

**Effects of Reamer-Irrigator-Aspirator Wastewater on Bone Regeneration**

*Derek J. Klaus, MD<sup>1</sup>; Douglas Crowder<sup>2</sup>; Ethan Scott, BS<sup>3</sup>; Steve Fening, PhD<sup>4</sup>; Fayez Safadi, PhD<sup>3</sup>; Eric T. Miller, MD<sup>1</sup>;*

*<sup>1</sup>Department of Orthopaedic Surgery, Summa Health System, Akron, Ohio, USA;*

*<sup>2</sup>Department of Biomedical Engineering, University of Akron, Akron, Ohio, USA;*

*<sup>3</sup>Department of Anatomy and Neurobiology, Northeast Ohio Medical University, Rootstown, Ohio, USA;*

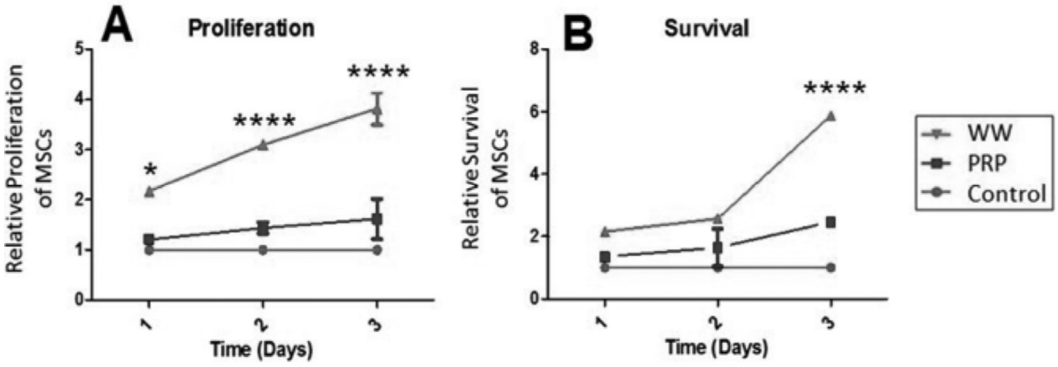
*<sup>4</sup>Austen BioInnovation Institute, Akron, Ohio, USA*

**Background/Purpose:** The reamer-irrigator-aspirator (RIA) device is capable of obtaining large quantities of autologous bone graft with significantly less donor site morbidity compared to iliac crest bone graft. The reamed femoral contents are aspirated and passed through a filter to separate the desired bone graft from the remaining wastewater (WW). The first aim of this study was to describe a method to concentrate osteogenic growth factors and viable mesenchymal stem cells (MSCs) from RIA WW. The second aim was to examine the effects of WW-derived growth factors on human MSCs in vitro as well as in a critical size defect (CSD) mouse calvarium model in vivo.

**Methods:** Twelve male patients scheduled for femoral RIA bone grafting procedures were enrolled. RIA WW and 50 cc of peripheral blood were collected. Peripheral blood was centrifuged to obtain platelet rich plasma (PRP). MSCs were extracted from the WW and the remaining aspirate was concentrated. MSCs were incubated in the presence of PRP or concentrated WW to assess cell proliferation, survival, and mineralization in vitro. 5-mm CSDs were made in the calvaria of immunodeficient mice and packed with a collagen sponge alone or a collagen sponge soak-loaded with PRP or WW. Four weeks post-surgery, the calvaria were harvested and examined using micro-CT to determine percent bone ingrowth.

**Results:** MSCs extracted from RIA WW remain viable after processing and retain multipotency. Concentrated WW yields comparable concentrations of osteogenic growth factors when compared to PRP. Concentrated WW significantly improved MSC proliferation by 4 times and survival by 3 times when compared to MSCs treated with PRP in vitro (Figure 1). MSCs treated with WW showed a 500-fold increase in mineralization after 2 weeks when compared to PRP. Significantly higher rates of bone ingrowth were observed in CSDs treated with WW (26%) compared to PRP (20%),  $P < 0.01$ .

**Conclusion:** When compared to PRP, concentrated WW was shown to (1) accelerate MSC proliferation, survival, and mineralization in vitro by 4, 3, and 500-fold, respectively, and (2) accelerate osteogenesis in a mouse calvarium CSD model in vivo.



**Figure 1.** MSCs were incubated 1 to 3 days with normal growth media (Proliferation, A) or nutrient deplete media (Survival, B). For both assays, the media was either left untreated (control) or supplemented with WW or PRP. Significance was calculated between WW and PRP-treated groups. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

- The FDA has not cleared this drug and/or medical device for the use described in this presentation (i.e., the drug or medical device is being discussed for an "off label" use). For full information, refer to page 600.