

A PRELIMINARY STUDY OF THE LIGHT INTENSITY INFLUENCE ON 4D PRINTED TEMPERATURE-RESPONSIVE NIPAM BASED HYDROGELS

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Abstract—4D printing managed to overcome some of the limitations of its predecessor, the 3D printing process, by replacing rigid structures with structures capable of changing their shape over time. The responsive nature of the 4D printed structures is of interest to several areas, including tissue engineering, which aims to restore, maintain, and improve damaged tissues or whole organs. Among the range of materials commercially available, poly (N-isopropyl acrylamide) (NIPAM) stands out as a thermo-responsive polymer compatible with different cell cultures. As much as there is already some consolidated knowledge about the material, there is still a lot to be explored in terms of 4D bioprinting technologies capable of efficiently generating NIPAM thermo-responsive structures. This work explores the impact of light incidence on a NIPAM based hydrogel to be processed by digital light processing (DLP). With the aid of a power meter, tests were performed regarding the variation of luminosity incident on the hydrogel. It was concluded that a waiting time of 20 minutes is necessary until the light source reaches a steady state of light intensity supply, and the ideal energy intensity for polymerization of a NIPAM based hydrogel using Irgacure 2959 as a photoinitiator is approximately 22mW.

Keywords-component; light intensity; photopolymerization; 4D printing; NIPAM

I. INTRODUCTION

The advent of 4D printing, in which time was added as the 4th dimension, urged a need to develop new materials able to respond to external stimuli in a controlled manner in such a way that their shape could be tunable with time [1]. In the specific case of 4D printing applied to tissue engineering, among the range of possible stimuli, temperature variations stand out by been a non-invasive, easy-to-vary stimulus that can be modified with considerable precision without affecting living cells [2], [3]. There are several polymers capable of changing their shape under thermal stimuli, such as polyethylene glycol diacrylate [4], polylactic acid [4], poly (N-isopropyl acrylamide-co-acrylic acid) [5], methacrylate polycaprolactone [6], among others.

The chemical composition is as important as its mechanical properties and its interaction with the media. Hydrogels are the

most desired structures because in this arrangement the networks of cross-linked hydrophilic polymer chains can swell 99% weight of its mass in water [7]. Hydrogel's porosity is high enough to enable exchanges with the external medium and allow cell infiltration and interconnectivity, since it mimics the behavior of the natural extracellular matrix, hydrogels provide an excellent growth medium for tissue engineering [8].

Several authors consider poly (N-isopropyl acrylamide) (NIPAM) the most promising temperature-responsive biocompatible monomer to produce bio hydrogels [5], [9], [10]. NIPAM based hydrogels have well-defined structures, tunable properties, and some research with long-term cell cultures proved their biocompatibility with several cell types [9]. Different methods can be used to obtain NIPAM hydrogels, one of them been radical-mediated photopolymerization. This polymerization method is fast, can allow spatial and temporal control, and obtain highly crosslinked, functional polymers [11]. Radical-mediated photopolymerization requires the formulation of a solution containing the desired monomer and a photoinitiator that will start the polymerization reaction when activated by light [12].

A wide range of photoinitiators is commercially available, among them 2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone, commercially known as Irgacure 2959, the most widely used UV light-sensitive photoinitiator in the field of tissue engineering [12]. Irgacure 2959 is a highly efficient, nonyellowing radical photoinitiator with low cytotoxicity, minimal immunogenicity, and moderate water solubility [13]. It is activated by ultraviolet (UV) light with a wavelength between 320 to 365 nm.

The joint use of Irgacure 2959 and NIPAM is already being explored by [14] – [16] to produce bio hydrogels, by [17] to produce a biomimetic flower structure, by [10] to produce drug delivery systems, among others. However, the manufacturing process must be adapted for each application and manufacturing technique employed. This work aims to analyze how UV light intensity affects the polymerization results of NIPAM based hydrogel to be processed by digital light processing (DLP).

II. METHODS

To produce the NIPAM based hydrogels, a solution of NIPAM, 1.50 mol% Irgacure 2959 (Sigma-Aldrich, >98%) and 1.47 mol% N,N'-methylenebis(acrylamide) (BIS) (Sigma-Aldrich, >99%), using deionized water as solvent was formulated and mixed for 30 minutes using a magnetic stirrer. After complete dissolution, the solution was taken to cure under the incidence of UV light (Everbeam 50 W and 365 nm). The curing process temperature was kept at 5 ± 0.5 °C utilizing an air-cooling system Temptronic's ThermoStream.

