Recovery of Mesenchymal Stem and Endothelial Progenitor Cells from Reamer Irrigator Aspirator (RIA) Waste Fluid using the SynGenX™-LAB.

Fernando Fierro PhD[1], Michael Lehmicke MSc[2], Jon Ellis[3], John Perea[3], Phil Coelho[3], Andrew Cicchetto[1], Mark Lee MD[4]
[1]UC Davis Institute for Regenerative Cures, Sacramento, CA, [2]Depuy Synthes Inc., West Chester, PA, [3]SynGen Inc., Sacramento, CA, [4] UC Davis Medical Center, Department of Orthopaedic Surgery, Sacramento, CA

Disclosures: F. Fierro: 5; Depuy Synthes Inc. M. Lehmicke: 3A; Depuy Synthes Inc. J. Ellis: 3A; Syngen Inc. J. Parea: 3A; Syngen Inc. P. Coehlo: 3A; Syngen Inc.. A. Cicchetto: None. M. Lee: 2; AO Trauma, AO North America. 3B; Depuy Synthes. 5; Depuy Synthes

Introduction: The Reamer/Irrigator/Aspirator (RIA) system (Depuy-Synthes, Inc.) has emerged as a viable tool for obtaining bone graft. The RIA devices cleared indications include harvesting bone graft using a primary graft filter. A large volume of fluid bypasses the primary filter and is normally discarded as waste. This fluid is known to contain a large number of viable cells. In a collaborative effort between Syngen™, Depuy-Synthes™ and the University of California Davis, as part of a non-significant risk (NSR) feasibility study, RIA waste fluid (hereafter called effluent) was collected from patients undergoing surgery. The RIA effluent was then processed in the SynGenX™-LAB System, a functionally closed method of harvesting mononuclear cells (MNCs). We hypothesized that a large number of viable MNCs, including mesenchymal stem cells (MSCs) and endothelial progenitor cells, could be recovered from the effluent with high efficiency. Cells were not re-introduced to the patients in this study. Cell recovery data from RIA effluent sourced from 8 patients are presented here.

Methods: IRB approval and patient consent were obtained to recover effluent from patients undergoing treatment for non-unions of the tibia, femur, and humerus. RIA graft material captured in the filter was obtained from the femur using a retrograde or antegrade approach as determined in each case by the operating surgeon. Effluent passing the graft filter was mixed with heparin and processed in the X-LAB System to obtain a concentrated MNC fraction. The X-LAB System is comprised of a disposable cartridge, control module and docking station, and employs a ThermoFisher Sorvall Legend XT centrifuge. During a single centrifugation run, the X-LAB stratifies the cell fractions according to density, depletes the RBCs, harvests the MNC fraction, and excess supernatant (fat particles, excess plasma and saline) remains in the central chamber of the cartridge. RIA effluent from patients 1 through 7 was spun for 20 minutes, and from patients 8 and 9 for 25 minutes. Cell counts from the depleted RBC and supernatant fractions, in addition to the harvested MNC fraction, were performed in order to reconcile the total MNC and CD34+ counts with the unprocessed RIA effluent. RIA effluent contains many atomized fat particles and bone chips that are of similar size to MNCs and are frequently inadvertently counted by hematology analyzers, so MNC and CD34+ cell counting was performed by single platform flow cytometry, in accordance with ISHAGE guidelines. Flow cytometry gating thresholds were established following analysis of patient 1 to exclude fat and bone chips from MNC and CD34+ enumerated populations. Data from patients 2-9 appear in the Results section. Cell viability was assessed by 7-AAD exclusion. Samples from the harvested and depleted fractions were then cultured for 10 days and average colony counts (CFU-f) were determined by 3 observers.

Results: The total amount of effluent processed ranged from 225 ml to 1000 ml and was processed in 1 to 4 cartridges. The X-LAB System was programmed to yield a harvest volume of 10ml per cartridge for the first seven patients, which resulted in a mean 24-fold volume reduction. For patients eight and nine, the program was changed to yield a harvest volume of ~6 mL per disposable cartridge, which resulted in a 42-fold volume reduction. The mean MNC recovery was 95.4% (83.1% - 102.6%), the mean CD34+ cell recovery was 98.7% (94.2%-103.1%), and there was no loss of cell viability due to processing (see Figure 1). The greater volume reduction of effluent from patients 8 and 9 did not reduce the cell recoveries or viabilities. CFU-f counts from the harvested MNC fraction were higher than counts in the unprocessed RIA effluent in every case except for those from patients 3 and 7, where the counts could not be determined due to confluence. Colonies were rare (<5) in the red blood cell and supernatant layers except for those from patient 7, where significant numbers of colonies were observed in all layers. The harvest fraction from patient 7 had the lowest MNC recovery (83.1%) of any of the samples; the missing MNCs were in the depleted RBC and supernatant fractions. Effluent from patients 8 and 9 were spun for 25 minutes and the MNC recoveries were 93.7% and 96.0% respectively.

Discussion: Previous studies of RIA waste effluent have processed a small sample (~ 50cc) by conventional lab centrifugation methods (e.g. Ficoll). In this feasibility study, we have demonstrated that up to 1 liter of effluent from a RIA procedure can be processed in as little as 25 minutes to obtain a smaller volume with a high concentration of MSCs and hematopoietic progenitor cells. The SynGenX-LAB System was able to isolate and concentrate MNCs with very high efficiency (>90% mean recovery). MSC recoveries correlated well with MNC recovery as evidenced by high CFU-f counts from the harvested MNC fraction and low counts from the red blood cell and supernatant fractions except in one case, where incomplete separation and a lower recovery efficiency were observed.

Significance: These preliminary data show that RIA effluent, which is currently discarded in clinical cases, may be efficiently processed to obtain a large number of viable MNCs in a concentrated volume. This highlights the future possibility of using RIA and the SynGenX Systems for intraoperative cell therapy.

Figure 1.

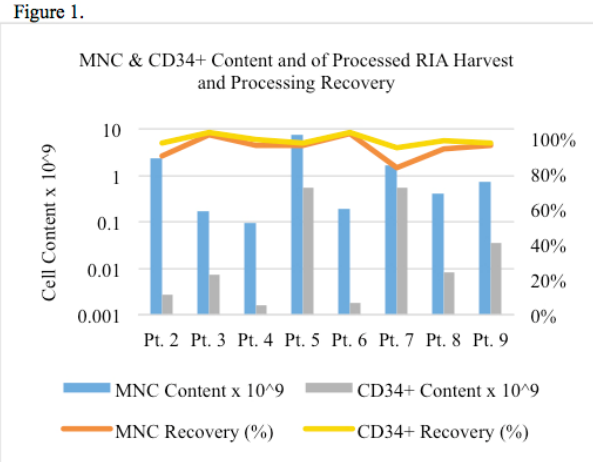

Figure 2.