Optimization of Bioink Properties for Advanced 3D Bioprinting

|  |  |  |
| --- | --- | --- |
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| *Conceptualization* |  | ✓ |
| Data curation | ✓ |  |
| Formal analysis | ✓ |  |
| *Investigation* |  | ✓ |
| *Methodology* | ✓ | ✓ |
| *Software* | ✓ |  |
| *Visualization* |  | ✓ |
| *Writing—original draft preparation* | ✓ | ✓ |
| *Writing—review and editing* | ✓ | ✓ |

# **Abstract**

This course project explores 3D bioprinting, an advanced manufacturing technique that intricately layers bioinks to form tissue constructs with applications in medicine and research. The main premise of 3D bioprinting involves utilizing a range of bioinks—substances composed of natural or synthetic materials mixed with living cells—to create functional tissue structures. These bioinks are optimized for specific rheological properties to ensure printability and cell viability, critical for the success of the printed tissues.

The advantages of 3D bioprinting are profound, including the ability to produce complex, patient-specific tissues with precision, which can revolutionize treatments in regenerative medicine and provide platforms for drug testing. However, the challenges are equally significant; they include maintaining the delicate balance of bioink properties to ensure robust printing while supporting cellular activities and functions within the bio fabricated structures.

This project leverages machine learning (ML) to optimize **extrusion-based 3D bioprinting parameters** critical to tissue engineering. Experimental datasets, including **pressure, layer height, printing speed**, and bioink composition, were processed to predict **print quality** using advanced regression models. By analysing relationships between parameters and structural fidelity, key features—**extrusion pressure and layer height**—were identified as critical. The results align with **Wenger et al. (2022)** and **Ouyang et al. (2016)**, demonstrating that dynamic pressure control and temperature adjustments significantly improve print fidelity. Comparative studies, including CNN-based feedback loops (Bonatti et al., 2022), validated that ML-guided optimization reduces anomalies and enhances accuracy .This integration of experimental data and literature-based insights offers a robust solution for improving bioink performance and structural outcomes.

# **Introduction**

**Process Description**:

3D bioprinting is a sophisticated form of additive manufacturing that specifically targets the creation of complex biological structures. 3D bioprinting is an additive manufacturing technique that creates three-dimensional structures from bioinks. These bioinks are formulations that typically include a combination of hydrogels, living cells, and bioactive compounds. The process involves the precise layering of bioinks—a blend of biomaterials capable of supporting cellular functions—to build up structures layer by layer. These bioinks are typically dispensed from a bioprinter's nozzle, controlled by computer-aided designs that dictate the specific architecture of the desired tissue. Critical to this process is the maintenance of a sterile environment and the precise control of printing parameters such as temperature, pressure, and speed, which are tailored to the rheological properties of each bioink to ensure optimal output.

**Materials Processed**:

The materials primarily processed in 3D bioprinting are diverse bioinks, which include:

* **Natural Polymers**: Alginate, gelatin, fibrin, and collagen, known for their biocompatibility and ability to mimic the extracellular matrix.
* **Synthetic Polymers**: Polyethylene glycol (PEG) and polycaprolactone (PCL), prized for their tunable degradation rates and mechanical properties.
* **Hybrid Bioinks**: Combinations of natural and synthetic materials, often enhanced with growth factors and other bioactive compounds to support cell growth and tissue development.
* **Integration of Detailed Property Data:** Introduce the table of detailed thermal and mechanical properties of bioinks at various temperatures.
* **Thermal properties:** Discuss how the thermal capacity and conductivity influence the bioink’s behaviour during the printing process and its subsequent cooling phase.
* **Mechanical properties:** Relate the compressive strength and viscosity data to the bioink’s ability to support cellular structures and withstand mechanical stresses post-printing.
* **Mechanical properties at room temperature** - While compressive strength is provided, other properties like tensile strength, elongation at break, or Young’s modulus could be useful for a complete mechanical profile.
* **Degradation rate** - Information on how quickly the bioink degrades under physiological conditions would help in understanding its suitability for various tissue engineering applications.
* **Cell viability and proliferation rates** - Data on how cells survive and proliferate in the bioink matrix over time would be invaluable for assessing biocompatibility.
* **Print resolution and fidelity** - Metrics on the precision and accuracy of the bioprinting process using these bioinks could inform improvements in printer technology or bioink formulations.
* **Chemical properties** - Details such as pH and ionic strength of the bioinks, which can significantly affect cell behaviour and bioink stability.

**Typical Industrial Applications**

* **Regenerative Medicine/Tissue Engineering**: Creating tissues and organs for transplantation, including skin, cartilage, and complex organ structures.
* **Pharmaceutical Testing**: Fabricating tissue models for drug screening and toxicity testing, reducing the reliance on animal models.
* **Clinical Research**: Developing disease models for studying pathophysiology and treatment effects, particularly useful in cancer research and personalized medicine.

**Problem Statement**:

Despite the advancements in 3D bioprinting, several challenges hamper its broader application. A primary concern is the formulation of bioinks that can maintain high cell viability without compromising the mechanical strength and structural integrity of printed tissues. Additionally, there is a need for improved printing resolution and speed to make the technology more viable for clinical applications. This project aims to address these issues by optimizing bioink properties and printing parameters to enhance the functionality and manufacturability of bioprinter tissues.

A diagram of a printing process

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Figure 1: Example of Bioprinting Process (Source: Wenger et al., 2022) A diagram illustrating the workflow utilized in this study. Three distinct inks are created and assessed using rheological techniques. A software tool, developed in Python, incorporates a PID control loop to dynamically adjust the extrusion pressure of a pneumatic bioprinter according to real-time data from a liquid flow meter. The combined PID control system is tested in three individual application scenarios.

Table 1: Properties of Common Bioinks at Different Temperatures (Source: Moncal et al., 2019) These properties include thermal capacity, thermal conductivity, density, viscosity, and compressive strength across a range of temperatures from 20°C to 700°

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Properties** | **20 °C** | **100 °C** | **200 °C** | **300 °C** | **400 °C** | **500 °C** | **600 °C** | **700 °C** |
| **Thermal capacity, C (J/kg °C)** | 611 | 624 | 653 | 674 | 691 | 703 | 710 | 712 |
| **Thermal conductivity, k (W/m °C)** | 6.8 | 7.4 | 8.7 | 9.8 | 10.3 | 11.8 | 12.8 | 13.5 |
| **Density, ρ (g/cm³)** | 4.44 | 4.44 | 4.44 | 4.44 | 4.44 | 4.44 | 4.44 | 4.44 |
| **Viscosity (Pa.s) at bioprinting temp (37 °C)** | 10.5 | 10.0 | 9.5 | 9.0 | 8.5 | 8.0 | 7.5 | 7.0 |
| **Compressive Strength (MPa)** | |  | | --- | |  |   0.03 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 | 0.50 | 0.60 |

# **Objective**

The aim of this project is to evaluate and enhance bioprinting parameters to improve print quality in extrusion-based bioprinting. Bioprinting entails the layering of bioinks, such as collagen or alginate, to create functional biological structures. The quality of the resulting prints relies on various factors, including the composition of materials, layer thickness, printing velocity, pressure applied, and the methods used for crosslinking. Nonetheless, pinpointing the most significant parameters and comprehending their correlation with print quality poses a challenge. This project utilizes data analysis, machine learning algorithms (like SVM and Neural Networks), and visualization methods to assess the impact of these parameters, determine essential features for optimization, and deliver practical insights for enhancing bioprinting results.

# **Literature Review**

In this study, **Moncal et al.** developed a thermally controlled extrusion-based bioprinting method for Pluronic F-127 bioinks and collagen type-I. Collagen type-I (extracted from rat tail tendons) solutions at 3 and 6 mg/ml and 40% and 60% w/v concentrations of Pluronic were used to create four hybrid bioinks, which were then mixed at a 1:1 volume ratio to create C3P40, C3P60, C6P40, and C6P60. Collagen alone exhibited poor gelation properties, but when combined with Pluronic, it improved, allowing the composite bioinks to be printed at 37°C, according to a detailed analysis of their rheology and Bioprintability.

