellular infiltration and subsequent enhanced scaffold vascularization.

In addition to the structural characteristics of the scaffold, the effective local delivery of angiogenic factors, including VEGF and PDGF, are necessary to promote blood vessel formation. For tissue regeneration, localized gene delivery can promote the expression of tissue inductive factors to guide tissue formation. Local gene delivery via hydrogel scaffolds has been studied for nearly a decade, primarily through the encapsulation of naked DNA during hydrogel formation [5,15–19]. Although naked DNA achieves gene expression and guided regeneration in vivo [5,15], limitations with low gene transfer efficiency and rapid diffusion of the DNA from the hydrogel scaffold motivated the use of DNA nanoparticles instead of naked DNA. DNA condensed with either cationic peptides, lipids, or polymers has previously been introduced into fibrin hydrogels [13,20–22], enzymatically degradable poly(ethylene glycol) (PEG) hydrogels [23,24] and PEG–hyaluronic acid hydrogels [25]. Poly(ethylene imine) (PEI) is a widely utilized cationic polymer for non-viral gene delivery; it is able to condense DNA through electrostatic interactions between the positively charged amines on the PEI and the negatively charged phosphates on the DNA, forming nanoparticles (polyplexes) in the range of 50 to 200 nm [26]. PEI has been successfully used in vivo to deliver DNA or siRNA to the brain [27,28], lungs [29–32], abdomen [33], and tumors [34–36].

Hydrogel properties, such as the type of natural or synthetic polymer used, can likewise be an important factor in the promotion of vascularization. While a synthetic polymer, such as PEG, can be biochemically inert, natural polymers, such as hyaluronic acid (HA), possess intrinsic qualities which can play a role in signaling to surrounding cells. HA, an anionic, non-sulfated

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