Optimizing a Cell Co-culture System to Understand Bone-Muscle Crosstalk

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INTRODUCTION: The elderly population experiences declines in musculoskeletal performance, demonstrated by decreased mobility, shuffling gait, poor balance, and an increased risk for falls and fractures[1,2]. The causes of these declines are not well defined yet are speculated to result from deterioration in the mechanical structures native to musculoskeletal tissues[3,4]. Improved understanding of these structural changes could lead to new targets for therapeutic treatments. While the coupling of skeletal muscles and bone has previously been considered primarily mechanical in nature, recent research demonstrates the two tissues interact through crosstalk signaling mechanisms important for the overall function of these tissues[5,6]. Because the factors involved in this crosstalk are not well defined, we developed a novel co-culture system to examine crosstalk between muscle satellite cells and bone osteoblasts.

METHODS: With approval of the NC State University IACUC, four 6-week-old Brown Norway (BN) male rats were euthanized; the femur, tibia, gastrocnemius, and tibialis anterior were dissected from one hindlimb. Bone marrow mesenchymal stem cells (MSCs) and muscle satellite cells (SCs) were isolated and expanded in cell-specific growth media on gelatin plates. Four media formulations (OM=osteogenic media, MM=myogenic media, 50%MM:50%OM, 75%MM:25%OM) and four plate coatings (gelatin, collagen, Matrigel®, none) were tested in mono- and co-culture for optimal stimulation of osteo- and myogenesis (Fig. 1). MSCs and SCs were mono- or co-cultured (Transwell, MSC on top, SC on bottom) in triplicate at 20,000 cells/well for 3 days in cell-specific growth media, then 14 days in one differentiation media formulation. Cell viability was assessed on Days 3, 7, 10, and 14 using alamarBlue®. Muscle-specific differentiation and cellular crosstalk were examined on Day 14 (n=4) with IGF-1 expression via ELISA. On Day 14, all cells were fixed and stained with alizarin red to assess bone-specific mineralization in MSCs and check for non-specific differentiation in SCs. The effects of plate coating and media formulation on the outcome measures were examined using two-way ANOVA with Tukey post-hoc tests (α=0.05, GraphPad).

RESULTS: Media formulation and plate coating had little effect on Day 14 cell viability for MSCs and SCs in either mono- or co-culture (Fig. 2). For media formulation, co-cultured SCs were more viable in MM than OM ($p=0.034$) yet remained similar for the mixed formulations. For plate coating, the only significant difference was that MSCs were more viable when grown on collagen than on Matrigel ($p<0.001$). Preliminary IGF-1 expression suggested higher and more varied expression with different media formulations than with plate coatings (data not shown). Qualitatively, alizarin red staining appeared greater in MSCs than SCs and was more pronounced in OM, as expected (Fig. 3). Furthermore, SCs had an elongated shape typical of differentiating myotubes only when in MM and not in other media formulations.

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DISCUSSION: The current co-culture system demonstrated the capacity for both MSC and SC proliferation and differentiation. Cell viability results were similar across all culture conditions, suggesting that rat MSCs and SCs did not prefer any particular media formulation or plate coating examined, contrary to other studies[7]. However, the lack of differentiation seen in cell morphology of SCs in non-MM media may be due to the presence of fetal bovine serum driving SCs towards proliferation and not differentiation[8]. Preliminary IGF-1 results suggest than crosstalk can altered with different media formulations, the extent of which is still being investigated. Therefore, when designing a co-culture system to understand bone-muscle crosstalk, plate coating may not be as important as the media provided to each cell type.

SIGNIFICANCE: We developed a novel co-culture system to understand the cellular crosstalk between bone and muscle cells. This system could facilitate the mechanistic investigation of bone-muscle crosstalk in clinical and non-clinical populations.

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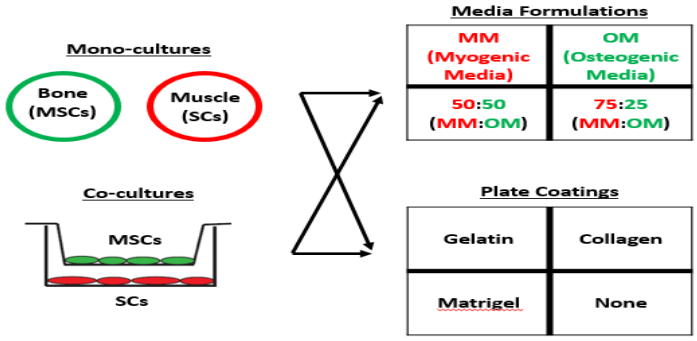


Figure 1. Study design: MSCs and SCs harvested and plated into mono- and co-cultures, and effects of media formulation and plate coating were tested.

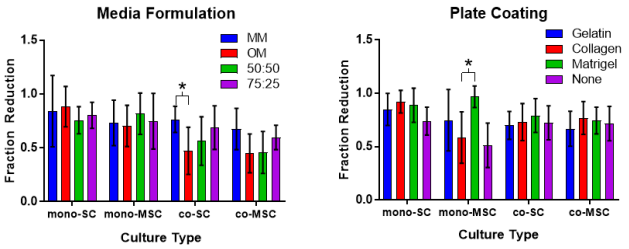


Figure 2. Cell viability (Day 14) was similar across media and plate coatings for MSCs and SCs in mono- and co-culture. MSCs had slight preference for Matrigel vs. collagen, and co-cultured SCs for MM vs. OM. *p<0.05.

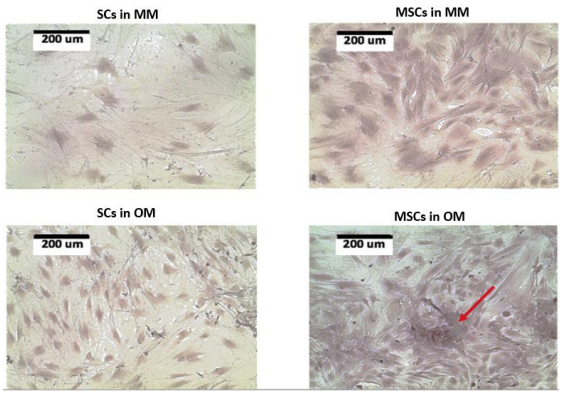
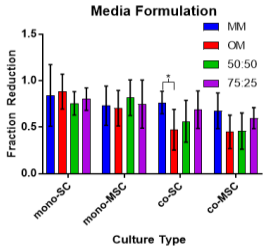


Figure 3. Mono-cultures: representative alizarin red images. MSCs in OM had slightly more staining than MSCs or SCs in other conditions. SCs had elongated cell shape typical of developing myotubes, while MSCs retained their fibroblastic appearance. Red arrow=high mineralization.


Media Formulation
MM
OM
50:50
75:25
Fraction Reduction
1.5
1.0
0.5
0.0
mono-SC
mono-MSC
co-SC
co-MSC
Culture Type

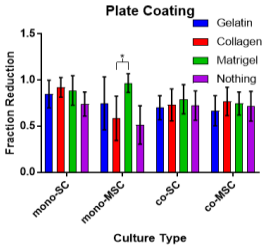


Plate Coating
Gelatin
Collagen
Matrigel
Nothing
Fraction Reduction
1.5
1.0
0.5
0.0
mono-SC
mono-MSC
co-SC
co-MSC
Culture Type