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RESEARCH ARTICLE

# Methodology for characterizing the printability of hydrogels

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## Abstract

Currently, the characterization techniques for hydrogels used in bioprinting are extensive, and they could provide data on the physical, chemical, and mechanical properties of hydrogels. While characterizing the hydrogels, the analysis of their printing properties is of great importance in the determination of their potential for bioprinting. The study of printing properties provides data on their capacity to reproduce biomimetic structures and maintain their integrity after the process, as it also relates them to the possible cell viability after the generation of the structures. Current hydrogel characterization techniques require expensive measuring instrument that is not readily available in many research groups. Therefore, it would be interesting to propose a methodology to characterize and compare the printability of different hydrogels in a fast, simple, reliable, and inexpensive way. The aim of this work is to propose a methodology for extrusion-based bioprinters that allows determining the printability of hydrogels that are going to be loaded with cells, by analyzing cell viability with the sessile drop method, molecular cohesion with the filament collapse test, adequate gelation with the quantitative evaluation of the gelation state, and printing precision with the printing grid test. The data obtained after performing this work allow the comparison of different hydrogels or different concentrations of the same hydrogel to determine which one has the most favorable properties to carry out bioprinting studies.

***Keywords:*** Hydrogel; Bioprinting; Printability; Biofabrication window; Characterization

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## 1. Introduction

Bioprinting is an important technique that is constantly evolving and is expected to bring about major breakthrough in the fields of medicine and research. In the future, it may help solve the problems associated with the shortage of organ and tissue donors, and provide a tool for testing new drugs against different diseases. Bioprinting, which is based on additive manufacturing technology, could create solid three-dimensional (3D) objects from digital models[1]. This additive manufacturing methodology is extremely

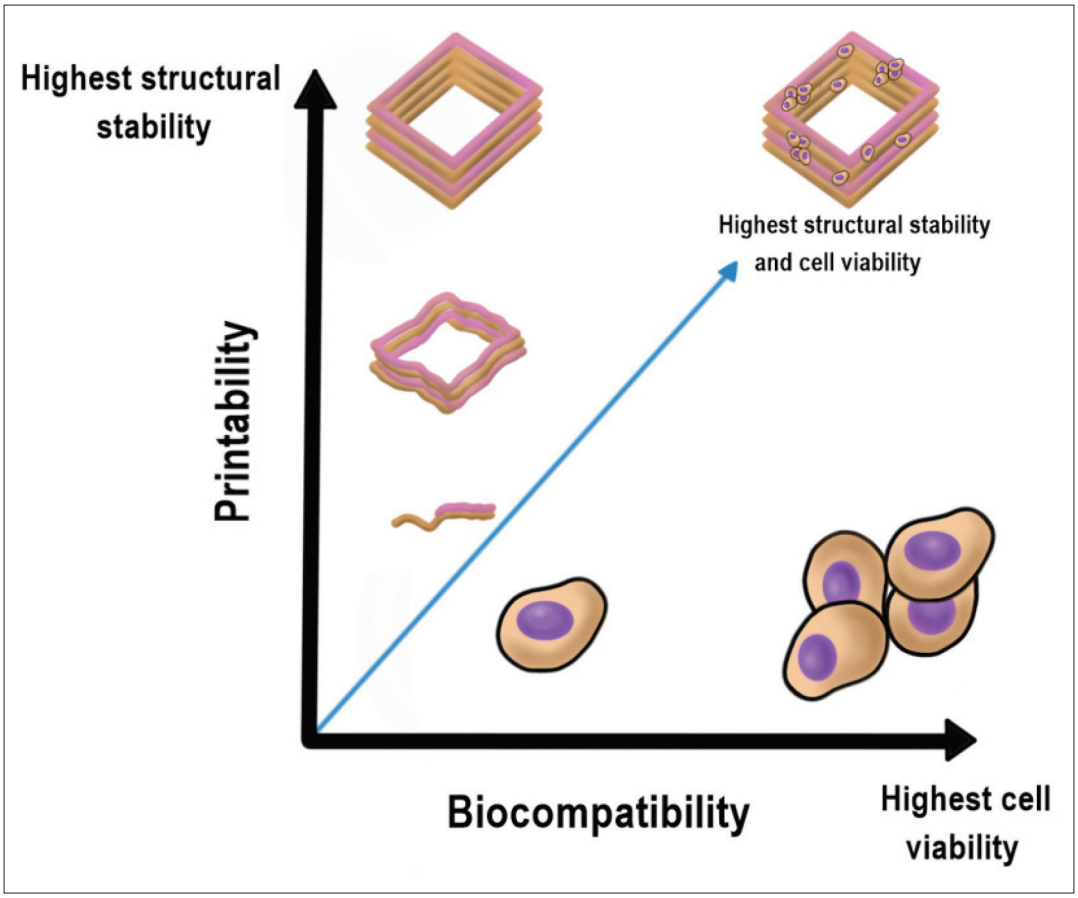


**Figure 1**. Biofabrication window. On the Y-axis, they improve printability and quality of the bioprinted scaffold, while on the X-axis, they improve biocompatibility and cell proliferation.

useful in the field of tissue engineering, defined as the science of designing and manufacturing new tissues for the functional restoration of altered organs and the replacement of structures damaged by trauma and disease[2].

