Incorporation of Osteogenic Protein-1 Plasmid into Chitosan Nanoparticles for Non-Viral Gene Transfer to Chondrocytes

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Statement of Purpose: The objective of the present study was to prepare chitosan nanoparticles incorporating the plasmid DNA encoding for osteogenic protein (OP)-1 for the transfection of adult articular chondrocytes. Most of the prior non-viral strategies directed toward the transfection of chondrocytes for cartilage repair have employed lipid or liposome-based methods. The reason for selecting OP-1 for this investigation is that several studies have demonstrated the favorable effects of OP-1 on chondrogenesis in vitro [1,2] and on cartilage repair *in vivo* [3,4]. While there have been several reports of OP-1 gene transfer to cells using viral vectors, there have been few reports of non-viral tranfection of cells with OP-1 plasmid DNA, and no studies investigating the use of nanoparticles as delivery vehicles for the gene. That the OP-1 plasmid that has been used is relatively large (~9-10 kb), suggested that it may represent a special challenge to incorporate into nanoparticles.

Methods: Chitosan working solutions with different concentrations were made from the stock solution (0.2%, w/v) by dilution with 5 mM acetate buffer (pH 5.5), and sterile filtered. The plasmid OP-1 (pW24, Cell & Molecular Technologies, Inc, Phillipsburg, NJ) working solution (200 μg/ml) was prepared with filtered 5 mM sodium sulfate. A complex coacervation method was used to make chitosan nanoparticles complexing the pOP-1. Increasing amounts of chitosan were mixed with a single quantity of pOP-1 (10ug), to yield the following weight ratios of chitosan to pOP-1: 0.5:1; 1:1; 2:1; 5:1; 10:1; 20:1; and 40:1. FITC was added to the nanoparticles along with the OP-1 plasmid. The plasmid encoding for enhanced green fluorescence protein (EGFP) was used as a reporter gene to transfect chondrocytes. Nanoparticles, each containing plasmids for both EGFP and OP-1, were prepared with the chitosan:total plasmid weight ratio of 10:1. The pEGFP:pOP-1 ratio was 7:3. The structural integrity of pOP-1 released from the nanoparticles was investigated by electrophoresis. Environmental SEM was used to evaluate the size and shape of the nanoparticles. The particle size distribution was determined by the dynamic light scattering technique, and the charge by zeta potential.
The nanoparticles were added to cultures of first passage adult canine chondrocytes. Nanoparticle uptake by the chondrocytes was examined by fluorescence microscopy and confocal laser scanning microscopy.

Results/Discussion: The positive charge of chitosan acted to condense the relatively large negatively-charged OP-1 plasmid such that it could be incorporated into nanoparticles. Incorporation of the plasmid into the chitosan nanoparticles did not affect the structural integrity of the plasmid as demonstrated by gel electrophoresis. The morphology and size of the nanoparticles were found to vary with the chitosan:plasmid weight ratio (Table 1). Nanoparticles formulated with a chitosan:plasmid ratio of 10:1 were of uniformly small size (less than 250 nm) and spherical shape. These nanoparticles had a positive charge of about 20 mV. FITC-labeled chitosan nanoparticles were found in virtually all of the cells after 24 hours of incubation with the nanoparticles, and confocal microscopy revealed FITC-related fluorescence in the nucleus of the chondrocytes. Transfection of the chondrocytes was demonstrated by the fluorescence of cells treated with chitosan nanoparticles containing the plasmid of EGFP along with the plasmid for OP-1.

Table 1
Light Scattering and Zeta Potential Results for Nanoparticles with Chitosan:Plasmid Ratios of 5:1, 10:1, and 20:1.

	5:1	10:1	20:1
Light Scattering			
Average diam., nm	546	240	325
Range, nm	114-2553	25-613	18-562
Zeta potential (mv)*	22.9±2.3	21.4±1.7	24.4±1.0

* The zeta potential of the original chitosan solution (pH 5.5) was 45.5 ± 1.1 mv. Mean ± std. error of the mean.

Conclusions: Chitosan nanoparticles incorporating plasmid OP-1 can be prepared with a range of diameters and morphologies by adjusting the chitosan:plasmid ratio. The OP-1 plasmid maintains its structural integrity after incorporation into the chitosan. A chitosan:plasmid weight ratio of 10:1 yields non-aggregating spherical nanoparticles of uniform size less than 250 nm. These particles can gain entry into adult articular chondrocytes and result in expression of the plasmid carried by the nanoparticles, even though the transfection efficacy is low.

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