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# Parameter optimization for 3D bioprinting of hydrogels

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ABSTRACT

Successful bioprinting of hydrogels relies on geometric accuracy and cell viability, both of which are influenced by a number of variable printing parameters. Despite much research aimed at the resulting quality of bioprinted structures, there is no standard method of comparing bioprint results. In this study, we have developed a simple method of assessing the bioprint results from a range of printing parameters in a standardized manner applicable to extrusion-based bioinks. The purpose of the parameter optimization index (POI) is to minimize the shear stress acting on the bioink, and thus on the encapsulated cells, while ensuring the maximum geometric accuracy is obtained. Here we demonstrate the use of the POI on a blend of 7% alginate and 8% gelatin, and test the printing achieved through 25, 27, and 30 gauge print nozzles at 1–6 mm/s print speeds, and at 100–250 kPa print pressures. In total, we tested 72 printing configurations. Our data shows that for this particular hydrogel blend, the optimum print is obtained with a 30 gauge nozzle, 100 kPa print pressure and 4 mm/s print speed. The POI is intuitive and easy to assess, and could be a useful method across a wide range of 3D bioprinting research and development applications.

## 1. Introduction

Three dimensional (3D) bioprinting relies on cell viability and geometric accuracy. Both these characteristics depend on the properties of the biomaterial being used and are both influenced by the bioprinting parameters [1–3]. Printing parameters include nozzle temperature, printing time, dispensing pressure, printing speed, and nozzle diameter [2,4], all of which directly influence the precision and accuracy of bioink deposition. Furthermore, these parameters also directly impact cell viability as they determine the shear forces within the bioink during the printing process. Increasing nozzle diameter decreases shear stress, therefore reducing cell injury, although this comes at the cost of reduced resolution [5]. Increasing the dispensing pressure has a similar effect to that of decreasing the nozzle diameter, although is proven to have an even more detrimental effect on cell viability [3–5].

Therefore, when bioprinting with hydrogels, much effort is required to determine the optimum printing parameters needed to ensure successful printing [6]. A simple method of assessing the results of different printing parameters could have benefit in the field. Standardized metrics to compare accuracy and understand error have long been in use in other areas of research. For instance, in computational fluid dynamics it is essential to determine the error introduced into simulations from the computational approach used. One such method is the grid convergence index (GCI) [7] where the GCI can be calculated for several versions of the same simulation, each using slightly different parameters, and the error can then be compared. This approach simplifies the assessment of error and thus the interpretation of the accuracy of the simulations. Furthermore, when these metrics are widely adopted, such as the case with the GCI [8], it can become a useful tool to assess and compare to the work of others.

The present study aimed to develop an optimization index for 3D bioprinting to facilitate researchers to use print parameters that maximise printing accuracy and minimise shear stress imparted on the hydrogel during printing. The resulting index is a convenient and standardized assessment metric.

## 2. Methods

### 2.1. Bioprinting process

We used a blend of 7% alginate (alginic acid sodium salt, low viscosity: 4 − 12 cP; Sigma Aldrich) and 8% gelatin (type A porcine skin; Sigma Aldrich) throughout the study. To improve visualisation of

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**Table 1**
Approximate influence of printing parameters on printing objectives.

| | Accuracy (%) | Shear Stress (kPa) |
|---|---|---|
| Increase print speed | Increases | – |
| Increase pressure | Decreases | Increases |
| Increase nozzle diameter | Decreases | Decreases |

the prints we added red food colouring to the hydrogel. Computer-aided design (CAD) software (Autodesk® Inventor®) was used to design a 40 mm long, 10 mm wide alternating line which was then imported into Repetier Host (Hot-World GmbH & Co. KG, Germany) six times as a stereolithography file, to create six equally spaced zigzag lines. The lines were converted into a G-Code with a line height of 0.4 mm using Slic3r. Each line was assigned a different print speed between 1 and 6 mm/s, in 1 mm/s increments. We then used the Inkredible+ bioprinter (CellInk, Sweden) to print the lines using three different diameter straight cylindrical nozzles (25G, 27G and 30G) and a range of pressures from 100 to 250 kPa, in 50 kPa increments. Therefore, we tested 72 combinations of printing parameters. All prints were performed at 37 °C and after printing, the lines were photographed with a scale bar and the dimensions of each line were measured at nine locations using ImageJ (NIH, USA).

