In vitro Testing of Adult Mesenchymal Stem Cells as an Adjunct Therapy for Treating Periprosthetic Joint Infections

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INTRODUCTION:
Current treatment regimens for periprosthetic joint infections (PJIs) in total joint replacements include repeat surgery and systemic antibiotic administration. These procedures are invasive and result in increased patient morbidity and contribute to the development of antibiotic resistant organisms. Adjunct antimicrobial therapies, particularly those utilizing naturally biocompatible agents, are thus desirable. Recently, it has been shown that adult tissue-derived mesenchymal stem cells (MSCs) have antimicrobial properties that inhibit bacterial growth in lung tissue. 1 In this study, we aim to test the hypothesis that the innate antimicrobial activity of the body’s endogenous MSCs may be exploited as an adjunct therapy for PJI. For this purpose, we have used an in vitro model consisting of bacteria-inoculated synovial fluid (SF) cultures to test the bactericidal effect of MSCs, combined with or without antibiotics. Our findings show that MSCs contribute measurable antibacterial activities in an SF setting, and suggest their possible utility as an adjunct therapy for PJIs.

METHODS:
The most common gram-negative organism present in septic SF after total joint replacements is Escherichia coli (E. coli). Cultures of E. coli HB 101 (ATCC 33694) were prepared by seeding a 100 μL aliquot in 5 mL of Luria-Bertani (LB) medium and grown overnight at 37°C in a shaker at 240rpm. The optical density (OD) at 600nm of the E. coli suspension was measured to determine E. coli concentration based on a standard curve (OD600 = 1.0 corresponds to 1.01 × 10^7 colony forming units CFU/mL). IRB approval was obtained for collection of MSCs and synovial fluid with signed patient informed consent. MSCs were derived from bone marrow as previously described. 2 Synovial fluid (SF) was obtained from the operating room with IRB approval under aseptic conditions prior to the capsular incision in a total knee arthroplasty to ensure minimal contaminants.

Bacterial growth inhibition was tested by placing a total volume of 200 μL in individual wells of a non-adherent 96 well plate with the following conditions in duplicate: (1) LB (control), (2) Culture medium (control), (3) SF (control), (4) MSCs (control), (5) Ampicillin (Amp) (control), (6) SF + MSCs, (6) SF + Amp, (7) E. coli, (8) E. coli + MSCs, (9) E. coli + Amp, (10) SF + E. coli, (11) SF + E. coli + Amp, (12) SF + E. coli + MSCs, and (13) SF + E. coli + MSCs. A total of 2.86 × 10^4 CFU of E. coli, 2 × 10^3 MSCs, and 0.01 mg of Ampicillin (Invitrogen 11593-027) were used in each designated well.

Cultures were incubated for 6 hours in a humidified 37°C chamber with gentle agitation. The plate was removed and 100 μL of fluid was aspirated from each well, and each well was cultured overnight on LB agar. Dilution series were performed on E. coli and E. coli and MSC samples prior to plating. Colonies were counted and the number of CFUs were determined. Data were compared using independent sample t-tests assuming unequal variances. A statistically significant result was considered where p < 0.05.

RESULTS:
All control samples demonstrated no bacterial growth. Incubation of E. coli alone resulted in an average of 2.86 × 10^4 CFU, E. coli with MSCs produced 2.87 × 10^3 CFU, and E. coli with Amp resulted in 1.14 × 10^7 CFU (Table 1). Culture of E. coli with MSC resulted in lower bacterial growth than E. coli alone (difference had trends towards statistical significance p=0.058), and E. coli with Amp resulted in even more reduced bacterial growth (difference was statistically significant p=0.030).

SF inoculated with E. coli produced an average of 32.5 CFU/100 μL. Treatment with Ampicillin, MSCs, and in combination reduced the amount of bacteria produced. SF infected with E. coli and treated with Amp produced an average of 6.5 CFU/100 μL (p=0.053 compared to E. coli alone), while SF infected with E. coli and treated with MSCs produced an average of 4 CFU/100 μL (p=0.011 compared to E. coli alone). The greatest reduction in bacteria occurred when SF inoculated E. coli was treated with both MSCs and Amp (4 CFU/100 μL, p=0.001 compared to E. coli alone) (Figure 1).

DISCUSSION:
Our results clearly demonstrated that MSCs are effective in reducing bacterial growth when added to E. coli cultures alone or to E. coli inoculated into SF. MSCs are not as effective as ampicillin for reducing bacterial growth in E. coli alone. However, in the presence of SF, MSCs show a measurable synergistic effect in decreasing E. coli growth. This observation is consistent with the fact that SF is bacteriocidal and has innate antimicrobial activity, which is effective for slowing bacterial growth until a certain threshold. 3 Combining MSCs and ampicillin as adjunctive antimicrobial therapy in our PJI in vitro system resulted in the most effective reduction of bacterial growth. It should be noted that a limitation here is the sensitivity of measuring bacterial growth using traditional plating methods. Future studies will utilize high sensitivity PCR-based methods to quantify viable bacterial load. 4

SIGNIFICANCE:
Current treatment methods of periprosthetic joint infections result in great morbidity and do not adequately resolve infection. Utilizing the body’s endogenous mesenchymal stem cells may prove to be an effective adjunctive therapy to treating PJIs in vivo.

ACKNOWLEDGEMENTS:
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REFERENCES:

Table 1: Effect of MSCs and Ampicillin (Amp) on E. coli

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<tr>
<td>E. coli + Amp</td>
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Figure 1: Effect of MSCs and Ampicillin (Amp) on E. coli in synovial fluid (SF) setting.