To further improve the outcomes of tissue, it is necessary to develop both cell culture techniques and scaffolds or 3D structures that allow cells to grow while generating biomimetic structures, i.e., while mimicking the 3D structure of tissue from different parts of the body. To this end, bioprinting can be used to generate scaffolds on which cells can grow in the desired 3D shapes. As an additive manufacturing technology, the bioprinting process involves the deposition of successive layers of biomaterials, taking into account the importance of including living cells under conditions that ensure their maximum survival and subsequent viability[3]. In bioprinting processes, materials such as polylactic acid (PLA), light curing resins, or hydrogels can be used as printing matrix, although hydrogels are the most recommended. Hydrogels provide a matrix for tissues to regenerate while controlling the diffusion of molecules and cells[4]. The printability of hydrogels is an important factor in choosing the most suitable one in bioprinting studies. Printability is the ability to form and maintain reproducible 3D scaffolds from bioink using the bioprinting technique[5].

In this study, hydrogels were used as printing matrix for printability studies as they are able to achieve high cell viability. For hydrogels to be considered optimal for use in extrusion bioprinting, there must be a balance between printability (or shape fidelity) and biocompatibility, so that the generated structure possesses sufficient structural stability while being able to achieve high cell viability. The range between high printability and high biocompatibility is known as the "biofabrication window"[6] (Figure 1). In the biofabrication window, the higher the viscosity of the hydrogel, the higher the quality of the bioprinted scaffold but the greater the damage to the cell membrane[7], so it is necessary to find a middle ground that offers high structural stability while allowing the highest possible cell viability. After adjustment of the bioprinting parameters and subsequent generation of structures with the bioprinter, the cell viability rate can be checked by means of different

**Table 1. Mechanical and regeneration properties required of a hydrogel suitable for bioprinting studies**

| Cell regeneration properties[10] | Mechanical properties[12] |
|---|---|
| Non-cytotoxic and non-immunogenic | Density |
| Mimic the extracellular matrix to achieve cell adhesion, propagation and osteogenic differentiation at the implantation site | Porosity |
| Degradable or hydrolyzable by endogenous enzymes | Stability |
| Structurally stable and mechanically strong | Adhesion |
| Adequate porosity (for the purpose of cellular interaction, control of bioactive factor release, nutritional and oxygen exchange) | pH and temperature |
| – | Biodegradability |

live/dead tests offered by commercial companies, and the use of image processing software such as ImageJ[8].

Hydrogels are hydrophilic and cross-linked polymers, which can absorb and swell in water and biofluids, and transform into insoluble 3D networks[9] that give them elastic properties when subjected to different stresses during bioprinting. Furthermore, they can be composed of either natural, synthetic, or hybrid materials[6]. When choosing a hydrogel for bioprinting that guarantees both cell viability and structural integrity, it is important to ensure that the hydrogel embodies a number of properties that make them suitable for cell regeneration[10]. Also, the mechanical properties of the hydrogel must be taken into account, which must be within certain ranges[11] (Table 1). Other properties such as concentration and viscosity have to be taken into account because some of the above-mentioned properties depend on them[12].

Certain tests can be carried out to determine the stability of hydrogels used in bioprinting, so as to provide an estimate of the mechanical stability and an approximation of the cell viability. Another important parameter to take into account when using hydrogels in bioprinting is their printability. Without good printability, hydrogels do not have the capacity to reproduce biomimetic structures. Often, the measurement of printability can be carried out by different rheometry and viscosity studies that require highly specific equipment, which is very expensive and consumes a large amount of the material to be studied. Rheometers are very precise measuring instruments that can determine and analyze the behavior of different materials in deformation and flow processes. Thus, they can provide useful data for the characterization of hydrogels used in bioprinting, such as viscosity, creep, shear strain, deformation, and shear rate. With the knowledge of these data, it would be possible to determine which hydrogel has the best printability characteristics for the printing conditions to be used in a very specific way. The disadvantages of using measuring instruments, such as rheometers or viscometers, include: (i) associated high cost, which has deterred many research teams from owning the instruments for determining the printability of hydrogels, and (ii) the need to spend large quantities of hydrogel, which is also expensive, for the study.

Both commercial ready-to-use hydrogels and reconstitution kits, produced under sterile and high-quality conditions, are very costly that not all research groups can afford to expend them just for testing purposes. In addition, cell viability tests are often expensive as well, so it is important to have cheaper methods that can easily determine whether a hydrogel has the necessary wettability to allow cell survival. Due to the high costs of both measuring instruments (rheometer or viscometer) and bioprinting materials to be studied (hydrogels), a methodology is proposed that allows printability studies to be carried out without the use of expensive equipment or large expenditure of material.

