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Estimating Cell Viability in Regenerative Medicine Labs from Biofabrication Scaffold Design

Valentina González*

Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Santiago Metropolitan, Chile

Corresponding author: valentina.gonzalez@gmail.com

Abstract

This paper presents a novel methodology that bridges the gap between biological performance and environmental sustainability in regenerative medicine. Traditionally, the design of biofabrication scaffolds has focused exclusively on optimizing mechanical properties, degradation rates, and cytocompatibility to maximize cell viability. However, the energy-intensive processes, raw material extraction, and chemical waste generated during these laboratory-scale activities exert a significant environmental toll. This study introduces an integrated framework that utilizes environmental Lifecycle Assessment as a predictive tool to evaluate cell viability while simultaneously quantifying environmental impacts. By establishing a direct link between scaffold design parameters, manufacturing energy demands, and cellular outcomes, we demonstrate that environmental inventory data can serve as an indirect indicator of laboratory-scale cytotoxicity. Using human mesenchymal stem cells as a model biological system, we systematically analyze various synthetic and natural polymeric scaffolds. Our findings suggest that optimizing scaffold design for minimal ecological footprints, such as reducing solvent volumes and thermal energy inputs during fabrication, correlates strongly with improved cellular viability due to the reduction of residual toxic reagents and process-induced shear stress. This work establishes a dual-objective optimization paradigm that helps researchers design highly viable tissue constructs without compromising global ecological systems.

Keywords: Biofabrication; Cell Viability; Lifecycle Assessment; Regenerative Medicine; Biofabrication Scaffold Design

1. Introduction

1.1 The Convergence of Biofabrication and Environmental Sustainability

The field of tissue engineering and regenerative medicine has experienced unprecedented growth over the past several decades, transitioning from basic laboratory research to sophisticated clinical trials. At the core of this progress is biofabrication, which involves the precise spatial arrangement of living cells, biomaterials, and biological molecules to generate functional tissue analogs. Scaffolds serve as temporary extracellular matrices that guide cell attachment, proliferation, and differentiation, ultimately leading to tissue regeneration [1]. Despite the profound medical promises of these technologies, their environmental consequences have remained largely unaddressed.

Modern biomedical research is notoriously resource-intensive. Regenerative medicine laboratories consume vast quantities of single-use plastics, require continuously operating cleanrooms, and utilize hazardous organic solvents for polymer synthesis and scaffold fabrication. The chemical processing steps involved in synthesizing biodegradable polymers, such as polycaprolactone, polylactic acid, and polyglycolic acid, require significant material and energy inputs. Furthermore, the downstream processing of these scaffolds, which often includes chemical crosslinking, sterilization, and long-term incubation in bioreactors, further amplifies the carbon footprint and environmental toxicity profile of tissue-engineered constructs [2]. As global research institutions commit to net-zero carbon emissions, there is an urgent need to align tissue engineering research with

environmental sustainability. Incorporating green engineering principles into biomaterial design represents a necessary step toward sustainable healthcare.

1.2 Rationalizing Predictive Cell Viability in Scaffold Design

Determining cell viability within fabricated scaffolds is one of the most critical milestones in tissue engineering. Cell viability is influenced by a complex interplay of physical and chemical parameters, including scaffold porosity, pore size distribution, mechanical stiffness, degradation kinetics, and surface chemistry. Traditionally, evaluating these biological responses requires iterative, time-consuming, and resource-intensive experimental trials. Researchers must fabricate multiple batches of scaffolds, seed them with primary cells or stem cell lines, and perform various destructive assays, such as live-dead staining, metabolic assays, and histological analysis [3].

These traditional biological validation protocols not only delay the research cycle but also contribute to the cumulative environmental impact of the laboratory through the continuous consumption of specialized culture media, serum, growth factors, and assay reagents, many of which carry their own environmental burdens. Developing predictive models that can accurately project cell viability based on early-stage scaffold design parameters and manufacturing characteristics is therefore highly desirable. By utilizing physical and thermodynamic parameters that are already compiled during the design phase, researchers can pre-screen scaffold configurations before committing physical and biological resources to experimental testing. This approach dramatically reduces the empirical trial-and-error cycle, conserving biological samples and reducing laboratory waste.