### *2.2. Parameter optimization index*

Optimizing the printing parameters between accuracy and shear stress required an understanding of the relationships between the variables. Printing accuracy is a direct result of the geometry of printed lines, and is obtained experimentally. The shear stress relationships, however, are associated with the fluid mechanics of extruding the hydrogel through a thin nozzle (flow through a cylinder) and we know that it is desirable to have low shear stress during the print process [5].

Therefore, from fluid mechanics we know that the shear stress in a fluid flowing through a straight cylinder is dependent on the pressure, whereby an increase in pressure results in increase in shear stress. On the other hand, an increase in nozzle diameter reduces the velocity gradient and thus reduces shear stress. These relationships form the basis of the printing parameter selection procedure, with a summary of the approximate parameter relationships presented in Table 1.

We used these relationships to develop the Parameter Optimization Index (POI). The POI aims to maximise printing accuracy and minimise theoretical shear stress (TSS), through a consistent procedure. The POI is calculated by:

$$POI = Accuracy \bullet \frac{1}{TSS} \quad (1)$$

Accuracy is inversely proportional to line thickness $t_{line}$ hence it can be expressed as:

$$Accuracy \propto \frac{1}{t_{line}} \quad (2)$$

As we cannot easily measure TSS experimentally, it can only be minimized by manipulating the printing parameter relationships. Therefore, the minimum TSS is found by decreasing pressure and increasing nozzle diameter.

$$TSS \propto \frac{1}{p} \bullet D \propto \frac{D}{p} \quad (3)$$

Nozzle gauge, denoted $D_G$, is inversely related to nozzle diameter, thus TSS becomes:

$$TSS \propto \frac{1}{D_G \bullet p} \quad (4)$$

Therefore the POI in a series of experiments using the same bioink can be expressed as:

$$POI = \frac{1}{t_{line} \bullet D_G \bullet p} \quad (5)$$

The POI for a specific bioink across a range of printing parameter can then be normalized relative to the maximum POI in the experimental series:

$$POI_i = \frac{POI_i}{POI_{MAX,n}} \quad (6)$$

Where $i$ denotes the POI for an individual set of parameters while ($MAX$, $n$) denotes the maximum POI of the entire range tested and $n$ is the number of discrete parameter combinations tested. The maximum normalized POI correlates to the set of parameters that maximize accuracy and minimize TSS.

**Table 2**
Mean ± standard deviation (range) measured strand width of all configurations tested. NA denotes configurations that could not create adequate prints and the best configuration with the most accurate strand width is shown in bold.

| | 1 mm/s | 2 mm/s | 3 mm/s | 4 mm/s | 5 mm/s | 6 mm/s |
|---|---|---|---|---|---|---|
| **100 kPa** | | | | | | |
| 25G | 2.25 ± 0.29 (1.99–2.85) | 1.57 ± 0.08 (1.49–1.72) | 1.49 ± 0.29 (1.19–2.14) | 1.34 ± 0.13 (1.15–1.51) | 1.30 ± 0.22 (0.95–1.70) | 1.13 ± 0.06 (1.04–1.12) |
| 27G | 0.70 ± 0.08 (0.62–0.86) | 0.54 ± 0.07 (0.43–0.64) | 0.52 ± 0.09 (0.37–0.69) | 0.44 ± 0.06 (0.35–0.55) | 0.39 ± 0.07 (0.28–0.47) | NA |
| 30G | 0.56 ± 0.06 (0.49–0.62) | 0.42 ± 0.06 (0.33–0.51) | 0.35 ± 0.05 (0.26–0.40) | **0.27 ± 0.05 (0.22–0.39)** | NA | NA |
| **150 kPa** | | | | | | |
| 25G | 4.32 ± 0.84 (3.91–6.31) | 2.73 ± 0.51 (2.26–3.68) | 1.82 ± 0.24 (1.59–2.38) | 1.59 ± 0.13 (1.36–1.75) | 1.31 ± 0.14 (1.10–1.56) | 1.14 ± 0.09 (0.98–1.28) |
| 27G | 1.39 ± 0.12 (1.14–1.53) | 0.93 ± 0.05 (0.86–1.04) | 0.79 ± 0.05 (0.72–0.86) | 0.69 ± 0.06 (0.61–0.77) | 0.63 ± 0.08 (0.53–0.77) | NA |
| 30G | 0.61 ± 0.07 (0.51–0.71) | 0.55 ± 0.04 (0.50–0.61) | 0.48 ± 0.07 (0.38–0.58) | 0.38 ± 0.05 (0.31–0.46) | 0.35 ± 0.06 (0.23–0.43) | NA |
| **200 kPa** | | | | | | |
| 25G | 5.08 ± 0.58 (3.35–5.53) | 2.83 ± 0.34 (2.33–3.41) | 2.18 ± 0.23 (1.83–2.55) | 1.89 ± 0.17 (1.63–2.18) | 1.76 ± 0.12 (1.58–1.90) | 1.59 ± 0.18 (1.33–1.86) |
| 27G | 1.61 ± 0.08 (1.50–1.74) | 1.17 ± 0.08 (1.07–1.30) | 1.00 ± 0.12 (0.84–1.17) | 0.83 ± 0.08 (0.69–0.94) | 0.73 ± 0.09 (0.59–0.87) | 0.73 ± 0.07 (0.62–0.81) |
| 30G | 0.79 ± 0.11 (0.70–1.0) | 0.59 ± 0.09 (0.41–0.70) | 0.51 ± 0.04 (0.44–0.57) | 0.49 ± 0.04 (0.44–0.54) | 0.49 ± 0.07 (0.37–0.59) | NA |
| **250 kPa** | | | | | | |
| 25G | 9.17 ± 1.06 (7.34–11.03) | 5.38 ± 0.76 (4.52–6.49) | 3.73 ± 0.39 (3.26–4.28) | 3.04 ± 0.49 (2.15–3.87) | 2.58 ± 0.28 (2.08–2.94) | 2.72 ± 0.26 (2.04–2.85) |
| 27G | 2.16 ± 0.26 (1.65–2.43) | 1.55 ± 0.14 (1.31–1.70) | 1.16 ± 0.16 (0.94–1.45) | 1.06 ± 0.12 (0.89–1.31) | 1.01 ± 0.06 (0.92–1.09) | 0.91 ± 0.11 (0.71–1.06) |
| 30G | 0.95 ± 0.10 (0.81–1.09) | 0.68 ± 0.08 (0.55–0.83) | 0.53 ± 0.07 (0.44–0.61) | 0.51 ± 0.10 (0.35–0.66) | 0.48 ± 0.06 (0.39–0.59) | NA |

