Surface-Mediated Delivery of Small Interfering Ribonucleic Acid Polyplexes
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Introduction
Many tissue engineering approaches utilize biomaterial scaffolds as a matrix for controlled cellular growth in an effort to support tissue regeneration. The application of nucleic acid-based therapy offers the potential to modulate cell behavior in regenerative and tissue engineering applications at the molecular level. While methods to deliver DNA from scaffolding materials have been described [1], there are few reports of the delivery of small interfering RNA (siRNA) from biomaterial scaffolds. Non-viral gene delivery systems such as polycation-nucleic acid complexes (polyplexes) are an attractive alternative to viral vehicles in part due to lower risk of immunological response and other safety concerns. Polyethylenimine (PEI) has been used as a polycation for delivery of nucleic acids to cells due to its ability to achieve endocytosis and endosomal escape. We have previously successfully delivered DNA from fibrin biomaterials via surface-mediated uptake with PEI polyplexes [2]. We now seek to characterize surface-mediated delivery of siRNA from PEI polyplexes as an approach to ultimately modulate cellular behavior.

Materials and Methods
Linear 25 kDa PEI and Green Fluorescent Protein siRNA or pDNA were each separately suspended in RNase free water. PEI and DNA or PEI and siRNA were thoroughly mixed and incubated to form polyplexes (referred to as DNA polyplexes or siRNA polyplexes). Separately, dissolved fibrinogen was placed in a 24-well plate and polymerized with thrombin resulting in a fibrin hydrogel. DNA polyplexes or siRNA polyplexes were then adsorbed to the fibrin gel. To visualize adsorbed polyplexes, samples were critical point dried and gold sputter coated to allow for Field Emission Scanning Electron Microscopy (FE-SEM). Polyplex shape, size and distribution on the surface of fibrin gels were analyzed using ImageJ software. In separate experiments, the level of uptake of DNA and siRNA polyplexes were determined by using fluorescently labeled DNA or siRNA, respectively. Polyplexes (DNA or siRNA) were adsorbed to gels as described above. S16 Schwannoma cells were seeded onto the scaffolds. After 4 hours in culture, the cells were trypsinized, and assessed for DNA or siRNA uptake by flow cytometry.

Results
FE-SEM confirmed polyplex formation and stability when adsorbed to a fibrin gel surface (Fig. 1). Diameters of both pDNA and siRNA polyplexes were found to center at 200nm on a histogram size distribution. Flow cytometry confirmed surface mediated uptake of both siRNA and DNA polyplexes (Fig. 2).

Fig. 1. FE-SEM images of fibrin gel surface (A) coated with siRNA polyplex (B) and pDNA polyplexes(C) highlighted by arrows. Profile view of siRNA polyplex adsorbed to fibrin gel surface (D).

Fig. 2. Flow cytometry data for S16 cells (A) which show positive uptake of YOYO-1 labeled pDNA polyplex(B) and Alexa488 tagged siRNA polyplex (C).

Discussion and Conclusions
Following fibrin gel deposition, polyplex stability is maintained and promotes cellular uptake. The ability to achieve adsorption and cellular uptake suggests the potential of this system to achieve knockdown of target RNA (and ultimately proteins) in a spatio-temporal fashion to allow material-based approaches to regulate cellular behavior.

Reference List

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