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**AUTOMATING THE PROCESS OF GENERATING MICROMOLDS IN
COMPUTER-AIDED DESIGN (CAD) FOR USE IN 3D CELL CULTURE
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Abstract. *In recent years, research in the field of cell culture has experienced significant growth due to its specialization, which can lead to important advancements in the treatment of various medical conditions such as Alzheimer's, diabetes, and cancer. In response to this growing demand, engineering has begun to develop micromolds, which are devices composed of micropins arranged on a flat surface. These devices are used to create microwells in suitable polymeric materials (hydrogels) for cell insertion. The micromolds presented in this work have a semi-spherical shape at their lower portion, where stem cells are cultivated, and a hexagonal shape at the upper portion, facilitating the manipulation of spheroids (cell aggregates). The size of these spheres influences the variability of the spheroids, making their dimensions crucial for scientists, as the experiment can be altered based on their size. Within this context, the objective of this research was to parameterize the dimensions of the micromold according to the dimensional characteristics of the cells for use in in vitro studies of 3D cell culture.*

Keywords: *parametrization, micromold cell culture, spheroid*

1. INTRODUCTION

Stem cells are undifferentiated cells capable of self-renewal, proliferation, and differentiation. In vitro, the standard procedure for stem cell culture is done in two-dimensional (2D) environments. However, this method is considered artificial and does not mimic the behavior of cells in vivo. As an alternative, the cultivation of three-dimensional (3D) cell aggregates, known as cellular spheroids, offers a more effective simulation of the natural microenvironment found in tissues and organs. This enables more precise cellular responses and is applicable to the development of biological tissues, drug responses, and studies of diseases such as cancer and Alzheimer's (Decarli *et al.* (2022)) (Białkowska *et al.* (2020))

For the cultivation of cellular spheroids, one of the methods employed is the use of devices known as micromolds. These devices consist of 3D micro pins with complex geometries designed to create a well-defined microwell matrix in a biocompatible hydrogel. Cells are deposited into these microwells, allowing the formation of spheroids through the influence of gravity (Decarli *et al.* (2021))

The customization of these micromolds allows for geometric control over the characteristics of the formed spheroids, an important aspect in reducing dimensional variability of these cellular aggregates used in clinical research. However, manual modeling of micromolds requires time and specialized labor. Therefore, the objective of this article is to generate a parameterized model of a micromold using CAD software for the fabrication of customized devices for in vitro cultivation of cellular spheroids.

2. METHODOLOGY

2.1 Mold Geometry

The geometry of the mold is formed by micropins with a complex structure consisting of a hexagonal-shaped region at its upper portion, connected by a hemisphere at its lower portion (Decarli *et al.* (2022)). These micropins are attached to a cylindrical geometry that serves as a support and facilitates handling during its use. The "Figures 1A, 1B and 1C" show the flowchart that presents the main steps used in the generation of the microtemplate for 3D cell culture.

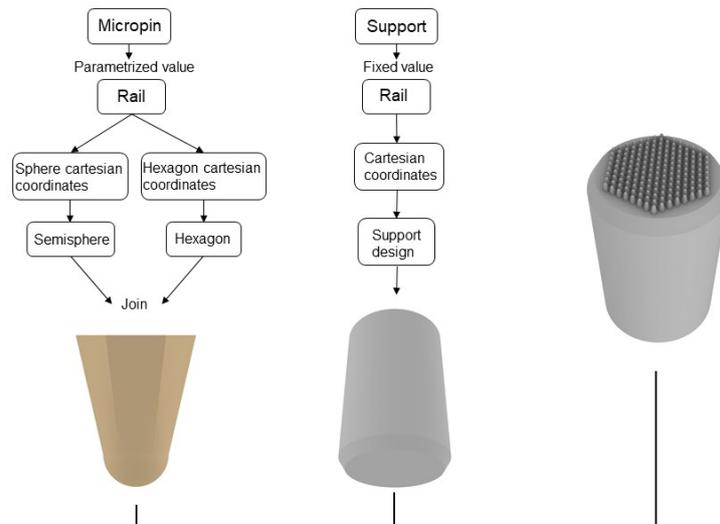


Figure 1. (A) Geometry of the micropin, (B) Geometry of the support, (C) Finished mold.

2.2 Parametrization

To initiate the project, it was necessary to initially define which parameters would be varied according to the user's interest and those that would be changed proportionally to the chosen number. In this case, it was determined that the pins would vary in a two-dimensional (2D) manner, only in the x and y directions, where the radius of the semisphere could be defined according to the user's required measurements, and consequently, the side of the hexagon would change proportionally to the radius of the semisphere. The intention was to create a single pin and multiply it throughout the entire length of the support.

For the parameterization process, an extension called Grasshopper, integrated into the Rhinoceros 7 software (Robert McNeel Associates, Seattle, USA), was employed to facilitate programming for CAD drawings. In this context, the adopted programming follows a more intuitive approach, where modules are represented by simplified modular elements, making it easier to understand the underlying language. However, it is worth noting that Grasshopper allows the use of the Python programming language within its context, which becomes necessary in the step of multiplying the pins along the entire length of the support.