To carry out the aforementioned studies, and after carrying out the sessile drop method to determine the possible cell viability in the bioprinted structure, Bio X bioprinter was used, allowing the ideal pressure and temperature parameters to be adjusted for each hydrogel within the parameters of cell viability, thanks to its integrated drop test. This test makes it possible, with a low material cost, to determine whether the hydrogel in question could achieve good printability. Thus, at ideal pressure and temperature conditions for each hydrogel, comparisons could be made between the different hydrogels, thanks to the combination of different tests that provide data on the printability of the same, to identify hydrogels that do not meet the required needs, with the aim of selecting the hydrogels with higher chance of printability.

In this paper, we propose a methodology combining different tests that characterize printability of different hydrogels, which help determine the hydrogels with the best mechanical properties and the best biological properties to allow cell survival with the least possible waste of material. Since there is currently no ISO standard for characterizing printability in terms of cell viability, the methodology described herein may be of great use to researchers in this field.

## 2. Objectives

The aim of this study is to develop a new methodology to configure and characterize hydrogels from different existing tests to achieve an optimal compromise between printability and cell viability in a process optimized to minimize material utilization.

This methodology enables the classification of new materials according to different characterization tests, such as sessile drop method, filament collapse test, quantitative evaluation of the state of gelation, and printing grid test. To this end, we developed the techniques mentioned and created a platform adapted to the Bio X bioprinter for filament collapse test. In the process, we assigned numerical values to help determine hydrogels with the most suitable characteristics for bioprinting.

## 3. Methodology

This methodology is designed to characterize hydrogels that are going to be used to bioprint cells inside them, i.e., those that can be printed at temperatures close to 37°C. First of all, the pressure and temperature parameters were adjusted by means of the droplet test of the extrusion-based bioprinter so that they were always within the ranges that allow cell viability. Therefore, as a reference, the temperature should not exceed 37°C, while the pressure should not exceed 30 kPa, according to some authors[13].

The bioprinter must be kept in a temperature- and humidity-controlled environment. In this study, the bioprinter was placed in a temperature- and humidity-controlled chamber manufactured by the team, using a PID temperature and humidity controller, a thermal resistor, and a humidifier. BIO X bioprinter from CELLINK was used to perform this methodology.

A battery of tests was carried out to test and process the hydrogels so that we were able to make precise comparisons between the different hydrogels we analyzed. In order to carry out the test on different hydrogels and to know which one is the most suitable for the bioprinting of biomimetic structures, it is necessary to study the printability together with other characteristics[14], such as resistance to traction or compression, and even the deformation that can be produced. In addition, the amount of material used was optimized in the methodology to reduce the relevant costs, while ensuring that quantitative and visual results showing which hydrogel presents the best structural characteristics could be obtained.

In addition, the proposed methodology can also provide data on cell viability of hydrogels, which helps decide whether to load hydrogels with cells. Sessile drop method allows discarding hydrogels with poor cell viability results, thereby obviating the need in such a case to perform biological assays. Cell viability can be further checked with tests such as the LIVE/DEAD® assay, or by assessing cell metabolic activity with MTT assay, among others.

This work aims to provide a unified methodology for characterizing the printability of hydrogels that will be loaded with cells for bioprinting. The paper also presents some examples with real data for better understanding.

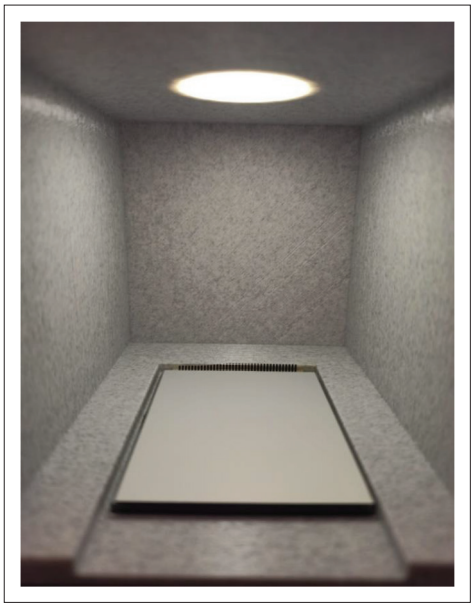

**Figure 2**. Chamber generated to carry out the sessile drop method using a non-absorbent bed and appropriate illumination.

### 3.1. Sessile drop method

The sessile droplet method is based on the contact angle of a hydrogel droplet on a surface, and provides data on its wettability[15] and on its ability to wet the surface of a solid. Contact angles between 0° and 90° have been found to indicate a wettable, hydrophilic surface, while an angle between 90° and 180° indicates a non-wettable, hydrophobic surface[16,17]. Hydrophilicity or wettability of biomaterials is considered a very important parameter for certain applications, such as cell adhesion in tissue engineering[18].

In order to measure the contact angle, a chamber was made, in which a glass plate was placed at its base, which does not absorb the material to be studied. At the same time, a light source was placed at the upper part of the chamber to generate a vertical illumination on the hydrogel drop without forming shadows that could hinder the image capture process (Figure 2).

