(YIA) **How to train your cells: cytokine priming to address ASC conditioned medium against inflammation**

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OBJECTIVES: The conditioned medium (CM) from Mesenchymal Stem/stromal Cells (MSCs) possesses promising features that can be exploited for regenerative medicine purposes. Here we characterized the composition of the CM obtained from adipose MSCs (ASCs), either naïve or primed with inflammatory cytokines, and compared their chemotactic properties and anti-catabolic effects.

MATERIALS AND METHODS: CM were produced from confluent ASCs after 72 hours in starving conditions. The priming step was performed by adding 10ng/ml IL-1β and/or TNFα to the cultures for 5 minutes and accurately rinsing cells before starvation. We concentrated the CM using 3kDa cut-off Amicon filtering units and analyzed the resulting products for protein content, particle concentration, and the levels of various immunoregulatory, anti-inflammatory, and pro-resolving factors using different techniques such as Nanoparticle Tracking Analysis (NTA), immunoassays, and mass spectrometry. We evaluated the effects of control and primed CM (CM and pCM) in vitro on THP-1 cells to assess cell attraction and on inflamed articular chondrocytes to evaluate the inhibition of matrix metalloproteinase (MMP) activity. We analyzed the data using one-way or two-way ANOVA and set significance at $p < 0.05$.

RESULTS: The characterization of CM and pCM highlighted an enrichment in the latter of total protein content and number of particles, together with higher levels of immunoregulatory, anti-inflammatory, and protective mediators (e.g. TGF-β1, PGE2, and CCL-2). Surprisingly, CM and pCM were equally effective in attracting THP-1 cells, although cytokine priming induces the accumulation of chemoattractants in pCM. At last, both products efficiently hampered the pathological activity of MMPs in TNFα-inflamed chondrocytes, even though the levels of TIMP-1 and -2 were reduced in pCM.

CONCLUSIONS: The comprehensive characterization of naïve and primed CM confirms the hypothesized empowering effect of the priming strategy, demonstrating that it could be a mighty tool to obtain a richer and pathology-tailored product. Both CM showed comparable abilities to attract cells and prevent catabolism, but their uncharted mechanisms and any real differences should be studied in more complex *in vitro* and *in vivo* models, which include other components of the immune system and consider inter-tissue crosstalk and repairing mechanisms