DNA DELIVERY INTO BONE TUMOR CELLS USING HYDROGEL ENCAPSULATION

INTRODUCTION:
Nonviral delivery vectors are attractive options for gene therapy approaches in tissue engineering. However they suffer from low transfection efficiency and short-term gene expression. Localized and sustained DNA delivery from polymers has the potential to provide long term gene therapy. The DNA encapsulation in the polymer protects it from nuclease degradation prior to its release from the matrix. Poly(lactic-co-glycolic acid) (PLGA) is one of the most common polymers for DNA delivery. However, low encapsulation efficiency and damage to DNA due to acidic matrix degradation products limit its application for DNA delivery. Natural hydrogels such as chitosan, alginate and collagen are alternatives to PLGA-based delivery systems. They have been successfully used for transfection of the cells in vitro and in vivo. DNA release from natural hydrogels occurs by enzymatic degradation or ionic exchange, which makes it difficult to control the DNA release kinetic. Alternatively, synthetic polymers allow greater control of the microspheres and scaffold properties and their degradation rates. Photocrosslinkable synthetic hydrogels are receiving increased attention for cell and protein delivery vehicles in tissue engineering due to the excellent spatial and temporal control over their crosslinking.

Recently, Nakayama et al reported the release of adenovirus-associated DNA from vascular stents coated with photocrosslinkable hydrogels. Other investigators demonstrated that photopolymerization was compatible with DNA encapsulation and through the use of protection agents the damaging effect of photoinitiated radicals could be reduced. We previously reported that oligo(polyethylene glycol) fumarate (OPF) could be crosslinked using long wavelength UV light and both the mechanical properties and swelling ratio of the hydrogels could be controlled by changes in the crosslinking levels. We also demonstrated that these hydrogels are biodegradable and that high viability was observed for chondrocytes encapsulated into the hydrogels. In the present study, we employed photocrosslinkable OPF hydrogels as a matrix for DNA delivery. We hypothesized that DNA could be released in a sustained manner as a consequence of the hydrogel’s degradation. We also suggested simultaneous entrapment of DNA and bone tumor cells to test the hypothesis that DNA, after undergoing the process of transfection of cells co-encapsulated with it.

MATERIALS AND METHODS:
Materials: DNA plasmid encoding green fluorescence protein (GFP) (Clontech, Palo Alto, CA) was amplified to sufficient quantities using plasmid Giga Kit (Quigen, Valencia, CA). OPF with weight average molecular weight of 16246±3710 was synthesized using PEG using plasmid Giga Kit (Quigen, Valencia, CA). OPF with weight average molecular weight of 10kDa according to a previously published method.

DNA encapsulation: 330 mg of OPF was dissolved in 1 ml PBS, and 500µg plasmid DNA was added to the macromer solution and frozen overnight at −80°C. The frozen DNA/macromer solutions were then lyophilized for 24 h. The freeze-dried DNA/macromer solutions were rehydrated to a concentration of 33% with PBS containing 7 µl N-vinyl pyrrolidinone (NVP) and 0.05% (w/w) Irgacure 2959 (Ciba-Specialty Chemicals). The macromer/DNA mixture was pipetted between glass slides with a 1 mm spacer and polymerized using UV light (365 nm) at an intensity of ~8mW/cm² for 10 min.

DNA release: Polymerized gels were cut into 7 mm diameter disks, and placed in 2.5 ml PBS at 37°C on an orbital shaker. Buffer was collected every 3-4 days, and DNA concentration was determined by the PicoGreen assay according to the manufacturer’s guideline. Parallel gels without DNA were used to eliminate the effect of hydrogel degradation products on the PicoGreen assay. Data present mean ±standard deviation, n=3. An unpaired Student t-test was used for statistical analysis of data at the 0.05 significance level.

Coencapsulation of cells with DNA: DNA was first complexed with Lipofectamine (Invitrogen, San Diego, CA) as transfection agent at 3 µl Lipofectamine/µg DNA for 20 min. One ml of 33% OPF macromer was added to the DNA/Lipofectamine solution and frozen overnight at −80°C, followed by lyophilizing for 24 h. The freeze-dried DNA/macromer solutions were rehydrated with PBS containing NVP and initiator to the volume of 1 ml as described above and mixed with a pellet of 25×10⁶ MG-63 cells (human osteosarcoma cell line, ATCC). This cell mixture was transferred to a glass mold with spacer and polymerized using 365 nm light at the intensity of ~8mW/cm² for 10 min. The resulting hydrogel-cell constructs (7mm in diameter and 1 mm in thickness) were placed into the 12-well plates with 2.5 of Dulbecco’s Modified Eagle’s Medium (DMEM) (Gibco BRL, Rockville, NY, USA) and Ham’s F12 medium (Nissui Pharmaceutical, Japan), supplemented with 10% Fetal Bovine Serum (Invitrogen), 2mM L-glutamine and 1.5 g/L sodium bicarbonate and incubated in a humid environment with 5% CO₂. The constructs were harvested after 1, 3, 7, 14 and 21 days and transfection of the encapsulated cells with entrapped plasmid encoding GFP was assessed by confocal laser microscopy using a FITC filter (excitation 480 nm, emission 535 nm). Viability of the encapsulated cells without DNA was determined using Live/Dead Kit (Molecular Probes, L3224).

RESULTS

Figure 1 shows DNA release profile for degrading OPF hydrogel over 21 days in vitro. An initial burst release of 57% was observed at day 3 followed by a sustained release over 21 days.

FIGURE 1: DNA release from photocrosslinkable OPF hydrogel.

DNA release profile for degrading OPF hydrogel are shown in Figure 1. The initial burst release of 57% was observed at day 3 followed by a sustained release over 21 days. The constructs were harvested after 1, 3, 7, 14 and 21 days and transfection of the encapsulated cells with entrapped plasmid encoding GFP was assessed by confocal laser microscopy using a FITC filter (excitation 480 nm, emission 535 nm). Viability of the encapsulated cells without DNA was determined using Live/Dead Kit (Molecular Probes, L3224). Figure 1 shows DNA release profile for degrading OPF hydrogel.

FIGURE 2: Viability and transfection of MG-63 cells by coencapsulated plasmid DNA encoding GFP after 21 days. The encapsulated cells expressed increasing GFP as the gel degraded and the encapsulated Lipofectamine-complex DNA was delivered to the bone tumor cells. These results show that the encapsulated DNA maintained its biologic activity and was capable of transfecting the bone tumor cells.

Cell Viability

Without DNA

With DNA

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References