Understanding Primary Human Myoblast Morphology & Behavior

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Introduction

Peripheral Arterial Disease (PAD) is the leading cause of amputation in the United States. PAD is characterized by narrowed arteries due to plaque buildup, which can lead to insufficient blood flow in distal limbs (ischemia) when the vessel becomes occluded. While surgery is an option, it only helps 50% of patients, leaving a clear need for alternative therapies. One option is using a cellular therapy that targets collateral vessels, which have natural abilities to enlarge and provide bypasses around blockages (arteriogenesis).

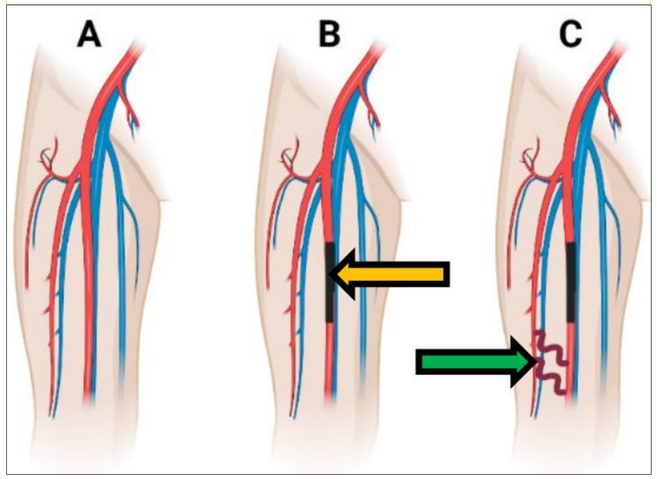


Figure 1. Unobstructed (A) and obstructed (B, orange arrow) distal limb arteries, with enlarged collaterals (C, green arrow) from arteriogenesis.

Myoblasts, muscle progenitor cells, are a compelling cell therapy candidate as they promote arteriogenesis in mice. Co-culturing mouse macrophages *in vitro* with primary mouse myoblasts shifted the macrophages to an M2 polarization, which is the regenerative phenotype responsible for revascularization via arteriogenesis. The current study explores the preliminary stages of translating these findings to human primary myoblast culture.

Methods

Primary Human Myoblasts (hMyo) were cultured and observed daily with a light microscope. Complete media changes occurred on day 2 and day 4 of culture, with cultures being passed and cryopreserved once 80-90% confluency was reached.

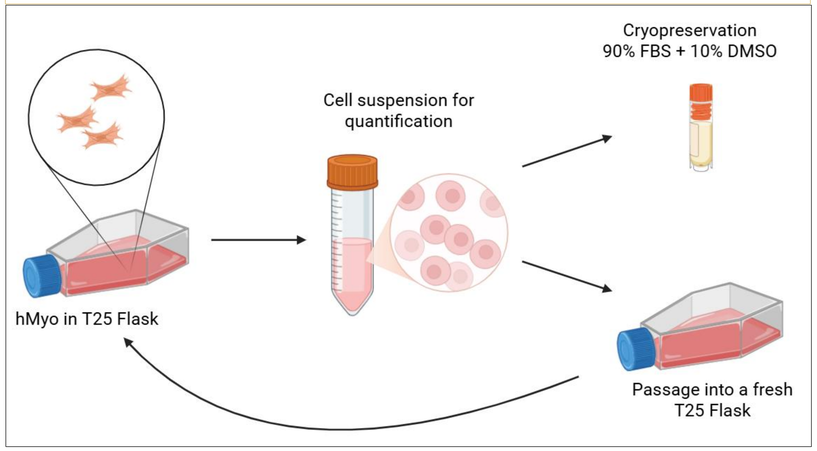


Figure 2. hMyo Cell Culture Workflow. The cells were cultured according to ATCC's Protocols, and various conditions of culture were tested, including surface coating, growth factor dosing, and seeding densities.

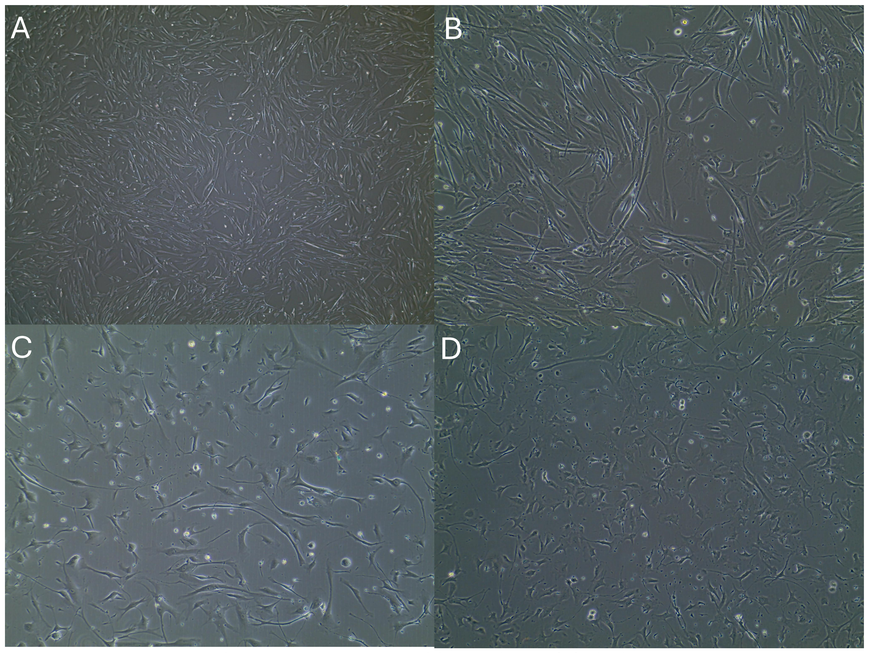
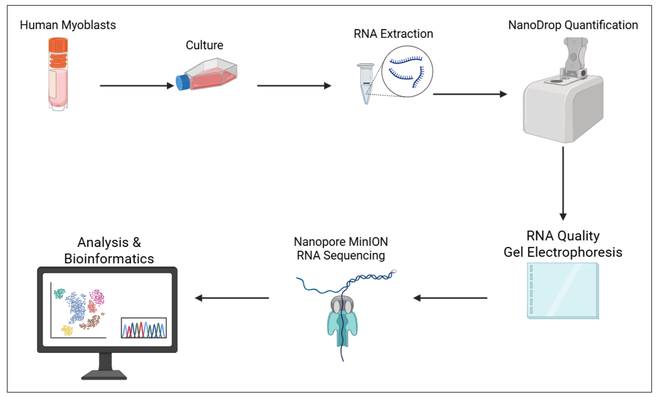


Figure 3. Progression of Primary Human Myoblast Culture. (A) Flasks were seeded at a density of 5000 cells/cm^2, and observed with a light microscope at 4X magnification on day 3 of primary initiation of culture. (B) 10X magnification of the same day 3 flask. (C) Day 2 culture of passage 5, at 10X magnification. (D) Day 3 culture of passage 10, at 10X magnification.

Future Directions / Next Steps

The aim is to study human myoblast behavior and expression in co-culture with human primary macrophages. Future studies will focus on identifying upregulated genes by sequencing extracted RNA, which will give insight into which genes and proteins likely control regenerative macrophage polarization.



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