

# 4D PRINTING OF EXTRACELLULAR MATRIX: A PILOT PROJECT FOR THE DEVELOPMENT OF COMPLEX TISSUES

*Impressão 4D de matriz extracelular: projeto piloto para o desenvolvimento de tecidos complexos*

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## ABSTRACT

**Introduction:** The shortage of organs and tissues for transplant is a great challenge in the medical field. The 3D printing emerges as a feasible solution for this problem. This study presents preliminary results of the first models of a 4D extracellular matrix (ECM) using a biomaterial.

**Methods:** This experimental multicenter study was conducted at the laboratory of simulation and digital fabrication of the Federal Institute of Pernambuco. A solution of sodium alginate at 2% was used to print the ECM on a 3D printer model Zmorph type fused deposition modeling.

**Results:** The geometric figure used to print the ECM was a square with 1cm<sup>2</sup> of area initially planned in a computer aided design and developed using the Voxelizer software. The biomaterial in gel was successfully printed with extrusion speed of 1mm/s and printing speed of 3mm/s. The uniform deposit of the material during printing resulted in the geometric figure designed.

**Discussion:** This paper shows preliminary results of the printing of a 4D ECM. The sodium alginate might be a promising biopolymer to the future phases of this study.

**Conclusion:** The variation of the nozzle area and extrusion speed highly influences the analysis of the 3D printer final parameters.

**Keywords:** Bioprinting; extracellular matrix; regenerative medicine; bioengineering; scaffolds.

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## RESUMO

**Introdução:** A indisponibilidade de órgãos e tecidos para transplante com o objetivo de atender a demanda existente é um dos grandes desafios na área médica. Uma solução viável é a impressão tridimensional de órgãos e tecidos. O presente estudo apresentou resultados preliminares dos primeiros modelos da matriz extracelular (MEC) 4D utilizando um biomaterial.

**Métodos:** Estudo experimental multicêntrico realizado no laboratório de Simulação e Fabricação Digital do Instituto Federal de Pernambuco. Uma solução de alginato de sódio a 2% foi utilizada para a fabricação da MEC em uma impressora 3D Zmorph do tipo fused deposition modeling.



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**Resultados:** A figura geométrica da MEC foi um quadrado com 1 cm<sup>2</sup> de área desenvolvida pelo software Voxelizer, projetado inicialmente no *computer aided design*. A seguir, o biomaterial em forma de gel foi impresso com uma velocidade de extrusão de 1 mm/s e uma velocidade de impressão de 3 mm/s. Obteve-se êxito na impressão. O depósito uniforme do material durante a impressão permitiu a obtenção da forma geométrica projetada.

**Discussão:** O presente estudo mostra resultados preliminares do processo de impressão de uma MEC 4D. O êxito da impressão qualifica o alginato como um dos principais biopolímeros a ser utilizado nas futuras fases desse estudo.

**Conclusão:** A variação da área da ponteira e a velocidade de extrusão têm grande influência na análise dos parâmetros finais na utilização da impressora tridimensional.

**Palavras-chave:** bioimpressão; matriz extracelular; medicina regenerativa; bioengenharia; tecidos suporte.

## INTRODUCTION

The shortage of organs for transplant is a major challenge in the medical field. Approximately six million people worldwide are in the final stage of organ failure,<sup>1</sup> and in several countries, high mortality rates affect patients on the waiting list for transplants. In the United States, around 40,000 patients receive transplants each year. However, 120,000 remain on the waiting list, and 7,600 die while waiting. Furthermore, the increasing demand for transplants might be proportional to the increased life expectancy and incidence of chronic diseases in the next years.<sup>2</sup>

The COVID-19 pandemic worsened this scenario, revealing a strong temporal association between the increased coronavirus infections and the reduced transplants of solid organs. In France and United States, the rate of transplants of deceased donors decreased by 90.6% and 51.1%, respectively. In addition, the organ procurement and transplant rates were reduced even in regions less affected by the pandemic, suggesting a global effect.<sup>3</sup> In Brazil, population aging follows the global trend, which combines with the rising number of patients with organ failure and increases the demand for transplants.<sup>4</sup>

