

Summary

Biological systems are intrinsically variable, exhibiting heterogeneity across molecular, cellular, material, and process scales. While such variability underpins adaptability and robustness, it represents a significant challenge in bioengineering contexts where reproducibility, predictability, and scalability are required. This thesis approaches biological variability not as experimental noise, but as a quantifiable and manageable property that can be studied and managed through quantitative characterization, data-driven methods, and controlled system design. The primary objective of this work was to investigate how biological variability can be characterized and managed across different bioengineering application contexts by integrating experimental models, material processing strategies, and predictive machine learning frameworks. Three distinct yet conceptually connected domains were examined: bone tissue engineering, decellularization processes for cellular agriculture, and industrial recombinant protein production.

In the context of bone tissue engineering, biological variability was investigated *in vitro* through the interaction between human mesenchymal stem cells (hMSCs), specific scaffold architectures, and a bone-like microenvironment characterized by fluid flow-induced shear stress. Homogeneous and graded three-dimensional (3D)-printed polycaprolactone (PCL) scaffolds were experimentally and computationally characterized to quantify permeability, flow field distributions, and fluid-induced shear stresses under direct perfusion conditions. Subsequently, hMSCs were cultured on the scaffolds under static and perfused regimes, and their osteogenic response was investigated using confocal microscopy and biological assays. The results demonstrated that, under perfusion culture, graded trabecular-like scaffold architecture effectively modulated transport properties and mechanical microenvironment while maintaining osteogenic performance comparable to the homogeneous design.

In the expanding field of cellular agriculture, significant variability arises from both the biological raw materials employed and the processing protocols adopted. In this context, plant-based materials are particularly attractive due to their intrinsic structural organization, wide availability, low cost, and edibility. In this work, an agitation-based decellularization strategy, employing a custom-designed sample holder, was developed and optimized for the treatment of leek tissue, with the aim of reducing process time and costs while preserving the anisotropic microarchitecture and mechanical integrity of the plant tissue. Structural, mechanical, and biochemical characterization, combined with preliminary biological tests using a fish myoblast cell line, demonstrated that process optimization was achieved, substantially reducing outcome variability while retaining key scaffold features and significantly shortening decellularization times. Overall, the proposed approach established a reproducible and scalable baseline that can be extended to other plant-derived scaffolds for cellular agriculture applications.

At the industrial scale, biological variability was investigated in terms of batch-to-batch fluctuations in recombinant protein production. A standardized upstream protein quantification workflow was established and integrated into a data-driven modeling framework. Using multi-year industrial data, the key drivers of variability were identified, and a machine-learning based model was developed to support yield prediction and constrained process optimization. This framework enabled the separation of upstream biological productivity from downstream processing effects, transforming historical production data into actionable information for practical decision support.

Overall, the research conducted during this doctoral program demonstrates that quantitative, multiscale, and data-informed approaches enable the systematic characterization and management of biological variability across diverse bioengineering domains. Rather than focusing on isolated process optimization, this work emphasizes reproducibility, robustness, and practical applicability, and proposes methodological frameworks that bridge experimental bioengineering research with translational and industrial implementation. At the same time, the results highlight that biological variability cannot be fully eliminated but can be constrained and managed within the intrinsic limits of complex biological systems.