Encapsulated mesenchymal stromal/stem cell and leukocyte immunomodulation had nominal impact on the elimination of established *Staphylococcus aureus* biofilms

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Statement of Purpose: The intrinsic immunomodulatory activity of mesenchymal stromal/stem cells (MSCs) has been utilized for the treatment of a number of inflammatory conditions and immune disorders[1]. In mice cecal ligation models, MSC administration improved animal survival by attenuating excessive inflammation associated with septic shock/multi-organ dysfunction while enhancing phagocytic cell-mediated bacterial clearance in the blood stream[2,3]. Prevention and early elimination of *Staphylococcus aureus* bacteria introduced during surgical implantation of medical devices is necessary to avert morbidity often associated with chronic implant-associated infections[4]. We have shown previously, that three-way interactions between encapsulated MSCs, adherent monocytes, and the biomaterial induced up-regulation of the M2 phenotype in monocytes, which was associated with attenuated inflammation and greater phagocytic activity[5,6]. *We hypothesize that MSCs can enhance neutrophil and/or monocyte/macrophage phagocytic function for the elimination of nascent S. Aureus biofilms.*

Methods: The MBEC™-HTP device was inoculated with *S. aureus* (ATCC 29213, 3.0 x 10^8 colony forming units (CFU)/mL) and incubated for either 6 or 24 hrs with continuous rocking (37°C, 10° inclination) to form biofilms. Pins removed from the MBEC™-HTP device were placed in transwell co-culture containing either monocytes or neutrophils (top chamber) and encapsulated MSCs (bottom chamber) with RPMI 1640 supplemented with either 10% or 1% autologous human serum for 2, 6, or 12 hrs isolated from healthy, human donors (depicted below). Bone marrow-derived MSCs (passages 4 - 6) were encapsulated in either collagen hydrogels or gelatin/poly(ethylene) glycol (Gel-PEG) interpenetrating biomatrices previously utilized in our laboratory[6]. The *S. Aureus* biofilms were sonicated and plated on tryptic soy agar for 24 hrs and bacterial colonies quantified by Log CFU/mL determination. MSCs, monocytes, or neutrophils were imaged for viability characterization after LIVE/DEAD® incubation (30 min).

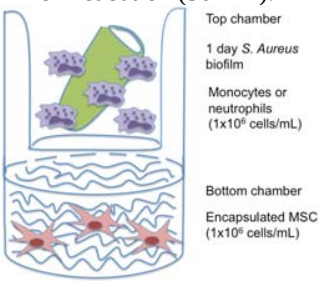

Figure 1. Transwell co-culture schematic.

Results: Biomatrix-encapsulated MSCs retained high viability for both materials (collagen and Gel-PEG) over various biofilm exposure times, whereas monocyte and neutrophil viability decreased with increasing biofilm exposure time (2, 6, or 12 hr) or biofilm maturity (6 or 24 hr). No significant decrease in Log CFU/mL was observed for the various culture conditions with either immune cells (neutrophils or monocytes), co-culture times (2, 6, or 12 hrs), or biofilm maturity (6 or 24 hrs) when compared to the biofilm only condition (with the exception of the minocycline positive control).

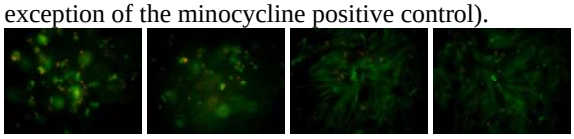

Figure 2. LIVE/DEAD® staining of encapsulated MSCs exposed to nascent biofilm (6 hr); left: [monoculture MSCs (Gel-PEG)]; middle left: [co-culture MSCs (Gel-PEG)]; middle right: [monoculture MSCs (collagen)]; right: [co-culture MSCs (collagen)].

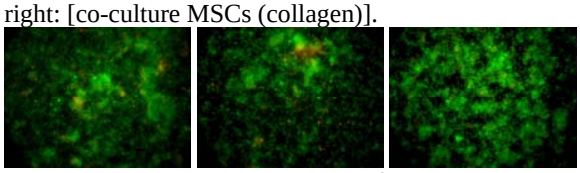

Figure 3. LIVE/DEAD® staining of polycarbonate-adherent neutrophils exposed to nascent biofilm (6 hr); left: (monoculture neutrophils); middle: [neutrophils with co-culture MSCs (Gel-PEG)]; right: [neutrophils with co-culture MSCs (collagen)].

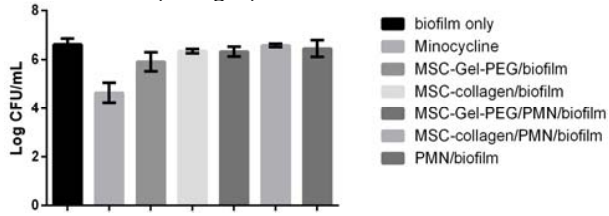

Figure 4. Bacterial burden comparison of nascent *S. aureus* (6 hr) biofilms in 2 hr monoculture or co-culture with neutrophils and/or encapsulated (Gel-PEG or collagen) MSCs (1:1, 1x10^6 cells/mL). Minocycline (2 mg/mL) was utilized as a positive control for bacteriostatic/bactericidal comparison.

Conclusions: Similar to implant-associated infection observed *in vivo*, prevention or early resolution of *S. aureus* colonization is needed as nascent biofilms proved highly resistant to both immune cells (monocytes and neutrophils) and immunomodulatory MSCs. Additional co-culture characterization will be undertaken to determine whether *S. aureus* planktonic bacteria can be eliminated prior to nascent biofilm formation.

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References: 1 Parekkadan B, et al. Annu Rev Biomed Eng 2010; 12:87-117. ; **2** Tyndall A, et al. Nature Med 2009; 15(1): 18-19. ; **3** Mei SHJ, et al. Am J Respir Crit Care Med 2010; 182: 1047-1057. ; **4** Busscher HJ, et al. Sci Trans Med 2012; 4(153): 153v10. ; **5** Kim J et al. Exp Hematol 2009; 37(12): 1445-1453. ; **6** Cantu DA, et al. Stem Cell Trans Med 2012; 1: 740-749.