Technologies inspired by regenerative medicine challenge the current allograft transplant model. The 3D printing of organs and tissues might be a feasible solution for the shortage of organs for transplant. Combined with tissue bioengi-

neering, regenerative medicine allows new approaches for replacing and regenerating tissues and organs, with the potential to correct congenital defects using laboratory-grown organs and tissues, known as bioartificial organs.<sup>5</sup>

A systematic review highlighted the revolutionary potential of 3D printing in patient counseling, preoperative and intraoperative surgical planning, and education in urology. Although time and costs of production remain as challenges, 3D printing might represent a step forward in meeting the needs of patients and surgeons.<sup>6</sup>

Despite experimental, regenerative medicine has already remarkable results. Atala et al.<sup>7</sup>, a world reference in 3D printing of biological material, developed a biocompatible tissue capable of fully or partially replacing the bladder; however, without functional contraction. The full implant of the organ developed was successful and significantly improved the quality of life of patients.<sup>7</sup> Atala is responsible for several other studies on regenerative medicine using 3D printing, such as the production of a human endothelialized myocardium from stem cells in a microfluidic hydrogel ECM.<sup>8</sup>

The 3D printing enables the fabrication of complex shapes with high precision by adding layers of smart materials. These materials change shape, color, or generate electrical currents in response to external stimuli, being bioactive or fulfilling a desired function. The 3D printing allowed the development of dynamic 3D struc-

tures, known as 4D printing.<sup>9</sup>

Tissues *in vivo* are dynamic structures composed of various cell types, an ECM, and a range of signaling molecules. The ECM is a crucial component of the cellular microenvironment, forming a complex 3D network.<sup>10</sup> In 3D printing, cells added to the ECM may be organized into complex structures, creating tissues or organs.<sup>11</sup> Currently, regenerative medicine aims to develop a technique of 4D printing for biological material capable of implantation in the human body, replacing tissues or organs while reducing the risk of rejection due to biological compatibility.

Therefore, this study aimed to fabricate 4D ECMs of sodium alginate using a 3D printer, with the purpose of adding cells hereafter originators of tissues and organs. This study seeks future application in the regenerative medicine with animals and humans.

ECMs were produced in the laboratory of simulation and digital fabrication at the research center of the Federal Institute of Pernambuco (Recife, Brazil). Thus, this study shows preliminary results of the printing, including training for printer operation and mechanical fabrication.

## METHODS

This multicenter experimental study involved the Faculty of Medicine of Olinda, the Federal Institute of Pernambuco, and the Federal University of Pernambuco. An extensive literature review was conducted to define the biomaterial, printer, extrusion nozzle adapted for bio-inks, software, geometric shape, and printing technique for the fabrication of ECMs.

The biomaterial selected for the initial tests was sodium alginate (Figure 1), defined as a class of biopolymers initially derived from the cell wall of brown algae from various species (e.g., *Laminaria*, *Macrocystis* and *Sargassum*).<sup>12, 13</sup>

