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ABSTRACT:

Bioprinting facilitates the generation of complex, three-dimensional (3D), cell-based constructs for a variety of applications. Although multiple bioprinting technologies have been developed, extrusion-based systems have become the dominant technology due to the diversity of substrate materials (i.e. bioinks) that can be accommodated, either individually or in combination. Each bioink has unique material properties and extrusion characteristics that limit bioprinting precision, particularly when generating identically shaped constructs from different bioinks. Here, we aimed to achieve high precision (i.e. repeatability) across samples by generating bioink-specific printing parameters using a systematic approach. We hypothesized that a Fuzzy system could be used as a soft computing method to tackle the inherent vagueness and imprecision in 3D bioprinting data and uncover the optimal printing parameters for a specific bioink that would result in high precision. Our Fuzzy model was used to approximate and quantify the precision and ease of printability for two common bioinks - type I collagen and Pluronic F127, with or without dilution in αMEM culture media. The model consisted of three inputs (pressure, speed, and bioink dilution percentage) and a single output (line width). Using this system, we introduce the Bioink Precision Index (BPI), a metric that can be used to quantify and compare the precision of any bioink regardless of bioprinting technique and environmental parameters. To validate BPI, we demonstrate a significant increase (+54%) in line width variation between parameter sets with high (16.6) and low (7.5) BPI. Finally, we estimate that printing with parameters optimized using BPI would increase the line width precision for collagen (+15%) and Pluronic F127(+29%) as compared to the manufacturer's recommended printing parameters.

KEY WORDS:
Extrusion Bioprinting; Fuzzy System; Precision; Collagen; Pluronic F127.

1. Introduction:

Bioprinting is a popular technique used in a variety of research areas, such as tissue engineering and drug delivery, and involves depositing biological material (bioinks) in a layer-by-layer fashion to produce a three-dimensional (3D) cell-laden construct [1]. Bioprinted 3D constructs have many novel applications, including visualization of cell-cell interactions in a biomimetic microenvironment in vitro for cancer research [2], production of 3D tissue for implantation [3], and in-vitro and in-vivo models for drug discovery [4]. Bioprinted constructs are frequently generated from computer-aided design (CAD) [5], and can be reproduced quickly from an expanding library of bioinks [6], [7]. As a result, fabrication methods that increase precision and facilitate reproducibility are particularly desirable [8], [9].

Extrusion-based bioprinting (EBB) is the most utilized bioprinting technique due to its compatibility with a large spectrum of bioinks, affordability, and ease of use [10]. In this method, bioinks are extruded from a small nozzle in a layer-by-layer manner using pressure generated from either a pneumatic, screw, or piston-based system [10], [11]. Bioprinting parameters, such as syringe pressure, nozzle gauge, printing speed, material temperature, and crosslinking status, affect the final printed construct [8]. Those parameters must be optimized for each bioink in order to generate a final construct that has the desired dimensions and appropriate material properties.

Type I collagen and Pluronic F-127 bioinks (also known as Poloxamer 407) are two widely used bioinks in biomedical research. Collagen bioink is a biocompatible, protein-based hydrogel composed of material commonly found in the extracellular matrix in a variety of tissues [12]. Importantly, collagen bioink must be extruded below 4 ˚C, since it irreversibly gels at higher temperatures and cannot be brought back to the liquid state [10], [12]. In contrast, Pluronic F-127 is a synthetic hydrogel consisting of a co-polymer tri-block structure with hydrophilic-hydrophobic-hydrophilic sequences [13]. Pluronic F-127 is aqueous at 4 ˚C, but gels at room temperature 20 ˚C. However, Pluronic F-127 can be returned to a liquid state by cooling the bioprinted construct, leading to its common use as a sacrificial support material for forming hollow structures or delivering cells [12]–[15].

Determining printability with universal parameters is challenging due to the variability of customized bioinks. Moreover, this complexity is increased in bioinks with multiple ECM components that affect the rheological properties and can result in non-linear relationships between input and output parameters. As a result, design of experiment (DOE) modelling is not practical in this case. Although machine learning (ML) techniques can be employed to identify and predict relationships in single or multiple component inks in an accurate way, it is important to choose the appropriate ML

method. In this study, we investigate the use of fuzzy systems as a potential method, given its significant robustness in parameter modelling and noise control in data, specifically for unseen data points [16].

Few systematic approaches have been developed to approximate optimized bioprinting parameters [17], [18]. The disadvantage of using mathematical modelling approaches is a lack of generalization to more than a specific number of inputs. Moreover, the model linearization may lead to imprecision and inaccuracy in output approximation for new bioinks formulations [19]. To overcome these shortcomings, we hypothesized that bioprinting parameter optimization in precision could be robustly executed by implementing a Fuzzy logic system, in which rules are defined to assign the input and outputs continuous values, rather than discrete values [20]. Fuzzy logic is an extension of standard logic, in which values can only be completely false or completely true. In contrast, Fuzzy values have a degree of truth, generally noted as a value between 0 and 1. As a result, this status provides a mathematical model to move from discrete to continuous values. Finally, a Fuzzy system implements the rules and membership functions necessary to translate inputs to outputs.

To test this hypothesis, we developed a Fuzzy system to optimize bioprinting parameters to achieve a higher precision for collagen and Pluronic F-127 bioinks with or without dilution in αMEM culture media. Our Fuzzy system consists of inputs of nozzle pressure, printing speed, and dilution percentage of bioink with a single output of line width. The results from our study suggest that this approach is useful for optimizing printing parameters and will improve reproducibility across diverse bioinks as well as provide an objective characterization of bioprinting precision for newly formulated bioinks.

