Stress-Relaxing Matrix Properties Impact Monocyte Derived Macrophage Polarization

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INTRODUCTION:
Macrophages are key players in tissue repair and regeneration, making them promising targets for therapy. Following injury, macrophages migrate to the damaged site to either promote or resolve inflammation based on local cues. A precise control of the inflammatory response, especially the shift from a pro- to an anti-inflammatory phase, is crucial for a successful healing outcome. Although the influence of local mechanics and immune compartment on the outcome of fracture healing is well described, little is known about the interaction of the two niches. Here, we investigate the influence of elastic and viscoelastic tissue properties on human monocyte-derived macrophages.

METHODS:
Immunologically inert alginate hydrogels were used to specifically mimic the elastic and viscoelastic properties of tissues. To ensure that there is a controlled cell-matrix interaction, the alginate polymer was modified with a cell adhesive motif (RGD) using carbodiimide chemistry. Peripheral blood mononuclear cells (PBMCs) are isolated from human peripheral blood using a density gradient, and monocytes are isolated using a commercially available magnetic bead isolation kit. Monocytes were cultured for 4 days with GM-CSF to generate monocyte-derived macrophages. Subsequently, either inactivated monocyte-derived macrophages, or LPS+IFN-y stimulated monocyte-derived macrophages were encapsulated in ionically crosslinked alginate hydrogels with distinct viscoelastic properties. After 48 hours, the expression of macrophage polarization markers was analyzed using flow cytometry.

RESULTS SECTION:
High molecular weight alginate (MVG) or low molecular weight alginate (VLVG) with Ca^{2+} concentration of 15mM or 27mM for crosslinking showed elastic modulus of ~ 12kPa. The stress relaxation half-life time ($_{t1/2}$) mainly depends on the molecular weight of the alginate. VLVG alginate hydrogels showed faster stress relaxation ($t_{1/2;\,VLVG}$ ~ 85s) than MVG alginate hydrogels ($t_{1/2}$, MVG ~ 1800s). In this study, we demonstrated that the mechanical niche influences macrophage polarization. Principal component analysis (PCA) showed distinct localization of monocyte-derived macrophages encapsulated in either a slow or a fast relaxing environment. In addition, uniform manifold approximation and projection (UMAP) plots of single cell flow cytometry data revealed the same impression: the localization of monocyte-derived macrophages was dependent on the stress-relaxation half-life time of the mechanical niche. As expected, the stimulation of monocyte-derived macrophages with LPS+IFN-y upregulated the surface expression of CD80 and CD64. Interestingly, the changes induced by the mechanical niche were persistent, regardless of the activation status of the monocyte-derived macrophages. In particular, 48 hours after encapsulation of macrophages in the alginate hydrogels, the expression of cell surface markers CD14, CD45, CD64, CD80, and CD86 seems to be modulated by the mechanical niche.

DISCUSSION:
Native materials are characterized by distinct material properties. Viscoelastic material properties have more recently been introduced as a relevant key parameter determining cell fate. Alginate hydrogels can be used to mimic a wide range of viscoelastic properties of biological tissues, and thus allow to investigate and control the impact of mechanical niche properties on cells to be studied more comprehensively. Here we show that the mechanical niche alters the macrophage phenotype in a different manner than the classical cytokines for macrophage polarization (IFN-y + LPS for M1 activation). Our data illustrates the potential of the extracellular matrix and its biophysical properties, specifically the viscoelastic properties, to influence macrophage polarization in their distinct 3D niche.

SIGNIFICANCE/CLINICAL RELEVANCE:
The influence of mechanics and the immune system have both been acknowledged as important factors for successful healing after a fracture. Thus, elucidating the mechanistic interaction between biomechanics and immunological processes has the potential to pave the way for the development of advanced therapeutic approaches grounded in biomaterials and mechanobiological principles.