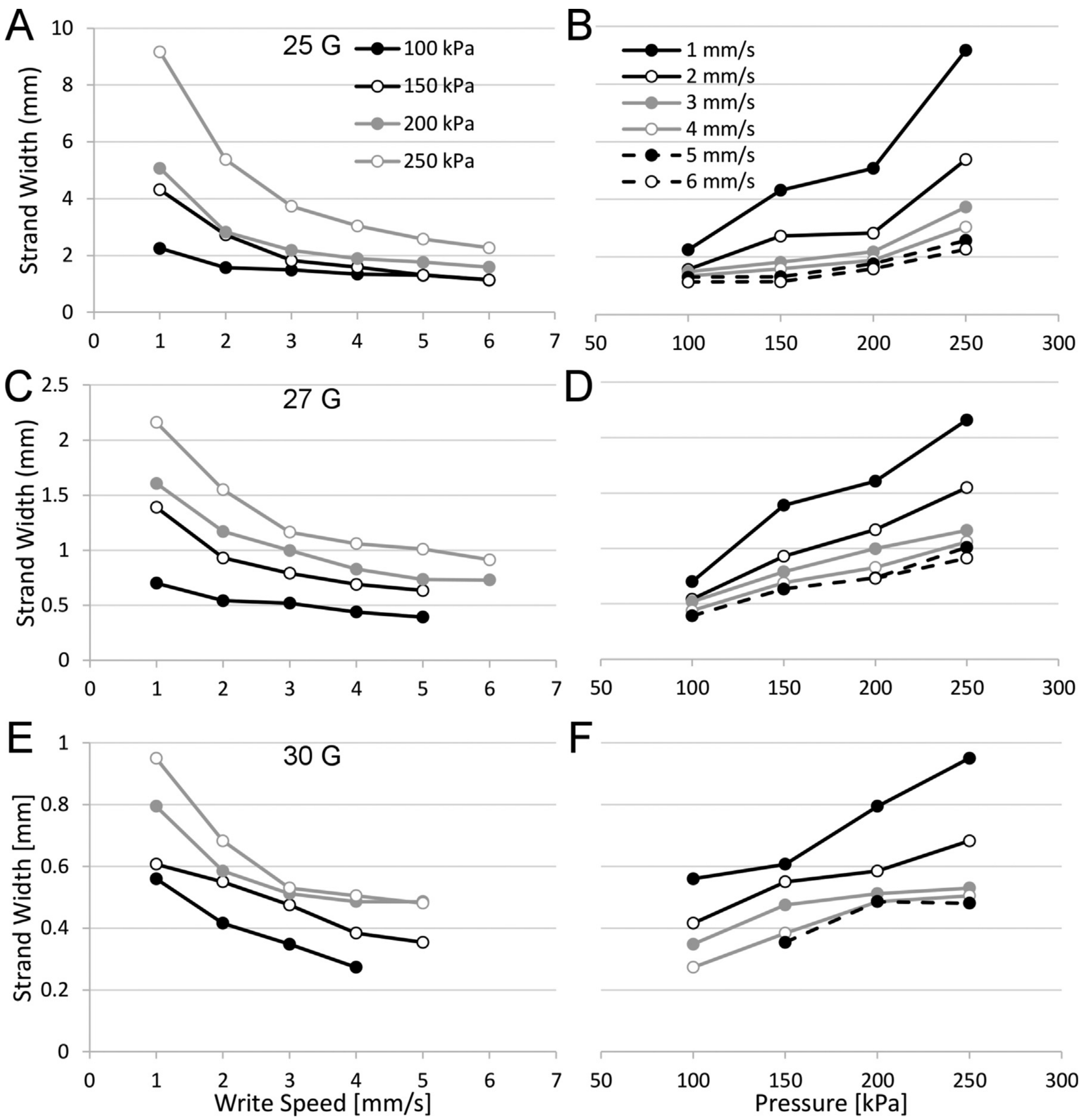


**Fig. 1.** Print test data at the range of write speeds *(left column, A, C and E)* and *pressures (right column, B, D, and F)*. Top row is data obtained using a 25G nozzle, middle row from a 27G nozzle, and bottom row from a 30G nozzle. Each data point is the mean of nine measurements and for clarity the error bars are not shown.

## 3. Results and discussion

From the experimental data presented in Table 2 and Fig. 1, we can see how the various print parameters impact results. At higher print pressures, the increase in write speed has a greater impact on strand width, and thus print accuracy. At the fastest print speeds, adequate prints were not possible for some combinations of gauge and pressure. Also from Fig. 1, we can see that the decrease in strand width, and thus increase in accuracy, is almost exponential with increasing print speed for the higher pressures, with a near linear relationship observed at lower print pressures.

From our experiments and theoretical estimates of shear stress, POI values for discrete combinations of printing parameters were obtained and are shown in Fig. 2. The mean ± standard deviation POI of the study was 0.218 ± 0.2. From the entire