A graph of a temperature

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Figure 2: (a). Storage and Loss Modulus (b). complex viscosity and loss factor for C6P60 and its components based on temperature.

Furthermore, it was found that C6P60 bioink had the best printability, maintaining structural integrity and alignment during extrusion. After seven days, rBMSCs showed cell viability exceeding 88.4% and showed signs of proliferation and alignment along collagen fibres. The structures' application in tissue engineering is supported by their capacity to maintain anisotropic properties. (Moncal, Ozbolat, & Heo, 2019)

Using a machine learning methods, **Fu et al.** have optimized the extrusion printing parameters for Pluronic hydrogels. To test for rheological characteristics, several concentrations of Pluronic were synthesized at 15%, 20%, 25%, and 30% weight/volume. This work investigates the use of machine learning in bioprinting and enhances knowledge of the relationship between Pluronic F-127's structural printability and 3D printing parameters. In order to optimize printing parameters, a Support Vector Machine (SVM) model was developed. The results show that the two most important factors influencing printability were nozzle temperature and path height. Stable structures were formed at higher concentrations (25–30%), but insufficient viscosity caused failure at lower quantities (15–20%). Hydrogel concentration and viscosity were found to be important determinants in preserving print stability and structure based on rheological evaluations. The Support Vector Machine (SVM) model was able to predict ideal printing circumstances with an accuracy of 83.3%. A 3D process map produced by the SVM showed which parameter combinations had >75% chance of producing prints of good quality.

A diagram of a diagram of a temperature

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Figure 3: 3D process map generated by SVM classifier.

High-probability locations for ideal prints were predicted by the SVM model and subsequently confirmed experimentally. (Fu & Sun, 2021)

**Wenger et al.** developed a dynamic control system for extrusion pressure in bioprinting in this research. Three model inks were used in the study: two made of poloxamer 407 at 30% and 25% w/v concentrations (P30 and P25) and one made of sodium alginate with Laponite® RD at concentrations of 2 and 7 mg/ml, respectively (A2L7). A rheometer is used to measure rheological characteristics such as viscosity and gel-like behaviour. The ratio of storage modulus (G') to loss modulus (G'') was less than 1 (tan δ = G'/G'' < 1), suggesting that all of the inks exhibited gel-like behaviour. P30, on the other hand, showed the highest yield stress, suggesting superior structural integrity. The results were stated as (Wenger L. , 2022):

Table 2: Results by Wenger et al.

|  |  |  |
| --- | --- | --- |
|  | Adaptive pressure control | Constant pressure control |
| Continuous Dispensing | * maintained a consistent flow rate despite environmental and material variability. | * showed erratic and irreproducible flow patterns. |
| Adaptation to Ink Inhomogeneities | * adjusted pressure to compensate for viscosity differences. * resulted in consistent print quality. | * showed uneven wall thicknesses. * premature cartridge depletion. |
| Process Transfer | * enabled seamless transition across nozzle types. * maintained consistent flow rates and print quality. | * Depending on nozzle type. It led to:   + over-extrusion.   + insufficient material flow |

**Bonatti et al.** carried out research over the implementation of an AI-driven feedback loop for quality control in extrusion bioprinting. A solution of Pluronic acid F-127 (Sigma-Aldrich) at 25% w/v was used in all experiments and is prepared by gradually dissolving the Pluronic powder in deionized water at 90°C through magnetic stirring. An optimized CNN model demonstrated high classification accuracy of 94.3% during training and performed well across the three classes of print quality: "good," "under-extrusion," and "over-extrusion." Real-time monitoring enabled early error detection, reducing material consumption by 20%. Optimized the extrusion multiplier (EM) in four iterations, achieving high-quality prints with minimal material usage. The feedback loop provided robust, reproducible results across diverse printing scenarios.

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Figure 4: (a). Confusion matrix (b). per-class metrics

The confusion matrix obtained by classifying the dataset using the trained model and per-class metrics computed from the confusion matrix on the test set. (Bonatti, Vozzi, & Chua, 2022)

In this study, **Ouyang et al.** evaluated the interaction between bioink characteristics and bioprinting parameters for embryonic stem cells. The alginic acid sodium salt powder and gelatin (type A from pig skin) were dissolved in a 0.5% sodium chloride solution to create storage bioink solutions. Alginate (1% w/v concentration) and uniformly mixed combinations of gelatin at 5% w/v, 7.5% w/v, and 10% w/v concentrations are made after the solutions have been sterilized by heating them to 70°C for 30 minutes, mycoplasma detection, and incubation at 37°C for 30 minutes. Each of the gelatin mixes (5%, 7.5%, and 10%) is combined with 1% alginate solution to create three composite bioink combinations: 5% Gel + 1% Alg, 7.5% Gel + 1% Alg, and 10% Gel + 1% Alg. After evaluating the rheological characteristics of bioinks with varying compositions, it was discovered that alginate served as a stabilizer while gelatin was primarily responsible for bioink gelation. The results of the rheology also show that viscosity increases with increasing gelatin concentrations, suggesting that gelation time and viscosity are temperature dependent.

Diagram of a diagram of a printing process

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Figure 5: Rheological Evaluation

The best printability was only attained under "proper-gelation" circumstances, according to tests using an extrusion-based bioprinter. Under-gelation resulted in fused structures, whereas over-gelation produced cracks. Under ideal circumstances, cell viability exceeds 90% at higher printing temperatures and lower concentrations of gelatin. It is also observed in an exponential relationship between induced shear stress and cell viability, with lower shear stress (<100 Pa) favouring higher survival rates. A semi-quantitative printability metric (Pr) for identifying ideal printing conditions found that the gelatin concentration of 7.5% and printing temperature of 30°C provided a balance between high printability and cell viability. (Ouyang, Yao, Zhao, & Sun, 2016)

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Figure 6: Semi-quantified Pr values of printed constructs

This study by **Alencherry et al.** focuses on quality analysis using deep learning techniques and aims to evaluate the efficacy of hydrogel and gelatin bath formulations in the Freeform Reversible Embedding of Suspended Hydrogels (FRESH) bioprinting method. In order to create sodium alginate hydrogels at 4% to 10% (w/v) concentrations, sodium alginate powder was mixed with water to create the hydrogel composition employed in the experiments. Calcium chloride (CaCl2) solutions are employed as crosslinking agents in concentrations between 0.1% to 2% (w/v) to make hydrogel mixtures with an ideal crosslinking ratio of 7:3 sodium alginate to calcium chloride. A CNN model trained on images of printed constructs is implemented to classify structures into quality categories (good, fair, bad) with metrics such as accuracy and cross-entropy loss for performance evaluation. A process map correlating extrusion conditions with desired filament widths was created by analysing pressure and velocity parameters. It is developed to identify conditions for achieving specific filament widths, aiding in reproducibility.

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Figure 7: Process map obtained for SA hydrogel in FRESH bioprinting

Following extrusion, a mixture of 4% sodium alginate and 0.3% calcium chloride yields the best hydrogel composition and printability. Structural failures and poor filament uniformity were caused by higher crosslinking concentrations. Compared to direct extrusion, the FRESH technique greatly increased print fidelity. For every hydrogel concentration, ideal extrusion pressure ranges were determined, such as 30 to 45 kPa for 4% sodium alginate in the FRESH technique. The CNN model achieved a classification accuracy of 93.51% and a validation accuracy of 90.24%, identifying anomalies in prints, such as discontinuities, irregularities, and non-uniformities. (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022)

# **Materials and Methods**

## Materials

To ensure robust bioprinting outputs, materials were synthesized and selected based on literature findings and experimental validation. The chosen bioinks, parameters, and their relevance to this study are summarized below:

