Immunomodulation towards regenerative wound healing by controlling the biophysical properties of biomimetic, defined matrices

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Cutaneous wound healing depends on interactions between cells, bioactive factors, and the extracellular matrix (ECM). Infiltrating immune cells, including blood monocytes and their differentiated progeny, macrophages, play key roles in initiating inflammation and promoting tissue repair. Macrophage dysregulation leads to persistent inflammation and impaired wound healing[1]. Macrophages are regulated by soluble mediators and cell-cell interactions, but also via mechanotransduction of biophysical microenvironmental stimuli. Yet, limited studies interrogate 3-dimensional biophysical effects on macrophage function.

Polyisocyano-peptide (PIC) is a reversibly thermoresponsive, 3-dimensional hydrogel system that mimics dynamic stress-stiffening mechanical properties observed in biological matrices. The mechanical properties can be controlled by altering polymer chain length, and the polymer can be functionalised with ECM peptide ligands for integrin mediated adhesion and mechanosignalling. PIC hydrogels were synthesised at a range of stiffnesses and then functionalised at different densities with collagen (GFOGER and DGEA) or fibronectin (cyclic RGDyk and linear GRGDS) integrin peptide ligands, to mimic native ECM cues in skin. We have employed this tunable hydrogel to de-couple effects of mechanical and integrin cues on monocyte differentiation and macrophage functional plasticity.

We demonstrated this system is conducive to immune evaluations by showing there was no material-associated inflammatory activation of macrophages cultured in PIC hydrogels. However, lipopolysaccharide-induced inflammatory activation was dampened in the presence of integrin ligands, compared to unfunctionalised PIC. Macrophages embedded in PIC hydrogels were able to proliferate and phagocytose E. coli bioparticles, and increased phagocytosis was seen in response to lower stiffness and critical stress mechanical properties. By contrast, the migration rate was greater through materials with higher mechanical properties, highlighting distinct effects of biophysical properties on macrophage functions. Human monocytes cultured in PIC hydrogels differentiated into macrophages in the presence of the homeostatic growth factor Colony Stimulating Factor 1 (CSF1) or Granulocyte Macrophage Colony Stimulating Factor (CSF2), key regulators of macrophage phenotype and function in inflammatory environments. In contrast to 2D culture, monocytes cultured in PIC hydrogels in the presence of CSF2 universally upregulated surface markers such as CD163 and CD169, associated with CSF1 differentiation and pro-regenerative wound healing. Comprehensive analysis of macrophage phenotype and secretome under these conditions is ongoing.

We have established a highly controllable and translatable PIC system, which has provided novel insights into the interplay between macrophage functions and specific ECM-associated biophysical cues. Ultimately, this improved understanding of macrophage immunoregulation will facilitate the development of novel biomaterials, with targeted biophysical properties that can potentially overcome biochemical stimulus, to promote pro-healing functions and phenotypes in chronic wounds.

References

[1]Raziyeva, K.; Kim, Y.; Zharkinbekov, Z.; Kassymbek, K.; Jimi, S.; Saparov, A., Immunology of acute and chronic wound healing. Biomolecules (Basel, Switzerland) **2021**, 11 (5), 700.