study, the lowest ranked POI was 0.014 (25G, 250 kPa, 1 mm/s) which resulted from a printed line width of 9.17 ± 1.06 mm, whereas the highest possible POI of 1.0 was found with the 30G nozzle at 100 kPa and 4 mm/s print speed, resulting in a line width of 0.27 ± 0.05. This is shown in bold in Table 2. The next highest POIs are the 30G, 100 kPa, 3 mm/s (POI = 0.78) and 27G, 100 kPa, 5 mm/s (POI = 0.77). The print accuracy of these parameters was 0.348 ± 0.048 mm (range 0.255 – 0.400 mm) and 0.392 ± 0.069 mm (range 0.277–0.448 mm). The slight increase in accuracy from the small diameter 30G nozzle compared to the 27G, is somewhat balanced by the increase in theoretical shear stress at the same print pressure.

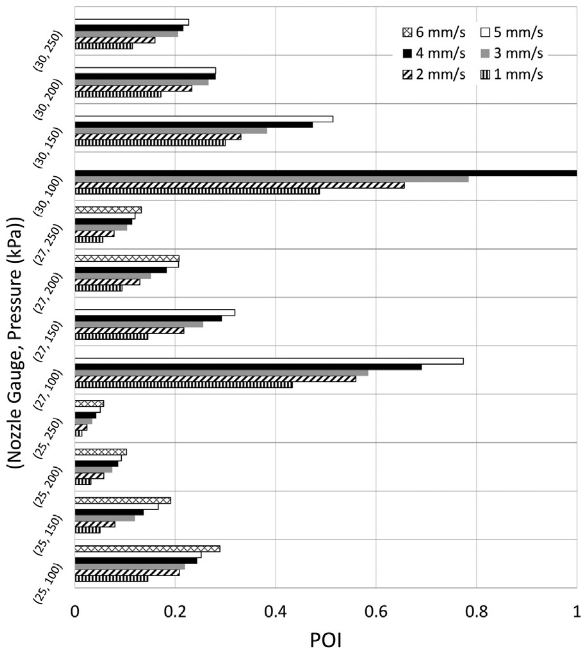


**Fig. 2.** POI for the combinations of printing parameters investigated.

Fig. 3 shows the physical difference between prints at different write speeds using the 27G nozzle and 150 kPa print pressure. The POIs for this combination of parameters deviate around the mean POI of the study and range from 0.15 to 0.32. However, the visible differences are apparent at slower print speeds where the resulting strand width is 1.39 ± 0.12 mm at 1 mm/s compared to 0.63 ± 0.08 mm at 5 mm/s.