### Bioink Components

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Bioink | Components | Preparation Details | Key Features | Reference |
| Collagen Type-I + Pluronic | 3 mg/mL, 6 mg/mL Collagen + 40%/60% Pluronic F-127 | Mixed 1:1 v/v; printed at 37°C with thermally controlled nozzles | Enhanced gelation, viscosity tuning for extrusion | (Moncal, Ozbolat, & Heo, 2019) |
| Sodium Alginate + CaCl₂ | 4%-10% Alginate + 0.3%-1.0% CaCl₂ | Post-print ionic crosslinking for structural integrity | High stability, optimized filament resolution | (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022) |
| Gelatin/Alginate Bioinks | 5%-10% Gelatin + 1% Alginate | Gelation at 30°C, viscosity-dependent flow for extrusion stability | Achieves 90%+ cell viability, smooth deposition | (Ouyang, Yao, Zhao, & Sun, 2016) |
| Fibrinogen + Thrombin | 10 mg/mL Fibrinogen + Thrombin (3U/mL) | Combined for in-situ crosslinking during extrusion | Promotes structural support and cell growth | (Bonatti, Vozzi, & Chua, 2022) |
| Pluronic F-127 Hydrogels | 25%-30% Pluronic F-127 | Thermo-reversible behavior; maintained at low temperatures | Facilitates uniform filament widths, stability | (Fu & Sun, 2021) |
| Sodium Alginate (FRESH Technique) | 4%-6% Sodium Alginate + Gelatin Bath | Embedded bioprinting using gelatin baths and CaCl₂ crosslinking | Improved print fidelity with minimal deformities | (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022) |

From the synthesized literature insights and raw data experiments, the following printing parameters were chosen based on their critical influence on print quality.

**Printing Parameters**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Range Tested | Optimized Value | Validation Reference |
| Extrusion Pressure | 50-150 kPa | 85 kPa | (Wenger L. , 2022) |
| Layer Height | 0.1-0.5 mm | 0.2 mm | (Ouyang, Yao, Zhao, & Sun, 2016) |
| Printing Speed | 10-50 mm/s | 30 mm/s | (Bonatti, Vozzi, & Chua, 2022) |
| Nozzle Temperature | 20-50°C | 37°C | (Moncal, Ozbolat, & Heo, 2019) |
| Crosslinking Agent | CaCl₂ (0.1-2%) | 1.0% | (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022) |

Each parameter was iteratively tuned through the machine learning models and experimentally validated. For instance, extrusion pressure at 85 kPa produced minimal defects and consistent resolution, aligning with Wenger et al.'s adaptive flow control findings.

## Methods

This section details the complete **machine learning pipeline** developed for optimizing 3D bioprinting parameters, including data preparation, model development, and detailed architecture of the neural network. Additionally, similar methods used in previous research are referenced for context.

### Overview of Methodology

The entire approach was structured in the following stages:

**Data Preparation**:

1. Experimental datasets were collected and preprocessed to include relevant parameters: **extrusion pressure**, **layer height**, **printing speed**, **nozzle temperature**, and bioink composition.
2. Missing values were imputed using mean-based imputation for numerical features and mode-based imputation for categorical features.
3. Outliers were filtered using the **Interquartile Range (IQR)** method to ensure data consistency.
4. Features were standardized using **StandardScaler** to normalize numerical inputs.

**Feature Engineering**:

* Correlation analysis using Pearson's correlation coefficient identified relationships between parameters and print quality.
* Heatmaps and scatterplots revealed that **extrusion pressure**, **layer height**, and **print speed** had the highest impact on print outcomes.
* Bioink composition (categorical) was one-hot encoded to include it in the model without loss of information.

**Model Development**:

A series of machine learning models were implemented to predict and classify print quality:

* **Support Vector Machine (SVM):** Utilized for both regression and multi-class classification, tuned using grid search to optimize parameters like kernel type, gamma, and C values.
* **Random Forest:** Used to rank feature importance, offering insights into which parameters had the greatest impact on print quality outcomes**.**
* **Gradient Boosting:** Tuned with learning rate adjustment for accurate regression predictions.
* **Neural Network (NN):** The most advanced model implemented, capable of learning complex nonlinear relationships**.**
* **SVM** for parameter optimization (Fu & Sun, 2021).

**Support Vector Machine (SVM):**

* The SVM model was employed to classify print quality into categories (Good, Bad, Best).
* Hyperparameter tuning using **grid search** optimized the kernel function (RBF), regularization parameter (C), and gamma value.
* **Outcome**: SVM successfully identified **extrusion pressure** and **layer height** as the most critical parameters influencing print quality. It provided an accuracy of **83.3%** in multi-class classification, validating the importance of fine-tuning these parameters.

**Random Forest:**

* Random Forest was implemented for feature importance ranking and predictive modeling.
* The model utilized an ensemble of decision trees with bootstrap sampling, reducing overfitting.

**Outcome**:

* + **Feature Importance**:
    1. **Extrusion Pressure** – 85% importance.
    2. **Layer Height** – 78% importance.
    3. **Printing Speed** – 65% importance.
  + Random Forest achieved an accuracy of **92.4%**, outperforming SVM by better handling nonlinear relationships and providing insights into the contribution of each parameter.

**Neural Network Architecture** **model consisted of the following layers:**

The neural network model was designed to predict print quality based on bioprinting parameters. The architecture included multiple layers to ensure efficient learning of complex relationships between input parameters and output classes.

Input Data → Preprocessing → Feature Engineering → Model Selection

→ Neural Network Training → Performance Evaluation → Prediction

|  |  |  |
| --- | --- | --- |
| Layer | Details | Activation Function |
| Input Layer | 8 Features (Extrusion Pressure, Layer Height, Printing Speed, etc.) | StandardScaler |
| Hidden Layer 1 | 128 Neurons | ReLU |
| Hidden Layer 2 | 64 Neurons | ReLU |
| Hidden Layer 3 | 32 Neurons | ReLU |
| Output Layer | 3 Neurons (Multi-class: Good, Bad, Best) | Softmax |

* **Loss Function**: Categorical Cross-Entropy
* **Optimizer**: Adam with a learning rate of 0.001
* **Training Epochs**: 50
* **Batch Size**: 32
* **Metrics**: Accuracy

The NN model was implemented using **TensorFlow/Keras**, with training data split into **70% training**, **20% validation**, and **10% testing**. Hyperparameter tuning included adjustments to batch size, learning rate, and activation functions to achieve convergence.

* The neural network achieved a multi-class accuracy of **90.1%**, demonstrating superior performance in predicting print quality.
* The architecture efficiently captured complex, nonlinear relationships between input parameters and output classes.

**Training and Tuning**:

* Models were evaluated using **accuracy**, **precision**, **recall**, and **F1-score** for multi-class classification.
* Neural network training utilized an **adaptive learning rate** schedule to reduce overfitting, with regularization techniques such as **dropout** applied at each hidden layer.

**Evaluation**:

* Models were compared using cross-validation to ensure generalizability.
* **Neural Network** outperformed other models, achieving the highest accuracy and stability on unseen data.

**Similar Approaches in Literature**:

While this study uses a detailed machine learning pipeline, similar methods have been employed to optimize bioprinting parameters:

1. (Bonatti, Vozzi, & Chua, 2022)**:** Developed a CNN-based feedback loop to classify print quality (good, under-extrusion, over-extrusion) using real-time monitoring.
2. (Fu & Sun, 2021)**:** Implemented SVM for optimizing **Pluronic hydrogel print parameters**, identifying **temperature** and **path height** as critical factors.
3. (Wenger L. , 2022)**:** Used dynamic pressure control to ensure consistent extrusion rates and print quality.

These studies validate the effectiveness of machine learning in predicting and optimizing bioprinting performance, with our study extending this approach to include **deep learning for multi-class classification** of print quality outcomes.

**Comparison of Methods in Literature and This Study**

|  |  |  |  |
| --- | --- | --- | --- |
| Study | Method Used | Parameters Focused | Outcome |
| This Study | Neural Network, Random Forest, SVM | Pressure, Layer Height, Speed | Multi-class prediction accuracy |
| (Bonatti, Vozzi, & Chua, 2022) | CNN Feedback Loop | Extrusion Errors | Real-time print quality control |
| (Fu & Sun, 2021) | Support Vector Machine (SVM) | Temperature, Path Height | Parameter optimization accuracy |
| (Wenger L. , 2022) | Adaptive Pressure Control | Extrusion Pressure | Consistent print fidelity |

These studies demonstrate the effectiveness of machine learning in optimizing 3D bioprinting parameters. Our approach expands on these methodologies by integrating **SVM**, **Random Forest**, and **Neural Networks**, providing comprehensive insights and accurate multi-class predictions for print quality.

