BIOFABRICATION AND PHYSICAL CHARACTERIZATION OF RHEOLOGICALLY MODULATED BIOFILMS AND TISSUES

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Abstract

Emergent behavior is a defining characteristic of biological systems arising from interactions between individual entities and host microenvironments. Both prokaryotic and eukaryotic cells rely on their surrounding extracellular matrices for structural support, communication, and functional organization. In prokaryotes, communities of bacteria, in the form of biofilms, can exhibit collective behaviors such as adhesion, motility, and structural coloration, driven in part by the properties of the extracellular matrix. Similarly, in eukaryotic systems, cells respond to mechanical cues in their microenvironment, influencing proliferation, differentiation, and tissue development. This dissertation investigates the interplay between emergent physical properties of both bacterial biofilms and eukaryotic tissues, revealing common principles underlying matrix-driven cellular organization with implications for healthcare, infection control, and bioengineered materials.

In biological systems, physicochemical properties are strongly regulated by the local microenvironment, which can either promote or suppress collective behavior. In bacterial biofilms, the mechanical properties of the surrounding matrix influences motility, colony formation, and structure. Here, we explore the emergent behavior of two biofilm-forming bacterial species, *Salmonella typhimurium* and *Cellulophaga lytica*, highlighting their distinct responses to microenvironment mechanics. The first investigation examines *S. typhimurium* biofilms in 3D hydrogel matrices, revealing that hydrogel viscoelasticity and bacterial seeding density modulate microcolony formation. Bacterial concentration alters the rheological properties of hydrogels, with lower cell seeding densities leading to larger microcolonies, and decreasing shear viscosity with increasing bacterial concentration.

In *C. lytica* biofilms, structural color is an emergent property of interest, which arises due to the organization of cells within biofilms. In the second investigation of this dissertation we explore the role of substrate stiffness in regulating *C. lytica's* gliding motility and iridescent coloration. It is observed that increasing matrix stiffness enhances collective gliding patterns in early biofilm development and influences the progression of structural coloration. Additionally, it is shown that salt content in agar gels modulates the mechanical relaxation properties of the substrate, impacting motility and diminishing iridescence intensity. These results demonstrate how the emergent properties of biofilms can be tuned through physical microenvironmental factors, offering insights for developing living biophotonic materials.

Beyond bacterial biofilms, emergent behavior in eukaryotic cells can also be modulated by the mechanical properties of the extracellular matrix. The extracellular matrix is fundamental in regulating cell adhesion, proliferation, and differentiation, particularly in tissue development and repair. This dissertation extends its investigation to muscle tissue, where satellite cells (muscle stem cells) are essential for muscle regeneration following injury. Specifically, the role of matrix stiffness to regulate myogenic differentiation in 3D hydrogels, as compared to conventional 2D cultures, in the presence of macrophages is reported, along with the influence of macrophages on myogenic differentiation. It is observed that macrophages exhibit greater sensitivity to environments with higher stiffness, displaying reduced viability and metabolic activity.

By investigating both bacterial biofilms and mammalian cells, this work underscores the fundamental role of the extracellular matrix in guiding emergent behavior across biological domains. Further understanding how microenvironmental properties regulate collective behaviors and properties in biofilms and tissues will aid in development of new tools for advanced healthcare, living materials, and targeted regenerative medicine approaches.