Local Delivery of Proteins and Antibiotics from Elastin-based Scaffolds

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Statement of Purpose: Elastin is a structural extra cellular matrix protein that is predominantly present in the connective tissue of vertebrates. Due to presence of crosslinks in elastin, the protein is highly insoluble and difficult to process into biomaterials. Consequently, elastin like polypeptides (ELPs), the genetically engineered soluble forms of elastin, are frequently used for drug delivery and tissue engineering. ELPs are biopolymers with pentapeptide repeat unit, VPGXG where V = valine, P = proline, G = glycine, and X, the guest residue, is any amino acid other than proline. ELPs are a class of stimulus responsive smart polymers that exhibit an inverse temperature phase transition behavior in response to changes in their solution environment[1-3]. The favorable properties of ELPs include biocompatibility, good mechanical properties, low immunogenecity, and the ability to self assemble[4]. Tissue engineering applications utilizing polymeric scaffolds benefit from the the sustained and controlled release of signaling molecules and growth factors essential for cell growth and proliferation. Therefore, this research investigated the release profiles of a model protein (albumin) and commonly used antibiotics (penicillin-streptomycin) from ELP scaffolds.

Methods: *Expression and Purification of ELP:* E.coli bacteria having synthetic gene for $(VGVPG)_{120}$ were multiplied in nutrient broth at 37°C for 24 h. The cells were then harvested and lysed by sonic disruption and cell debris separated by centrifugation. ELP was purified by an inverse transition cycling method involving atleast 3 repeated cycles of cold and warm centrifugations at 4°C and 40°C respectively. The solution was dialyzed against deionized (DI) water to remove buffer salts. The purified ELP was then lyophilized.

Gel formation by Sonication: Gels were prepared by adding 1 and 2% bovine serum albumin and 10% penicillin-streptomycin (penstrep) to 50 mg/ml ELP solutions in DI water. Additionally base ELP gel (50mg/ml) was made with no loading of protein or antibiotic. All the solutions were sonicated for 2 min using 40% amplitude setting on a Branson 450 sonifier with a 1/8" diameter-tapered microtip. The suspension was transferred to 24 well tissue culture polystyrene plate and incubated at 37°C for 72 h to allow gelation and film formation.

Analysis of Gels: Films were washed with phosphate buffered saline (PBS) and 1ml of fresh PBS was added to each well. The amount of ELP, penstrep, or albumin released into the PBS was analyzed at specific time intervals for upto 72 h by measuring the UV absorbance at 270-280 nm with a NDS 1000 spectrometer. All experiments were performed at least in triplicate. Results reported as mean ± 95% confidence intervals.

Results: Fig. 1 shows the calibration curves for various concentrations of albumin and penicillin streptomycin for calculating the release profiles. Fig. 2 shows the release profiles of the base ELP gel, the ELP gels loaded with albumin (1 and 2%) and penicillin-streptomycin (10%). For the base ELP gel, an initial phase of rapid release was observed which gradually plateaued to a value of 12%. This result showed that there was about 12% dissolution of ELP in PBS over the 72 h period. Albumin loaded ELP gels showed a gradual time dependent release of up to 30% of the initially loaded albumin over the 72 h period. This release kinetics seemed independent of the initial albumin loading over the first 72 h. Our preliminary results showed that penicillin-streptomycin was not released from ELP gel over the first 72 h and we plan to continually monitor its release over an extended period.

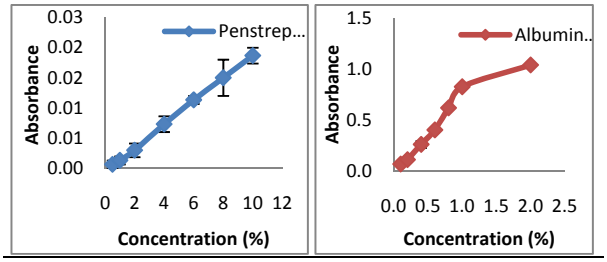

Fig.1 Calibration for penicillin-streptomycin and albumin

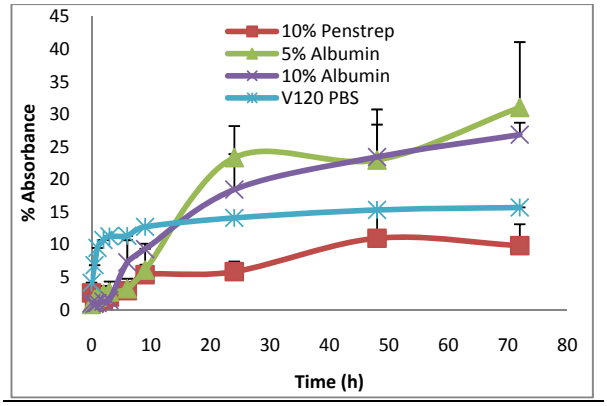

Fig.2 Release profiles for base ELP gel, and the ELP gels loaded with penicillin-streptomycin and albumin

Conclusions: Here, we have demonstrated a controlled and sustained release of a model protein (albumin) from biocompatible ELP gels. Further refinement of experimental parameters is expected to provide better control of the ELP hydrogel characteristics to obtain sustained antibiotic release. This study provides a good starting point to evaluate the local delivery of various proteins and antibiotics critical to tissue engineering.

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