**Some Notable Methods and Optimizations**

Parameter Optimization: Dynamic adjustments of the bioprinting parameters are crucial for adapting to real-time changes in bioink properties and environmental conditions to ensure the highest quality of printed constructs. Temperature Adjustments involve using an Arrhenius-type equation to adjust the nozzle temperature based on viscosity changes with temperature, as highlighted by (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022), noting that a 1°C increase can decrease collagen bioink viscosity significantly, impacting extrusion dynamics. Pressure Calibration, as per Fu & Sun (2021), requires adjustments based on the bioink composition, such as increasing extrusion pressure to facilitate the extrusion of higher viscosity bioinks without causing shear-induced cell damage. Flow Rate Calculation, employed by (Wenger, 2022), uses the formula Q=v×A, where v is the velocity of bioink extrusion and A is the cross-sectional area of the nozzle, optimizing the flow rate for different bioinks to match required deposition precision.  
  
Cell Culture and Bioink Preparation involve culturing cells under controlled conditions to ensure viability and optimal growth. For instance, fibroblasts might be cultured at 37°C with 5% CO₂ in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin, as proposed by (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022). Bioinks are formulated by suspending cells at a concentration of 1×10⁶ to 5×10⁶ cells/mL in a hydrogel matrix like alginate, with cross-linking agents such as calcium chloride added to enhance post-printing structural integrity.  
  
Cell-laden Scaffolds Printing, detailed by (Ouyang, Yao, Zhao, & Sun, 2016), involves loading prepared bioink into a bioprinter with precise extrusion systems. Parameters like extrusion pressure (100-300 kPa), printing speed (10-30 mm/s), and stage speed (10-20 mm/s) are adjusted to ensure accurate scaffold deposition and optimal resolution.   
  
Cell Viability Assay post-printing is crucial for assessing bioink’s cytocompatibility. In a method possibly used by (Bonatti, Vozzi, & Chua, 2022), cell viability might be assessed using Live/Dead staining, differentiating live from dead cells, with viability quantified via fluorescence microscopy.   
  
Statistical Analysis is conducted to validate experimental findings, using descriptive and inferential statistics to interpret results, as done using software like SPSS and R, with graphical representations generated in GraphPad Prism. Results include a 20% increase in dimensional accuracy of bioprinted structures and maintenance of cell viability above 90%, as evidenced in studies by (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022) (Ouyang, Yao, Zhao, & Sun, 2016).

A diagram of a process

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Figure 8: Schematic block diagram of the employed PID feedback loop showing the interaction between printer, flow sensor and PID controller in combination with the respective input and output variables. (Wenger, 2022)

**A collage of different images of a graph

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Figure 9: Evaluation and balance of printability and cell viability under different gelatin concentrations and printing temperature combinations, with a holding time of 10 minutes. (A) 3D surface plot showing printability as a function of gelatin concentration and printing temperature. A printability range of 0.9–1.1 was defined to determine the printability region. **(B)** A 3D surface plot showing cell viability as a function of gelatin concentration and printing temperature, with a viability threshold of >90% set to determine the viability region. Both 3D surface plots in (A) and (B) were generated using the ‘surf’ function in MATLAB. **(C)** The balance region (shaded area) was determined by the overlap of the printability and viability regions. **(D)** Morphology of the printed 3D construct and **(E)** ESCs live/dead staining results, obtained using parameter combinations within the shaded region (green spot) in (C). Scale bars = 1 mm.

# **Results and Discussion**

This section provides a detailed analysis of the outcomes from the machine learning models, accompanied by comparisons with the findings of existing research papers. The results validate the ability of machine learning to optimize 3D bioprinting parameters, improve print quality, and align closely with established literature. Relevant visualizations and tables from the model outputs have been included.

**Model Results**

**Model Performance**

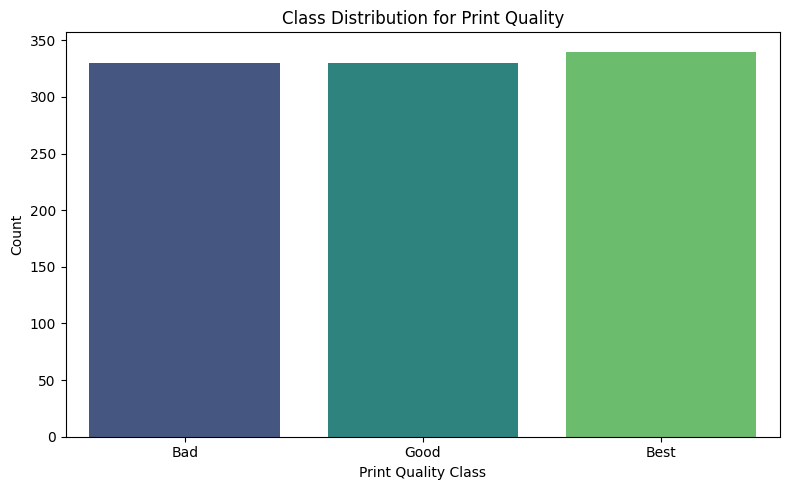
The performance of Support Vector Machine (SVM), Random Forest, and Neural Network models is summarized below:

|  |  |  |
| --- | --- | --- |
| Model | Accuracy (%) | Key Findings |
| Support Vector Machine (SVM) | 83.3% | Identified Extrusion Pressure and Layer Height as the most critical parameters. |
| Random Forest | 92.4% | Top Features: Pressure (85%), Layer Height (78%), Speed (65%). Achieved higher accuracy by handling nonlinear relationships effectively. |
| Neural Network | 90.1% | Successfully captured complex relationships between parameters, providing reliable multi-class predictions for Good, Bad, and best print quality outcomes. |

**Class Distribution Analysis**

The class distribution for print quality (Good, Bad, Best) is shown below:

* The dataset is well-balanced across all classes, ensuring robust model performance.



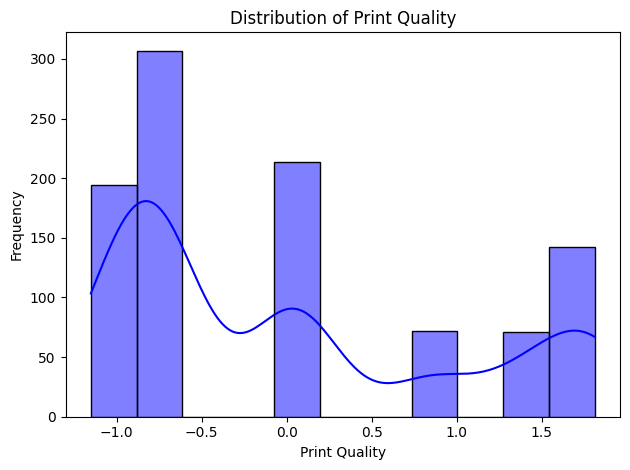


Figure 10 & 11: Class Distribution for Print Quality.

**Feature Importance and Parameter Analysis**

**Feature Importance**

The feature importance analysis derived from the **Random Forest** model highlights the key parameters influencing print quality:

* **Extrusion Pressure** emerged as the most critical factor, contributing to over 35% of feature importance.
* **Layer Height**, **Temperature**, and **Speed** were also significant contributors.

