Matrix Metalloproteinase Degradable PEG Hydrogels with High DNA/PEI Loading for Tissue Engineering

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Introduction: Gene delivery for tissue engineering is a promising approach to guide tissue formation. Our previous research showed that DNA/poly(ethyleneimine) (PEI) polyplexes could be encapsulated into matrix metalloproteinase **(**MMP) degradable poly(ethylene glycol) (PEG) hydrogels while maintaining their activity (Lei Y. Biomaterials. 2009;30:254-265). Cells migrating in these gels were transfected multiple times by the encapsulated polyplexes resulting in sustained transgene expression. However, only a limited dosage of DNA could be loaded because aggregation occurred when high polyplex concentrations were encapsulated. Here, we report a process that allows high DNA/PEI polyplex loading into MMP degradable PEG hydrogels.

Materials and Methods

DNA loaded hydrogel formation: Plasmid DNA was complexed with PEI in the presence of sucrose. The polyplexes were then lyophilized in the presence of a polysaccharide. The dry polyplexes were reconstituted with 4-arm-PEG-vinyl sulfone premixed with a MMP sensitive peptide crosslinker (GCRDGPQGIWGQDRCG) in 0.3M TEOA buffer. The solution was gelated at 37ºC for 30 mins. Gels were swelled in PBS buffer. Confocal microscopy was used to visualize polyplex distribution by acquiring images within 2 µm of the gel after staining with 2µM ethidium homodimer-1.

In vitro and *in vivo* characterization of hydrogel: The bioactivity of the encapsulated DNA/PEI was tested *in vitro* through degrading the hydrogel and using the released polyplexes to transfect adherent cells. *In vivo* activity was tested using the explanted Chicken Chorioallantoic Membrane (CAM) assay. Fertilized chicken eggs were incubated at 37ºC for 3 days before embryos were released into tissue culture dishes and incubated at 38°C until day 10. PEG hydrogels with encapsulated pVEGF/PEI or pBeta-galactosidase (βGal)/PEI polyplexes were placed on the CAM at day 10. At day 13, the embryos were perfused with FTIC dextran and blood vessels within and around the gel area were assessed. Standard βGal staining was used to characterize βGal expression.

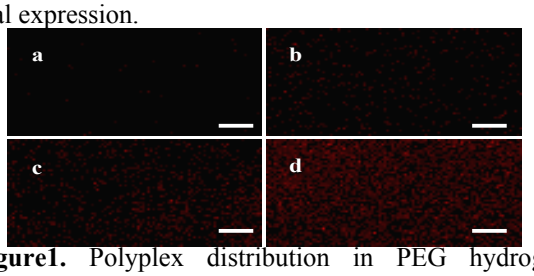

Figure1. Polyplex distribution in PEG hydrogel. Polyplexes including 0, 50, 100 and 300µg DNA were encapsulated into 100µL hydrogel (a), (b), (c) and (d) respectively. Scale bar = 500nm.

Results and Discussion: Addition of sucrose, polysaccharides, freeze-drying and gelation did not affect polyplex size significantly. After hydrogel formation and polyplex staining, the distribution of DNA polyplexes could be observed using confocal microscopy. The polyplex density in the gel was found to be proportional to the DNA loading density (i.e. **Fig 1d** > **Fig 1c** > **Fig 1b**). Polyplexes distributed throughout the hydrogel without aggregation (**Fig. 1**).

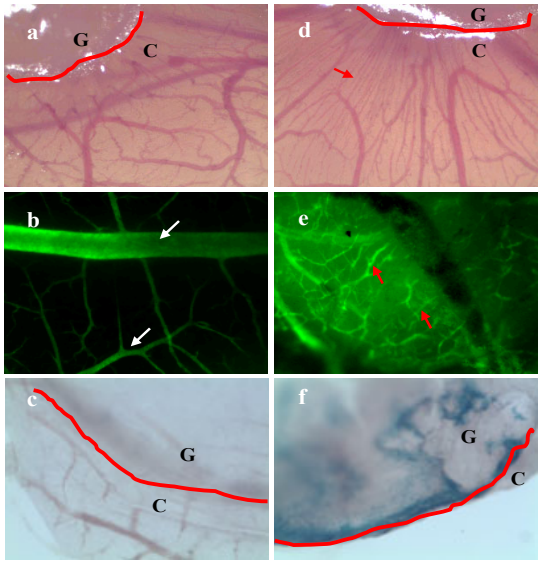

Figure 2. CAM assay with PEG gels encapsulating DNA/PEI polyplexes. Negative control gels w/o DNA (a,b,c), gels with pVEGF (d,e) and pβgal (f) (100 µg DNA/100 µL gel). Stereo microscope (a,d), fluorescent microscope (b,e) and β-gal staining (c,f) pictures. (G: gel area; C: CAM around the gel; red line: edge of the gel)

A CAM assay was used to study the ability of pVEGF/PEI loaded hydrogels to induce blood vessel growth around the hydrogel (**Fig 2**). pVEGF/PEI loaded hydrogels resulted in enhanced angiogenic activity (**Fig 2d**, red arrow) compared gels that did not contain DNA (**Fig 2a**). pVEGF/PEI loaded hydrogels promoted the formation of immature vessels (**Fig 2e**, red arrows), which leaked perfused FITC-dextran resulting in high background fluorescence (**Fig 2e**). In contrast, only mature vessels, which existed in the CAM before placing the gel, were found in the control gels (**Fig 2b,** white arrows). DNA loaded hydrogels that encoded for ß-Gal showed that the encapsulated polyplexes remain active due to the positive β-Gal staining (**Fig 2c vs. Fig 2f**).

Conclusions: High DNA/PEI loading into PEG hydrogels was achieved using lyophilized polyplexes. pVEGF/PEI loaded hydrogels were shown to induce new blood vessel formation in the CAM assay and result in positive ß-gal staining indicative of active polyplexes. The technique described here provides an efficient strategy to deliver genes from scaffolds.