

Recapitulating The Effects Of The Osteoarthritis Inflammatory Environment On Mesenchymal Stem Cell Chondrogenesis

Krishna Jayaram¹, Chiebuka Okpara¹, Zachary Weiser¹, Lisette Werba¹, Lesley W. Chow^{1,2}, Tomas Gonzalez-Fernandez¹

¹Department of Bioengineering, Lehigh University, Bethlehem, PA, USA and ²Department of Materials Science and Engineering, Lehigh University, Bethlehem, PA, USA

Introduction

- Osteoarthritis (OA) is a degenerative disease involving damage to articular cartilage in the knee joint with risk factors such as age, sex, and obesity¹.
- Extracellular matrix (ECM) degradation leads to inflammation which can be regulated by pro-inflammatory M1 macrophages.
- In order to treat OA with anti-inflammatory therapies, we must develop a robust model of inflammation for our *in vitro* studies.
- **The objective of this study is to explore how inflammation affects human mesenchymal stem cell (hMSC) chondrogenesis by incorporating cell pellets in macrophage conditioned media (MCM).**

Experimental Design

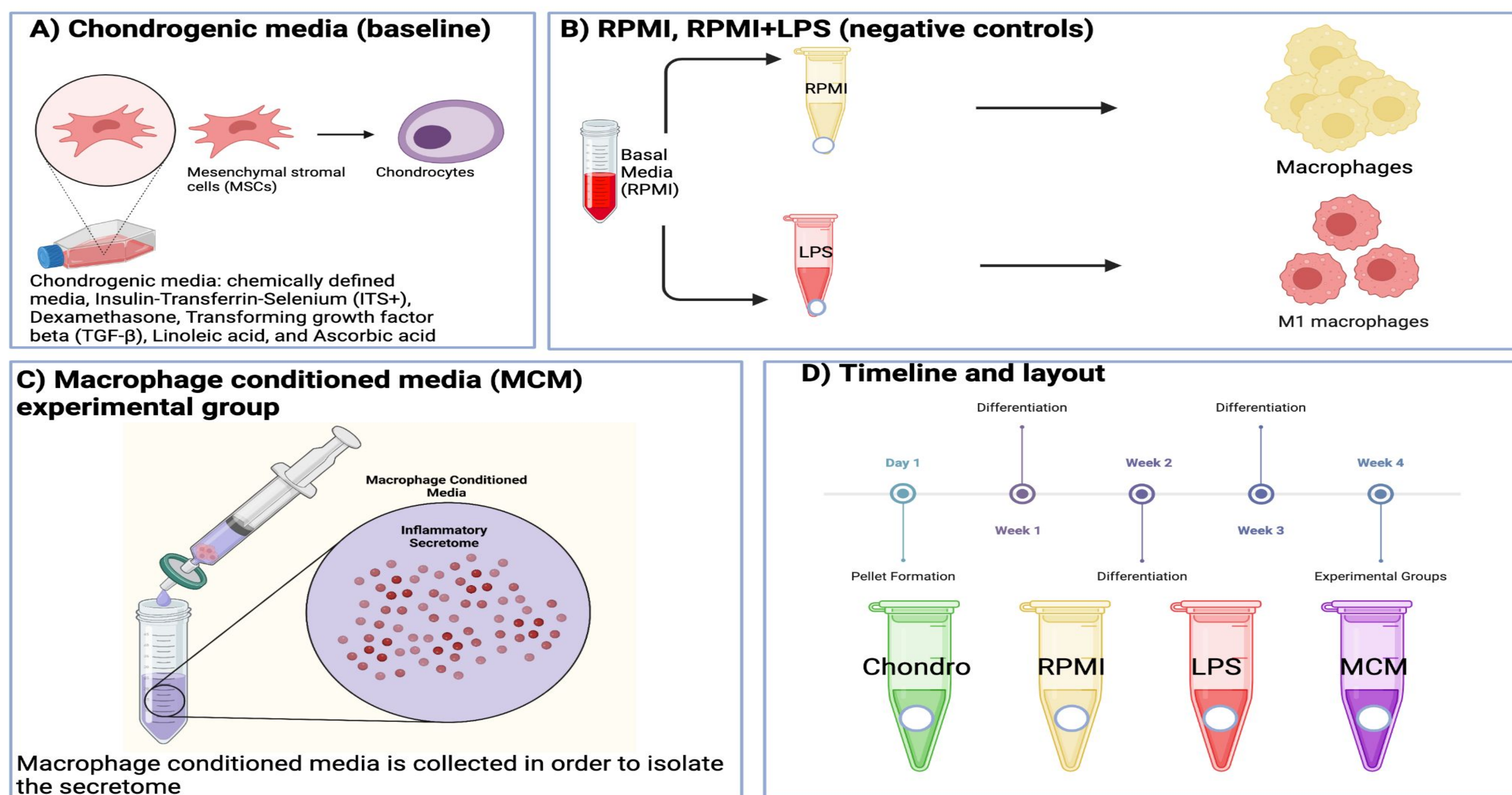


Figure 1: Experimental groups

A) Chondrogenic media induces chondrogenesis **B)** Basal media is used to grow macrophages, LPS and RPMI induce a proinflammatory phenotype (negative controls) **C)** Macrophage conditioned media is isolated in order to collect inflammatory secretome which produces pro-inflammatory markers (experimental group) **D)** Timeline and layout. All groups contain chemically defined media (CDM). CDM contains high glucose media, penicillin/streptomycin (pen/strep), L-proline, and bovine serum albumin (BSA).

