Mesenchymal stromal cell metabolic activity and GAG production is rescued by co-culture with nucleus pulposus cells in IVDD-like pH

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INTRODUCTION: In the context of mesenchymal stromal cell (MSC) regeneration of the intervertebral disc (IVD), the role pH has in influencing MSC differentiation and subsequent matrix synthesis, metabolic activity, and viability is an emerging area of interest , as the pH of the degenerating IVD is extremely harsh [1]. Wuertz found that rat bone marrow derived MSC cell number and viability decrease when cultured in a pH similar to that found in IVDD [2]. Low pH has also been linked to increased MSC stress and premature senescence, which would affect their metabolic activity and matrix synthesis [3]. While these studies have laid the initial groundwork for investigating the impact of pH on MSC fate, further study is warranted. For example, it is unclear if MSCs derived from different tissue sources exhibit the same response to IVDD-like pH. Prior studies by our group and others have demonstrated that MSC tissue source plays a key role in dictating MSC response to the same microenvironmental conditions. [4,5]. A final characteristic of the microenvironment that should be considered, is the resident cells of the IVD. It has been previously shown that nucleus pulposus cells (NPCs) are able to drive MSC differentiation and increase their matrix synthesis [6,7]. However, these studies were conducted in a physiological healthy pH (7.4) which is not representative of the environment that the MSC would be injected into. To our knowledge, it is unknown how the interaction between NPCs and MSCs is impacted by low (6.5) pH. Thus, the objective of this research was to investigate the influence of IVDD-like low pH conditions on MSC metabolic activity and matrix synthesis, how the MSC tissue source alters these responses and the impact of co-culture of NPCs and MSCs on these outcomes. It was hypothesized that: 1. Decreasing pH would detrimentally impact MSC metabolic activity and matrix production. 2. MSCs from different tissue sources would exhibit significantly different responses to IVDD-like pH. And 3. NPC conditioned acidic media would rescue MSC metabolic activity and GAG production.

METHODS: MSCs from three different sources: adipose (AD-), amnion (AM-), and bone marrow (BM-) MSCs were cultured in alginate beads, at $2x10^6$cells/mL, within 6-well plates with 5 beads per well. Cells were cultured in DMEM (10% FBS, and 1% ab/am) with pH adjusted to either 6.5 or 7.4. Additionally, one other media group of conditioned media was added. This conditioned media was generated from the culture of NPC at a pH of 6.5. After 3 days, the media was directly transferred from the NPCs to the MSCs. A total of 5 different medias were used through the course of this study: fresh media pH 7.4 (0% N), fresh low pH 6.5 media (0% L), 50% fresh media:50% NPC conditioned media (50% N and 50% L) and 100% conditioned media (100% L). At days 1,6, and 12 of culture cell metabolic activity was determined (AlamarBlue, n=6) and after 12 days of culture bead sulfated glycosaminoglycan (s-GAG) (DMMB, n=6) and total collagen (hydroxyproline assay, n=6) content was determined.

RESULTS: MSC metabolic activity was negatively affected by acidity but with increasing concentrations of NPC conditioned media, the metabolic activity is restored for both AD-MSCs and BM-MSCs. Interestingly AM-MSC metabolic activity was not significantly altered by the media conditions studied, however, at the 100% L media condition the metabolic activity of the cells was significantly lower than both AD- and BM-MSCs. The DMMB assay showed similar results for all three cell types with a general trend of low pH significantly decreasing s-GAG production, and the introduction of conditioned media restored the s-GAG content to that of the normal pH for both AD- and BM-MSCs. The hydroxyproline assay showed that the collagen content of each alginate bead was not altered by media condition across all MSC tissue sources.

DISCUSSION: The overall finding of this study is that IVDD-like low pH decreased MSC metabolic activity and GAG production, however co-culture with NPC conditioned low pH media restored these measures. Furthermore, this response was dependent on the tissue source of the MSC. This protective effect of NPCs on MSCs in low pH could be attributed to the cytokines that the NPCs are releasing into the media. It has been shown that NPCs stimulate local cell GAG production via cytokines such as TGF-b, HIF-1a, and BMP-2 [9]. These same cytokines may elicit a similar response in MSCs resulting in restored metabolic activity and GAG production even in this harsh IVDD-like pH, however further study of the mechanisms involved are warranted.

SIGNIFICANCE: Understanding how implanted cells will react to the harsh microenvironment in the degenerating IVD, as well as, how they will be influenced by resident IVD cells is important for increasing the efficacy of cellular therapy of IVDD. Our results indicate that pH, the tissue source of the MSC and the resident cells of the IVD all are important factors to consider in the optimization of cellular therapy of the IVD.

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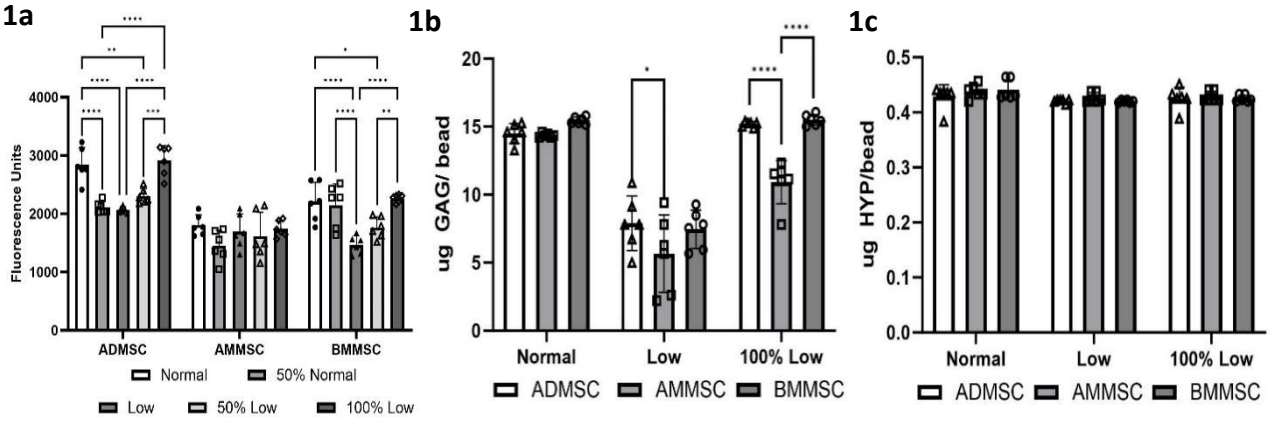

Figure 1 Day 12 Metabolic activity and matrix production: 1a. AlamarBlue assay depicting metabolic activity of adipose (AD-), Amnion (AM-). And bone marrow (BM-) derived MSCs. Legend: fresh normal(7.4) pH media (Normal), fresh low (6.5) pH media (Low), 50% fresh media, 50% conditioned media(50% Normal and 50% Low) and 100% conditioned media (100% Low). **1b.** DMMB assay depicting s-GAG content in 1 alginate bead. **1c.** Hydroxyproline assay depicting total collagen content in 1 alginate bead. All data is represented as mean ±SD with $P < 0.05$ for n=6 for each MSC source and media condition. Asterisk connecting two bars represent a significant change.