Exploring Myoblast-Macrophage Interactions as a Mechanism for Promoting Arteriogenesis in Peripheral Arterial Disease

Elisabeth Stodola, Gabrielle Melamed. Dr. Trevor Cardinal PhD
Biomedical Engineering Department, California Polytechnic State University, San Luis Obispo

CAL POLY
Bailey College *of* Science & Mathematics

Introduction

Peripheral Arterial Disease (PAD) is an atherosclerotic condition characterized by arterial plaque buildup, leading to impaired blood flow, ischemia, and tissue necrosis in lower extremities[1]. Affecting over 10 million individuals in the U.S., PAD is the primary cause of limb amputation[2].

Background:
Cellular therapies have attempted to combat the need for amputation by promoting the formation of natural bypasses around occluded arteries but have resulted in minimal improvement in patient disease[3].

- Our lab has identified myoblasts as a novel cell therapy candidate for increasing arteriogenesis.
- *In vivo*, the recruitment and polarization of macrophages from a pro-inflammatory (M1) to a pro-regenerative (M2) phenotype is critical in orchestrating arteriogenesis[4].

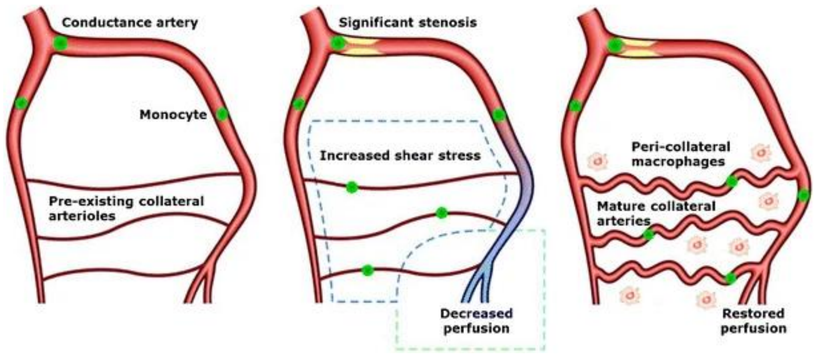

Figure 1. Mechanism of arteriogenesis. Shear stress on the collateral vessels downstream of a stenosis stimulates macrophage-mediated vessel remodeling allowing the restoration of blood flow[5].

Hypothesis and Goals

Hypothesis: The coculture of primary murine myoblasts with RAW 264.7 macrophages will drive the polarization of M0 and M1 macrophages to a pro-regenerative, M2 phenotype.

Goals:

- Quantify macrophage phenotype based on cell morphology following coculture with myoblasts.
  - M1 – large cell area
  - M2 – high axial ratio
- Determine if the coculture of myoblasts with macrophages increases M2 polarization.

Methods

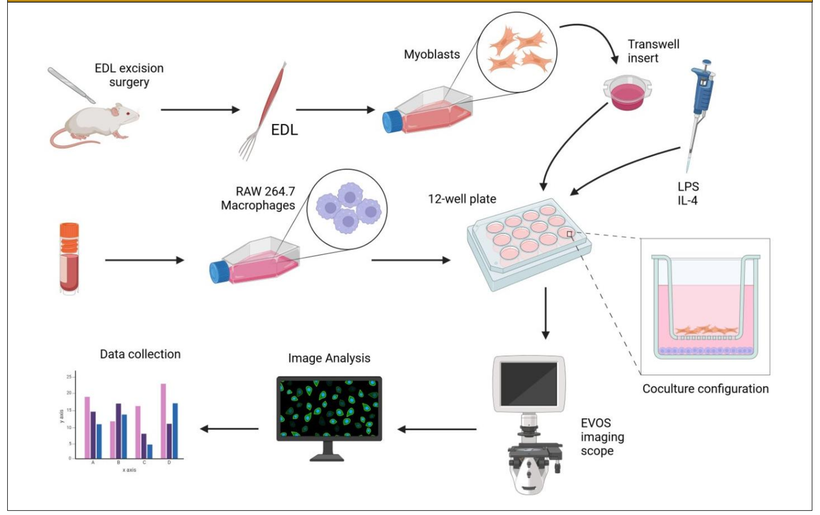

Figure 2. Myoblast-macrophage coculture methods. A 9-day experiment was conducted, beginning with myofiber isolation and RAW 264.7 macrophage culture. LPS and IL-4 were used to polarize macrophages to the M1 and M2 phenotype, respectively. Myoblasts were seeded on transwell inserts and cocultured for 3 days. Macrophage morphology was quantified on days 4 and 7 using ImageJ, and statistical comparisons between experimental groups were performed using one-way ANOVA with Tukey post-hoc analysis.

