In Vitro Studies of Silica Xerogels for Controlled, Sustained Gene Delivery

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Statement of Purpose: Recent scientific breakthroughs in the genomics field have advanced gene therapy as a method with great potential to prevent or treat inherited or acquired diseases [1]. The non-viral delivery is a safe method but only results in transient expression, limiting its application for gene therapy. To treat chronic or prolonged diseases, a long-term gene expression is critical. Currently, only few effective solutions exist. One could conceivably expose plasmids to target tissues over extended durations [2]. Thus, a prolonged, localized gene release system with a well controlled release profile can improve the efficacy of gene therapy. Herein we pursue this approach by incorporating and releasing DNA from a delivery vehicle. The delivery vehicle that we selected in this study is a highly biocompatible oxide material synthesized using a sol-gel processing methodology. Previously, we have demonstrated that a wide variety of therapeutic molecules can be released from silica xerogel [3, 4]. Given that the sizes of DNA (50-2000bp) or DNA/nanocarrier complexes (~200nm) are well above the pore size of silica sol-gels, we decide to pursue the degradational mechanism to achieve release. In this study, silica xerogels with different degradation behavior were synthesized for controlled gene delivery.

Methods: Silica xerogels were prepared by partially substituting the precursor tetraethoxysilane (TEOS) with other silanes. Four different precursors were studied; 3-aminopropyltriethoxysilane (APTES), Methyl(triethoxy)silane (METES), Ethyl(triethoxy)silane (ETES), and triethoxysilylpropyl succinic acid (TESPSA). The percentage of the substituting precursor was varied in order to adjust the degradation behaviors of resulting xerogels. DNA or DNA/Chitosan nanoparticles were added to the hydrolyzed sol. Then the solution was poured onto petri dishes and allowed to dry completely overnight in a 37°C oven. The resulting xerogel was crushed and sieved to produce granules with a size 40-100 μm.

DNA/chitosan nanoparticles were synthesized as described previously [5]. Briefly, the chitosan solution (0.02% in sodium acetate buffer, pH 5.5) was mixed with DNA solution. Both solutions were preheated at 55°C before mixing. The size and zeta potential of the DNA/chitosan nanoparticles was measured using a Zetasizer (Malvern Instruments). In addition, gel electrophoresis was used to check whether the DNA was condensed by chitosan.

DNA or DNA/chitosan nanoparticle release was studied by immersing the silica xerogel granules in PBS. 75mg xerogel granules loaded with DNA or DNA/chitosan nanoparticles were immersed in 15ml PBS solution. The degradation and release experiment was performed in a 37°C incubator. Control groups included silica xerogel granules without DNA or DNA/chitosan nanoparticles. The PBS was collected every 3 days for 3 weeks and the amounts of released DNA/chitosan nanoparticles in the PBS were measured by UV-visible spectrophotometry at a wavelength of 260 nm. The samples were dried completely for 24 hours in 37°C oven and subsequently weighted.

Results: The METES and ETES substituted sol-gels did not degrade sufficiently over 3 weeks. On the other hand, the TESPSA and APTES substituted sol-gels displayed the adjustable degradation behavior by simply varying their relative content. The TESPSA substituted sol-gels were chosen over the APTES substituted sol-gels by virtue of harsh synthesis conditions with APTES.

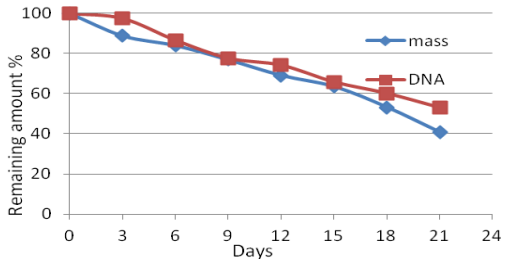

Figure 1 DNA/chitosan release profile of sol-gel containing 18% TESPSA

Figure 1 shows that sol-gel degradation and DNA/chitosan release were synchronized. The mass degraded 59.3% in 21 days, and released 47.2% of total DNA loaded in the sol-gel system. At higher TESPSA content (Fig. 2), both degradation and DNA release rate were accelerated.

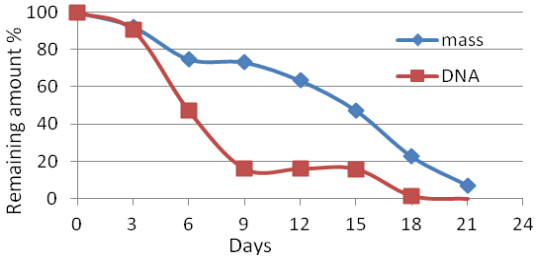

Figure 2 DNA/chitosan release profile of sol-gel containing 30% TESPSA

Conclusions: An array of substituted sol-gels was synthesized and tested for prolonged delivery of DNA and DNA/chitosan nanoparticles for gene therapy. The degradation kinetics of TESPSA substituted sol-gel can be easily adjusted. Both the sol-gel degradation rate and DNA release rate increased with higher TESPSA content.

References:

[1] Somia N, Verma IM, Nature Reviews Genetics. 2000;1: 91-99.[2] Jang H, Rives CB. *et. al*. Mol Ther 2005;**12**:475-483.[3] Santos EM, Radin S, *et al.* Biomaterials 1999;20:1695 -1700.[4] Costache MC, Qu, H, *et al.* Biomaterials 2010;*31:* 6336-6343.[5] Mao HQ, Rao K, *et. al.* Journal of controlled release. 2001;70:399-421.