Magnesium Oxide Nanocomposites for Improving Orthopedic Tissue Regeneration

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Statement of Purpose: Where ligaments meet bone, there exists a transitional region of heterogeneous tissue that is graded from hard, highly mineralized fibrocartilage at the bone interface to un-mineralized soft tissue at the ligament. This structure, called the enthesis, disperses stress concentrations that arise due to the vastly different mechanical properties of bone and ligaments. However, in the event that a ligament is injured and requires surgery, the tendon-to-bone insertion site (TBI) must be destroyed and is not easily restored due to avascularity in the region and because of slow and disorganized healing within heterogeneous tissues. It is believed that this loss of enthesis functionality following joint reconstructive surgery is a leading cause of high failure rates for such surgeries (5-25% failure rates for ACL surgery) [1]. Therefore, there is considerable interest in the development of a nanostructured biomaterial that is capable of regenerating the TBI.

In this study, magnesium oxide (MgO) nanoparticles were used to mineralize poly(l-lactic acid) (PLLA) and tested for their ability to improve the attachment and growth of TBI-related orthopedic tissue. Magnesium is an essential mineral in bone which is thought to regulate the size and density of hydroxyapatite (HA) crystals, and further, Weng and Webster demonstrated that nano-rough MgO increased bone cell density three-fold compared to bulk MgO [2]. Presently, the ability of these materials to promote tissue growth at the TBI was characterized via cell adhesion and proliferation experiments with fibroblasts and osteoblasts. Materials were also tested for their mechanical properties, and further characterization was performed using SEM, TEM, XRD, FTIR, EDS, and contact angle tests.

Methods: Hydroxyapatite (HA) nanoparticles were synthesized following the method reported by Zhang *et al.* [3]. Poly (l-lactic acid) (PLLA) (MW=50,000), (Polysciences, Warrington, PA) was dissolved in 10 mL of chloroform to reach 3 wt% PLLA in chloroform. HA nanoparticles and MgO nanoparticles (particle diameters of 20 nm, US Research Nanomaterials Inc., Houston, TX) were added to separate vials in concentrations ranging from 5-20 wt% in PLLA. Samples were heated to 55 °C and sonicated for 1 hour, then poured into 60 mm diameter pyrex petri dishes (Sigma Aldrich, St. Louis, MO). Samples were heated at 60 °C for 1.5 hours to evaporate excess chloroform, and then were cut into strips for further study.

Samples were cut into 1 cm x 3 cm rectangular strips for tensile testing with a uniaxial tensile tester equipped with a 10-lb. load cell and material analysis software (ADMET, Norwood, MA). This arrangement was used to obtain the elastic modulus, material elongation, and maximum load endured for each sample. Cell adhesion and proliferation tests were performed by seeding 3500 cells/cm^2 of fibroblasts and osteoblasts (American Type Culture Collection, Manassas, VA) onto 1-cm^2 samples and culturing for 4, 24, 72, and 120 hours under standard conditions. Cell numbers were quantified using MTS assays (Promega, Madison, WI). Experiments were conducted in quadruplet and repeated three times. Data was analyzed using Student's t-tests.

Results: Results indicated for the first time that MgO nanoparticles in plain PLLA or PLLA/HA composites significantly increased osteoblast and fibroblast adhesion on PLLA (Figure 1). SEM images showed considerable differences in nanoscale surface topography between samples. Mechanical tensile testing revealed that the addition of HA nanoparticles to plain PLLA hardened the polymer, reducing the material elongation and increasing its elastic modulus. Moreover, the observed changes in the mechanical strength of PLLA seemed to be dictated by the size and shape of its secondary nano-phase.

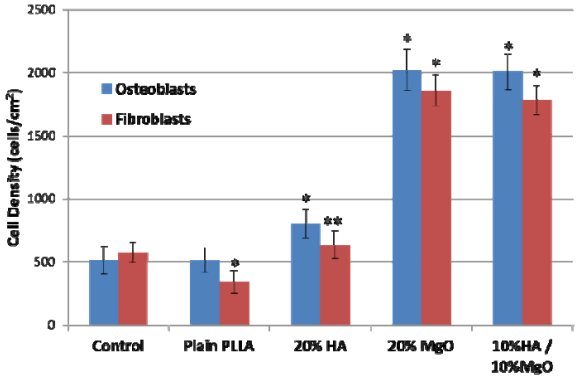

Figure 1. Adhesion of fibroblasts and osteoblasts on pure PLLA and PLLA mineralized with nanoparticles (20% HA, 20% MgO, and 10% HA/10% MgO). Control is empty cell culture well. Data represents mean ± SEM. N=3. % = weight%. *P<0.005, **P<0.05 compared to controls.

Conclusions: Here, MgO nanoparticles have been shown for the first time to increase osteoblast and fibroblast cell adhesion on PLLA, and should therefore be further investigated as a material to promote bone tissue growth at one end of the tendon-to-bone insertion site, and fibrous tissue growth at the other end of the TBI. Moreover, the addition of MgO nanoparticles allowed for the tailorability of PLLA mechanical properties for bone or ligament tissue.

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References:

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