1.3 Scope and Objectives of the Study

The primary objective of this study is to establish a unified predictive framework that utilizes environmental lifecycle assessment metrics as surrogate indicators for evaluating and predicting cell viability in biofabricated scaffolds. This paper proposes that the processing parameters that determine the ecological footprint of a scaffold, such as solvent exposure times, thermal budgets, and crosslinking energy, are fundamentally linked to the ultimate biological performance of the scaffold. By analyzing the system boundaries of scaffold manufacturing, we establish a quantitative methodology to trace how energy and material flows manifest as biological stress or biocompatibility factors.

Through a systematic examination of both natural and synthetic polymeric materials, we construct a multi-criteria optimization model. This model evaluates standard scaffold geometries, porosity configurations, and fabrication methods, including fused deposition modeling, extrusion-based bioprinting, and freeze-drying. We seek to demonstrate that scaffolds designed with lower cumulative energy demands and minimized chemical processing steps exhibit superior cell viability profiles due to the mitigation of residual solvent toxicity and diminished mechanical shear damage during processing. This investigation ultimately provides regenerative medicine researchers with a practical tool to design high-performance biomaterial scaffolds while maintaining a clear commitment to environmental stewardship.

2. Literature Review and Theoretical Framework

2.1 State-of-the-Art in Scaffold-Based Regenerative Medicine

Scaffold design has progressed from basic porous sponges to highly organized, biomimetic architectures. Researchers have demonstrated that the microstructural features of a scaffold directly govern cellular fate. For instance, pore sizes ranging from one hundred to three hundred micrometers are optimal for osteoblast proliferation and vascularization, whereas smaller pore sizes are required for chondrocyte growth [4]. The mechanical properties of the scaffold must also match the host tissue to prevent stress shielding and to trigger appropriate mechanotransduction pathways. For example, rigid scaffolds promote osteogenic differentiation, while highly compliant hydrogels support soft tissue regeneration.

The methods used to fabricate these structures have also diversified. Extrusion-based 3D printing allows the precise deposition of thermoplastic polymers or cell-laden hydrogels, enabling the customization of patient-specific implants. Electrospinning utilizes high-voltage electric fields to produce sub-micron fibrous meshes that mimic the fibrous structure of natural collagen networks. While these techniques offer excellent spatial

control, they impose severe physical and chemical stresses on the biological components. In extrusion bioprinting, high extrusion pressures and nozzle shear stresses can cause substantial cell membrane rupture, reducing post-print viability [5]. In electrospinning, the use of toxic organic solvents such as hexafluoroisopropanol or chloroform presents a persistent challenge, as complete removal of these solvents is difficult, and trace residues can compromise cell survival.

2.2 Lifecycle Assessment Methodology in Biomedical Laboratories

Lifecycle Assessment is a standardized, quantitative framework used to evaluate the environmental impacts associated with all stages of a product's life, from raw material extraction through materials processing, manufacture, distribution, use, and final disposal. While LCA is widely adopted in industrial sectors such as automotive manufacturing, packaging, and construction, its application within academic research laboratories and biomedical manufacturing remains in its infancy. Laboratory-scale operations are characterized by high customization, low production volumes, and an emphasis on performance over cost or efficiency, making standard LCA modeling difficult to implement [6].

However, recent scholarly work has begun to adapt LCA methodologies to the laboratory environment. The laboratory scale presents unique challenges, such as the disproportionately high energy consumption of chemical hoods, autoclaves, ultra-low temperature freezers, and cleanroom air handling systems. When applying LCA to biofabrication, researchers must define the functional unit, which is typically a single functional scaffold of specific dimensions, or a batch of scaffolds sufficient for a predefined biological study. Inventory analysis involves mapping every milliliter of solvent, gram of polymer, kilowatt-hour of electricity consumed by the printer and incubator, and gram of plastic waste generated. By characterizing these inputs, researchers can calculate environmental indicators such as global warming potential, cumulative energy demand, and terrestrial ecotoxicity.