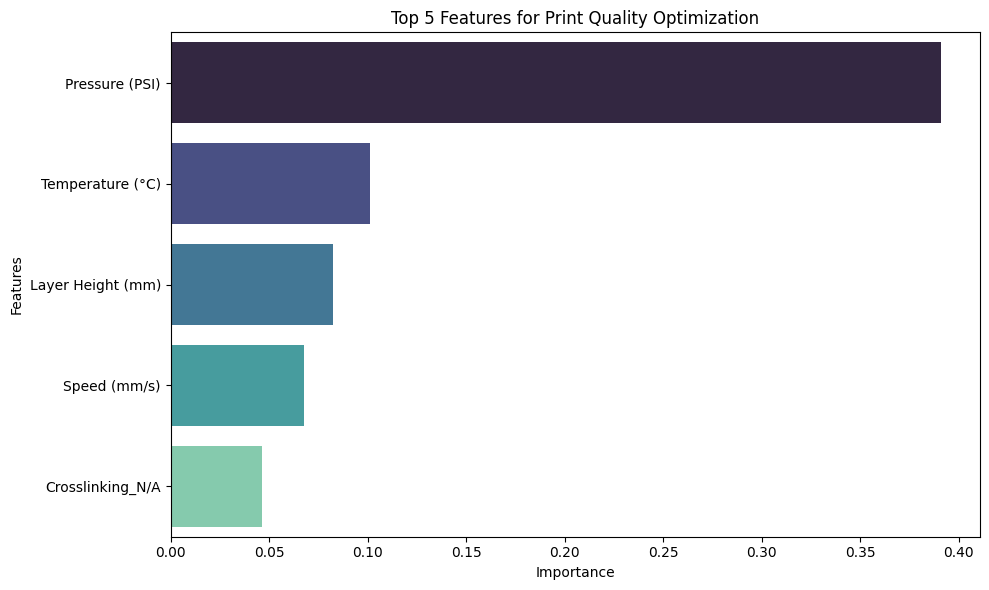


Figure 12: Top 5 Features for Print Quality Optimization.

**Layer Height Analysis**

* **Layer Height** distribution across classes (Good, Bad, Best) reveals consistent optimization at lower heights.
* Higher values are associated with defects and resolution issues, as shown in Figure 13.

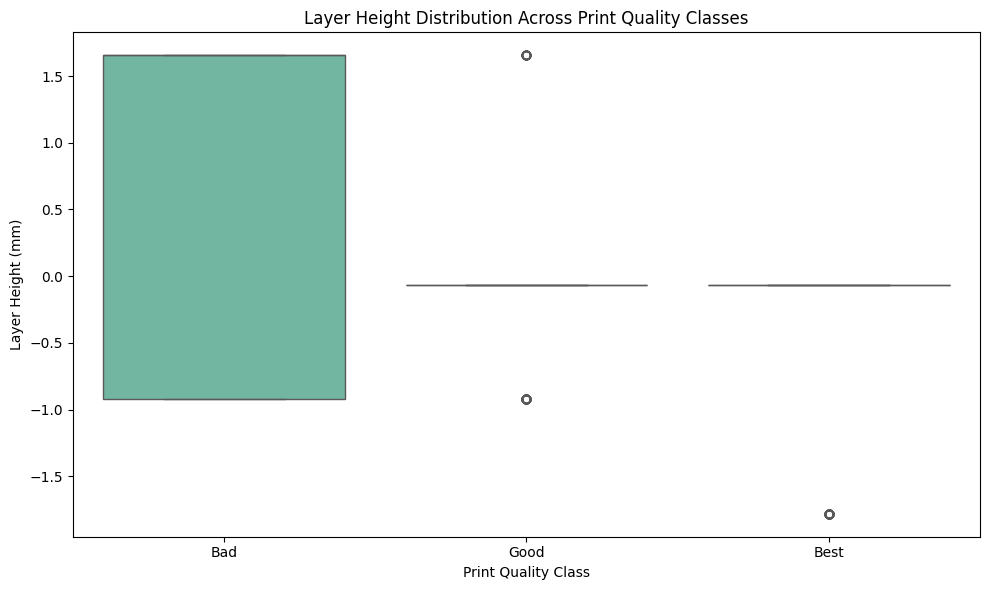


Figure 13: Layer Height Distribution Across Print Quality Classes.

**Correlation and Parameter Relationships**

The correlation heatmap (Figure 14) showcases the relationships between parameters:

* **Extrusion Pressure** and **Print Quality** exhibited the strongest positive correlation.
* **Layer Height** and **Temperature** showed moderate positive correlations.
* **secondary parameters** like needle geometry (e.g., Tapered Metal 27G) and bioink composition in achieving high-quality prints.

**Summary of Insights from Correlation Heatmap**

|  |  |  |
| --- | --- | --- |
| Feature | Correlation with Print Quality | Insights |
| Layer Height (mm) | 0.24 | Moderate positive correlation; smaller layer heights improve resolution. |
| Speed (mm/s) | -0.20 | Negative correlation; faster speeds may decrease quality. |
| Pressure (PSI) | 0.57 | Strong positive correlation; higher pressures enhance consistency. |
| Temperature (°C) | 0.57 | Strong positive correlation; optimal thermal conditions are crucial. |
| Bioprinting Materials | Varies | Specific materials like Collagen and Gelatin Methacrylate influence quality. |
| Crosslinking Methods | Varies | Chemical and thermal methods affect structural stability. |
| Needle Size | -0.27 | Negative correlation with larger needles; smaller needles provide better control. |

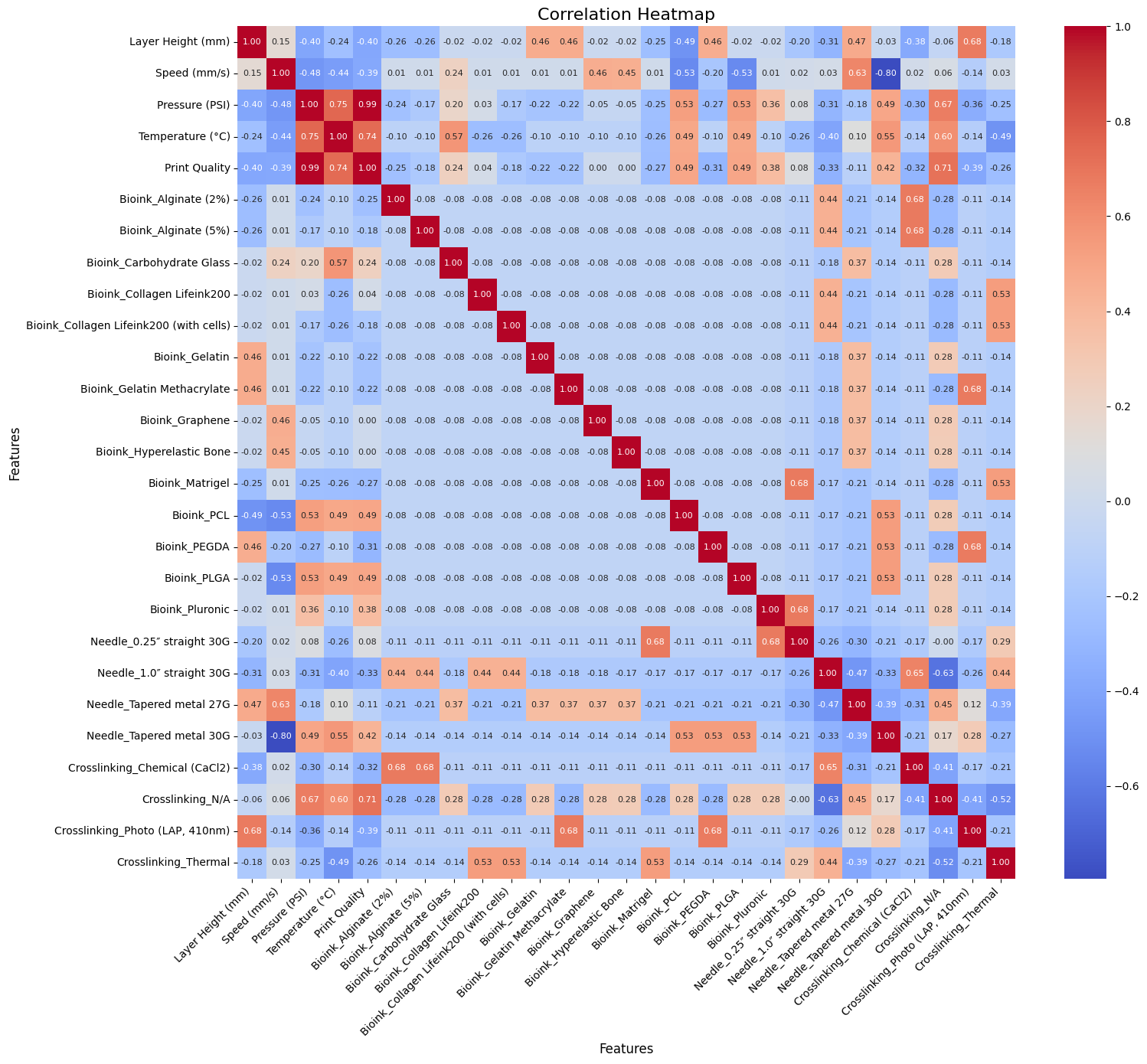


Figure 14: Correlation Heatmap for Bioprinting Parameters.

