Digit Amputation Level Influences Macrophage Polarization

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Introduction

Macrophages play a critical role in the body's healing process. Early after injury, macrophages exhibit an inflammatory activation state. These inflammatory macrophages (M1) create a sterile environment and recruit new blood vessels into the healing tissue[1-3]. Inflammatory macrophages transition to a resolution state (M2) in which they reduce inflammation and promote collagen deposition, bone mineralization, and myoblast differentiation[1-4]. Without the proper balance of macrophages, injuries will not heal [2,4]. Thus, macrophages make promising therapeutic targets to improve wound healing.

Objectives

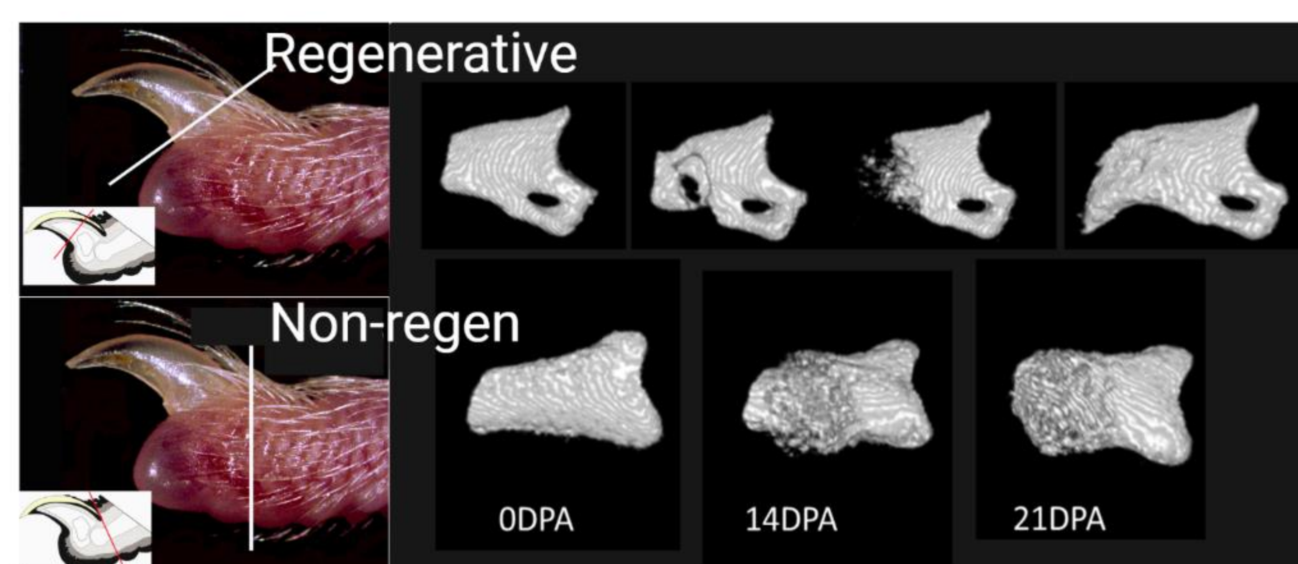
Macrophage activation states in two types of injuries are being studied: a distal amputation (P3) that fully regenerates, and a more proximal one (P2) that forms scar tissue. Previous studies suggest that an M1 P2 injury are glycolytic and express high levels of TNF and IL1b. An M1 P3 injury demonstrates fatty acid oxidation (FAO) and lower levels of TNF and IL1b. We hypothesize that microenvironmental factors control the metabolic and transcriptomic state of the macrophage. To test this hypothesis, we expose unstimulated macrophages (M0) to the paracrine factors from the homogenized tissue and analyze changes in chemical markers.

