Gene Transfer in and from Hydrogels

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Introduction: Gene delivery from hydrogel scaffolds is promising to guide and induce tissue engineering and regeneration. We have developed an approach to introduce highly active DNA/cationic polymer polyplexes into hydrogel scaffolds without aggregation. In this study, we investigated how the N/P ratio, DNA dose and cell number affect non-viral gene transfer to NIH/3T3 cells seeded inside fibrin hydrogel scaffolds, parameters that are traditionally studied for cells seeded at 2D surfaces but that have not been thoroughly and successfully investigated for cells seeded in three-dimensions.

Materials and Methods: Plasmid DNA encoding for secreted alkaline phosphatase (pSEAP) was complexed with polyethyleneimine (PEI) in the presence of neutral disaccharides & polysaccharides and lyophilized. The polyplexes were reconstituted with fibrinogen and cells, and gelation was induced with thrombin. In vitro gene transfer was analyzed by measuring the amount of SEAP protein in the cell medium. Gene transfer from hydrogel *in vivo* was tested with the chicken chorioallantoic membrane (CAM) assay. Fibrin gels 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.

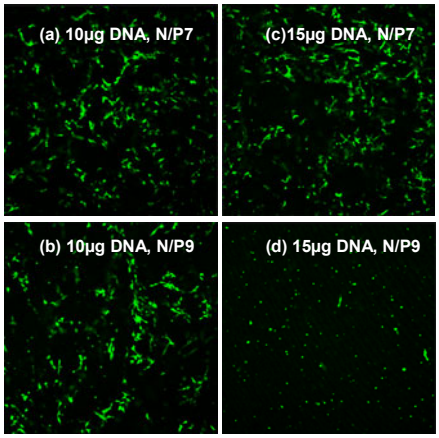

Figure 1. Cell viability in fibrin hydrogel with DNA/PEI polyplexes. Polyplexes and 100k NIH 3T3 cells were co-encapsulated into 100μL fibrin (5mg/mL) hydrogel. Cells were stained with Live (green)/Dead (red) kit at day 3.

Results & Discussion: We hypothesize that factors affecting gene transfer in and from hydrogels for a cell type include the cell proliferation, migration and density in the gel, the polyplex dosage and N/P (molar ratio of amines from PEI over phosphate from DNA), the gel density and degradation rate. No significant cell apoptosis was found in hydrogels with polyplexes as showed by the Live/Dead cell assay (Fig1). However the spreading of cells was inhibited when 15μg DNA at N/P 9 was loaded in 100μL gel (Fig1d) indicating the PEI in the hydrogel has to be limited below a certain concentration.

The N/P significantly affects gene transfer in 3D fibrin

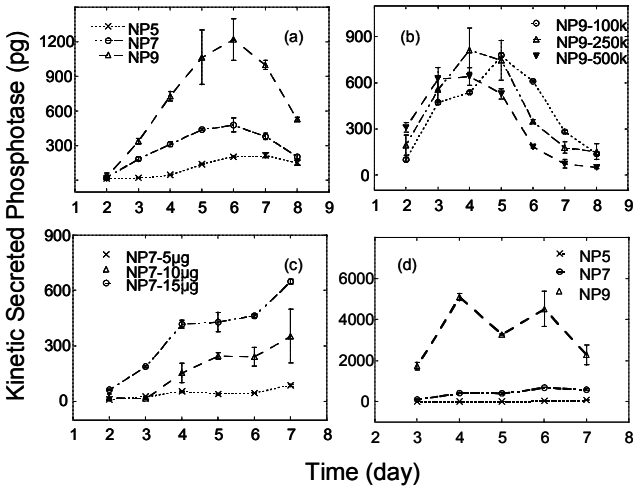

Figure2. Transgene expression in vitro. 100k NIH 3T3 cells (a,c, d) were plated on (d) or encapsulated in (a,b,c) 100μL fibrin gel with 10μg (a, b, d) pSEAP/PEI. SEAP in medium at different day was quantified.

hydrogel (Fig2a) and the order of gene transfer efficiency is: N/P 9>7>5. The expression increased at the beginning, peaked around day 6 and dropped afterward due the severe degradation of the fibrin scaffold, which led to cell aggregation. The transgene expression in 3D was dosage dependent (Fig2c). It was related to the cell proliferation and migration as well (data not shown). Unexpectedly, higher cell density only shifts the expression peak to the earlier days without increasing the protein expression (Fig2b). Cells plated on top of fibrin hydrogel could be transfected by the polyplexes encapsulated inside the hydrogel as well. And the gene transfer efficiency increased as the N/P was raised from 5 to 9 (Fig2d). However, its transgene expression profile was different from the profile of 3D transfection. The bioactivity of fibrin hydrogel with encapsulated polyplexes were tested Chick Chorioallantoic Membrane (CAM) Assay (data not shown). pVEGF released from the hydrogel induced extensive radial blood vessel growth around the gel. Within the gel area, pVEGF promoted the formation of immature vessels. In contrast, only mature vessels, which existed in the CAM before placing the gel, were found in the control gels.

Conclusions: Gene transfer in & from hydrogels were quantitatively studied for the first time. Factors affecting the transfer came from cells, hydrogel scaffolds and the gene delivery nanoparticles. An effective gene delivery in & from hydrogels require a optimized combination of all these factor