

# MgO Nanoparticles Enhance Cell Functions and Reduce the Adhesion of *Staphylococcus aureus* on Hydroxyapatite-PLLA Nanocomposites for Orthopedic Tissue Engineering Applications

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**Statement of Purpose:** Regeneration of complex orthopedic tissues (such as ligament, bone, and the tendon-to-bone insertion site) is problematic due to a lack of suitable biomaterials with the appropriate chemical and mechanical properties to elicit the formation of tissues with similar structure, organization, and functionality to natural tissues. Additionally, a non-trivial fraction of implanted biomaterials acquire bacterial infections, which can lead to implant failure, secondary surgeries, and the spread of infection to other tissues throughout the body. To address these issues, the current study investigated magnesium oxide (MgO) nanoparticles as novel materials to improve orthopedic tissue regeneration and reduce bacterial infection.

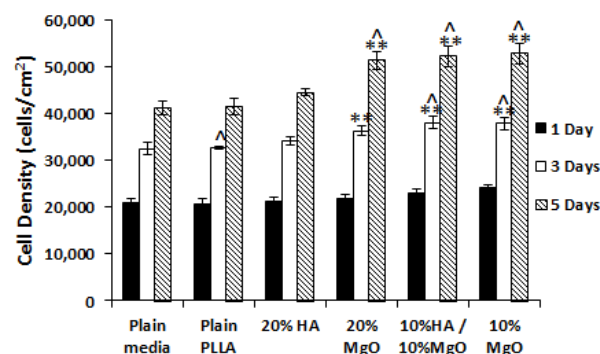
**Methods:** Hydroxyapatite (HA) nanoparticles were synthesized following the method reported by Zhang et al. [1]. MgO nanoparticles (particle diameters of 20 nm, US Research Nanomaterials Inc., Houston, TX) and the synthesized HA nanoparticles were combined with poly (l-lactic acid) (PLLA) (MW=150,000, Polysciences, Warrington, PA) and dissolved in chloroform with sonication. Polymer solutions were cast to glass petri dishes and heated to evaporate excess solvent. The resulting polymer sheets 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 50,000 cells/cm<sup>2</sup> of fibroblasts and osteoblasts (American Type Culture Collection, Manassas, VA) onto 1-cm<sup>2</sup> samples and culturing for 4, 24, 72, and 120 hours under standard conditions. Cell numbers were quantified using MTS assays (Promega, Madison, WI).

Nanocomposite antibacterial efficacy was assessed by seeding approximately 10<sup>7</sup> cells of *Staphylococcus aureus* (ATCC 12600) onto 1-cm<sup>2</sup> nanocomposites and culturing for 24 and 48 hours under standard culture conditions. Samples were then rinsed with PBS and transferred to 15 mL centrifuge tubes to be vortexed, releasing attached bacteria from the samples. Three dilutions of this bacteria-PBS solution were then plated onto agar and counted manually after 12 hours of incubation. Experiments were conducted in quadruplet and repeated three times. Data was analyzed using Student's t-tests.

**Results:** HA nanocomposites exhibited the highest Young's modulus while retaining considerable ductility compared to plain PLLA. The addition of MgO nanoparticles to HA nanocomposites did not significantly alter their mechanical properties. MgO nanocomposites supported enhanced fibroblast adhesion and proliferation,

and MgO nanoparticles dispersed within PLLA in combination with HA nanoparticles showed improved osteoblast adhesion and proliferation compared to plain PLLA and composites containing only HA nanoparticles (Figure 1). Further, the addition of MgO to these scaffolds drastically reduced bacterial viability. These results together indicate the promise of MgO nanoparticles as antibacterial materials for the fabrication of optimized scaffolds for orthopedic tissue engineering.



**Figure 1:** Proliferation of primary human osteoblasts on the indicated nanocomposite samples. Data represents the mean  $\pm$  SEM. \*\* $p < 0.05$  compared to plain media;  $\Delta p < 0.05$  compared to 20% HA.

**Conclusions:** Here, MgO nanocomposites showed excellent bactericidal efficacy in addition to their ability to enhance the adhesion and proliferation of fibroblasts and osteoblasts. Moreover, the addition of MgO nanoparticles allowed for the tailorability of PLLA mechanical properties for bone or ligament tissue. Therefore, MgO nanoparticles should be further investigated as an antibacterial material to promote orthopedic tissue regeneration.

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## References:

1. Zhang L *et al.*, International Journal of Nanomedicine, 3 (2008), 323-34.