2. Methods

2.1. Bioprinting

Pluronic F-127 and type I collagen bioinks (Allevi Collagen Lifeink® 200) were prepared for analysis by diluting with alpha Minimum Essential Medium (αMEM) to contain 0, 20, or 40% of media, to mimic potential applications in which cell suspensions are mixed into each bioink. Dilutions were performed by mixing between two syringes connected with a female-to-female Luer Lock coupler for 20 minutes at room temperature. To confirm the material was homogenously mixed, the Pluronic F-127 used for this study was dyed using 600 uL of commercially available food dye.

Each bioink was used to print a 10x10 mm square shape with a line width and height of 200 microns (Fig. 1A). For each bioink, we used a 6.33 mm straight 25 gauge nozzle. Our initial experimentation was performed with undiluted bioinks. For the Pluronic F-127, we tested four extrusion pressures (60, 70, 80, 90 psi) and four printing speeds (12, 13, 14, 15 mm/s). For the collagen bioink, we tested three extrusion pressures (15, 20, 25 psi) and five printing speeds (8, 10, 12, 14, 15 mm/s).

Next, to determine the effect of dilution, we used a constant printing speed (15 mm/s). For the collagen bioink, we tested 20% and 40% diluted collagen at three extrusion pressures (8, 10, 12 psi). For the Pluronic F-127, we tested three extrusion pressures at 20% dilution (40, 45, 50 psi) and 40% dilution (20, 25, 35 psi). All samples were performed in triplicate and an average of each experiment group is reported in each table output. Since collagen needs to be printed at 4˚C (39 ˚F) or below, an ice pack was secured to the extruder throughout the print (Fig. 1B). For all experiments, we used an Allevi 2 bioprinter (*Allevi, USA*) located inside a sterile biosafety cabinet.

We choose the initial parameter set to be the manufacturer's recommended bioprinting parameters. For an unknown or novel bioink, the initial parameter set should be generated based on similar bioink printing parameters. Alternatively, the physical limitations of the bioprinter can be used as the upper and lower bounds for parameterization.

2.2. Imaging and Analysis

Each bioprinted sample was imaged using brightfield microscopy (Nikon E800) at 4x objective size (Fig. 1C). Images were analyzed in FIJI [21] to determine actual bioprinted line width by quantifying the width of the line at the midpoint of each side of the square. The values from four independent trials were averaged to obtain the final

value for each sample. These measurements were then utilized as the outputs for the inference engine in the Fuzzy system (Fig. 1D).

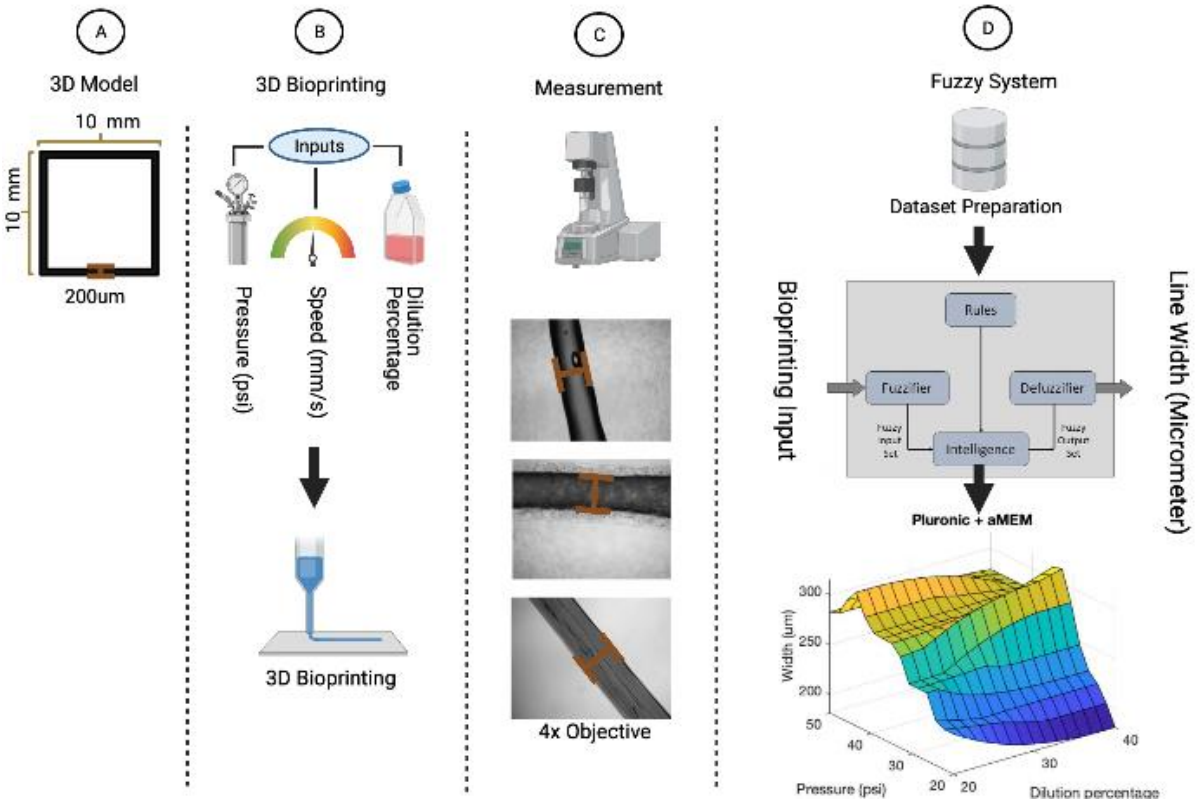

Figure 1. General workflow of the proposed study. (A) is the first step that the 3D model is designed and with three different input parameters the sample is printed by an extrusion based bioprinter (B). In step (C) data is measured, processed and fed into the Fuzzy system rules, then the final 3D surface is generated to calculate the BP for the preferred points (D).