Results

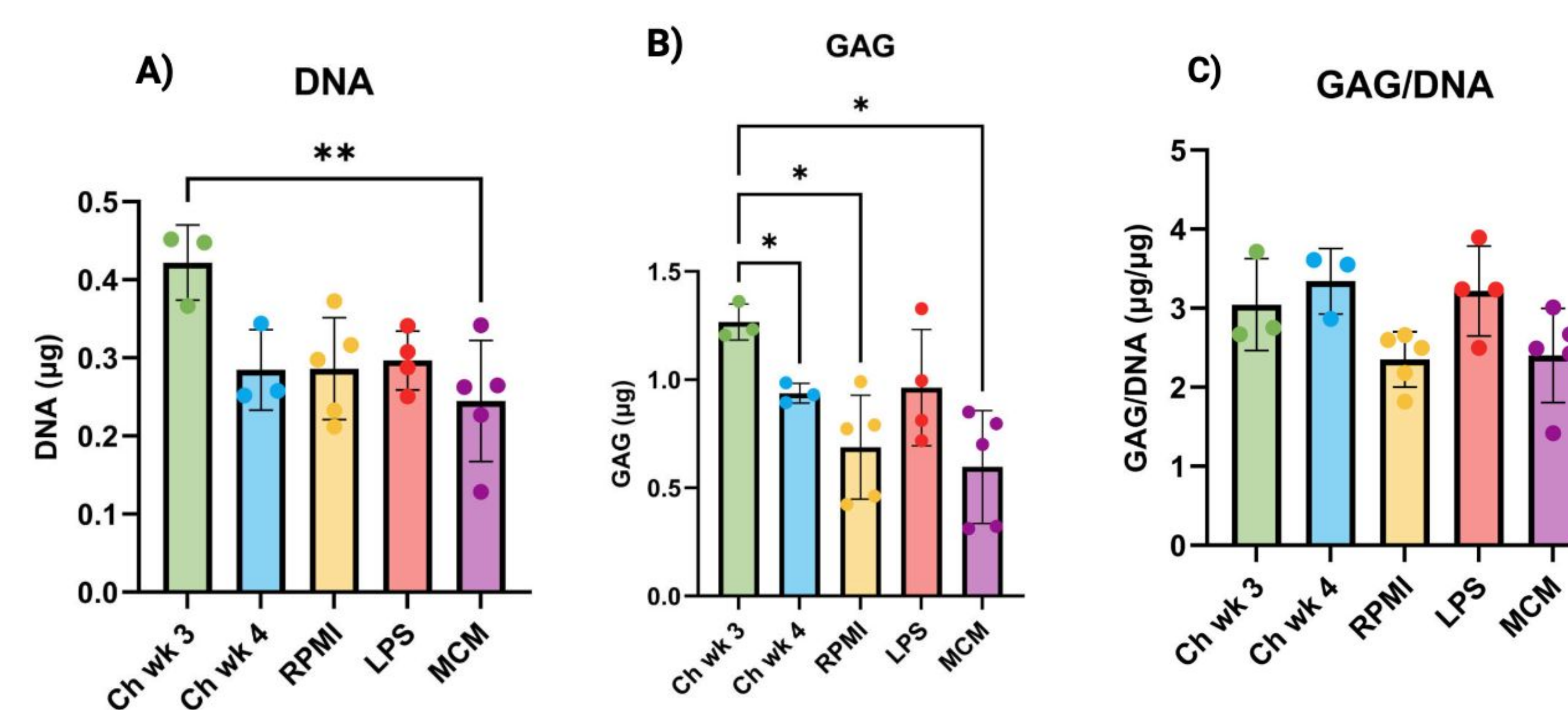


Figure 2: Biochemical quantification of **A)** DNA **B)** GAG **C)** GAG/DNA using Picogreen and Dimethyl-Methylene Blue assays, for pellet culture, respectively.

One-way ANOVA was used with Tukey's or Dunnett's T3 post-hoc test for multiple comparisons. $p < 0.05$ was considered significant (* $p < 0.05$, ** $p < 0.01$).

Discussion/Conclusion

- The data suggests that macrophage conditioned media (MCM) is stalling chondrogenesis and mimicking an osteoarthritic environment.
- MCM attempts to degrade the extracellular matrix in pellet culture. However, this effect is counterbalanced by chondrogenically differentiated hMSCs, resulting in no observable levels of degeneration or chondrogenesis.
- The current study used conditioned media from a murine cell line, but future studies will use a murine primary cell line with a greater potential for pro-inflammatory phenotype.

References

1. Katz et al., *Jama*, 2023
2. Chen et al., *Am J Transl Res*, 2020
3. Kennedy et al., *Tissue Engineering part A*, 2023
4. Spiller et al., *Experimental Cell Research*, 2016

Acknowledgements

The authors acknowledge the generous support by Lehigh University Department of Bioengineering and the P.C. Rossin College of Engineering & Applied Science through the Rossin College Doctoral Dean's fellowship and Maida's fellowship (C.O.), Clare Boothe Luce Research Award (K.J.), Presidential Fellowship Award (L.W.) and start-up funds (T.G.-F.). This work was generously supported by National Science Foundation (NSF) through a Faculty Early Career Development (CAREER) award (DMR1944914) and the National Institutes of Health (NIH) Grant (1R21AR079117) (L.W.C).