After adding the drop to be studied in the chamber, images were captured in a perpendicular angle from the glass plate using a USB 40× to 100× digital microscope, and software (AMCap) was used to digitize the image on the computer. In this way, precise images could be taken of all the hydrogel droplets from the same angle. These images must be processed by an image processing program, such as ImageJ or Fiji, to measure the contact angle. They can also be measured by computer-aided design (CAD) software, which can also measure the contact angle.

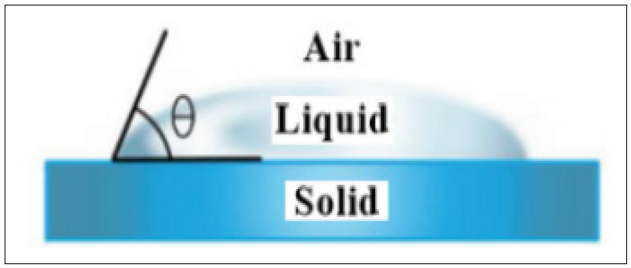


**Figure 3**. Contact angle at the solid–air interface.

After taking four measurements of each hydrogel droplet, the following results were obtained[19]:

(i) A contact angle of less than 35° indicates that the surfaces are too hydrophilic, which prevents interactions with cells.

(ii) A contact angle of greater than 80° indicates that the surfaces are too hydrophobic, which can lead to protein denaturation.

(iii) A contact angle between 35° and 80° is ideal for a hydrogel for moderate wettability property (Figure 3).

The study can be carried out at different temperatures for each hydrogel, which makes it possible to analyze the behavior of the hydrogels as a function of the bioprinting temperature. This study is designed for the subsequent introduction of cells into a hydrogel under human body temperature conditions, so temperatures at around 37°C have been chosen, which is the optimum temperature for maximum cell viability.

An example of hydrogel characterization using the sessile drop method is given in Table 2. In this example, Matrigel and ColMA (Cs = 10) were used. Matrigel is a material that requires low temperature for its maintenance as it polymerizes at room temperature. Therefore, its temperature must be low both when performing the sessile drop method and when using it as a bioink in the bioprinter. Although due to its low bioprinting temperature, this hydrogel is not suitable for cell-loaded bioprinting, the good results obtained in the sessile drop method indicate that it is suitable for subsequent loading of cells into the bioprinter-generated structure because the contact angles are between 35° and 80°. It can also be seen in Table 2 that the ColMA material (Cs = 10), with a contact angle of 32° and 31° at 9°C and 15°C, respectively, did not perform well in the sessile drop method. As a consequence, the cells started to die 3 days after printing.

This method does not determine the actual cell viability of a hydrogel, but rather provides information on the wettability of the hydrogel, related to cell adhesion and propagation[20].

**Table 2. Example of hydrogel characterization using the sessile drop method**

| Material | Temperature (°C) | Contact angle |
|---|---|---|
| Matrigel | 9 | 45 |
| | 15 | 48 |
| ColMA (Cs = 5) | 9 | 32 |
| | 15 | 31 |

### 3.2. Filament collapse test

Filament collapse test allows the deflection of the hydrogel filaments to be determined as they pass through pillars spaced at different distances. These distances span from shorter to longer range, with deflection being more likely to occur at longer distances.

To obtain the collapse rate, the hydrogel must be deposited on top of the platform pillars (Figure 4), so that it passes through the least spaced pillars first and ends at the most spaced pillars.

From the differences between the theoretical area and the real area, the collapse rate is obtained ($C_f$) using the equation below[21]:

$$C_f = \frac{A_t^c - A_a^c}{A_t^c} \cdot 100\% \quad \text{(I)}$$

where $A_t^c$ is the total area, and $A_a^c$ is the area generated after depositing the filament (real area).

In this way, if the real area and the theoretical area coincide while the filament does not collapse, the collapse coefficient is 0%. Using an image processing program such as ImageJ, or a vector drawing program such as AutoCAD that allows the measurement of angles by scaling images, the total area ($A_t^c$) of the square formed by the adjacent columns and the area generated after depositing the filament ($A_a^c$) are calculated (Figure 4).

The collapse of each separation of the pillars is calculated individually, starting with the end of the platform with the smallest separation between the pillars and maintaining the consecutive order in which the measurements are taken ($C_{f1}$, $C_{f2}$, $C_{f3}$...). With the data obtained, a table is made in which after calculating the $C_f$ for each separation, the exact point at which the hydrogel collapses completely or partially can be observed.

With the data obtained from this test, a table is obtained that allows the comparison of different hydrogels, thus providing a quantitative method for determining the mechanical stability of hydrogels, which allows them to be compared with greater precision.