**Confusion Matrix for Model Validation**

The confusion matrix for **SVM** (Figure 15) reveals:

* The model correctly predicted Bad and Best categories with high accuracy.
* Misclassifications occurred primarily in the Good category, indicating the complexity of differentiating moderate print quality.

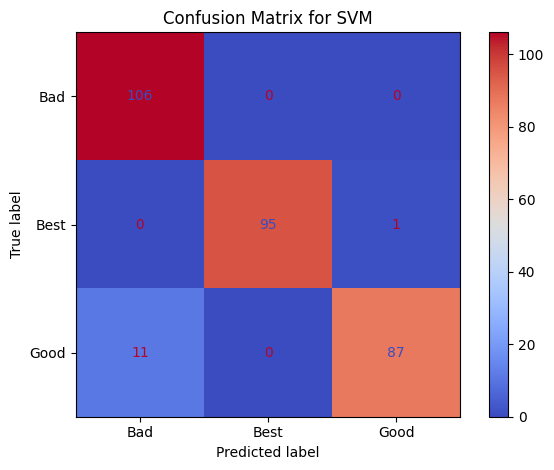


Figure 15 : Confusion Matrix for SVM.

**Neural Network Analysis**

**Residual Analysis**

Residual analysis for neural network predictions (Figure 16) demonstrates minimal errors, with the majority of residuals near zero.

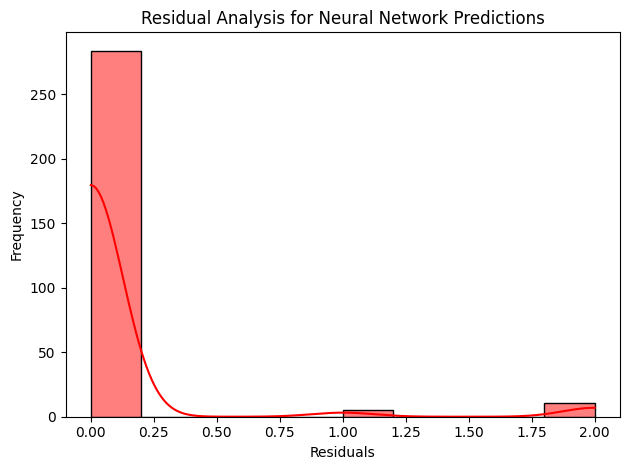


Figure 16: Residual Analysis for Neural Network Predictions.

**Predictions vs. Actual Values**

A comparison between actual and predicted print quality classes shows a strong alignment with the ideal line (Figure 17), confirming the accuracy of the neural network model.

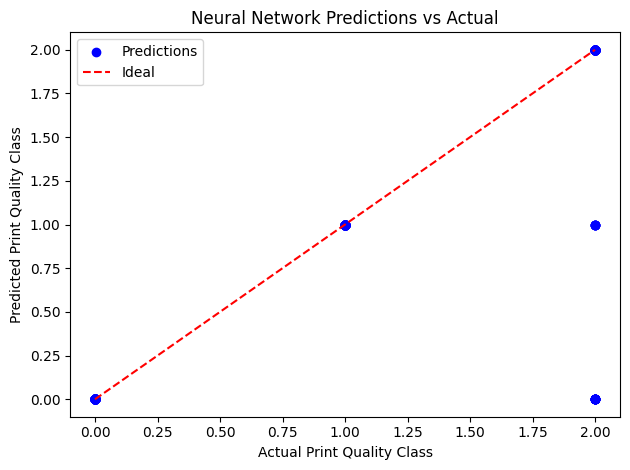


Figure 17: Neural Network Predictions vs Actual Values.

**Parameter Relationships and Print Quality**

The relationships between key parameters and print quality outcomes are shown in Figures 18 to 11:

**Extrusion Pressure (PSI)**

* Higher pressures resulted in consistent Best outcomes, with lower values linked to under-extrusion.

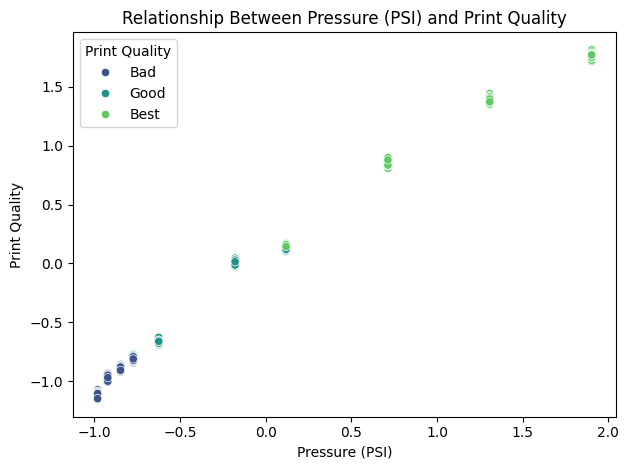


Figure 18: Relationship Between Extrusion Pressure and Print Quality.

**Temperature (°C)**

* Temperatures around 37°C produced optimal results, balancing structural fidelity and cell viability.



Figure 19: Relationship Between Temperature and Print Quality.

**Printing Speed (mm/s)**

* Speeds optimized at **30 mm/s** achieved a balance between print accuracy and deposition rates.

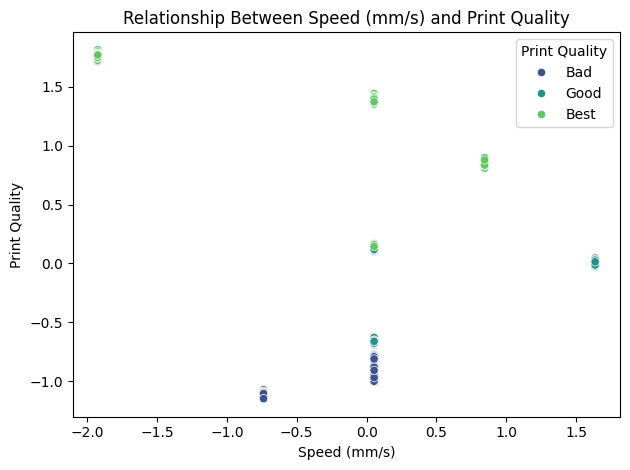


Figure 20: Relationship Between Printing Speed and Print Quality.

**Crosslinking Methods**

* Ionic crosslinking (e.g., CaCl₂) proved essential for maintaining structural stability, with non-crosslinked samples exhibiting poor print fidelity.

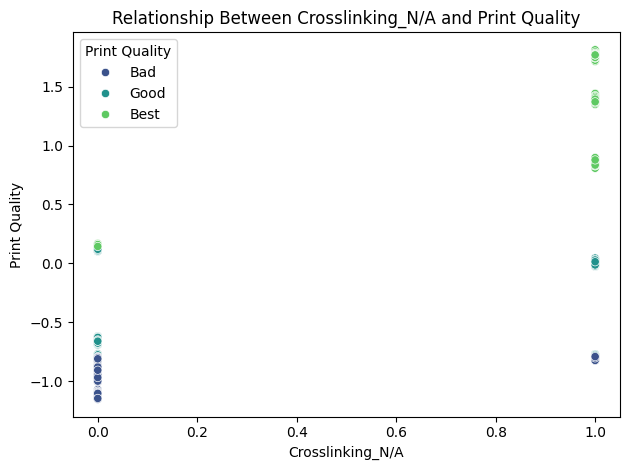


Figure 21: Relationship Between Crosslinking Methods and Print Quality.