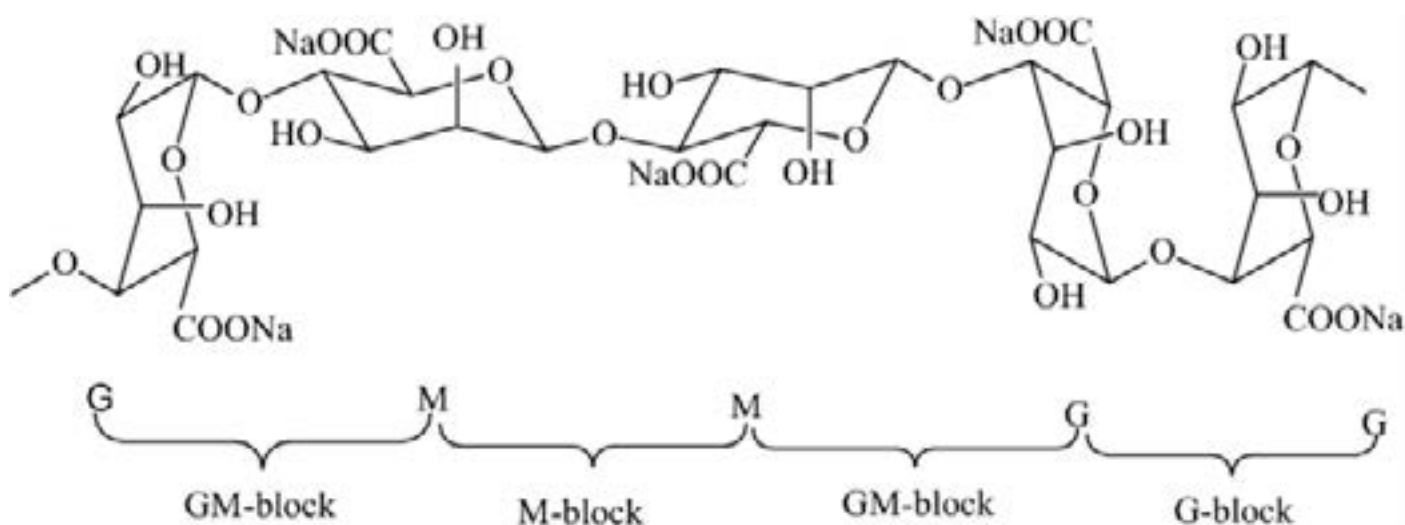


Figure 1. Different blocks of sodium alginate linked by 1→4 glycosidic bonds.

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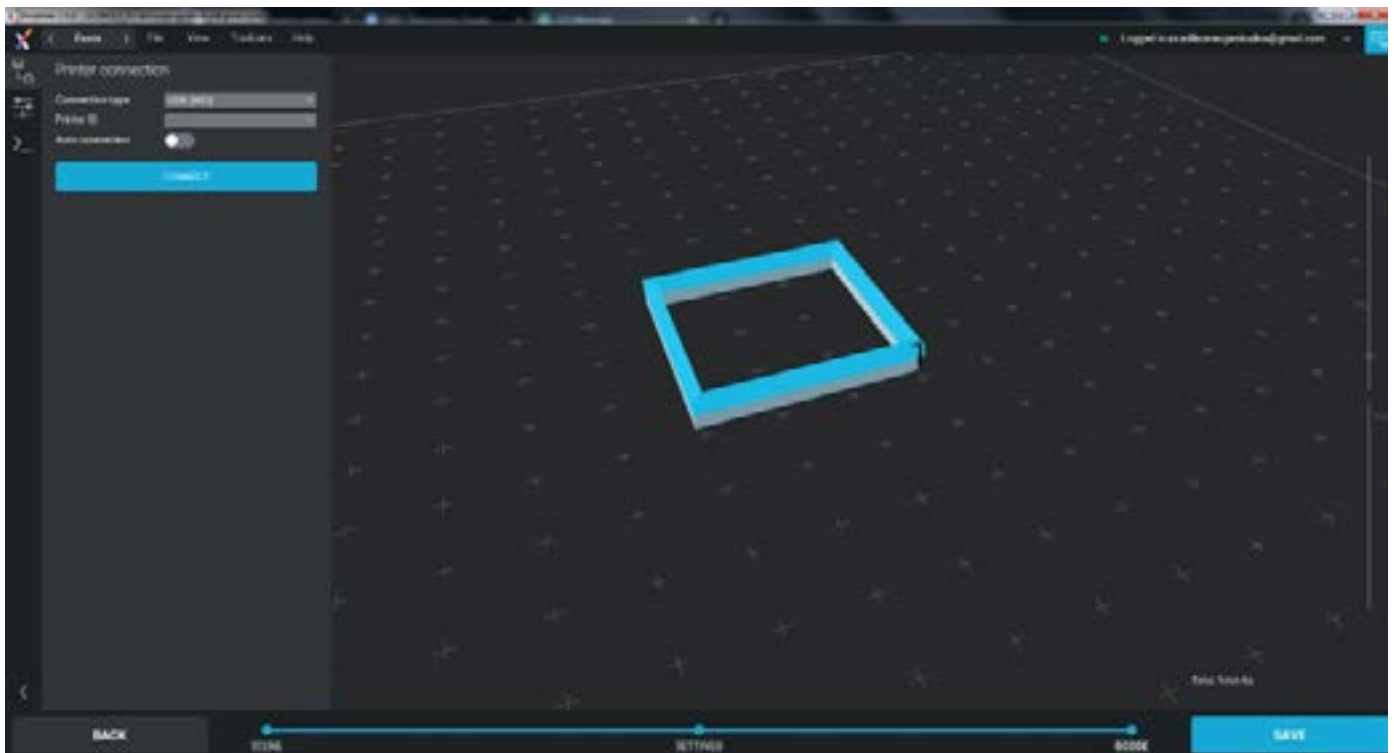
For ECM fabrication, a gel was produced consisting of a solution containing 2% sodium alginate and 0.25% sodium chloride diluted in water. Under mechanical stirring and heated to 80° C, calcium chloride was added at a concentration of 0.25% in a ratio of 1:5 mL, respectively.