An example of a hydrogel exposed to the filament collapse test can be seen in Table 3. The hydrogel used

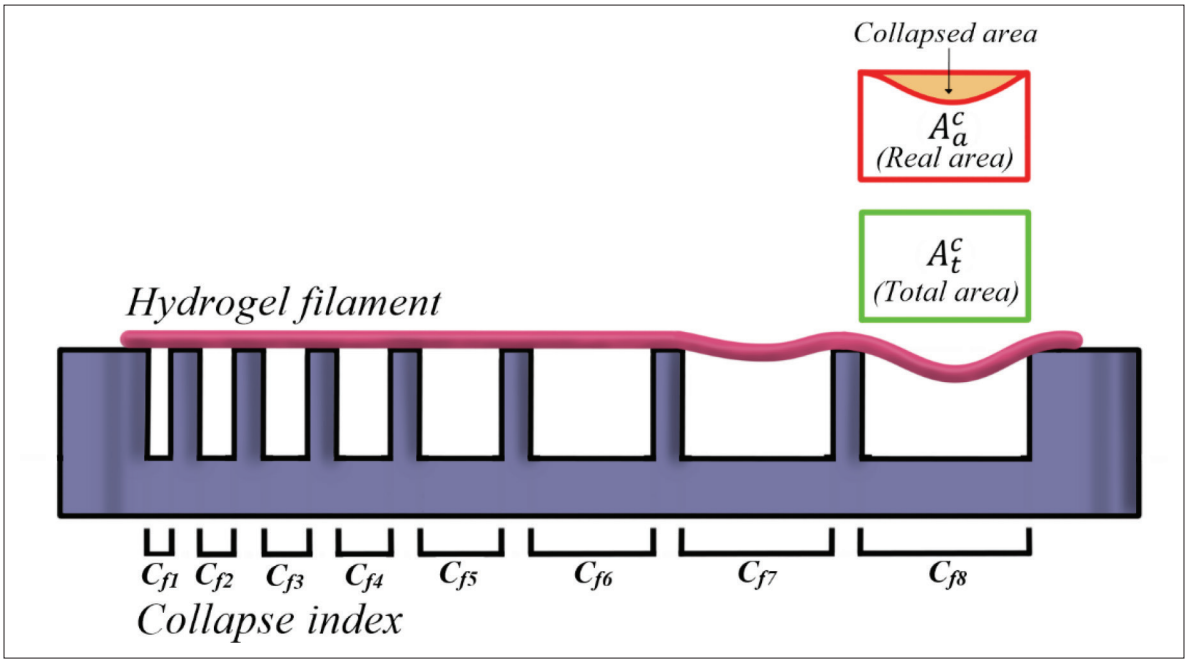


**Figure 4**. Calculation of the collapse coefficient on the basis of the actual area and the theoretical area.

**Table 3. Filament collapse test for CELLINK START**

| Hydrogel | $C_{f1}$ | $C_{f2}$ | $C_{f3}$ | $C_{f4}$ | $C_{f5}$ | $C_{f6}$ | $C_{f7}$ | $C_{f8}$ |
|---|---|---|---|---|---|---|---|---|
| **CELLINK START** | 0 | 0 | 0 | 0 | 2.43 | 5.64 | 9.83 | 10.30 |

The data obtained after calculations for each Cf.

was CELLINK START, a sacrificial hydrogel with excellent mechanical properties that is used for testing with a bioprinter and can avoid the waste of commercial bioinks.

The results obtained indicate that from $C_{f5}$, a notable collapse begins to occur, so this test is a comparison guide between different hydrogels and an indication of the point at which the hydrogel can fail when generating a structure. To carry out this study, we propose the development of a platform with the same shape of a Petri dish that the bioprinter detects, but with pillars inside that are spaced one unit further apart each time (Figure 5). The use of this platform makes it possible to control:

(i) Extrusion pressure of hydrogels

(ii) Speed of movement during the application of pressure and therefore during printing

(iii) Temperature during the test

(iv) Temperature of the plate on which the test is carried out (an important factor because some hydrogels may crosslink at certain temperatures, resulting in misleading results)

(v) Application of UVC rays (short-wave ultraviolet C rays), which causes crosslinking in some hydrogels

(vi) Amount of hydrogel to be bioprinted (to standardize amount of hydrogel in all tests and allow reliable comparison among the hydrogels to be studied)

This platform has been digitally designed using Inventor software and adjusted to the bioprinter bed. A 3D printer is used for printing with PLA. Afterward, all the pores generated by the PLA were covered with resin.

### 3.3. Quantitative assessment of the gelation state

Quantitative assessment of the gelation state allows us to determine the printability of the different hydrogels to be studied. In this study, it is possible to determine whether a hydrogel has a good state of gelation and, therefore, whether it has a smooth surface and a constant width in the three dimensions, facilitating the bioprinting of regular matrices with square holes[22].

In this test, feed speed, printing distance, and air pressure affected the print quality of the web, and in addition to these parameters, line distance and line intersection area also affect web quality[23]. For this purpose, a regular matrix with square holes was bioprinted, and the data necessary to carry out the study were obtained using an image processing program such as those mentioned in the above.