Light intensity measures from a constant distance of 35 mm from the light source, were taken every 30 seconds for 2 hours, to identify the behavior of the light intensity over time. To map the differences in light intensity regarding the distance between sample and source, measurements were made from the top of the light until 300 mm apart from the source. Measurements were taken every 10 mm with a light capture time of 3 seconds for each measurement. For both tests, the light intensity was

measured using an optical power meter (ThorLabs – PM100D), with a high-resolution thermal power sensor (ThorLabs – S401C). The sensor has a resolution of 1 μ W and the uncertainty of the measurement for the applied wavelength was $\pm 5\%$. The tests were carried out in a dark room to avoid the incidence of ambient light, however, it was not complete darkness, as there were cracks of light and there were also the panel lights from the equipment that were being used.

To verify which light intensity was ideal for the proposed composition, experimental tests were made by curing the hydrogel solution at distances of 25 mm, 30 mm, and 50 mm between the sample and the light source.

III. RESULTS AND DISCUSSION

Figure 1 shows the variation of the luminous intensity of the UV light source during a period of 2 hours.

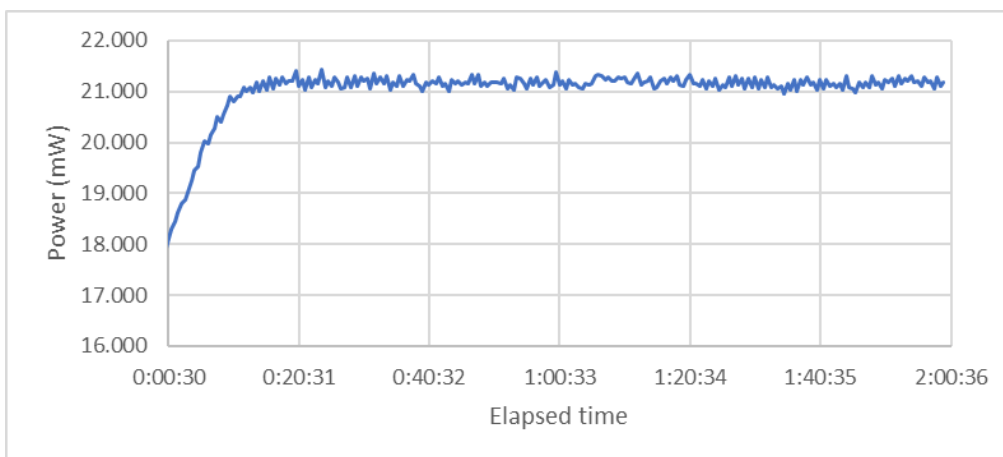


Figure 1- Variation of light intensity over time. Measurement was performed at 35 mm from the light source.

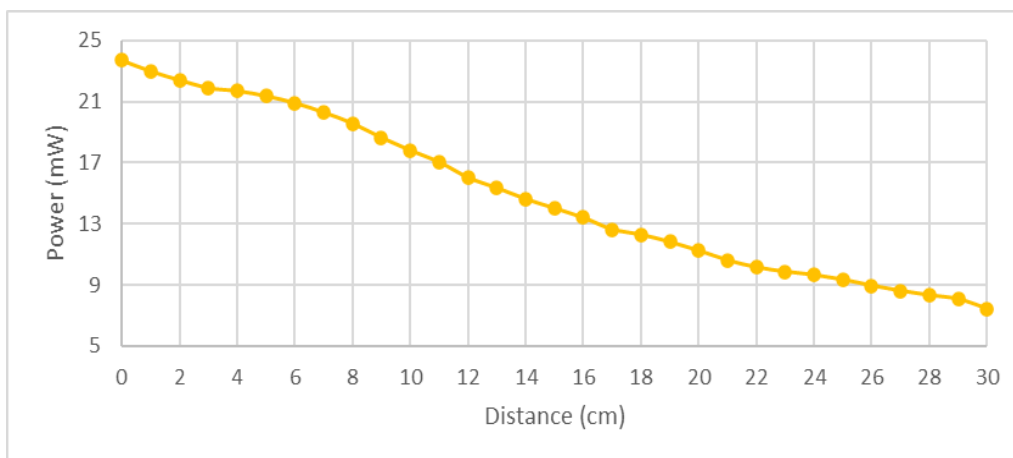


Figure 2 - Light intensity in relation to the distance from the light source

Since the light source utilizes UV surface mounted light-emitting diodes (SMLEDs) it can be seen during the first 20 minutes an upward curve until the stabilization of light intensity. After this time, the luminous intensity reaches its apex of approximately 21.18 ± 1 mW, and remains constant, at least for the next 1 hour and 45 minutes, for which the test was performed.

This test was not replicated for other distances because it can be extrapolated that the results will be the same only reaching different maximum peaks of luminous intensity.

The second test aimed to analyze, for the specific light source that was being used, how its luminosity varied with the distance from the source. The results are shown in Figure 2. The graph shows a maximum of 23.71 mW in the position where the sensor has direct contact with the light source (distance = 0mm) and then the light intensity is reduced approximately linearly to the minimum measured value of 7.43 mW at 300 mm from the light source. Based on the results present in Figure 2 it was possible to correlate the following results between the distance of the hydrogels and the UV light source.

