# DNA Polyplex Loaded Hyaluronic Acid Hydrogel Scaffolds for Local Gene Transfer

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**Statement of Purpose:** Local delivery of DNA from hydrogel scaffolds would increase the applicability of gene therapy in tissue regeneration and cancer therapy. The incorporation of high concentration DNA/poly(ethylene imine) (PEI) polyplexes into hyaluronic acid (HA) is limited by polyplex aggregation, which is caused by the hydrogel precursor solution. Before gelation, the highly negatively charged HA polymer competes with DNA for the positively charged PEI causing aggregation (**Fig 1A**). In a previous study by our laboratory [1] we developed an approach, termed caged nanoparticle encapsulation or CnE, to load high concentration of DNA/PEI polyplexes into poly(ethylene glycol) hydrogels. The goal of this study was to determine if CnE could also enhance encapsulation of DNA/PEI polyplexes into HA hydrogel scaffolds, resulting in low aggregation and active polyplexes.

**Methods:** _CnE process and polyplex encapsulation:_ plasmid DNA encoding for secreted alkaline phosphatase (pSEAP) or gaussia luciferase (pGLUC) was complexed with polyethyleneimine (PEI) in a dilute solution (20µg/mL) the presence of neutral disaccharides (sucrose) and polysaccharides (agarose) and lyophilized. The polyplexes were then reconstituted with the gel precursor solution in the presence of cells for the *in vitro* studies or without for *in vivo* studies and allowed to gel at 37°C. _Hydrogel synthesis_: Acrylate groups (AC) were introduced to the HA backbone by first modifying the HA backbone with adipic acid dihydrazide to introduce hydrazide functionalities to the backbone and then reacting the hydrazides with NHS-Acryalte. HA-AC is crosslinked into a hydrogel using Michael addition of a dithiol-containing peptide with a sequence that is degraded by MMPs to the acrylates in the HA backbone. _In vitro gene transfer_: gels were placed in tissue culture plates and at predetermined time points the media was removed and replaced with fresh media. The collected media was assessed for transgene expression using SEAP or gaussia assay kits. _In vivo gene transfer_: Gene transfer from hydrogel *in vivo* was tested with the chicken chorioallantoic membrane (CAM) assay. Hydrogels with pVEGF/PEI or pBeta-galactosidase (βGal)/PEI were placed on the CAM at day10. At day13, 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.

**Results:** The delivery of DNA/cationic polymer nanoparticles (polyplexes) using hydrogels has not been successful partially due to the aggregation and inactivation of polyplexes inside hydrogel scaffolds. To overcome this challenge, we developed a strategy to introduce unaggregated and highly active DNA/PEI polyplexes to hydrogel scaffold. Specifically, we have found that sucrose was able to retain the activity of polyplexes during lyophilization and agarose could prevent polyplex aggregation during gelation by increasing the viscosity of the gel precursor solution. Polyplexes were not free to diffuse inside the hydrogels as shown by the fact that no DNA was released from the HA hydrogel in PBS (**Fig 1B**). Thus hydrogel degradation was required for the gene transfer from hydrogels to cells, which allowed highly localized gene delivery *in vivo*. Polyplexes encapsulated inside the hydrogels through the CnE process were found highly active *in vivo* as shown by the ß-galactosidase expression (**Fig. 1C**). To ensure that the expressed transgene was present at sufficient concentration to induce an angiogenic response in and around the hydrogel, plasmid DNA encoding for VEGF was entrapped within the hydrogel using the CnE. An angiogenic response was observed in all CAMs that contained a hydrogel with pVEGF/PEI polyplexes entrapped through CnE. The angiogenic response extended out in a radial orientation toward the CAM (**Fig 1D left**), suggesting that the expressed VEGF diffused out of the hydrogel area and created a VEGF gradient with the highest concentration near the hydrogel. In contrast, the vessels observed at the hydrogel area are highly branched neovessels without preferred orientations (**Fig 1D right**), suggesting that the VEGF concentration at the gel area was high and relatively constant.

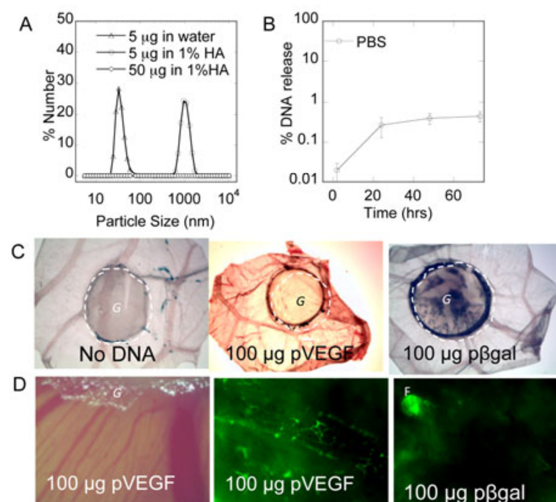


**Fig.1** (A) DLS of DNA/PEI polyplexes in the presence of HA, (B) Release of DNA/PEI polyplexes from HA hydrogels, in vivo CAM assay for (C) beta gal gene expression and (D) angiogenesis.

**Conclusions:** We were able to introduce high concentrations of DNA/PEI polyplexes (up to 300µg DNA) into hyaluronic acid hydrogel scaffolds. We showed that the polyplexes do not release from the hydrogel scaffold and that the polyplexes were active *in vitro* and *in vivo*. *In vivo* we showed that the transgene is expressed in sufficient quantity to induce an angiogenic response.

**References:**

1. Lei Y Biomaterials 2010 31(34):9106-1


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