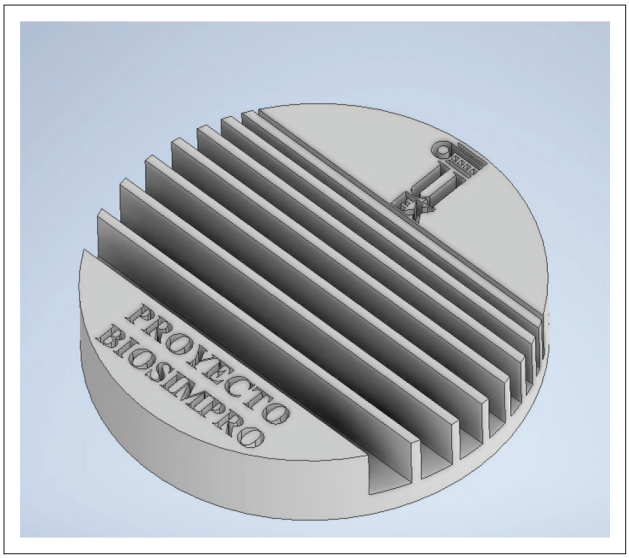


**Figure 5**. Platform designed to carry out the collapse test of the hydrogel filaments extruded with bioprinter.

Thus, printability (*Pr*) is defined by the following equation[24]:

$$Pr = \frac{\pi}{4} \bullet \frac{1}{C} = \frac{L^2}{16A} \quad \text{(II)}$$

Where *C* (circularity) is:

$$C = \frac{4\pi A}{L^2} \quad \text{(III)}$$

Where *L* is perimeter and *A* is area.

*Pr* = 1 implies the square printing shape of the holes in the matrix and thus presents a perfect gelation condition[25]. Thus, the closer the value obtained is to 1, the higher the printability of the hydrogel. On the other hand, a hydrogel that obtains a printability value lower than 1 will exhibit low gelation, and a hydrogel that obtains a value above 1 will exhibit excessive gelation (Figure 6).

The data can be used to study the gelation properties of the same hydrogel when the concentrations of the different elements that form it are modified, or even to compare different types of hydrogels according to these properties in order to determine which one has better printing characteristics.

The images to observe the *Pr* value are obtained using a USB 40× to 100× digital microscope placed perpendicularly to the surface where the regular matrix is located, which, as already mentioned, allows sharp images to be captured and processed directly on the computer. The platform used to support the microscope and allow the images to be obtained from the same position and angle was designed with Inventor.

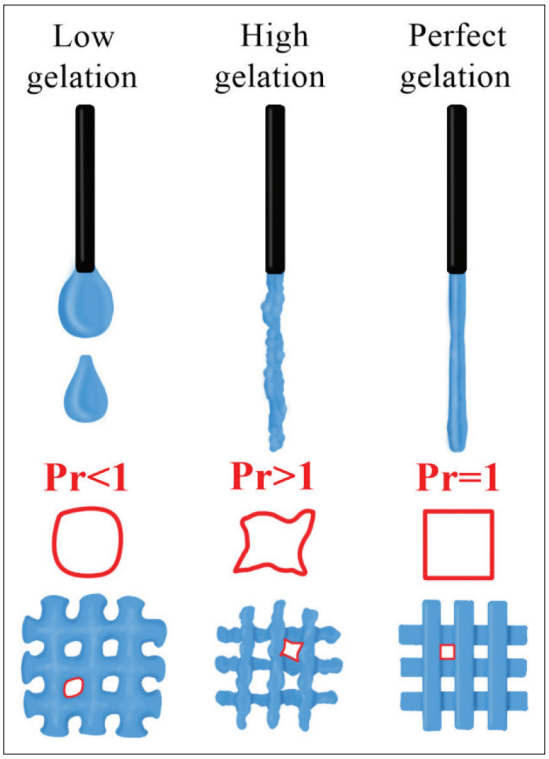


**Figure 6**. Visualization of a hydrogel with low gelation (A), with perfect gelation (B), and with excessive gelation (C).

**Table 4. Characterization of the printability of HAMA 5% using the quantitative assessment of gelation state**

| Hydrogel | Image | Pr | State of gelation |
|---|---|---|---|
| HAMA 5% |  | <1 | Low |

In the following example (Table 4), the printability of HAMA 5% was observed. According to the scheme presented above in Figure 6, and depending on the amorphous shape of the squares in the matrix, the value of *Pr* is lower than 1 (*Pr* < 1), indicating that the printability is poor.

### 3.4. Printing grid test

Printing grid test allows to determine the capacity of a hydrogel to reproduce a given pattern and its tendency to generate accumulations or clusters[26]. It can be used to check whether the hydrogels under study have sufficient mechanical properties to generate a grid or pattern of squares and rectangles of different dimensions like the one in Figure 7.