Results

In the M1 and M2 conditions, coculture with myoblasts significantly increased macrophage axial ratio compared to macrophages cultured alone.

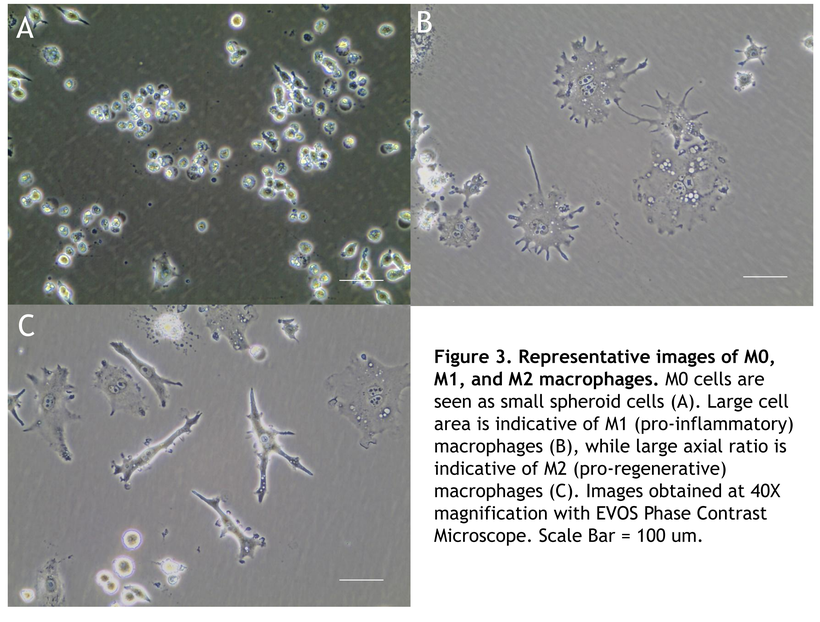

Figure 3. Representative images of M0, M1, and M2 macrophages. M0 cells are seen as small spheroid cells (A). Large cell area is indicative of M1 (pro-inflammatory) macrophages (B), while large axial ratio is indicative of M2 (pro-regenerative) macrophages (C). Images obtained at 40X magnification with EVOS Phase Contrast Microscope. Scale Bar = 100 um.

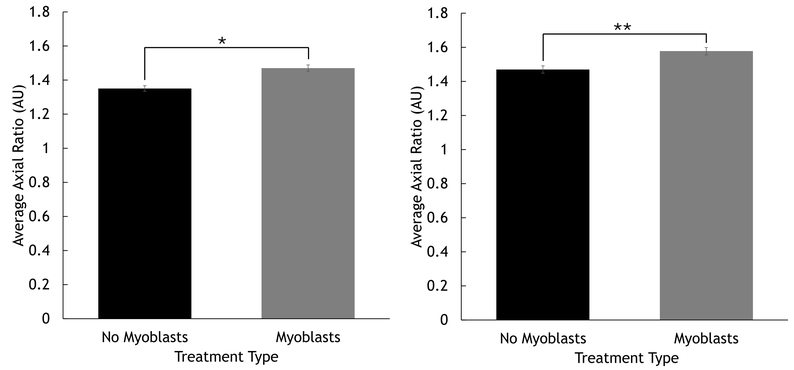

Figure 4. Differences in macrophage axial ratio between each treatment type on day 7 of experiment. Macrophages polarized to both M1 phenotype (left) and M2 phenotype (right) show significant difference in average axial ratio between the treatment of no myoblasts (n_1 = 4) to myoblasts (n_2 = 4) at a significance level of $p \leq 0.05$.

Conclusions / Discussion

- Coculture with myoblasts significantly increased M2 macrophage morphology compared to M2 macrophages cultured alone, suggesting enhanced macrophage polarization via myoblast interaction.
- These findings implicate myoblast-macrophage relationships as a potential mechanism promoting M2 polarization and arteriogenesis, relevant for improving regenerative therapies in PAD.

Future Directions/Steps

- Replicate the coculture study with human cells to enhance translational relevance and advance potential applications in human cell therapy.
- Integrate flow cytometry to improve cell characterization based on surface marker expression within the coculture system.

References

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Acknowledgements

We would like to thank Dr. Trevor Cardinal, our colleagues in the MaVR lab, and our friends and family for supporting us.