For the 7% alginate and 8% gelatin hydrogel blend tested here, our POI suggests that the optimum parameters are:

- Nozzle Gauge = 30G
- Printing Pressure = 100 kPa
- Printing Speed = 4 mm/s

Although there is much effort aimed at improving the accuracy and function of bioprinted objects, with the current state of the art recently reviewed by Murphy and Atala [5], there is still much work needed. When printing with materials such as hydrogels there is often a delicate balance between printing parameters (e.g. print speed, nozzle diameter, temperature), each affecting print results. Therefore, when beginning to bioprint with a specific material, a thorough investigation is often needed to fully understand the influence of each printing parameter. Previous studies have investigated the printability of hydrogels [6] and typically report methods that compare measurements of physical printed objects to the original design. There is no standardized metric to describe print data and we believe a simple method would have benefit; similar to the impact that accuracy metrics have had in computational mechanics. Furthermore, the POI presented here can be tailored so that the measure of accuracy can be the preferred method of the user, and so does not necessarily have to be determined using strand width but could be overall volume, surface area, or another measure.

There are of course some limitations to the POI. We assume that temperature is maintained constant throughout the printing. While this is not unreasonable, it can be difficult to achieve without strict control. Change in temperature can often affect print results and in future work temperature could be incorporated into the POI. We also only investigated straight print nozzles and without further research and modification, the POI is not applicable to conical nozzles. In conical nozzles, the theoretical shear stress will not be constant along the nozzle at constant pressure, and so would need to be further incorporated into the index. However, despite these limitations, we feel the POI could be a useful tool in bioprinting developmental research.

## 4. Conclusions

We have presented a simple method of determining optimum printing parameters for extrusion based 3D bioprinting. The parameter optimization index (POI) has been designed on the basis that increased accuracy and cell viability are both highly desirable features in bioprinting. We demonstrate the use of the POI on a hydrogel blend of 7% alginate and 8% gelatin. The POI shows that the optimum print parameters for this blend are the use of a 30G print nozzle, 100 kPa print pressure and 4 mm/s print speed, which result in strand widths of 0.27 mm while ensuring the shear stress acting on the fluid (and thus any encapsulated cells) is minimized. The POI could be a useful method to compare print data and understand the influence of print parameters on bioinks.

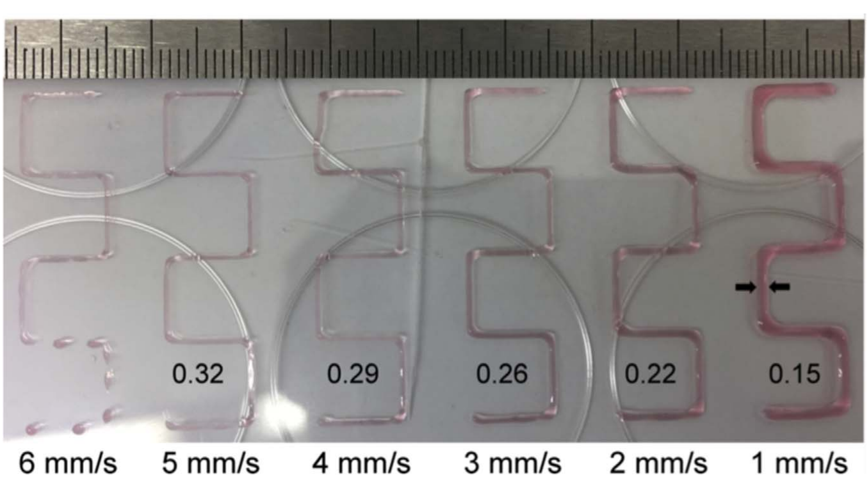


**Fig. 3.** Printed lines using the 27G nozzle at 150 kPa pressure. Print speed is indicated under each line and the resulting POI is next to each line. At a print speed of 6 mm/s the final print is not complete and therefore not included in analyses (denoted NA in Table 2). The arrows indicate the midsection where strand width is measured. There were nine measurements per strand; one measurement at each section of the printed line.

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