Abstract

Living tissues are mechanically dynamic systems whose response to physical stimuli arises from the interplay between solid matrix deformation, fluid transport, internal structural rearrangements, and cellular mechanotransductive responses. These coupled mechanisms operate across multiple spatial and temporal scales, generating mechanical environments that continuously evolve under physiological loading. Despite this intrinsic complexity, the mechanical characterization of soft tissues and biomaterials is still frequently based on a limited set of descriptors, often obtained under simplified and heterogeneous experimental conditions. Consequently, such measurements may only partially capture the material response and are not always directly comparable across studies, particularly when time-dependent phenomena are involved.

This doctoral thesis addresses this challenge by proposing a methodological framework for the systematic characterization of the mechanical behavior of biomimetic hydrogels that more accurately reflects better reflects biologically relevant conditions, accounts for scale-dependent effects, and captures their time-dependent nature.

The work focused on the mechanical characterization of several formulations of gelatin methacryloyl (GelMA), a widely used biomaterial exhibiting strong biochemical similarity to the native extracellular matrix (ECM) and high tunability. Despite its extensive use, the mechanical data reported in literature for this material remain highly heterogeneous, complicating direct comparison across studies.To overcome these limitations, in this thesis an integrated and comparative approach was adopted. Rather than focusing on a single formulation or isolated testing method, multiple GelMA formulations were systematically investigated by independently varying degree of methacrylation and polymer concentration. All experiments were conducted under consistent experimental conditions, enabling mechanical trends to be compared across scales while minimizing variability associated with the experimental setup. In particular, a specific GelMA formulation was mechanically examined against native soft tissues, such as porcine and human lung tissue, using a nanoindentation-based protocol. This comparison enabled direct assessment of mechanical properties at the cellular length scale and demonstrated that GelMA can reproduce stiffness ranges comparable to those of native lung tissue. However, the analysis revealed that matching the elastic modulus alone is insufficient to ensure mechanical equivalence, particularly when local heterogeneity and time-dependent responses are considered, emphasizing the importance of biological reference systems in the interpretation of hydrogel mechanics.

Subsequently, a systematic multiscale analysis of multiple GelMA formulations was conducted using uniaxial tensile tests, unconfined compression tests, and microscale nanoindentation. At the macroscale, stiffness was found to be primarily governed by polymer concentration, while the influence of the methacrylation degree appeared less pronounced. In contrast, microscale measurements revealed a greater sensitivity to methacrylation degree, particularly at higher polymer concentrations, indicating that local mechanical properties at the cellular scale capture compositional effects that are partially averaged out in bulk measurements. These findings demonstrated that bulk mechanical descriptors cannot always be representative of the mechanical environment at the cellular level and reinforce the necessity of scale-aware characterization strategies.

The final part of the thesis focuses on the investigation of the time-dependent mechanical behavior of GelMA hydrogels at the microscale. Stress-relaxation nanoindentation experiments were analyzed using viscoelastic, poroelastic, and coupled poroviscoelastic modeling frameworks applied to the same dataset. All investigated formulations exhibit measurable stress relaxation, arising from the combined effects of polymer network rearrangement and fluid-mediated transport. While the Young's modulus varied substantially across formulations, the relative magnitude of stress relaxation remained within a narrower range, suggesting that composition primarily governs instantaneous stiffness, whereas dissipation mechanisms are less sensitive within the explored parameter space.Importantly, both viscoelastic and poroelastic models were able to fit subsets of the experimental data with high goodness of fit, yet yielded distinct global behaviors. This finding reflects the coexistence of viscoelastic and poroelastic mechanisms acting on comparable timescales. Under these conditions, fitted parameters were interpreted as effective descriptors suitable for comparative analysis rather than intrinsic material constants.

In conclusion, this doctoral thesis provides a systematic and integrated investigation of GelMA mechanical behavior across multiple length scales and the associated time-dependent response. This approach contributes to a deeper understanding of how composition, scale, and relaxation mechanisms jointly shape the mechanical microenvironment experienced by cells, offering a solid foundation for the rational design and interpretation of ECM-mimetic hydrogels in tissue engineering and mechanobiology.