2.3. Implementing Fuzzy System

We implemented a Fuzzy system to utilize our experimental data to optimize bioprinting parameters for each bioink, as previously described [22]. Briefly, we defined our crisp input values to be pressure, speed, and dilution percentage with line width as a single output value. This study used 44 data point in three independent experiments (total of 132 data points). The process of converting these crisp inputs to Fuzzy values is known as fuzzification. Here, we used Gaussian membership functions due to their smoothness, concise notation, and similarity to a variety of biological processes [23]. The standard deviation (SD) of Gaussian membership functions for each input and output are reported in Tables 1-4. Next, mapping from a given Fuzzy input to a Fuzzy output was performed using a Mamdani Fuzzy inference system (Fuzzy Logic Toolbox, Matlab R2020a). In this process, we imported a series of *If-Then* rules (experimental

results) to the inference engine (Tables 1-4). Finally, a single value for the output (line width) is generated as the aggregate of the Fuzzy values in a process known as defuzzification. A schematic of our Fuzzy system approach is illustrated in Figure 2. Note that this Fuzzy

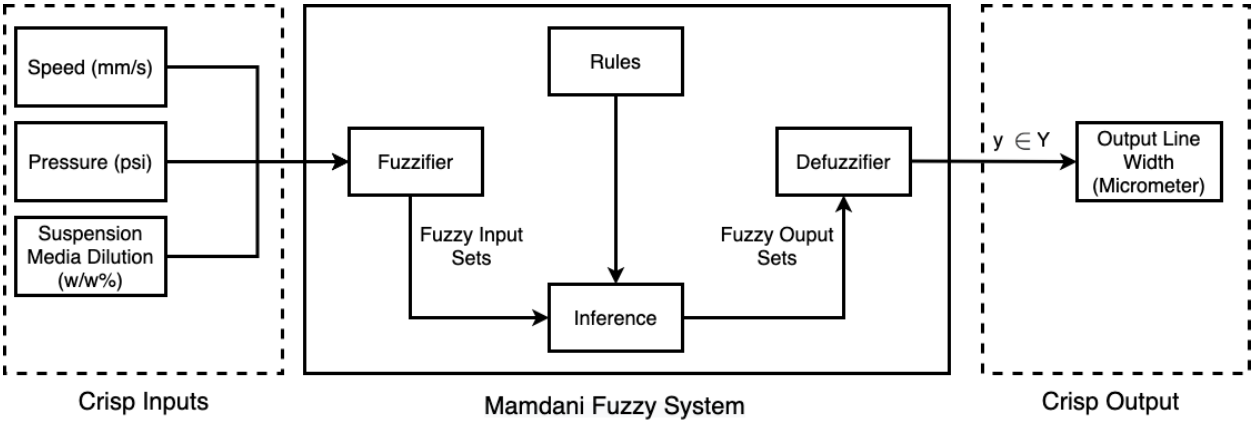

Figure 2. Type-1 Fuzzy Logic Algorithm and Study Design. General overview and different features of the Type-1 Fuzzy system including fuzzification, rules, inference engine, and defuzzification. It includes three inputs (speed, pressure, and Suspension Media Dilution Factor) and one output (line width).

system is based on the following assumptions: (i) the output material is incompressible, (ii) the pressure drop during extrusion is negligible, (iii) the flow is steady and laminar.

2.4 Measuring Bioprinting Precision Index

Our Fuzzy system approach to bioprinting parameter optimization enables us to introduce the Bioink Precision Index (BPI), a new metric for evaluating bioink precision that is defined as the gradient of the Fuzzy 3D surfaces. The standard calculation for a gradient of a 3D surface is:

$$f(x,y) \approx f(x_0,y_0) + (\nabla f)_x(x_0,y_0)(x-x_0) + (\nabla f)_y(x_0,y_0)(y-y_0)$$

Thus, BPI is calculated from the sum of the squared error of the above equation:

$$BPI = \sqrt{(\nabla f)_x(x_0,y_0)^2 + (\nabla f)_y(x_0,y_0)^2}$$

Where x_0 and y_0 are the inputs in our system with two inputs (speed-pressure, or dilution-pressure).

3. Results

3.1. Imprecision in undiluted bioink is primarily associated with extrusion pressure

First, we utilized our Fuzzy system to visualize the line width (output) as a function of the inputs, which were printing speed (mm/s) and nozzle pressure (psi) in collagen bioink and Pluronic F-127 that were not diluted (Fig. 3). As expected, we observe that increasing the extrusion pressure generally increased the line width for any given printing speed for both materials. In contrast, the output line width does not significantly change when altering the printing speed for either Pluronic F-127 (Fig. 3a) or collagen bioink in the range of recommended printing speed (Fig. 3b).