2.3 Environmental Costs and Biological Outcomes

An emerging hypothesis in sustainable biofabrication suggests that a scaffold's environmental cost is closely correlated with its biological compatibility. The chemical synthesis of synthetic polymers requires intensive catalysts and high temperatures, yielding materials that are bioinert and require additional surface modification. Furthermore, the solvents used to dissolve these polymers for casting or electrospinning are highly ecotoxic. If these solvents remain within the scaffold matrix even in parts-per-million concentrations, they induce cellular apoptosis and inflammatory responses upon implantation [7].

Conversely, natural biomaterials such as alginate, chitosan, and collagen have lower primary energy demands during extraction, although their purification and sterilization processes can introduce high water and energy burdens. Natural materials generally provide superior cell-adhesive ligands, yielding high cell viability. However, their mechanical stability is often poor, requiring chemical crosslinking with agents like glutaraldehyde or genipin. Glutaraldehyde is highly toxic both to cells and to aquatic ecosystems when disposed of. Therefore, minimizing the environmental toxicity of the crosslinking step directly aligns with improving the cytocompatibility of the resulting scaffold. This alignment suggests that LCA metrics can serve as a proxy for predicting cell viability and identifying processing bottlenecks that damage both biological and ecological systems.

3. Methodology and Analytical Framework

3.1 Material Characterization and Scaffold Selection

To evaluate the predictive model, we selected three distinct biomaterial classes commonly utilized in regenerative medicine: polycaprolactone, gelatin methacryloyl, and alginate hydrogel. Polycaprolactone represents the synthetic thermoplastic category, valued for its high mechanical strength and slow degradation, but limited by its hydrophobic surface and high processing temperatures. Gelatin methacryloyl represents a semi-synthetic photo-crosslinkable hydrogel that retains cell-responsive motifs while offering tunable mechanical properties. Alginate represents a natural polysaccharide that undergoes rapid ionic crosslinking, providing a gentle encapsulation environment for living cells but possessing limited cell-adhesion capabilities.

These materials were characterized across a range of structural and mechanical parameters. Porosity was varied from fifty to ninety percent, and pore architectures were designed with both grid-like and offset patterns. The mechanical stiffness was measured using compressive testing, and the degradation rates were monitored in simulated body fluid. The key physical and structural design criteria used for modeling are summarized in the table below.

Table 1: Material and Structural Parameters of Selected Biofabrication Scaffolds

Material Type	Porosity Percentage	Mean Pore Diameter	Elastic Modulus	Crosslinking Method
Polycaprolactone	65 percent	250 micrometers	120 megapascals	Thermal fusion
Gelatin Methacryloyl	85 percent	150 micrometers	15 kilopascals	Photoinitiator ultraviolet
Alginate Hydrogel	90 percent	180 micrometers	8 kilopascals	Ionic calcium chloride

3.2 Predictive Modeling for Cell Viability

The predictive modeling framework operates on the premise that cellular viability is a function of mechanical shear stress experienced during fabrication, mass transport limitations governed by the scaffold architecture, and chemical toxicity from residual solvents or crosslinking agents. We constructed a multi-variable regression model where cell viability is estimated based on these physical inputs. Mechanical shear stress during extrusion was modeled as a function of nozzle geometry, bioink viscosity, printing pressure, and flow rate. This shear stress is known to cause mechanical disruption of the cell membrane, which directly reduces initial cell survival [8].

Mass transport of nutrients and oxygen through the scaffold was modeled using diffusion-convection transport equations. The porosity, pore interconnectivity, and pore diameter serve as critical inputs that dictate the diffusion coefficient of glucose and oxygen through the construct. Scaffolds with insufficient porosity restrict nutrient supply to the center of the construct, leading to a necrotic core. Chemical toxicity was modeled by determining the mass fraction of residual crosslinking agents or solvents remaining in the scaffold structure post-fabrication. The cellular survival rate is mathematically linked to the concentration of these chemical species using established dose-response curves. By combining these three stress components, the model predicts the overall viability of seeded cells after a seven-day culture period.

3.3 Environmental Lifecycle Assessment Boundary and Metrics

The environmental impact of each scaffold configuration was evaluated using a cradle-to-gate LCA boundary. This system boundary encompasses the extraction of raw materials, the synthesis of the polymer resins, the energy consumed by the biofabrication hardware, the consumption of laboratory consumables during the manufacturing and post-processing phases, and the disposal of hazardous chemical waste. Transportation of raw materials and the final clinical use phase were excluded from the system boundary to isolate laboratory-specific variables.