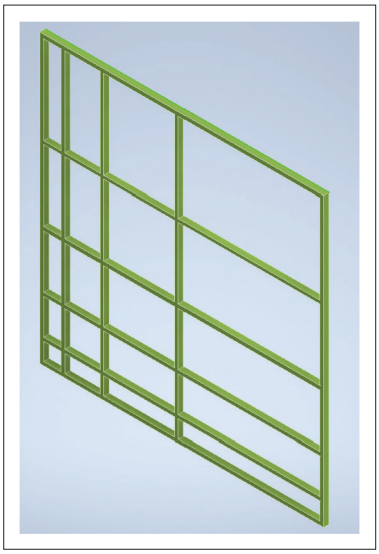

**Figure 7**. Grid or pattern of squares and rectangles of different dimensions.

After bioprinting the designed pattern and knowing the real size of the generated structure, using an image processing program, the difference between the real value and the theoretical value in $mm^2$ of the squares generated within the grid can be established. The real value is obtained by measuring the area of each of the squares. To do this, the bioprinted grid is first compared with a millimeter standard to obtain the measurement of the sides of each square. Subsequently, using an image processing software and knowing the measurement of the sides of the squares, the real value of each of them can be obtained. The theoretical value is the one that is established for each square during the design of the grid by means of computer programs such as AutoCAD. Both values can be compared with each other using the statistical analysis of the standard deviation, which provides data on their degree of equality. According to the outcome, the lower the standard deviation, the closer the theoretical and real values, and therefore, the higher the printability of the hydrogel (Table 5). The data obtained from this test can be useful for comparisons of printability between different hydrogels.

### 3.5. Quantitative assessment of gelation state and printing grid test

In order to optimize the process of characterizing the printing properties of hydrogels, it is necessary to carry out the quantitative evaluation of the gelation state and the printing grid test together. For this purpose, the printing of the grid is carried out by measuring all sides of the grid, obtaining the actual values in order to compare them with the theoretical values after analysis using image processing software, and then calculating the standard deviation. At the same time, the printability is measured using the Equations II and III described in section 3.3 (Figure 8).

**Table 5. Example of calculation for the printing grid test**

| | Real value ($mm^2$) | Theoretical value ($mm^2$) | Standard deviation |
|---|---|---|---|
| **Row 1** | 17.33 | 20.41 | 2.18 |
| **Row 2** | 36.33 | 40.83 | 3.18 |
| **Row 3** | 55.08 | 61.24 | 4.35 |
| **Row 4** | 74.40 | 81,.66 | 5.13 |
| **Row 5** | 98.68 | 102.06 | 2.39 |

The hydrogel used in this test was GelMA 5%. In this table, from the bottom (row 1) to the top (row 5), the data of the largest squares of the grid (right) have been included.

## 4. Discussion

Printability applied to the field of bioprinting is a parameter that measures the accuracy of bioprinters in generating biomimetic 3D structures with biomaterials. For a bioink to have adequate printability, it must have certain properties that provide optimal printing results and biomimetic fidelity. Some of the parameters that determine these properties are concentration and viscosity, which, on the one hand, ensure the reproducibility of the experiment and increase the printability of the hydrogel, and on the other hand, determine its flowability[14].

When printing a hydrogel, the printing parameters must be adjusted. The printing parameters must be chosen according to the composition of each hydrogel in order to ensure the highest possible printability in each case. Good printability with good cell viability properties will result in structures that are suitable for construction of biomimetic tissue for future medical applications. The most relevant printing parameters are temperature, pressure, and printing speed. Therefore, these parameters will be adjusted before different tests of this methodology are carried out so as to allow subsequent comparisons between different hydrogels.

Bioprinting of cell-loaded bioink has been shown to have a high rate of cell viability[27-29]. The conceptualization of this methodology takes into account the possibility of loading cells into the bioink for the bioprinting of scaffolds with cells inside, although it can also be applied to bioinks that are going to be used for the generation of scaffolds that will subsequently be seeded with cells on their surface.

Before using this methodology, the best conditions for bioprinting hydrogel under minimum cell viability conditions can be determined with the help of droplet test of the bioprinter. Specifically, data pertaining to the most suitable pressure for this bioink at the temperature of 37°C

