Elastin like Polypeptide Based Hollow Spheres for Gene Delivery
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Introduction
The elastin protein is found in extra cellular matrices of the arteries, lungs and skin of most vertebrates. Elastin like polypeptide (ELP) can be produced by recombinant genetic engineering technique, containing pentapeptide repeat Val-Pro-Gly-Xaa-Gly and undergoes an inverse temperature phase transition. They are soluble in aqueous solutions below their transition temperature (Tt) but hydrophobically collapse and self assemble to form solid nanoparticles at temperatures greater than Tt. The nanoparticles are unstable and coacervate to form microparticles (1). The overall goal of the present study is to fabricate hollow spheres that can be loaded with plasmids and investigate cellular internalisation of these spheres. The hypothesis behind this study is that sulfonated polystyrene (PS) beads act as a template on which ELP hollow spheres of defined size can be fabricated. These hollow spheres can be loaded with plasmid and can be internalised by endothelial cells through endocytosis.

Methods and Materials
ELP based, hollow spheres were produced using template based method (2) Briefly PS beads of defined size were sulfonated to create a negative charge on the sphere. EP of ~35 kDa was used for coating. 1: 20 ratio of EP to PS beads was dissolved in phosphate buffer solution and finally incubated for 10 mins at 37°C. The coated beads were then centrifuged twice at 9000 rpm and characterized for protein adsorption using the bicinchoninic acid (BCA) assay. The coated beads were then cross-linked using 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) activated star-PEG. Coating was characterized using zeta-sizer, scanning electron microscope (SEM). Fourier transform infrared spectroscopy (FTIR) was used to characterise cross-linking. Finally PS beads were dissolved using tetrahydrofuran (THF) to get the hollow spheres. The spheres thus produced were observed under transmission electron microscope (TEM) and SEM. Cy3 labeled plasmid was loaded in to the spheres by agitating a mixture of plasmid/spheres/THF/water and characterized using fluorescence microscope and agarose gel electrophoresis. Cell viability studies of these spheres were done with HUVEC using MTT and Picogreen assay for 24 hrs time period. Fluorescein isothiocyanate labeled spheres were incubated for 6, 12 and 24 hrs time point with HUVEC. The cells were stained with rhodamine-phalloidin and DAPI and then observed under fluorescence microscope.

Results and Discussion:
The BCA assay shows around 65 percentage of coating of ELP on the beads after the washes, with no significant difference for incubation time (Fig. 1). The zeta potential shows an increase in surface charge and size after the coating step (Table). The SEM image shows rough surface morphology after coating indicating a layer of ELP over the PS bead. TEM micrograph after washing with THF shows transparent spheres without PS beads (Fig. 2A). Fluorescence microscopic image shows the Cy3 labelled plasmid co-localised inside the sphere. The cell viability is comparable to control and spheres were seen in the cytoplasm-surrounding nucleus (Fig. 2B).

Table representing zeta potential and size of PS beads only and ELP coated PS beads (n=3)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Size (nm)</th>
<th>Zeta Potential</th>
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<tbody>
<tr>
<td>PS Bead</td>
<td>320 ± 6</td>
<td>-37 ± 6 mV</td>
</tr>
<tr>
<td>EP Coated Bead</td>
<td>430 ± 20</td>
<td>-8.3 ± 2 mV</td>
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Figure 1: Adsorption of ELP on PS beads with different time points at 37°C. Results are mean ± standard deviation(n=3, p < 0.05)

Figure 2. (A) Arrow indicates ELP hollow sphere probed by TEM (B) Confocal micrograph of HUVECs with 300 nm spheres at 24 hr. Nucleus is stained with DAPI (blue) and red colour represents cytoskeleton. Arrow pointing yellow represents co-localisation of spheres inside the cell.

Conclusion
We have successfully fabricated hollow spheres from elastin like polypeptides. The template method for fabrication of hollow spheres gives a defined structure whereas cross-linking with NHS activated star PEG along with EDC provides stability and prevents coacervation. ELP spheres can be loaded with plasmid and can be internalized within HUVEC by endocytosis.

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References