3-DIMENSIONAL POLYMER-DNA-CALCIUM PHOSPHATE MATRICES FOR NON-VIRAL GENE TRANSFECTION

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INTRODUCTION

Peptide encapsulated within a biodegradable, polymeric matrix or adsorbed to a polymeric surface has been demonstrated to establish a localized system capable of sustained protein delivery [1,2]; however, polymer processing techniques and *in vivo* implantation have been shown to induce protein unfolding and subsequent inactivity [3]. An alternative strategy to peptide delivery is the delivery of a gene encoding for the factor. Calcium phosphate (Ca-P) mediated gene transfer is an efficient non-viral gene delivery strategy [4]. In this study, we hypothesized DNA/Ca-P co-precipitates could be combined with a 3-dimensional (3D) biodegradable polymeric matrix of poly(lactide-*co*-glycolide) (PLAGA) to serve as an efficient non-viral gene delivery system. Lambda DNA, which serves as a model DNA, was incorporated into a Ca-P co-precipitate and adsorbed to the surface of a 3D-[50:50] PLAGA (Mw = 63k) scaffold. The release profile demonstrated an initial burst release over the first 48 hours. Cellular transfection by the gene delivery matrix was completed using the osteoblast-like human osteosarcoma cell line, SaOS-2. Cells seeded on the 3D-PLAGA matrix with adsorbed DNA/Ca-P incorporated via endocytosis the DNA within 48 hours as determined by polymerase chain reaction (PCR) analysis. To develop a PLAGA/DNA/Ca-P gene delivery system with a sustained release profile, DNA/Ca-P co-precipitates were incorporated in PLAGA microspheres of [50:50], [75:25] (Mw = 41k), and [80:22] (Mw = 350k) and sintered to form a 3D structure. The release kinetic profile demonstrated these matrices have a significantly reduced initial burst release as compared to the scaffold with adsorbed DNA/Ca-P.

MATERIALS AND METHODS

SCAFFOLD FABRICATION - A modification of the calcium phosphate coprecipitation technique described by Schenborn, et al. [4] was used to fabricate matrices capable of gene delivery. Polymeric matrices with DNA/Ca-P adsorbed to the surface were formed by fabricating microspheres using a solvent evaporation technique and 1% poly(vinyl alcohol) as the emulsifying solution [5]. Microspheres, 425-600 μm in diameter, were placed into a mold and sintered to form a porous, 3D, matrix. [50:50] PLAGA scaffolds with adsorbed DNA were formed by pipetting the DNA (10μg)/Ca-P co-precipitates onto the matrices, placing the matrices at -20ºC for 24 hours, and vacuum drying for 24 hours. Matrices with DNA (10μg)/Ca-P incorporated into the microspheres were fabricated by pipetting the co-precipitates into the dissolved polymer and vortexing the mixture for 15 min before pouring into the emulsifying solution.

RELEASE STUDIES - Matrices were individually placed in vials containing PBS + 1% antibiotics (pH = 7.4) and incubated in a shaker bath at 37ºC. At the appropriate time point, the PBS solution was collected for pH measurement and a colorimetric assay for calcium (Sigma, Kit #578M) was used to quantify precipitate release from the matrices.

CELLULAR TRANSFECTION - 3D-PLAGA/Ca-P matrices containing 10μg of lambda DNA were plated with SaOS-2 cells at a seeding density of 200,000 cells/scaffold. Cells were cultured on the matrices in M-199 supplemented with 10% FBS and 1% P/S for 48 hours and cell lysates collected. Cellular uptake of lambda DNA was confirmed using PCR (Gibco Life Technologies, Kit #11904-018). The sense and antisense primers for lambda amplification are 5'GATGAGTTCGTGTCCGTACACTGG3' and 5'GGTTATCGAAA TCAGCCACAGCG CC3', respectively.

STATISTICAL ANALYSIS - A power analysis assuming a 2% standard deviation, α=0.05, 2 sides, and a power of 0.90 indicated an effect size of 6 matrices per group for the release study. Data is presented as mean $\pm$ standard deviation.

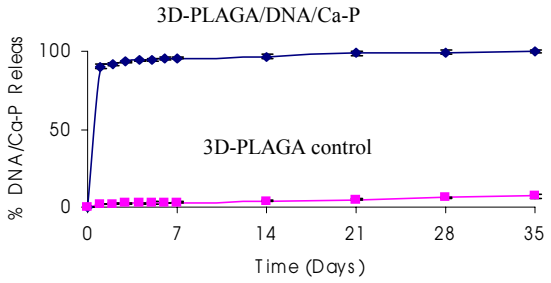
RESULTS AND DISCUSSION

In these studies, DNA was encapsulated in the Ca-P co-precipitates for adsorption to the polymeric scaffolds or incorporation into the polymeric microspheres. Release studies performed using DNA/Ca-P co-precipitates adsorbed to 3D-PLAGA [50:50] matrices

demonstrated an initial burst release, over the first 48 hours (93% release), followed by a slower release over 35 days (Graph A).