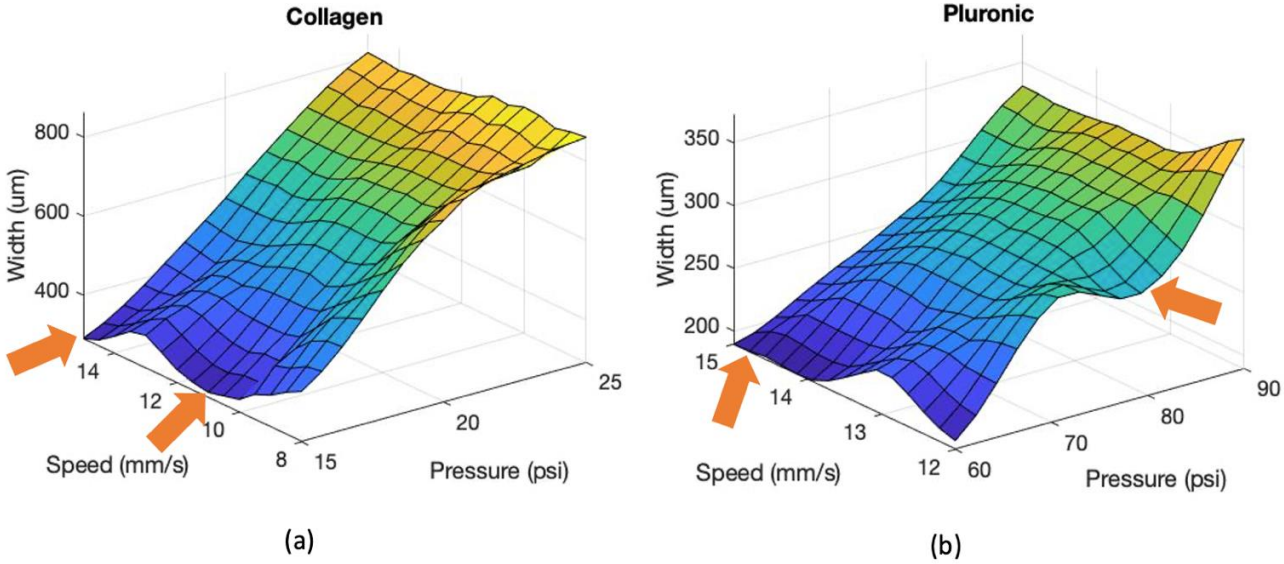

Figure 3. Fuzzy system output for undiluted Pluronic F-127 and collagen bioink. The general shape of the 3D graphs of Collagen (a) and Pluronic (b) shows that increasing the pressure will increase the output line width. However, at the same pressure level on the 3D graphs of Pluronic and Collagen, the output line width does not change significantly by expanding the bioprinting speed ratio.

3.2 Diluted bioinks are more precise for bioprinting

Next, we simulated the use of each bioink when laden with cells by diluting each bioink to 20% or 40% with αMEM culture media. To overcome the technical difficulty of implementing a Fuzzy system with three inputs (pressure, speed, and dilution percentage), we utilized our observation that printing speed for both the collagen bioink and Pluronic F-127 had essentially the maximum correlation between inputs at high speed (Fig. 4). Correlation analysis shows that the pressure and line width are highly correlated ($r>0.99$) and statistically significant ($p<0.001$) at speed 15 mm/s. In particular, an analysis of the undiluted Fuzzy output indicates maximum linearity with a

printing speed of 15 mm/s for both collagen bioink (Fig. 4a) and Pluronic F-127 (Fig. 4b).

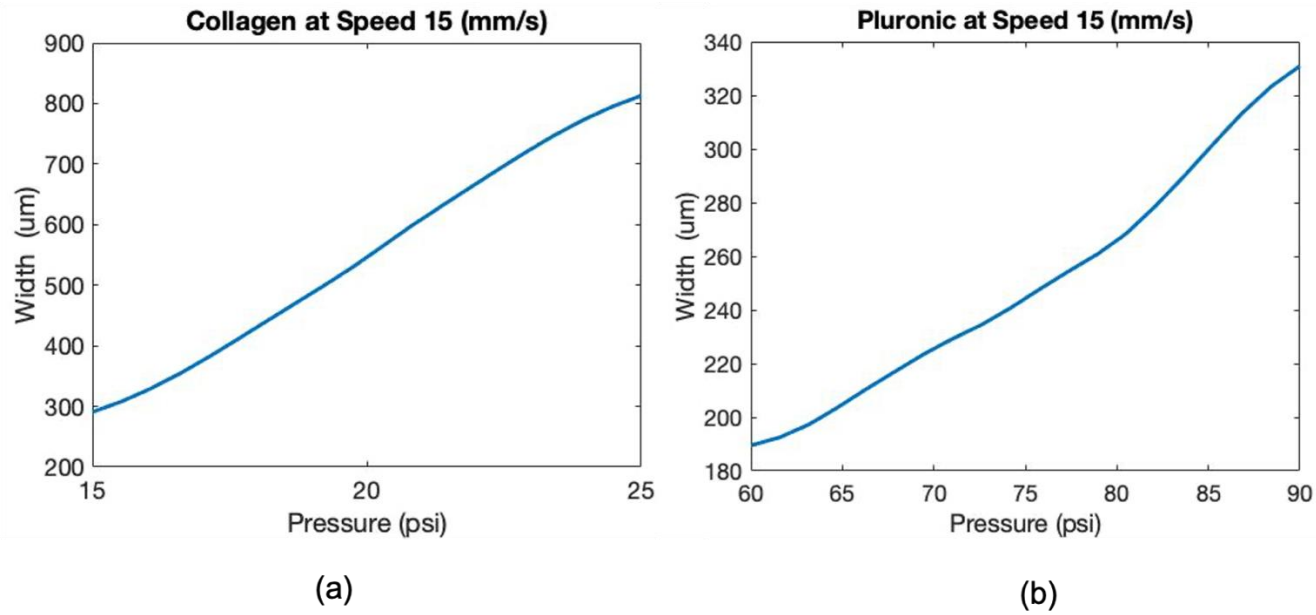

Figure 4. The Pressure linearity at speed 15 mm/s in Collagen and Pluronic. The 2D section of Collagen (a) and Pluronic (b) at speed 15 mm/s show that it has the minimum impact on the output line width because of the linear relationship between pressure and line width.

As a result, we constructed a Fuzzy system by maintaining a constant printing speed at 15 mm/s and visualized the Fuzzy system output for the diluted bioink experiments.