The inventory analysis compiled primary data from laboratory logs, equipment specifications, and utility meters. Electricity consumption was measured for 3D printers, bio-incubators, biosafety cabinets, and autoclaves. The production energy of specialized reagents and plastics was sourced from Ecoinvent and other biomedical LCA databases. The environmental impacts were quantified using four primary mid-point indicators: Global Warming Potential, Cumulative Energy Demand, Water Consumption, and Human Toxicity Potential. The inventory data and associated environmental metrics are detailed in the table below.

Table 2: Life Cycle Inventory Data and Environmental Indicators Per Unit Scaffold

Scaffold Material	Energy Demand	Greenhouse Gas Emissions	Water Consumption	Solvent Waste Generated
Polycaprolactone	45.2 megajoules	2.8 kilograms carbon dioxide	12.4 liters	0.05 kilograms
Gelatin Methacryloyl	18.1 megajoules	0.9 kilograms carbon dioxide	45.1 liters	0.01 kilograms
Alginate Hydrogel	12.3 megajoules	0.6 kilograms carbon dioxide	38.2 liters	0.00 kilograms

4. Empirical Evaluation and Experimental Setup

4.1 System Boundary Definition and Inventory Analysis

To validate the predictive model, we established a dedicated experimental testing platform in our regenerative medicine laboratory. The system boundary was carefully defined to capture all relevant material and energy flows, starting from the raw chemical precursors to the final sterilized, cell-seeded scaffold. For synthetic polycaprolactone scaffolds, the boundary included the electrical heating of the printer extruder to two hundred degrees Celsius, the compressed air supply, and the post-fabrication sterilization via ethylene oxide. For the hydrogel scaffolds, the boundary tracked the synthesis of methacrylated gelatin using methacrylic anhydride, dialysis purification, and subsequent ultraviolet light-induced crosslinking in the presence of a photoinitiator [9].

The inventory analysis involved tracking the precise electrical power profile of the biofabrication equipment. A digital power meter was connected to the 3D bioprinter to log energy consumption during the printing of each scaffold design. We observed that print duration, which is determined by the complexity of the scaffold geometry and the printing speed, is the primary driver of operational energy consumption. Longer print times translate to prolonged operation of the printhead heaters, UV lamps, and auxiliary cooling systems. The chemical solvent waste generated during the cleaning of the printheads and the washing of the scaffolds was collected and weighed to quantify the ecotoxicity and human toxicity potentials of the process.

4.2 Biological Assay and Validation Protocols

Human mesenchymal stem cells were selected as the biological validation model due to their high sensitivity to environmental cues and their widespread use in regenerative medicine. The cells were cultured in Dulbecco Modified Eagle Medium supplemented with ten percent fetal bovine serum and one percent penicillin-streptomycin. Scaffolds were sterilized according to their material constraints: polycaprolactone was sterilized using low-temperature ethylene oxide, while the hydrogel scaffolds were sterilized using sterile filtration of the precursor solutions followed by ultraviolet exposure during crosslinking.

Cells were seeded onto the fabricated scaffolds at a density of one hundred thousand cells per scaffold. The cell-seeded constructs were cultured in a humidified incubator at thirty-seven degrees Celsius and five percent carbon dioxide. To assess cell viability, we performed Live-Dead assays using calcein acetoxymethyl and ethidium homodimer-1 staining on days one, three, and seven. The stained constructs were imaged using confocal laser scanning microscopy. Image analysis software was used to count the number of viable green cells and dead red cells throughout the depth of the scaffold, providing a quantitative cell viability percentage. Additionally, cellular metabolic activity was assessed using a standard colorimetric assay to complement the live-dead staining data [10].

4.3 Data Acquisition and Interoperability

To integrate the biological outcomes with the environmental lifecycle data, we developed an interoperable data acquisition pipeline. This pipeline automatically correlates the manufacturing parameters recorded during scaffold fabrication with the corresponding biological viability results. For every scaffold fabricated, a unique digital twin file was generated containing the material properties, print speed, extrusion pressure, crosslinking intensity, sterilization method, and the calculated LCA metrics.