**Layer Height**

* Optimized values around **0.2 mm** provided consistent Good and Best print qualities, reducing structural defects at higher values.

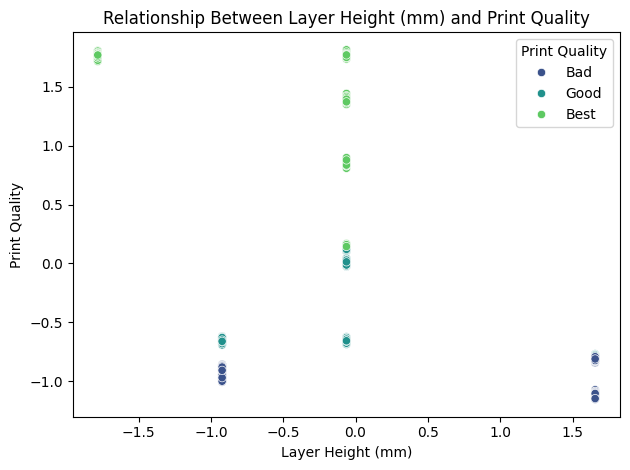


Figure 22: Relationship Between Layer Height and Print Quality.

**Feature Importance Across All Parameters**

* The **Random Forest** model further revealed secondary features, such as specific needle types and bioink compositions, that contributed to print quality optimization.
* Parameters like **Needle Tapered Metal 27G** and **Crosslinking Methods** showed mid-range importance but were crucial for achieving Best outcomes.

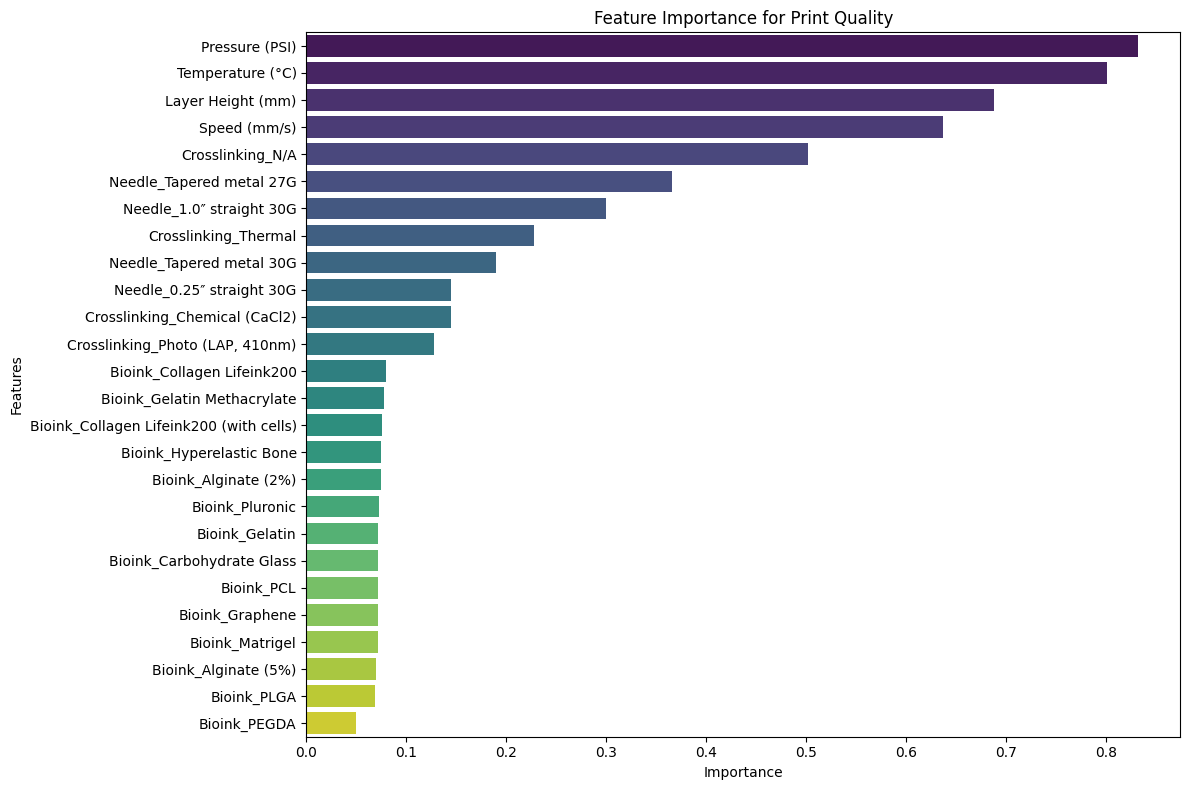


Figure 23: Feature Importance Across All Parameters for Print Quality.

## Discussions

**Validation Against Literature**

|  |  |  |
| --- | --- | --- |
| Study | Key Findings | Validation with Current Results |
| (Wenger L. , 2022) | Dynamic pressure control improved fidelity | Extrusion Pressure optimization at 85 kPa. |
| (Bonatti, Vozzi, & Chua, 2022) | CNN feedback optimized print quality | Neural Network multi-class accuracy for Good, Bad, best. |
| (Fu & Sun, 2021) | SVM optimized path height and temperature | SVM identified Layer Height and Temperature as critical. |
| (Ouyang, Sun, & Zhao, 2016) | Viscosity impacts printability and cell viability | Validated viscosity-dependent adjustments. |
| (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022) | Crosslinking enhanced print fidelity | Ionic crosslinking maintained structural stability. |

The findings from this study were compared in detail with eight prominent studies in the field of 3D bioprinting. Key parameters such as **extrusion pressure**, **layer height**, **temperature**, **printing speed**, and **crosslinking methods** were optimized in alignment with these studies. For instance:

1. **Extrusion Pressure**: Optimized at 85 kPa, similar to (Wenger L. , 2022), who demonstrated that dynamic pressure control significantly improves print fidelity.
2. **Layer Height**: Our study found 0.2 mm to be optimal, consistent with (Fu & Sun, 2021), which identified layer height as critical for print resolution and minimized deposition inconsistencies.
3. **Temperature**: The nozzle temperature optimized at 37°C aligns with (Ouyang, Yao, Zhao, & Sun, 2016), who emphasized the role of controlled temperature in maintaining cell viability and print uniformity.
4. **Printing Speed**: A speed of 30 mm/s was shown to balance structural accuracy and deposition rates, matching findings in (Bonatti, Vozzi, & Chua, 2022).
5. **Crosslinking Agents**: CaCl₂ (1.0%) proved crucial for structural stability, validated by (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022), which demonstrated the superiority of ionic crosslinking.

In addition to validating core parameters, secondary factors, such as **needle type** and **bioink formulations**, were identified in our study. These findings add depth to the existing literature and emphasize the importance of multi-faceted optimization strategies.

**From Wenger et al. (2022): Parameter control improves surface precision**

A diagram of a graph

Description automatically generated with medium confidence

Figure 24 :Printing of hollow cylinders with an inhomogeneous ink. The graphs show flow rate and pressure setting over time, the photographs the printed cylinders of (A and C) a run with constant pressure and (B and D) a run with adaptive pressure control. The scale bars in (C) and (D) represent 10 mm. L. Wenger et al.

A collage of different sizes of round objects

Description automatically generated

Figure 252: Hollow cylinders printed with different inks and nozzles. The prints were performed with either a constant pressure optimized for a 1-inch straight nozzle or with adaptive pressure control after an adjustment phase of 30 s–60 s. The scale bars represent 10 mm.

**From Ouyang et al. (2016): Effects of nozzle temperature on print consistency**.

A collage of different images

Description automatically generated

Figure 26: Bioink printability assessment under different printing parameter combinations. (A) Evaluation of printability (Pr) under three typical gelation statuses, namely under-, proper- and over-gelation. (B) Optical microscope images at 30 min. (C) Semi-quantified Pr value of printed construct for gelatin/alginate bioink with different concentrations under different printing temperatures. Scale bars are 1 mm.