In contrast to the undiluted bioinks, the Fuzzy output for collagen bioink diluted with αMEM reveals two potential parameter sets that yield a desired line width of 200 μm (Fig 5a, illustrated by arrows). The first solution required a high dilution of 40% and a low extrusion pressure of 8 psi. In contrast, the lower dilution of 20% required a slightly higher extrusion pressure of 10 psi to obtain a 200 μm line width (Fig. 5a). In general, we observed that the line width increases with increased dilution percentage and extrusion pressure.

We observed that the printing precision of diluted collagen bioink was less sensitive to changes in extrusion pressure, in opposition to the undiluted bioink. In fact, the increase in extrusion pressure from 15 psi to 25 psi increases line width by 37 percent in the undiluted collagen bioink as compared to 9 percent in the diluted collagen bioink (Fig. 5c). In total, these results indicate that diluted bioinks are more precise for bioprinting, yielding greater reproducibility. The surface irregularities in Fig. 5c are a result of the experimental data (Table 4), and not due to the approximation modeling by our fuzzy system.

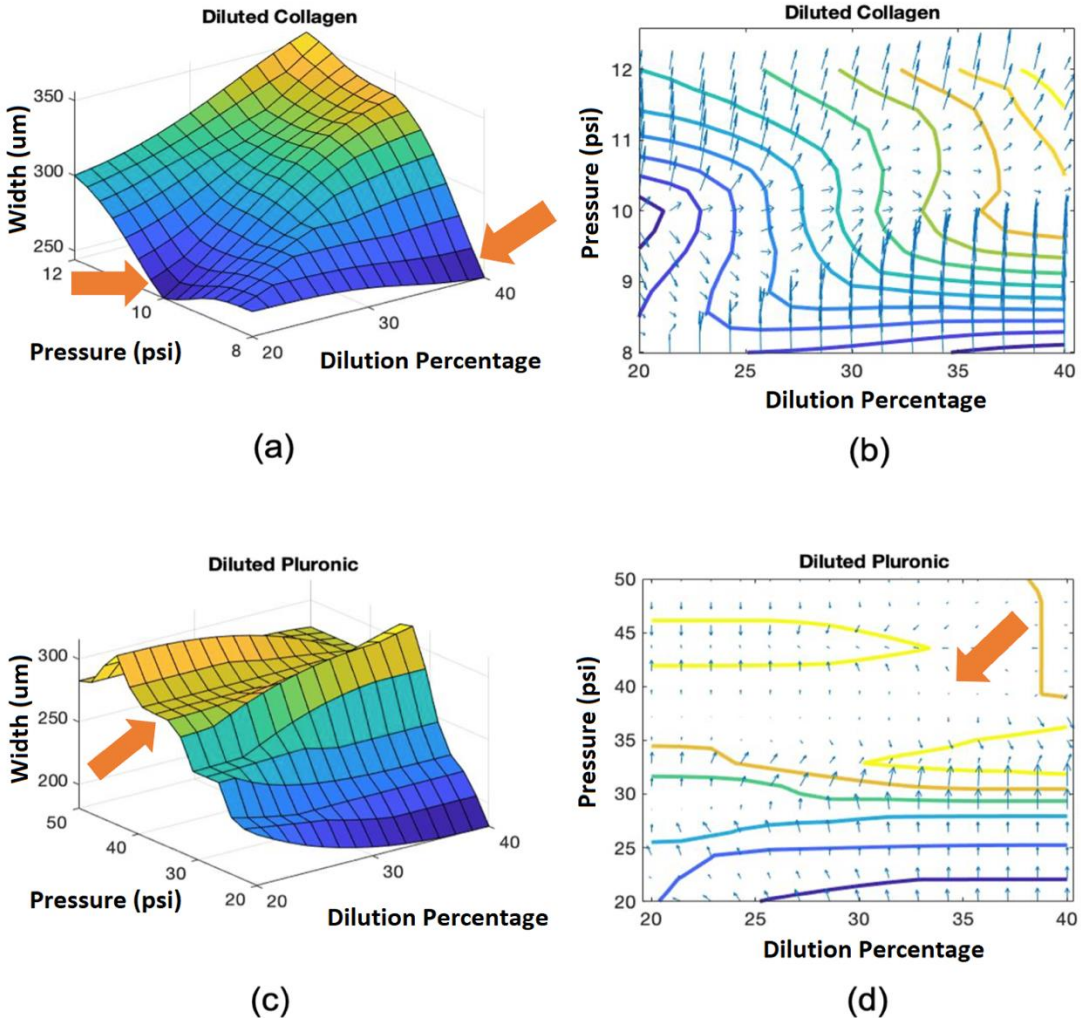

Figure 5. The general shape of the 3D graphs of diluted Collagen and Pluronic with aMEM. The general shape of the 3D graphs of diluted Collagen (a) and diluted Pluronic (c) shows that increasing the pressure will increase the output line width. (b) and (d) show the gradient based on the explained equation. The smaller arrow is in length, the bioink has higher printing precision. The flat surface in (c), which is the area between two orange lines (d), indicates a high precision and robust input parameters to print.

3.3. Bioink Precision Index for novel bionks

BPI is a metric for evaluating bioink precision that is defined as the gradient of the Fuzzy 3D surfaces (Fig. 5a and c). Thus, a smaller numerical value of BPI indicates a more precise parameter set for that bioink. In Fig. 5b and d, BPI is indicated by arrow length at each position. The manufacturer's suggested printing parameters for the

collagen bioink (6 mm/s and 15 psi) results in a BPI of 42.4, but our optimized parameter set (11 m/s and 15 psi) decreases the BPI to 36, resulting in an approximately 15% increase in BPI. The line width accuracy is also increased by 36% (from 350 um to 240 um). Similarly, the BPI for undiluted Pluronic F127 using the manufacturer's suggested printing parameters (12 mm/s and 80 psi) is 14.2, much greater than the BPI of 4.5 obtained using our optimized parameter set (15 mm/s and 60 psi), improving BPI by 68 percent. As a result, the line width precision is improved by 29% (from 260um to 190um). Finally, we note that the optimized BPI for collagen is significantly higher than the optimized BPI for pluronic, indicating that collagen is a more challenging bioink to use for high precision bioprinting.

