An arginine-based polycation/heparin matrix for the controlled delivery of growth factors

Blaine Zern, Anh Nguyen, and Yadong Wang
Department of Biomedical Engineering, School of Chemistry & Biochemistry, and Petit Institute for Bioengineering & Biosciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

Statement of Purpose: Coronary heart disease, the accumulation of fatty deposits in the arterial wall that results in tissue ischemia, is one of the leading causes of mortality in the United States. Therapeutic angiogenesis is the development of new blood vessel formation from pre-existing vasculature under the direction of exogenous mediators and is an attractive approach to treat this disease[1]. There are a multitude of factors that have been shown to induce angiogenesis and fibroblast growth factor-2 (FGF-2) is one that has been studied extensively. In order for FGF-2 to be introduced safely & efficiently, it has to be delivered in a local and controlled manner. In this approach, a positively charged, biodegradable polymer has been synthesized and used to self-assemble with a negatively charged polysaccharide, heparin, to form non-covalent networks. These networks have then been used to deliver therapeutic growth factor with a controlled, localized release.

Methods: The arginine-based polymer was synthesized via polycondensation reaction of equimolar amounts of diglycidol succinate and arginine ethyl ester. The resultant polymer (PSR, **Figure 1A**) was characterized via FTIR, NMR, and DSC. To ensure PSR was not cytotoxic to cells, MTT, Caspase-3 levels, and Live/Dead was performed using baboon smooth muscle cells. PSR's ability to interact with heparin was characterized by SEM and dynamic light scattering experiments. The electrostatic network's aptitude to load and release growth factor was investigated using I-125 FGF-2.

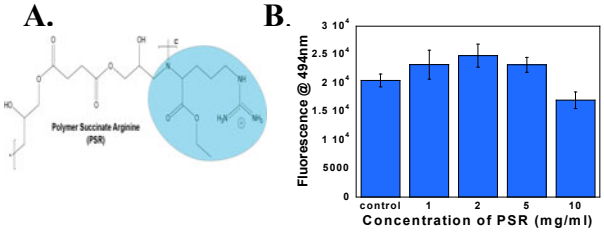

Figure 1: A. The chemical structure of PSR (arginine highlighted in light blue). **B**. Cytotoxicity profile of PSR via Live/Dead Assay.

Results/Discussion: PSR was a pale yellow powder soluble in water, alcohols, and DMF. The spectroscopic characterization of PSR indicated that both arginine and the diglycidol ester were incorporated into the polymer in a 1:1 molar ratio. MTT assay was performed on baboon smooth muscle cells to investigate the cytotoxicity of PSR. It was found that PSR had negligible cytotoxicity up to a concentration of 5 mg/ml. Apoptotic levels and Live/Dead (**Figure 1B**) were also used to examine *in vitro* cytotoxicity. These experiments showed that PSR (up to 10 mg/ml) did not cause increased levels of cell death or apoptosis relative to control cells. The ability of PSR to form non-covalent networks with heparin was examined by SEM and dynamic light experiments. These experiments showed networks composed of a majority of fiber-like domains along with a minority of globular-like domains. Fibers of the networks average ~ 1 μm and globular domains range from 5-20 μm in diameter. Release kinetics of the matrix were evaluated using I-125 labeled FGF-2. It was shown that using high molecular (HMW) weight PSR that an initial burst was followed by a slow sustained release. This release resulted in ~20% of loaded FGF-2 released over 4 weeks. In contrast, low molecular weight (LMW) PSR yielded a similar release pattern but released ~65 % of loaded FGF-2. It should also be noted that the low molecular weight PSR incorporated 25% less FGF-2 compared to the high molecular weight. These results indicate the capability of the networks to have adaptable release kinetics based on molecular weight of the polycation.

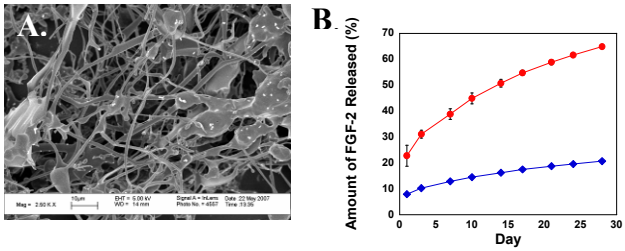

Figure 2: A. SEM image of PSR/heparin matrix **B.** Release profile of HMW & LMW PSR FGF-2 from network.

Conclusions: We have designed and synthesized a biocompatible polymer with integrated arginine functional groups. This polymer has proven to be positively charged thus enabling PSR to interact with heparin electrostatically. This strategy to deliver growth factor has demonstrated a sustained release of FGF-2 over a four week period. Furthermore, PSR/heparin networks have demonstrated the capacity to adapt release rates of growth factor from the network according to molecular weight of the polycation. Also, the versatile design of PSR allows for a tunable polymer that can be modified for optimal transfection efficiency. Future investigation includes bioactivity of released FGF-2 & altering polycation and polyanion weight ratios to examine how this affects the release profile of the matrix.

References:

1. Silva, E. & Mooney, DJ. Spatiotemporal control of VEGF delivery from injectable hydrogels enhances angiogenesis. Journal of Thrombosis and Haemostasis, 5, 3, 590-598.