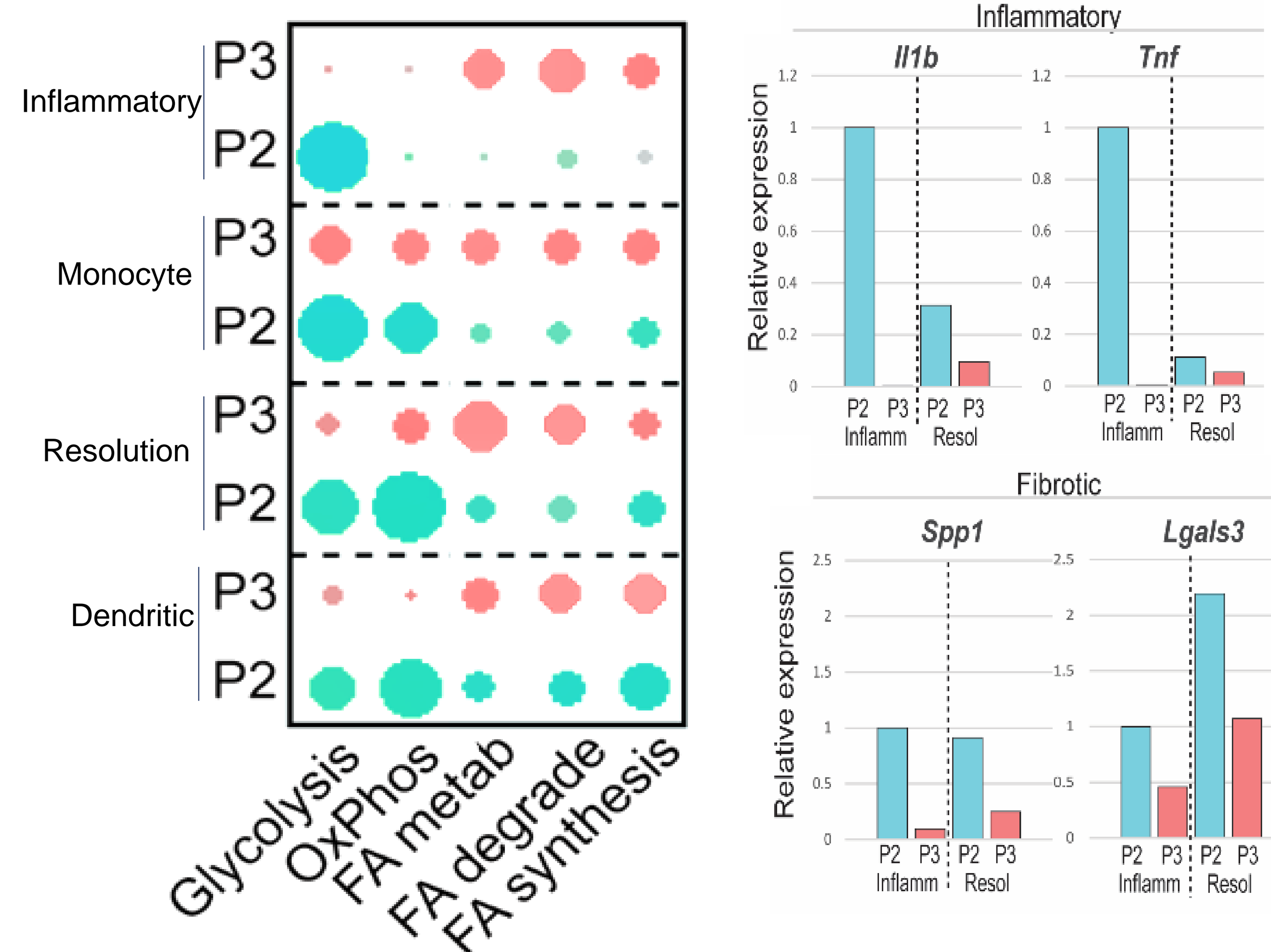
As we are interested in how different biochemical factors could influence macrophage polarization, we have chosen to use itaconate as an experimental factor. This metabolite both increases wound healing while also reducing macrophage polarization, which appears contradictory [10,11].

Methods

In this experiment, bone marrow was derived from the hind limbs of two euthanized mice to produce undifferentiated macrophages (M0). These were plated on L929 media, which promotes macrophage differentiation. Two groups of eight mice each underwent amputations at the P2 and P3 digits. The amputated tissue was homogenized and added to the M0 plates. After a resting period for differentiation, the samples were filtered to separate cellular structures from secreted factors. The Seahorse XF96 was used to analyze metabolic characteristics, such as extracellular acidification and oxygen consumption rates, to determine the predominant macrophage population. This allowed for a comparison between the P2 and P3 media.

Preliminary Data – P2 and P3 macrophages are metabolically distinct

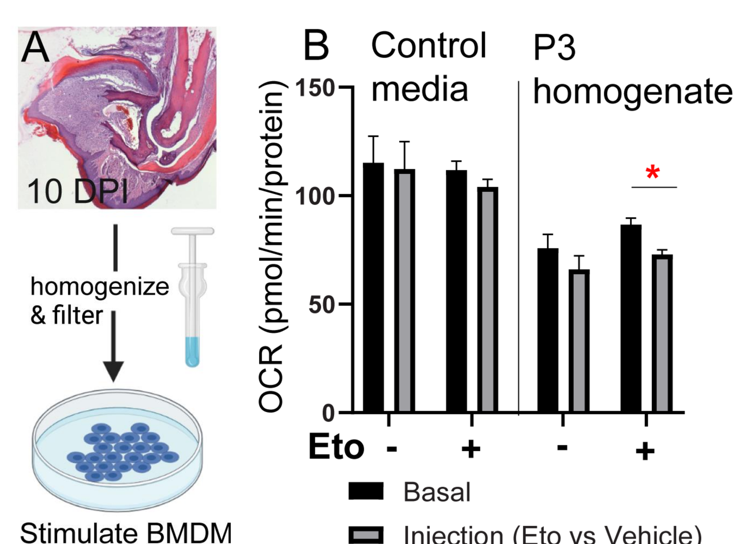




Analyzing a single cell RNAseq dataset from P2 and P3 at 10 days, we find 4 populations of mononuclear phagocytic cells. In all 4 clusters, cells from P2 show an increase in glycolytic genes whereas P3 cells are more likely to display genes for FAO.