3.4. Fuzzy systems identify robust and precise printing parameters sets

This Fuzzy system approach can identify printing parameter sets that are insensitive to the small perturbations routinely encountered during bioprinting, such as inconsistent experimental dilutions or fluctuations in extrusion pressure during printing. One such parameter set can be visualized by the flat surface in Fig. 5c (arrow), which results in the same 250 μm line width for any dilution between 40% and 50% and extrusion pressure between 20 psi and 40 psi.

To illustrate these areas of Fig. 3a, we separately analyzed sections illustrating the speed-width relationship for a given a pressure (Fig. 6a) and pressure-width relationship for a given speed (Fig. 6b). Since BPI can be reduced to one dimension (one input-output system) or higher dimensions (multiple input/output parameters), we calculated the BPI for a one dimension model (pressure-width). The figures are color-coded with green, yellow, or red, where the green zone has the most precision and least sensitivity to fluctuations, resulting in the most precise outputs (BPI < 10). In contrast, the yellow (10 < BPI < 20) and red (BPI > 20) zones are progressively more challenging to achieve a high level of precision over time. Similar regions have been identified for undiluted Pluronic F-127 (Fig. 6c,d), diluted collagen bioink (Fig. 6e, f), and diluted Pluronic F-127 (Fig. 6g, h).

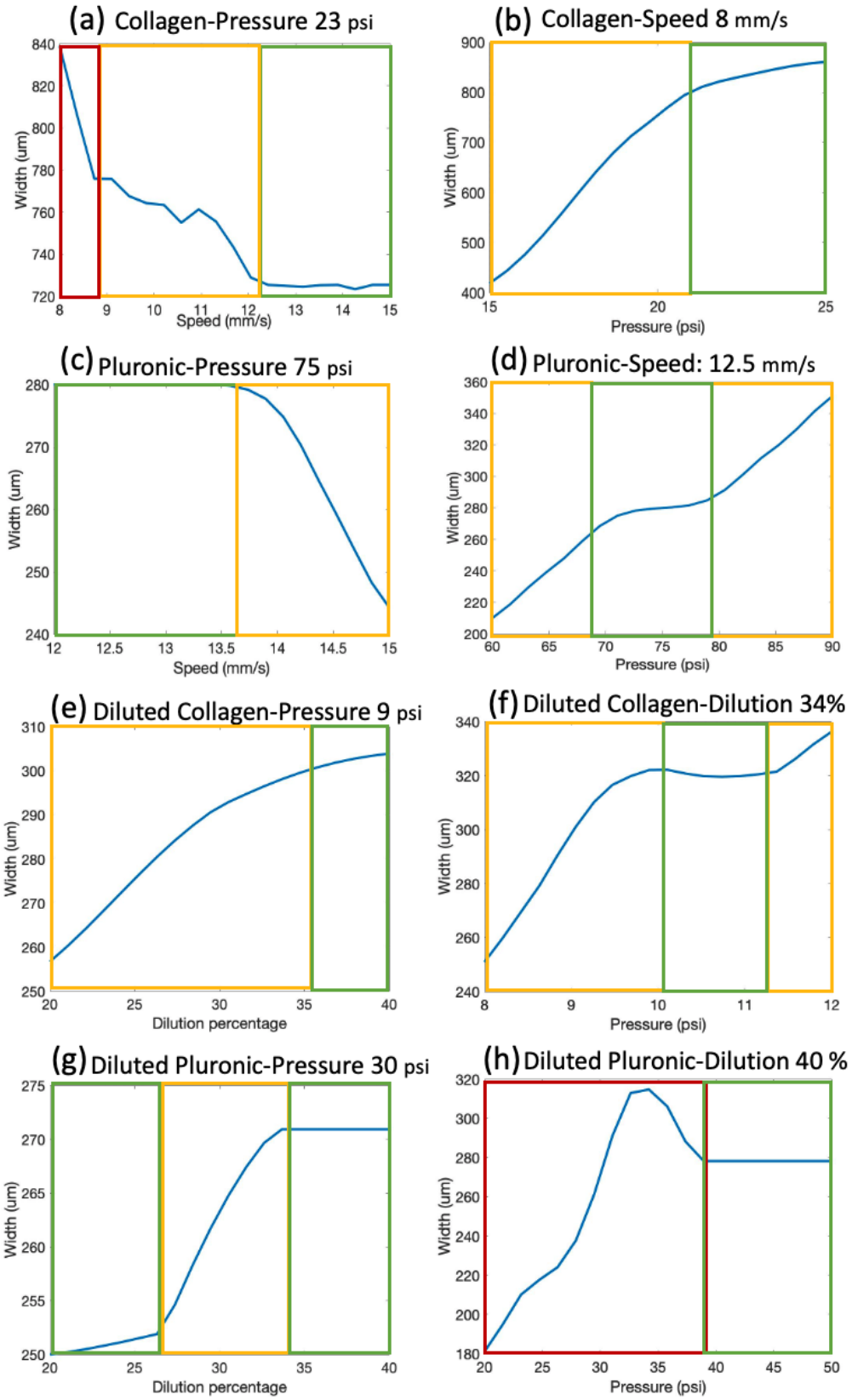

Figure 6. The 2D slices of Collagen, Pluronic, diluted Collagen, and Pluronic with aMEM. This figure indicates the 2D slices in x and y direction at specified value written as the label for the Collagen (a and b), Pluronic (c and d), diluted Collagen with aMEM (e and f), and diluted Pluronic with aMEM (g and h).