To determine the ability of the released DNA/Ca-P co-precipitates to transfect cells cultured on the gene containing matrices, SaOS-2 cells were plated on 3D-PLAGA matrices with adsorbed DNA/Ca-P, cultured for 48 hours, collected, and examined for lambda DNA uptake. PCR analysis verified cellular transfection and the presence of a single band confirmed maintenance of DNA structural integrity (Figure 1).

To overcome the rapid initial burst release observed by DNA/Ca-P co-precipitates adsorbed to the 3D-PLAGA matrix surface, an alternative method, which incorporated the DNA/Ca-P co-precipitates into the polymeric microspheres, was developed using PLAGA microspheres of various co-polymer ratios. The release kinetics of these matrices demonstrated a sustained release over 35 days (Graph B). The [50:50] and [75:25] PLAGA/DNA/Ca-P scaffolds demonstrated a reduced initial burst release, as compared to scaffolds with adsorbed DNA/Ca-P, while the [80:20] PLAGA/DNA/Ca-P scaffold had a minimized initial release. The [50:50] and [75:25] PLAGA/DNA/Ca-P matrices demonstrated a steady release until the onset of rapid polymer degradation, indicated by the rapid drop in pH at day 21 (Graph C and D), which subsequently accelerated DNA/CA-P release. A drop in pH accompanied by an accelerated DNA/Ca-P release was not observed by the matrices fabricated with the higher molecular weight polymer, [80:20] PLAGA (not shown).



Graph A: The release profile of DNA/Ca-P co-precipitates adsorbed to 3D-PLAGA scaffolds in PBS, at 37ºC. Note the initial burst release over the first 48 hrs. Scaffolds with no DNA/Ca-P served as controls (n=6).

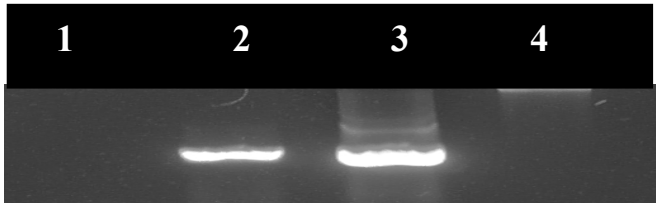
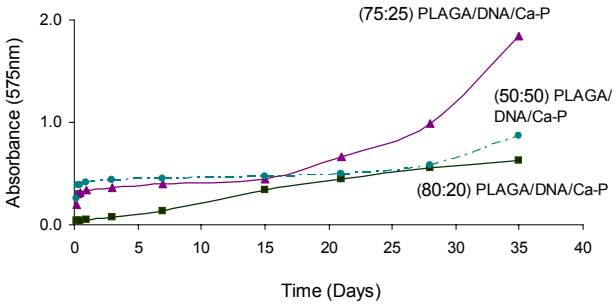
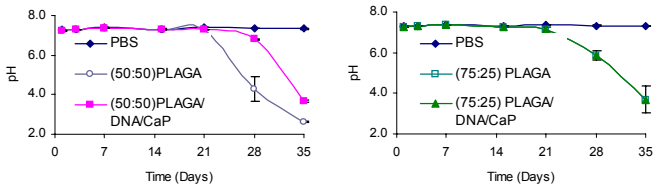


Figure 1: PCR analysis for cellular transfection following lambda DNA release from a DNA/Ca-P precipitates adsorbed to the 3D-PLAGA matrix. Lane 1: Negative control for PCR, Lane 2: SaOS-2 cells transduced with lambda DNA released from a 3D-PLAGA/DNA/Ca-P matrix, Lane 3: Lambda DNA (positive control) Lane 4: Non-transduced SaOS-2 cells (negative control)



Graph B: Release profile of DNA/Ca-P co-precipitates incorporated in 3D-PLAGA scaffolds of various co-polymer ratios and molecular weights (n=6). Note the rapid initial burst release by DNA/Ca-P co-precipitates adsorbed to the polymer surface (Graph A) is absent.



Graph C and D: pH of the PBS degradation solution following incubation with 3D- [50:50] and [75:25] PLAGA/DNA/Ca-P scaffolds. Note the rapid drop in pH of the matrices at day 21 corresponds to the rapid release of DNA/Ca-P observed in the release profile (Graph B).

CONCLUSIONS

As the use of naked plasmid DNA delivery is impaired by its low cellular transfection efficiency and susceptibility to nuclease degradation, we present here the first studies to develop a novel, non-viral gene delivery system by combining DNA containing calcium phosphate co-precipitates with a biodegradable, polymeric matrix. Release studies of the adsorbed DNA/Ca-P co-precipitates demonstrate a rapid initial burst release, while DNA/Ca-P co-precipitates incorporated within polymeric microsphere matrices demonstrate a controlled, sustained release. PCR analysis verified the ability of the released DNA containing co-precipitates to transfect SaOS-2 cells cultured on the scaffold. Under controlled conditions, we have found these matrices suitable for non-viral gene therapy strategies. Future studies will examine the transfection efficiency of the DNA/Ca-P co-precipitates incorporated within the 3D-PLAGA microsphere matrix.

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