Figure 3 presents the obtained results from curing the hydrogel solution under 3 different distances from the light source. In Figure 3 (a) the sample was kept 20 mm distance from the light source, Figure 3 (b) and Figure 3 (c) were kept 30 mm and 50mm distance from the light source, respectively. Which can be read as 22.5 mW, 21.9 mW and 21.2 mW light intensity for (a) (b) and (c) respectively. The samples were kept under light incidence as long as necessary to observe any changes on the solution, with a maximum of 2 hours.

Figure 3 (a) shows the obtained result after 15 minutes of light incidence. Due to the high incidence of light, the photoinitiator undergoes a very rapid cleavage process, releasing a large number of radicals that quickly react with the unreacted monomer around it. This process yields a high degree of polymerization promoted mainly by intramolecular crosslinking. However, the excess of intramolecular connections, makes the polymer excessively rigid, with a low degree of mobility. For this reason, the polymer obtained cannot expand its chains when immersed in water and therefore does not characterize a hydrogel.

Figure 3 (b) shows the obtained result after 30 minutes of light incidence. The result obtained was a translucent-looking gel, with whitish regions. The distancing of the solution in relation to the light source reduced the efficiency of the polymerization process. Thus, initially, the radicals released by the photoinitiator promoted intramolecular crosslinking, however, with the reduction of the available unreacted monomer, intermolecular connections started to be made. The result obtained for this light intensity has all the characteristics that define it as a hydrogel. Even so, there were regions with an excess of intramolecular bonds, which may indicate the non-

complete solubilization of the solution with regions with excessive concentration of Irgacure 2959.

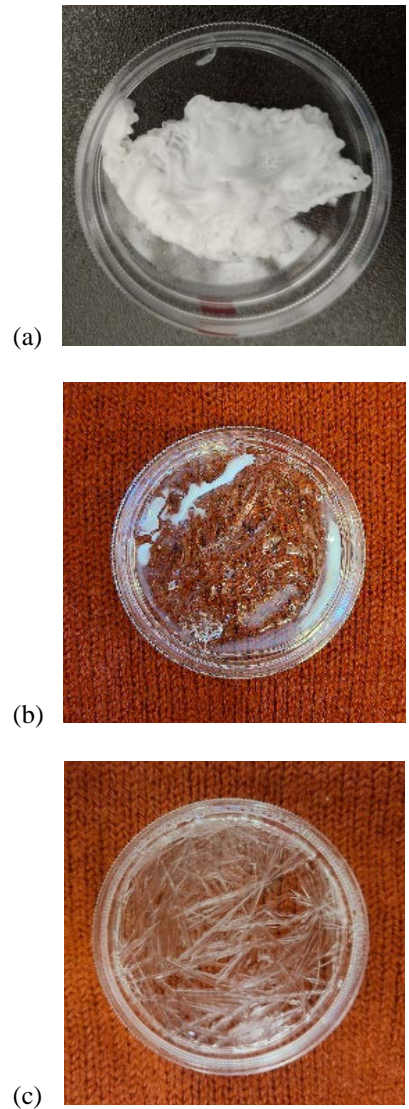


Figure 3 – Hydrogels obtained when curing at (a) 20 mm (b) 30 mm and (c) 50 mm from the light source

Figure 3 (c) shows the obtained result after 2 hours of light incidence. Even with a high time, the light power was not enough to start the cleavage reaction in the photoinitiator, and therefore there was no reaction. Due to the high time at a low temperature, the formation of crystals was noticed due to the freezing of water in the solution. The same test was carried out at room temperature to avoid the formation of ice crystals, however, as expected, after two hours under the incidence of light there was no change in the solution, which characterizes the non-polymerization of the hydrogel.

IV. CONCLUSIONS AND FUTURE WORK

The possibility of changing a structure's shape over time due to external stimuli represents a great advance for the tissue

engineering field since responsive behavior is the desired characteristic found in organic structures such as organs and tissues. 4D bioprinting offers the possibility of creating such morphing structures, requiring for so the use of biocompatible responsive materials and processes adapted to manufacturing them. NIPAM is one of the most promising materials to be used by 4D bioprinters, however, it is manufacturing by technologies such as DLP still requires further studies. This work analyzed how light intensity affects the manufacturing process of NIPAM based hydrogels using Irgacure 2959 as the photoinitiator. It was concluded that luminous intensities should be maintained at approximately 21.9 mW since under this intensity results were obtained that meet the requirements of a hydrogel, under 22.5 mW and higher, the excess of intramolecular crosslinking generates excessively rigid structures, while intensities of 21.2 mW and below are unable to initiate the photopolymerization process.

For a better understanding of the processing of NIPAM based hydrogels by DLP, other parameters still need to be studied, such as how printing temperature may affect the responsive temperature, and printing time optimization.

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