We also found that M1 and M2 from the P2 region express higher levels of pro-inflammatory cytokines (Il1b, Tnf) and pro-fibrotic genes (Spp1, Lgals3) compared to macrophages from P3.

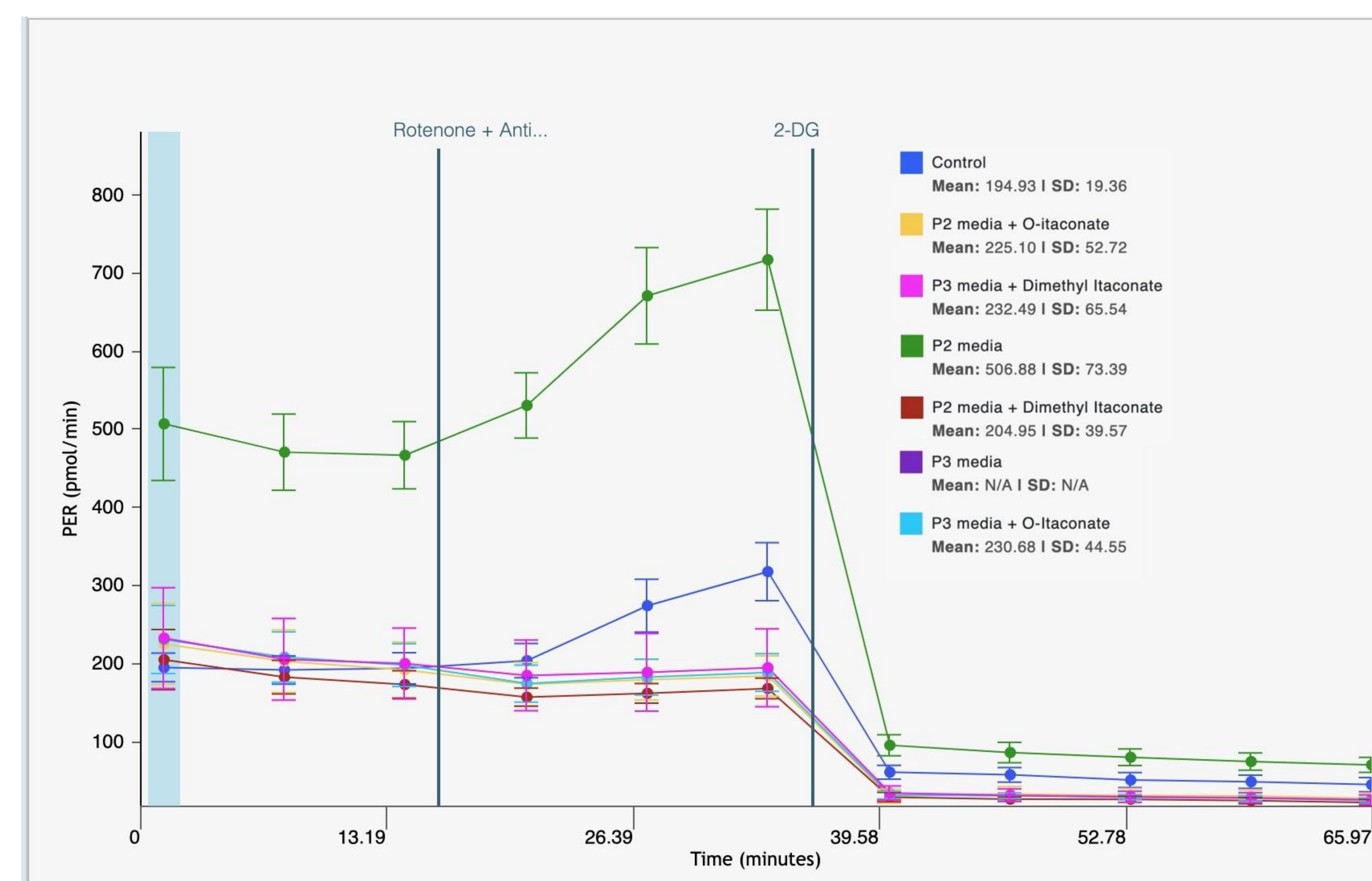
Results



In response to extract from P3 10 days after amputation, BMDM increased reliance on FAO (*p<0.05, n=10/group).

OCR decreases when FAO is blocked in BMDM exposed to P3 homogenate but not when exposed to normal media.

Results



FAO increased in macrophages exposed to P3 tissue. In contrast, high levels of ECAR (signified by the proton efflux rate, PER) due to increased glycolysis was observed in P2 tissue. Cells lost glycolytic capacity in response to itaconate.

Conclusion

These results supported our hypothesis that factors within the P2 tissue favor the M1 macrophage type while the P3 favors the M2 type. The deleterious effects of itaconate on the macrophage populations was unexpected and should be further explored to determine if this was an error in application or if this chemical inhibits polarization.

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