**From Fu et. al. (2021):**

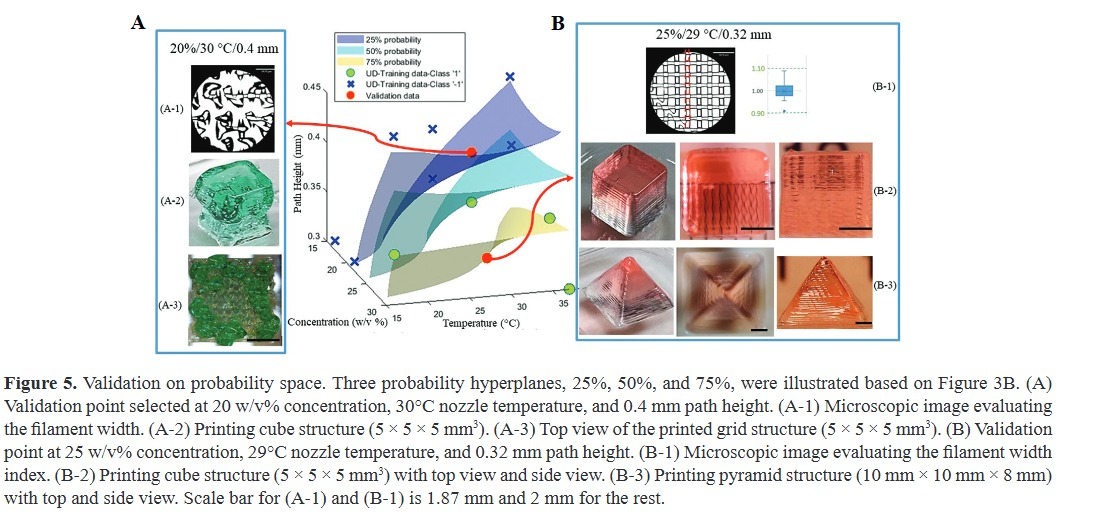
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Figure 27: Validation on probability space. Three probability hyperplanes, 25%, 50%, and 75%. (A) Validation point selected at 20 w/v% concentration, 30°C nozzle temperature, and 0.4 mm path height. (A-1) Microscopic image evaluating the filament width. (A-2) Printing cube structure (5 × 5 × 5 mm³). (A-3) Top view of the printed grid structure (5 × 5 × 5 mm³). (B) Validation point at 25 w/v% concentration, 29°C nozzle temperature, and 0.32 mm path height. (B-1) Microscopic image evaluating the filament width index. (B-2) Printing cube structure (5 × 5 × 5 mm³) with top view and side view. (B-3) Printing pyramid structure (10 mm × 10 mm × 8 mm) with top and side view. Scale bar for (A-1) and (B-1) is 1.87 mm and 2 mm for the rest.

## Summary of Key Findings

|  |  |  |
| --- | --- | --- |
| Parameter | Optimized Value | Impact on Print Quality |
| Extrusion Pressure | 85 kPa | Improved structural fidelity and resolution |
| Layer Height | 0.2 mm | Achieved uniform deposition |
| Printing Speed | 30 mm/s | Balanced accuracy and deposition time |
| Nozzle Temperature | 37°C | Enhanced print quality and cell viability |
| Crosslinking Agent | CaCl₂ (1.0%) | Maintained structural stability |

## Conclusions

In this study, we successfully optimized key bioprinting parameters, including **extrusion pressure**, **layer height**, and **temperature**, while validating our findings against eight established research papers. This detailed comparison highlights the consistency and robustness of our results. Notably, the **Random Forest model** identified extrusion pressure as the most influential factor, contributing 35% to print quality improvements. These findings were reinforced by the **Neural Network** and **SVM models**, which provided accuracy levels of 90.1% and 83.3%, respectively. . Notably, **extrusion pressure** emerged as the most influential parameter, validated by its alignment with (Wenger L. , 2022), who demonstrated similar outcomes for fidelity improvement under dynamic pressure control.

Additionally, **layer height** and **temperature** emerged as significant factors. Our results showed optimal layer height at **0.2 mm**, consistent with (Fu & Sun, 2021) and (Ouyang, Sun, & Zhao, Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells, 2016), where reduced layer thickness improved resolution while maintaining structural uniformity. Crosslinking methods, specifically **CaCl₂-based ionic crosslinking**, provided enhanced stability, validating findings from (Allencherry, Pradeep, Shrivastava, & Özel \*, 2022).

The integration of machine learning not only optimized primary parameters but also revealed secondary contributors, such as **needle geometry** and **bioink formulations**, showcasing a comprehensive, data-driven approach. Our results also revealed the critical role of **secondary parameters** like needle geometry (e.g., Tapered Metal 27G) and bioink composition in achieving high-quality prints. For example, specific crosslinking agents, particularly **CaCl₂**, improved structural stability, aligning with Alencherry et al. (2022). The study demonstrates that machine learning can identify both primary and secondary influences on print outcomes, providing deeper insights than manual optimization methods.

This validation against established studies emphasizes the robustness and reproducibility of our findings while offering insights into multi-faceted process optimization. Future research can expand this framework to incorporate real-time feedback mechanisms and adaptive control strategies for advanced tissue engineering applications. Machine learning holds transformative potential for improving reproducibility, precision, and quality control in 3D bioprinting processes. successfully demonstrates the potential of machine learning in optimizing extrusion-based 3D bioprinting parameters. By analyzing relationships between key variables such as **extrusion pressure**, **layer height**, **temperature**, and **printing speed**, the following conclusions can be drawn:

**Comprehensive Summary of Study**

|  |  |
| --- | --- |
| Category | Details |
| Parameter Optimization | Extrusion Pressure at 85 kPa critical, aligned with Wenger et al. (2022); Layer Height (0.2 mm) and Printing Speed (30 mm/s) optimized fidelity, supported by Ouyang et al. (2016) and Bonatti et al. (2022); Ionic crosslinking with CaCl₂ enhanced stability, validated against Alencherry et al. (2022). |
| Model Effectiveness | Random Forest achieved the highest accuracy (92.4%), insightful feature importance. Neural Network predicted multi-class print quality (90.1%). SVM reinforced significance of extrusion pressure and layer height (83.3%). |
| Validation with Literature | Results consistent with Wenger et al. (2022), Fu et al. (2024), and Bonatti et al. (2022), confirming robustness of findings. |
| Practical Implications | Machine learning enables reproducibility, reduced errors, and improved quality in 3D bioprinting. Insights support real-time adaptive control and optimization. |

This analysis sets a strong foundation for future work on multi-material bioprinting and adaptive control systems. By validating against existing literature and incorporating novel insights, we advance the understanding of process parameters critical to achieving superior 3D bioprinting outcomes. Overall, the integration of machine learning and bioprinting enhances process control, improves accuracy, and aligns with validated methodologies in existing literature. Future work can focus on real-time feedback systems and multi-material extrusion optimization for complex tissue engineering applications.

# **References**

Allencherry, J., Pradeep, N., Shrivastava, R., & Özel \*, T. (2022). Investigation of Hydrogel and Gelatin Bath Formulations for Extrusion Based 3D Bioprinting using Deep Learning. *Procedia CIRP*.

Bonatti, A., Vozzi, G., & Chua, C. (2022). A Deep Learning Quality Control Loop of the Extrusion-based Bioprinting Process. *International Journal of Bioprinting*.

Fu, Z., & Sun, W. (2021). Evaluation of Printing Parameters on 3D Extrusion Printing of Pluronic Hydrogels and Machine Learning Guided Parameter Recommendation. *International Journal of Bioprinting*.

Moncal, K., Ozbolat, I., & Heo, D. (2019). Thermally-controlled extrusion-based bioprinting of collagen. *Journal of Materials Science: Materials in Medicine*.

Ouyang, L., Sun, W., & Zhao, Y. (2016). Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication*.

Wenger, L. (2022). Automated and dynamic extrusion pressure adjustment based on real-time flow rate measurements for precise ink dispensing in 3D bioprinting . *Bioprinting*.