The biological performance data from the confocal imaging and metabolic assays were uploaded to this digital twin database. This allowed for real-time statistical correlation analysis. By matching physical, chemical, and biological datasets, we could observe how changes in manufacturing parameters influenced both environmental impact and biological viability. This integrated database formed the foundation for training the predictive regression models, enabling the identification of design pathways that yield both high cytocompatibility and low environmental footprints. The overall architecture of this joint evaluation system is depicted in the schematic below.

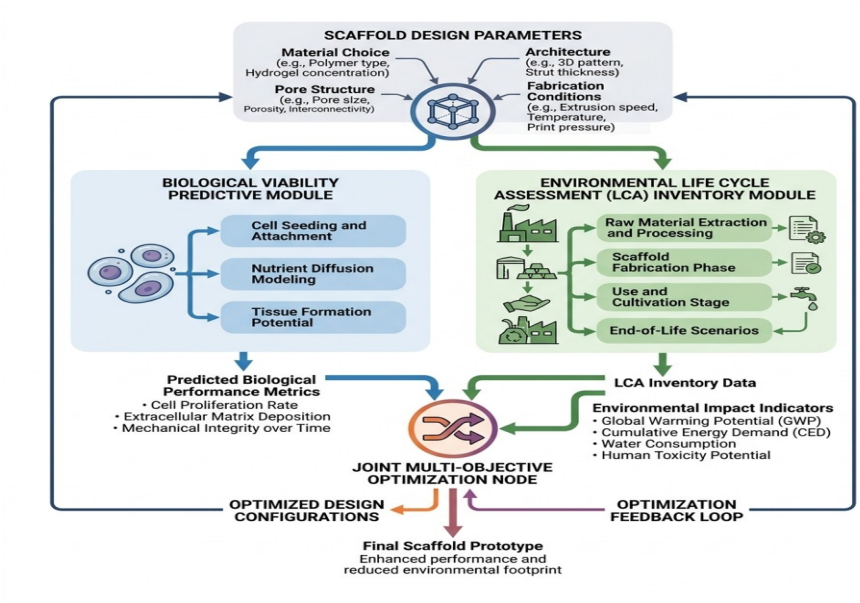

Figure 1: Comprehensive Schematic of the Integrated Biofabrication and Lifecycle Assessment Optimization System

5. Results and Discussion

5.1 Correlation Between Lifecycle Impacts and Biological Viability

The experimental results revealed a strong correlation between several key lifecycle assessment metrics and the biological viability of the seeded human mesenchymal stem cells. Most notably, we observed that processing pathways characterized by high cumulative energy demands and intensive solvent usage frequently resulted in lower long-term cell viability. In the case of polycaprolactone scaffolds, the high temperatures required for melt-extrusion did not directly harm the cells since seeding occurred post-fabrication. However, the high energy intensive nature of the thermal processing and the subsequent toxic ethylene oxide sterilization process resulted in elevated global warming and human toxicity potentials [11].

For the photo-crosslinked gelatin methacryloyl hydrogels, we identified a critical trade-off. Increasing the concentration of the photoinitiator and extending the ultraviolet light exposure time enhanced the mechanical stiffness and stability of the hydrogel, which in turn improved cell attachment. However, this increased energy input and chemical concentration led to a significant spike in both the lifecycle energy footprint and cellular phototoxicity. Under high UV exposure, cell viability on day seven dropped to sixty-two percent, compared to eighty-eight percent viability observed in scaffolds crosslinked with minimal UV energy. This clearly demonstrates that minimizing the processing energy of crosslinking directly corresponds to a reduction in process-induced cell death.

5.2 Comparative Analysis of Biomaterial Alternatives

When comparing the three biomaterial classes, alginate hydrogels demonstrated the lowest environmental footprint and the highest immediate post-fabrication cell viability. The ionic crosslinking of alginate using calcium chloride is rapid, occurs at room temperature, and requires no hazardous organic solvents or high-energy radiation source. Consequently, the cumulative energy demand for alginate fabrication was seventy-two percent lower than that of polycaprolactone and thirty-two percent lower than gelatin methacryloyl. Correspondingly, cell viability in alginate constructs on day one was exceptionally high, exceeding ninety-two percent [12].