3.5. BPI Validation and Fuzzy system Error

To validate the BPI metric, we printed Pluronic F127 at speed 12.5 mm/s (Fig. 6D) using two extrusion pressures - 73 psi (BPI: 7.5) and 87 psi (BPI: 16.63). Consistent with our hypothesis, we observed that using a parameter set with a lower BPI results in higher precision (Fig. 7). Specifically, the standard deviation of line width for 73 psi (58 um) was significantly different than the standard deviation of line width for 87 psi (111 um) by F-test ($p = 0.0487$). We note that the mean was not statistically different between groups ($p > 0.05$), although line width accuracy was improved by an average of 30 um by printing at 73 psi.

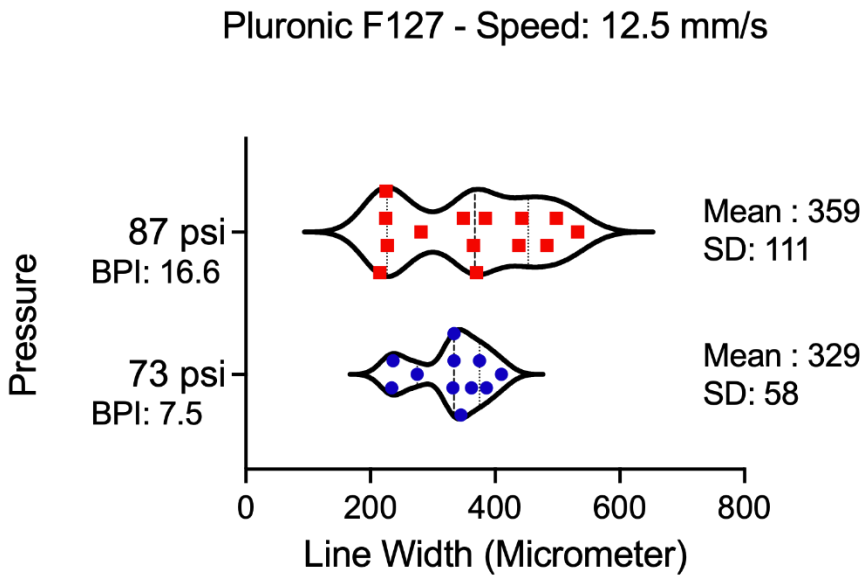

Figure 7. BPI Validation. Pluronic F-127 was printed at 12.5 mm/s speed with extrusion pressure of either 73 psi (BPI: 7.5) or 87 psi (BPI: 16.6). The variance in line width is significantly different between groups ($p < 0.05$).

We reported the Root Mean Squared Error (RMSE) in Tables 5-6 for the four experiments (Collagen, Pluronic, Diluted Collagen, and Pluronic with aMEM). The RMSE shows the quality of the Fuzzy system approximator with the given dataset. However, in this paper, we aimed to measure the precision (BPI value), but the overall accuracy for the Fuzzy system predictor is acceptable based on the bioprinter sensitivity. It is shown that Pluronic performed more accurately in approximation than collagen.

4. Discussion

To our knowledge, no published methods exist to evaluate precision in extrusion bioprinting. Here, we show that Fuzzy systems can be used to identify optimized printing parameter sets that improve the printing precision of existing or newly formulated bioinks. Specifically, we observed that Fuzzy optimization improved precision in collagen bioink by 15% and Pluronic F127 by 68%, as compared to the manufacturer's recommended printing parameters. Furthermore, we have introduced and validated a new standardized metric (BPI) that can be used as a tool comparing repeatability between bioinks. Here, we used BPI to illustrate that the collagen bioink is more challenging for precision printing than pluronic bioink and that diluted bioinks are more precise than non-diluted bioinks.

BPI is a dimensionless factor to measure precision. In this study, we used a simple output of line thickness to analyze bioprinting precision, but this technique can be applied towards any 2D or 3D shape parameter. For example, the inputs or outputs in this system could include a 3D shape factor, the volume of the extruded material, a rheology parameter, or other desired design parameters.

Scientists utilize ML algorithms in additive manufacturing to optimize the material or predict the outputs [24]–[26]. ML and Fuzzy systems are two independent subsets of soft computing. Fuzzy logic, which is inherently based on a fuzzy set, improves system robustness to respond to unpredictable changes in parameters. The rule-based process helps the user to modify the system easier than ML methods, such as Neural Networks (NN). Moreover, the fuzzy system is explicitly defined as compared to the Neural Network algorithm, which is based on the learning from the dataset. The Fuzzy System assists with pattern recognition, which is essential in additive manufacturing to increase system precision. On the other hand, NN help to perform pattern predictions.

Another advantage of a Fuzzy system is that system knowledge can be extracted from the inference engine. In Neural Networks, it is challenging to extract information from the system inference engine with transparency. Nonetheless, a disadvantage of a fuzzy system is that it is not based on learning as a stand-alone system. However, hybrid methods as Neuro-fuzzy systems can be utilized to add these features to a fuzzy system.

Additionally, another potential ML method is Decision Tree modeling, which utilizes a set of if-then rules. The Decision Tree is based on crisp input and generates crisp output data. For an unknown parameter (not in the training dataset), decision tree is less robust in approximating the final output. The fuzzy system uses crisp data that is

converted to a fuzzy set with the fuzzification method (see Supplemental methods). This procedure increases the robustness for unknown or out-of-range data approximation. Moreover, since the inference engine uses Gaussian membership functions, the approximation may continue outside the input range data. However, a hybrid model of decision tree modeling based on a fuzzy system could be investigated in future work [27].

