Analysis of Osteoactivin in Reamer-Irrigator-Aspirator (RIA) Wastewater as an Osteogenic Factor for Bone Regeneration

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Disclosures:

Introduction: Non-unions and critical-size bone defects are challenging problems that can be successfully treated with a combination of rigid fixation and autologous bone graft. Historically, harvesting iliac crest bone graft (ICBG) has been the gold standard for massive bone grafting procedures; however, ICBG harvest is limited by the amount of graft available as well as a relatively high complication rate. Recently, the Reamer-Irrigator-Aspirator (RIA) device has become a popular method to obtain large amounts of autologous bone graft, with less donor site morbidity compared to ICBG. The RIA is an intramedullary reaming device capable of retrieving finely morselized corticocancellous bone in excess of 90 cm3 that has osteogenic properties comparable to ICBG. The reamer head is constantly cooled via sterile saline irrigation which decreases thermal osteonecrosis. In addition, continuous suction removes bone debris from the femoral canal and decreases intramedullary pressure, which has been linked to pulmonary fat embolism. The aspirated femoral contents are passed through a filter to collect and separate the desired bone graft from the remaining filtrate or wastewater (WW). Traditionally, the WW is discarded; however, recent studies have demonstrated the presence of growth factors and viable cells within WW. This study is the first to examine the presence of osteogenic factors in the WW and to test their effects on RIA-derived growth factors on mesenchymal stem cell (MSC) differentiation in vitro. We also demonstrate the presence of the osteogenic factor osteoactivin (OA) - a downstream mediator of BMP-2 discovered by our lab - in the WW. Local injection of the recombinant OA protein induces bone formation in vivo. We also developed a method of concentrating these growth factors via filtration, which can be used clinically and has not been described previously. For all studies, platelet rich plasma (PRP) was used as a positive control since it is currently used in myriad medical applications to promote healing. Our hypothesis is that the concentrated WW would stimulate more osteogenesis than PRP in both in vitro and in vivo models.

Methods: All procedures were approved by the IRB of Summa Health Systems and the IACUC of NEOMED. Nine male patients scheduled for femoral RIA bone grafting procedures were enrolled in the study (mean age, 48.2±18.2). Approximately 1L of WW from each patient was transported on ice to the laboratory facilities at NEOMED, as well as approximately 65mL of peripheral blood with sodium citrate as an anticoagulant. To obtain PRP, whole blood was centrifuged, and then the plasma/platelet layer was centrifuged again to concentrate the platelets. WW was centrifuged to pellet cells and then optionally passed through a 3kDa MWCO filter to concentrate. The concentration of OA in the WW and PRP was evaluated using ELISA sandwich assay. In order to test the effects of the WW in vitro, we isolated primary MSCs from the WW. The cell pellet was subjected to density gradient centrifugation using Ficoll-Paque according to the manufacturer’s instructions and then further purified using differential plating. Cells were differentiated into osteoblasts in the presence of ascorbic acid 2-phosphate, β-glycerolphosphate, and dexamethasone and were stained for alkaline phosphatase (ALP). Cells were also differentiated into adipocytes in the presence of IBMX, ITS, dexamethasone, and indomethacin and were stained with Oil Red O. MSCs were treated with concentrated WW, and their proliferation and survival were assayed according to the manufacturers’ specifications (CyQuant and Alamar Blue assays, respectively). For Alamar Blue assay, cells were serum starved. To test the WW in vivo, 5mm critical-size defects were made in the calvaria of immunodeficient mice. The defect was either left with no further treatment or packed with a collagen sponge. The sponge was loaded with either PBS, PRP, unconcentrated WW, or concentrated WW. 4 weeks post-surgery, the calvaria and serum were harvested. The serum samples were tested to identify biomarkers of bone formation. The calvaria were examined using micro-CT.

Results: As revealed by our studies, OA can be found in greater concentrations in the concentrated WW than in the PRP (Figure 1).
MSCs extracted from the wastewater demonstrated multipotency by differentiating along the osteogenic and adipogenic pathways (Figure 2).

**Figure 1**: In order to demonstrate that the concentrated wastewater has more osteogenic proteins than the platelet rich plasma, the concentration of osteoactivin in each fluid was measured using a sandwich ELISA assay. As can be seen, for each patient, there is a higher concentration of osteoactivin in the concentrated wastewater than in the platelet rich plasma. *p<0.05, **p<0.01, ***p<0.001, unpaired t-test, error bars represent mean±SEM.
WW was concentrated for use in cell culture; the final concentration of OA in the WW-supplemented media was 2ng/mL. The WW was added as a supplement to actively proliferating MSCs, which demonstrated 80% increased proliferation over untreated controls. The WW was also added as a supplement to cells undergoing serum starvation. By day three, cell survival of WW-supplemented MSCs was 80% higher than untreated controls (Figure 3).

**Discussion:** The described studies have demonstrated that concentrated RIA WW has potential as an osteogenic supplement that promotes MSC proliferation, survival, and differentiation and could aid in healing large bone defects. In order to confirm...
these studies, we are currently quantifying the concentrations of other osteogenic proteins within the WW. We will also examine the ability of concentrated WW to induce MSC differentiation into osteoblasts compared to PRP. We have developed an in vivo model to characterize the ability of the WW to promote healing of critical-size defects in athymic mice. These studies are currently ongoing. Our hypothesis is that defect bone ingrowth will be significantly increased in mice treated with concentrated WW when compared to those treated with carrier alone or with PRP. We are also working to develop a biodegradable carrier that can be used intraoperatively to capture/selectively bind WW the proteins for use in the same patient.

**Significance:** Previous studies by others demonstrated the presence of several angiogenic proteins in the WW as well as the presence of bone morphogenetic proteins and viable MSCs. Our studies have demonstrated the presence of OA in the WW which has not been previously described. Our study is also the first to demonstrate that proteins in the WW remain capable of inducing MSC differentiation, even after being processed. By stimulating MSC proliferation and survival, the WW may accelerate healing. It may also be possible to harvest MSCs, prime them for osteogenic differentiation, and pack them into the defect where they can act as sources of both osteogenic cells and proteins. Eventually, it is our goal to translate these techniques into real-time procedures in the operating room.

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**References:**