The 3D printer model selected was the Zmorph type fused deposition modeling. For the initial tests, The Voxelizer software version 2.0(Zmorph S.A., Wrocław, Poland) was used to convert the computer-aided design (CAD) files into GCODE format (i.e., the language used to describe how the 3D printer should execute a prin-

ting). The printer used a paste extrusion nozzle in aluminum of 4 mm, operating at a flow rate of 5 mL/s in a linear format to construct squares with 1 cm<sup>2</sup> of area. Initially, polylactic acid (PLA) and cellulose were used to define printing parameters.

## RESULTS

The preliminary results included training in printer operation and mechanical fabrication. Initially, the structure of ECMs was designed in CAD (Figure 2), and the Voxelizer signaled the printer to execute the printing (Figure 3).



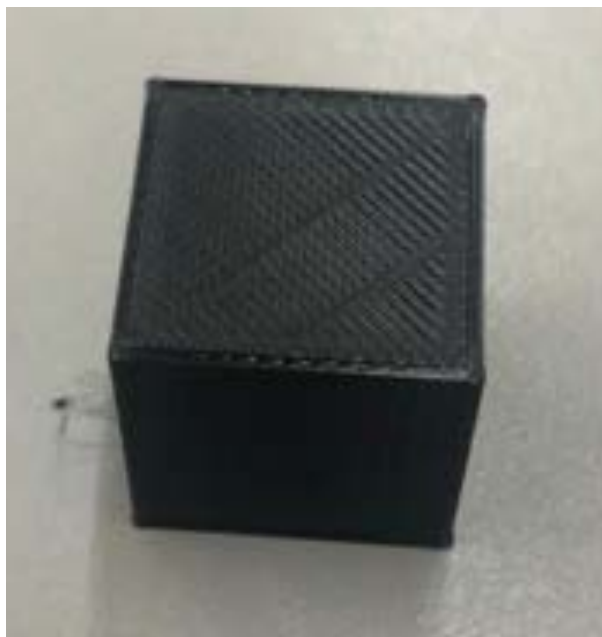
**Figure 2.** Design of the ECM structure.



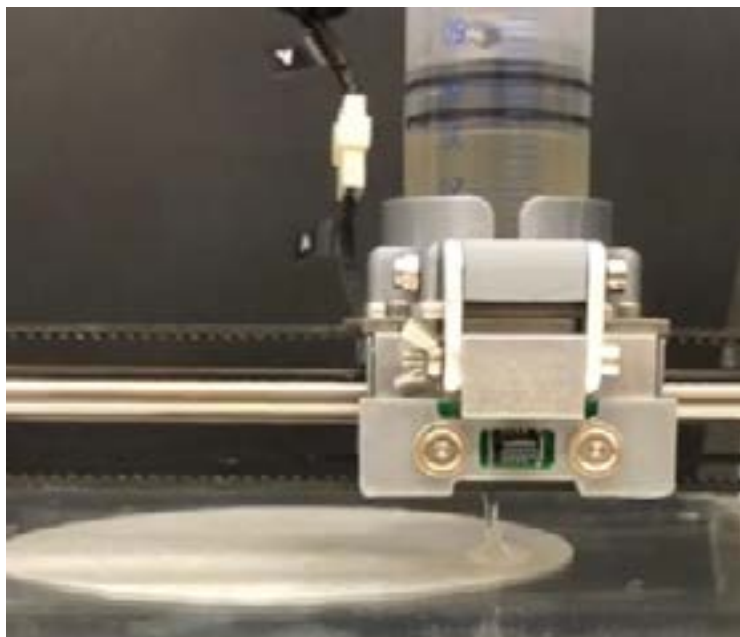
**Figure 3.** Zmorph 3D printer, type fused deposition modeling initiating the printing.