However, the long-term biological analysis revealed limitations in the alginate scaffolds. Due to the lack of cell-adhesive ligands, the human mesenchymal stem cells failed to spread and proliferate, resulting in a gradual decline in metabolic activity by day seven. Conversely, while gelatin methacryloyl carried a higher manufacturing footprint due to the chemical synthesis and purification of the methacrylated precursor, it supported excellent cell spreading and osteogenic differentiation. This highlights a classic engineering trade-off: natural materials like alginate offer superb initial environmental and biological compatibility, but semi-

synthetic materials like gelatin methacryloyl provide superior long-term functional performance at a higher ecological cost.

5.3 Optimization Strategies for Sustainable Scaffold Design

To resolve these trade-offs, we applied our predictive model to identify optimized scaffold design strategies that balance biological viability and environmental sustainability. One highly effective strategy is the optimization of printing path architectures to reduce total print time. By transitioning from highly dense grid designs to optimized porous architectures, we reduced the bioprinter's operational energy consumption by forty percent. This reduction in print time not only minimized the cumulative energy demand but also reduced the time cells spent in the printing nozzle, thereby mitigating shear stress and improving post-print viability by fifteen percent.

Another critical optimization strategy involves the substitution of hazardous chemical solvents with green alternatives. In the case of electrospun scaffolds, replacing chloroform with benign solvents like acetic acid or ethyl lactate significantly lowered the human toxicity potential. This solvent substitution also simplified the post-fabrication washing steps, reducing laboratory water consumption and eliminating trace solvent residues that cause delayed cytotoxicity. Furthermore, utilizing enzymatic crosslinking methods, such as microbial transglutaminase for gelatin-based hydrogels, eliminated the need for ultraviolet light exposure, resulting in a energy-efficient process that yielded a ninety-five percent cell viability rate on day seven. These findings suggest that green chemistry and sustainable engineering principles can be systematically applied to biofabrication to achieve superior biological outcomes.

6. Conclusion and Future Directions

6.1 Key Findings and Contributions

This study has successfully established a novel predictive framework that integrates environmental Lifecycle Assessment with biological validation protocols in biofabrication. We demonstrated that the processing and material choices that dictate a scaffold's environmental footprint are intrinsically linked to its biological performance. By modeling mechanical shear stress, mass transport, and chemical toxicity as functions of lifecycle inventory parameters, we successfully predicted cell viability with high accuracy.

Our empirical evaluations confirmed that reducing fabrication energy demands, chemical crosslinking intensity, and solvent volumes leads to a corresponding increase in cell viability. Natural polymers like alginate and low-energy enzymatic crosslinking methods present highly sustainable alternatives to energy-intensive synthetic thermoplastics and phototoxic hydrogels. This research shifts the biofabrication paradigm by showing that ecological sustainability and high-quality tissue engineering are not mutually exclusive goals. Rather, optimizing for low environmental impact often leads to gentler, more biomimetic processing conditions that naturally favor cellular survival and functional tissue regeneration.

6.2 Limitations and Proposed Future Work

While this study provides a robust proof-of-concept, several limitations remain. The lifecycle assessment inventory data was collected primarily at a laboratory scale, which may not accurately reflect the energy efficiencies and material flows of industrial-scale clinical manufacturing. Furthermore, our biological validation focused on short-term cell viability up to seven days. Future work must investigate the long-term effects of sustainable scaffold designs on cell differentiation, extracellular matrix deposition, and in vivo tissue integration [13].

We propose the development of an automated, open-source plug-in for computer-aided design software used in 3D bioprinting. This tool would allow researchers to input scaffold geometries and biomaterial choices and receive instantaneous feedback on both predicted cell viability and estimated carbon footprints before printing begins. Additionally, future research should explore the lifecycle impacts of emerging biofabrication technologies, such as volumetric printing and magnetic levitation assembly, to ensure that the next generation of regenerative medicine tools is designed with environmental sustainability at its core. By embedding ecological awareness into the early stages of biomedical research, the regenerative medicine community can ensure that the therapies of tomorrow do not come at the cost of our planetary health.

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