

A novel *in vivo* model to screen degradable magnesium alloys for bone fixation devices

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Statement of Purpose: Bone fractures are an extremely common injury, with over six million occurring each year in the U.S. Treatment of these fractures often requires the use of internal fixation devices such as plates and screws to facilitate healing. Traditionally, these devices have been made with permanent metals such as titanium alloys; however these materials can cause numerous long-term complications, and may necessitate a secondary, invasive removal surgery [1-4]. To circumvent these complications, resorbable polymers have been tried; however their mechanical limitations render them inadequate for most load bearing applications. Furthermore foreign body reactions have been reported. For these reasons, there remains a need to develop novel materials for bone fixation.

Unlike permanent metals and resorbable polymers, magnesium (Mg) alloys can provide an ideal balance of degradation and strength. Recent studies have demonstrated their biocompatibility, bone-like mechanical properties, and osteointegration [5-9]. Furthermore, it has been suggested that Mg ion, a degradation product of Mg alloys, may enhance bone regeneration. As numerous Mg alloys are developed, an efficient method to screen their biocompatibility and osteogenic behavior is needed [6-10]. For these reasons, we have developed a novel 3D model to assess degrading Mg alloys and their effect on human stem cells and tissue *in vivo*.

Methods: A 3D scaffoldless model was developed to assess Mg alloys *in vivo*. To do so, human bone marrow stromal cells (hBMSC) were cultured into confluent cell sheets. Cell sheets were gently detached and wrapped around cleaned and sterilized Mg alloys. Two Mg alloys were compared, commercially available 99.9% pure Mg, and custom alloy WXX410 (courtesy of Dr. Kumta, University of Pittsburgh). All constructs were prepared immediately before surgical implantation, and kept sterile until surgery. Constructs were implanted subcutaneously in immunocompromised mice and explanted upon sacrifice four and eight weeks post-operative. Alloy degradation was assessed with micro-computed tomography (microCT). Biological effect of the degrading implant was assessed through histological staining and immunohistochemistry. Mg alloys without human cells were used as controls.

Results: MicroCT analysis showed no significant difference in net volume loss for pure Mg and WXX410 after four and eight weeks *in vivo*. Localized regions of pitting corrosion were observed on pure Mg samples, while dense deposits were observed on WXX410 samples. Hematoxylin and Eosin staining revealed normal tissue organization surrounding the alloys. Human cells were identified at the alloy-tissue interface by

immunohistochemistry with human specific Ku80 antibody. Von Kossa and Alizarin Red stainings showed a thin calcium and phosphate rich layer surrounding all degrading alloys.

Conclusions: In this study, we established and tested a novel model to screen degradable Mg alloys for bone fixation. We compared commercially available pure Mg with custom alloy WXX410, optimized for orthopedic applications. We observed no significant difference in net volume loss for both materials; however observable morphological distinctions were made after four and eight weeks *in vivo*. For pure Mg, we observed pitting corrosion, contributing to a net volume loss over time. Alternatively, for WXX410, we observed dense deposits, likely due to corrosion product accumulation. These discrepancies suggest differences in the materials' degradation behavior despite similar net volumes. Hematoxylin and Eosin staining revealed no adverse effects of the material degradation on surrounding tissue. Furthermore, human cells were observed at the alloy-tissue interface, indicating that they were retained and were not adversely affected by the degrading alloys. Consistent with prior studies, we observed a calcium and phosphate rich precipitate surrounding the degrading alloys, which has previously been hypothesized to contribute to Mg's osteointegration. These results suggest that both pure Mg and WXX410 may be suitable materials for bone fixation devices, however additional studies should be conducted to better understand their long-term degradation and associated mechanical properties.

In conclusion, this model can be used as an efficient method to screen Mg alloys based on corrosion behavior and subsequent biological effect, allowing candidate alloys for craniofacial and orthopedic device applications to be identified.

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