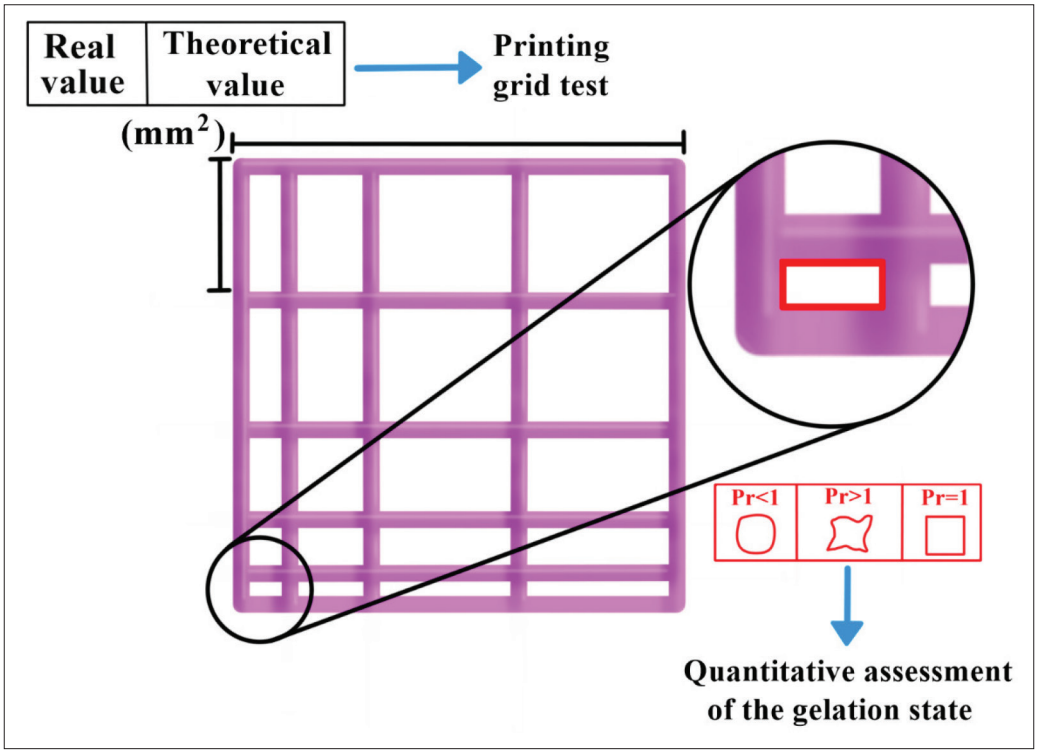


**Figure 8**. Simultaneous performance of the grid test and quantitative assessment of the gelation state.

can be obtained. The rest of the tests of the methodology are based on previous characterization.

The proposed methodology starts with the sessile drop method. In this method, the contact angle generated by a hydrogel drop on the surface on which it is deposited is measured. This angle can provide data on the wettability of the hydrogel and therefore whether it has sufficient moisture conditions to allow cell viability. With the data obtained from this test, the hydrogels that do not have an adequate wettability to allow cell viability due to their concentration or composition can be discarded.

Once the wettability of the hydrogels has been determined and therefore, those with the best wettability properties for cell survival have been selected, the filament collapse test can be performed. With this test, the stability of the filament can be checked by measuring the deflection at mid-span of a suspended bioink filament[30], and the speed parameter can be adjusted. This test can be used to compare different hydrogels and to compare different concentrations of the same hydrogel, allowing the selection of a combination of optimum printing requirements in each case.

After performing the filament collapse test, the quantitative assessment of the gelation state and the printing grid test can be performed together. With the quantitative assessment of the gelation state, the printability of a hydrogel can be measured on the basis of the circularity of the squares of a matrix of squares. The closer the squares of the matrix to the shape of a perfect square, the closer the value is to 1. With excessive gelation, the result will be greater than 1, while with poor gelation, the result will be less than 1. On the other hand, the grid test measures printability in terms of the degree of similarity of the surface area in $mm^2$ of the squares of a bioprinted grid (real value) to that of the squares of the digitally designed version (theoretical value). Thus, when comparing the two data, lower standard deviation indicates higher printability. As both tests are based on the measurement of parameters related to squares, it has been proposed that both tests should be jointly carried out while printing a grid on which the surface area of its squares is measured for subsequent comparison with its digital version and the circularity of the squares is measured.

Cell viability tests can be performed after choosing the hydrogel with the best results using different viability tests, such as the live/dead test that uses calcein-AM and propidium iodide (PI)[31]. After performing the different tests proposed, it is possible to determine which hydrogel is best suited to the required printability parameters. In this case, the bioprinting conditions for each hydrogel have been adapted to have the best possible printability at 37°C to allow cell-loaded printing.

## 5. Conclusions

The characterization of hydrogels in bioprinting can be expensive due to the high cost of the hydrogels and the necessary analytical instruments. In addition, the cell inclusion process can also be costly and tedious due to the high cost of the components to be used, the complicated procedures prior to obtaining viable cultures and the difficult conditions that must be maintained to ensure cell survival. Therefore, it is necessary to carry out studies before using the hydrogel in order to increase the probability of success, both in terms of cell viability and structural integrity.

After the adjustment of the temperature and pressure parameters, studies such as sessile drop method, filament collapse test, quantitative evaluation of gelation state, and printing grid test allow fast and simple evaluation of the hydrogels to be loaded with cells, with low material waste.

With these studies, the behavior of the hydrogels after the bioprinting process can be predicted to a large extent, making it possible to discard those formulations that do not perform well before carrying out the cell inclusion process. The proposed methodology saves time and money in bioprinting research, bringing researchers closer to a positive result. The development of this methodology for characterizing the printability of hydrogels in the area of bioprinting is not possible without the INMA group's experience in the analysis of hydrogels[14,32-35].

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## Conflict of interest

The authors declare no conflicts of interest.

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## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data

Not applicable.

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