Mesoporous PLGA-Silicon Microparticles for the Treatment of Orthopedic Degenerative Disc Disease

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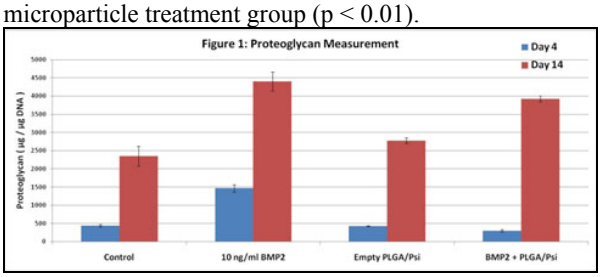
Purpose: Low back pain caused by degenerative disc disease remains a significant cause of morbidity and mortality. Polymers such as poly(lactic-*co*-glycolic acid) (PLGA) have been investigated as alternatives compared to conventional approaches. We describe a novel drug delivery system utilizing PLGA-coated mesoporous silica microparticles for the delivery of rhBMP2 to bovine nucleus pulposus (NP) cells for the treatment of degenerative disc disease.

Methods: ***Particle synthesis:*** Mesoporous silicon microparticles were fabricated with a mean diameter of 3.2±0.2 µm, pore diameters 6.0±2.1 nm. Heavily doped p++ type (100) silicon wafers were used as the base source and by deposition of a 200 nm layer of silicon nitride followed by standard photolithography was used to pattern the surface using an EVG 620 contact aligner. A current density of 320 mA/cm^2 was applied. Particle surface was further oxidized using H_2O_2. The suspension was heated to 100-110˚C, and resuspended in isopropyl alcohol. PLGA-coating was prepared by a modified solid-in-oil-in-water (S/O/W) emulsion method. rhBMP2-loaded particles were suspended in 10% PLGA solution, and the organic phase was mixed with 2.5% w/v PVA. ***Cell culture:*** NP cells were cultured in complete media at 37˚C, 5% CO_2. Encapsulation of NP cells into alginate beads was accomplished by the addition of cells into a 1.2% alginate solution. Beads were cultured in chondrogenic media and were allowed to equilibrate for 72 hrs prior to rhBMP2 treatment. Cells were treated with 10 ng/ml rhBMP2, 1x10^6 empty PLGA/Psi microparticles, chondrogenic media, or 1x10^6 BMP2-loaded PLGA/Psi microparticles. After 14 days, beads were collected and dissolved. Alginate-cultured NP cells were recovered on 24-well plates, and lysed for proteoglycan analysis. MTT assay was used to assess cellular viability and proliferation. Proteoglycan expression was measured using the dimethylmethylene blue (DMMB) assay. Bovine trachea provided with the kit was used as a standard. DNA picogreen assay was used to normalize values. ***Staining and image analysis:*** Alcian blue and Safranin-O staining were used to stain for expression of the cartilage phenotype. Images were taken with an inverted Zeiss Axio microscope. Histogram analysis was performed to quantitate staining differences. Data are expressed as mean ± SD. P-values < 0.05 were determined to be statistically significant.

Results: ***Proliferation/cytotoxicity:*** No significant differences in proliferation or cytotoxicity were observed after 3 days. ***Proteoglycan production (Figure 1):*** The greatest amount of proteoglycan was measured in cells treated with 10 ng/ml of BMP2. However, after 14 days the greatest change in proteoglycan was measured in cells treated with BMP2-loaded PLGA/Psi microparticles (12-fold increase, p = 0.005). This effect was significant compared to untreated cells and the unloaded microparticle treatment group (p < 0.01).



Cartilage staining/image analysis: Significantly greater collagen staining was observed in cells treated after 10 days. Image analysis demonstrated that cells treated with BMP2-loaded PLGA/Psi showed the greatest increase in cartilage staining (37.54 ± 12.09%, p < 0.001), followed by cells treated with BMP2 alone (27.66 ± 7.37%, p < 0.001). Cells treated with empty PLGA/Psi showed the lowest staining intensity (14.79 ± 6.64%, p < 0.002). Comparison of phenotypic expression of cartilage in the BMP2-loaded microparticle group with controls were statistically significant (p < 0.05).

Conclusions: We demonstrate that PLGA can be used to coat the surface of mesoporous silicon microparticles and control the rate of release of rhBMP2. The particles did not have any proliferative or cytotoxic effects in bovine NP cells after 72 hours. The greatest change in proteoglycan was measured in cells treated with BMP2-loaded PLGA/Psi microparticles after 14 days. Additionally, the greatest change in cartilage staining intensity was measured after 10 days. These results demonstrate a novel approach using PLGA-coated porous silicon microparticles capable of delivering biotherapeutic molecules for the long-term, controlled, non-invasive treatment for degenerative disc disease.

References: 1. Tasciotti, E., Liu, X., Bhavane, R., Plant, K., Leonard, A.D., Price, B.K., Cheng, M.M., Decuzzi, P., Tour, J.M., Robertson, F., Ferrari, M. *"Mesoporous Silicon Particles as a Multistage Delivery System for Imaging and Therapeutic Applications"*. **Nature Nanotechnology** 3, 151-157(2008). 2. Chiappini, C., Tasciotti, E., Fakhoury, J.R., Fine, D., Pullan, L., Wang, Y., Fu, L., Liu, X., Ferrari, M. *"Tailored Porous Silicon Microparticles: Fabrication and Properties"*. **Chem Phys Chem** 11 (5), 1029-1035 (2010).