For the experimental analysis of the printing, five tests were conducted using PLA, cellulose, and sodium alginate to configure parameters, which included extrusion and printing speeds, described as follows:

- 1) PLA: utilization of a nozzle with an area of 0.4 mm<sup>2</sup>, extrusion speed of 40 mm/s, and printing speed of 40 mm/s. Successful printing of the geometric figure (Figure 4).
- 2) Cellulose: utilization of a nozzle adapted for bio-inks with an area of 13.2 mm<sup>2</sup>, extrusion speed of 7 mm/s, and printing speed of 10 mm/s. During the test, the extrusion value associated with the deposition speed applied to the material was too high, hindering the printing of the geometric figure.
- 3) Sodium alginate: utilization of a syringe with tips of 35.7 mm and 4.0 mm in diameter, extrusion speed of 1.5 mm/s, and a printing speed of 1 mm/s. A significant material deposition occurred at one pole, hindering the printing of the geometric figure.
- 4) Sodium alginate: utilization of a syringe with the same characteristics as the previous, extrusion speed of 1 mm/s, and printing speed of 0.5 mm/s. Similarly to the previous test, a high material deposition occurred at one pole, hindering the printing of the geometric figure.
- 5) Sodium alginate: utilization of a syringe with the same characteristics as test 3, extrusion speed of 1 mm/s, and printing speed of 3 mm/s. The material was uniformly deposited and the geometric figure was successfully printed (Figure 5).



**Figure 4.** Model printed with polylactic acid.



**Figure 5.** Model printed with sodium alginate.

## DISCUSSION

The shortage of organs and tissues for transplant is one of the major challenges in the medical field. Furthermore, the COVID-19 pandemic negatively impacted worldwide transplant rates, decreasing procedures and organ donors, and increasing mortality among patients on the waiting list.<sup>3, 14</sup>

This scenario requires innovative therapies, such as regenerative medicine, which is crucial for replacing or restoring human cells, tissues, and organs. However, the fabrication of ECM (a pillar of regenerative medicine) represents one of the technological obstacles to tissue production. ECMs are structures made from synthetic or natural materials on which new tissues may grow to replace damaged tissues.<sup>15</sup> Creating a similar environment to the human ECM in which cells may attach and maintain their proper metabolism is complex.

This study shows preliminary results of the 4D printing of ECMs, including training for printer operation and mechanical fabrication. The equipment choice was defined by optimizing the existing resources since ECMs fabrication using 3D printers incurs high costs.<sup>16</sup>

The sodium alginate was selected as biopolymer for being one of the most used biomaterials in bioengineering due to its molecular similarity to polysaccharides. Sodium alginate enables the production of gels that incorporate stem cells and mimic an ECM microenvironment, facilitating tissue bioengineering and basic research.<sup>17</sup>

The first test was conducted with PLA, which presents superior thermomechanical characteristics compared to the gel of sodium alginate. The PLA has greater mechanical strength and a lower coefficient of thermal expansion, enhancing its printability and reducing biases during the production process.<sup>18</sup> In the second test, extrusion and deposition speeds were excessive, hindering the printing. Consequently, parameters were defined using sodium alginate.

Successfully printing the ECM was not possible using parameters previously obtained with the different materials, as showed in tests 3 and 4. However, these tests indicated that reducing the extrusion speed to 1 mm/s and increasing

the printing speed to 3 mm/s would enable the intended printing. After applying the parameters, a uniform material deposition was achieved, resulting in the successful printing of the geometric figure.

This study had limitations. Statistical analyses were hindered by the absence of physicochemical analyses for a 4D ECM and the challenges associated with stabilizing the extruded filament according to the tested material. However, our findings are preliminary and foresee advancement in regenerative medicine and tissue bioengineering.

## CONCLUSION

The variation in the nozzle area and the extrusion speed influenced the outcome of the 4D ECM printing. The ease of handling and the accessibility of sodium alginate were observed during the printing. Sodium alginate proved to be highly useful in tissue bioengineering and should be considered in future studies aimed at stabilizing the extruded filament.

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