The Fuzzy system developed in our study identified one or multiple sets of optimized printing parameters for each bioink. We note that the target line width for our model was 200 μm. As illustrated in Fig. 3a (arrow), collagen bioink would meet this requirement at the low pressure of 15 psi and speed of 10 or 15 mm/s. In contrast, printing undiluted Pluronic F-127 at 200 μm line width is possible with two different parameter sets, as illustrated in Fig. 3b (arrows). Here, we observed that it is possible to meet this requirement with either high pressure (80 psi) and low speed (12 mm/s) or low pressure (60 psi) and high speed (15 mm/s). Generally low pressure is preferred due to increased cell viability [28], so this observation could significantly improve printing time and experimental outputs when using Pluronic F-127. Investigators are likely to make similar observations if this approach was applied during novel bioink development.

Previous attempts to improve bioprinting precision have focused on developing novel bioprinting techniques, such as the miniaturized progressive cavity pump method to replace the extrusion-based method [29]. However, utilizing our soft computing Fuzzy system technique increases bioprinting precision without hardware changes. This method helps to maintain a robust quality control in bioprinting process which is a multi-parameter nonlinear environment. Moreover, the Fuzzy surface may be useful for understanding tradeoffs between precision and other biological constraints (e.g. cell viability). Nonetheless, our model consisted of three inputs (pressure, speed, and dilution) and one output (line width) that are directly related to the extrusion bioprinting method.

One of the limitations in this study, is the lack of experiments on multiple nozzle gauges. In this study, we only used a 25 g nozzle, which is directly related to flow rate and output pressure. Furthermore, we did not assess temperature, which directly affects bioink viscosity. Since the BPI is specifically a measure of the output precision, utilizing a parameter set with optimal BPI may not result in high accuracy. This limitation may result in a necessary tradeoff between accuracy and precision in choosing a parameter set for bioprinting.

Future work may extend this approach to other parameters that affect bioprinting or its experimental outcomes, such as cell viability or biocompatibility. Nonetheless, we note

that BPI is independent from input dimensions/units and will remain a useful metric for comparison between bioinks as bioprinting technology evolves.

5. Conclusion

Obtaining high precision in bioprinting is a necessary step towards mass production of bioprinted constructs for use in research and medicine. Here, we have demonstrated that a Fuzzy system approach can be used to approximate line width given a set of bioprinting parameters, including printing speed, extrusion pressure, and media dilution percentage, as well as determine bioprinting parameter sets that maximize precision. Furthermore, we have defined the Bioink Precision Index (BPI) that can be used to quickly compare the ease of reproducibility across the wide variety of bioinks currently available.

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Tables:

Table 1. Type-1 Fuzzy system rules for Pluronic experiment

Rule	Pressure (psi) MF SD: 4.24	Speed (mm/s) MF SD: 0.42	Output line width (micrometer) MF SD:16.99
1	60	12	172
2	60	13	223
3	60	14	187
4	60	15	177
5	70	12	316
6	70	13	286
7	70	14	262
8	70	15	224
9	80	12	296
10	80	13	309
11	80	14	337
12	80	15	295
13	90	12	429
14	90	13	371
15	90	14	342
16	90	15	362

Table 2. Type-1 Fuzzy system rules for Collagen experiment

Rule	Pressure (psi) MF SD: 2.12	Speed (mm/s) MF SD: 0.74	Output line width (micrometer) MF SD:42.4
1	15	8	368
2	15	10	264
3	15	12	237
4	15	14	356
5	15	15	267
6	20	8	868
7	20	10	697
8	20	12	671
9	20	14	541
10	20	15	562
11	25	8	1041
12	25	10	1009
13	25	12	959
14	25	14	910
15	25	15	879

Table 3. Type-1 Fuzzy system rules for Diluted Collagen with aMEM experiment

Rule	Dilution Percentage MF SD:8.43	Pressure (psi) MF SD: 0.84	Output line width (micrometer) MF SD: 21.24
1	20	8	251
2	20	10	204
3	20	12	336
4	40	8	210
5	40	10	350
6	40	12	498

Table 4. Type-1 Fuzzy system rules for the Diluted Pluronic with aMEM experiment

Rule	Dilution Percentage MF SD: 8.49	Pressure (psi) MF SD: 2.54	Output line width (micrometer) MF SD: 28.3
1	20	40	302
2	20	45	330
3	20	50	303
4	40	20	169
5	40	25	244
6	40	30	360

Table 5. Type-1 Fuzzy system output vs Measured value for Pluronic and Collagen experiments

Pluronic		**Collagen**	
Measured output line width (micrometer)	Approximated output line width (micrometer) with Fuzzy System	Measured output line width (micrometer)	Approximated output line width (micrometer) with Fuzzy System
201	196	368	418
223	222	264	323
187	189	307	323
177	189	356	333
316	287	267	290
286	259	868	751
262	259	697	619
224	228	671	601
296	272	636	544
309	300	562	545
353.	301	1149	860
295	274	1009	862
429	372	959	823
371	334	895	804
342	332	879	813
362	330	-	-
RMSE = 26.98		**RMSE = 108.84**	

Table 6. Type-1 Fuzzy system output vs Measured value for diluted Pluronic and Collagen with aMEM experiments

Diluted Pluronic with aMEM		**Diluted Collagenwith aMEM**	
Measured output line width (micrometer)	Approximated output line width (micrometer) with Fuzzy System	Measured output line width (micrometer)	Approximated output line width (micrometer) with Fuzzy System
302	283	251	260
330	303	243	243
303	282	336	300
169	181	236	243
244	216	340	339
360	284	569	356
RMSE = 37.37		**RMSE = 88.66**	