In the creation of the pin, the procedure was divided into two parts: the lower part, which consists of a semisphere, and the upper part, which is a hexagon. The first step was to create the lower part, the semisphere. For this, a number slider was created to adjust its radius. This tool allows controlling the size of the semisphere according to the project's needs. Next, coordinates were used to construct 1/4 of the semisphere. After completing this step, the polar array command was used to multiply it by 4, resulting in a complete semisphere. The "Figures 2A and 2B" show the commands used to create the quarter of the semi sphere, and the "Figure 2C" shows the result.

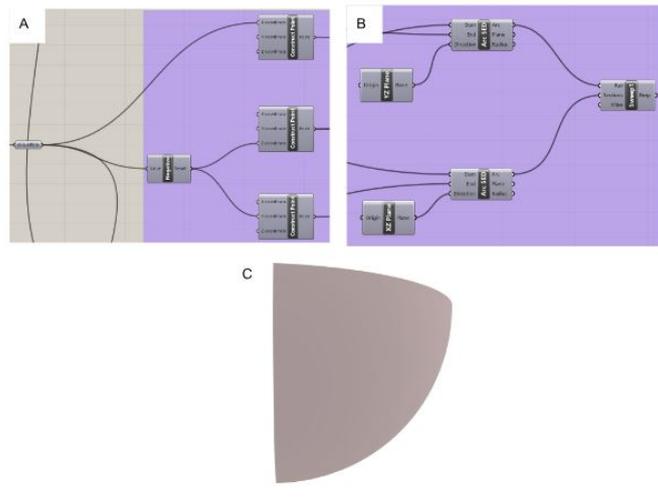


Figure 2. (A) Coordinates based on the radius of the mold, (B) Arcs made from the coordinates, (C) Result of the operation.

Then, with polar array it was possible complete the entire half sphere, as shown in “Figure 3A and 3B”.

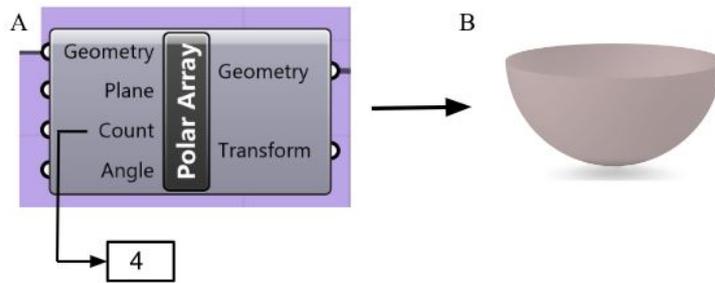


Figure 3. Finishing the lower portion.

In the procedure for creating the hexagon, the base was established as the previously created semisphere. The process involved dividing the hexagon into six equal parts since a regular hexagon has six sides. Next, one of these parts, or 1/6 of the complete hexagon, was created using coordinates that provided the position of the vertices of the hexagon geometry. After creating the side of the hexagon, the "polar array" command was used again, but this time multiplying this shape by six. This resulted in obtaining the complete geometry of the hexagon. The flowchart in “Figure 4” illustrates the process used to create the upper portion of the micropin.

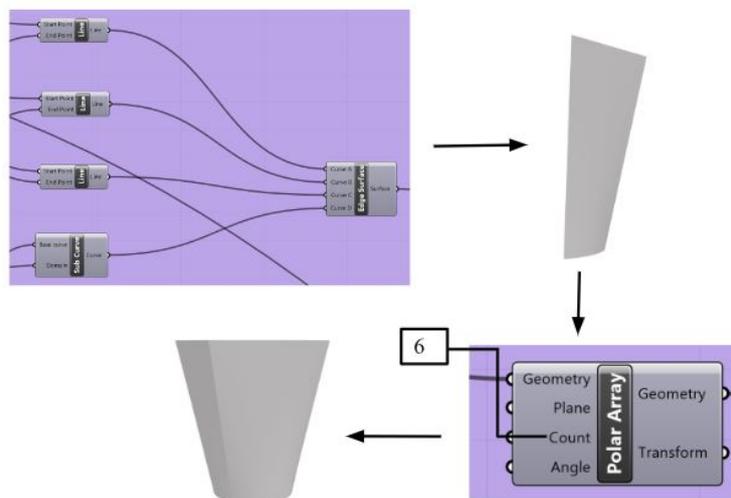


Figure 4. Formation of the hexagon.

For the creation of the support, a radius was defined for the extension of the sphere where the pins would be attached. Subsequently, the shape of the support was drawn in Grasshopper, as having it ready would allow use of the revolution command to complete the shape. "Figure 5A" shows the commands used on Grasshopper, while "Figure 5B" shows the result of the shape.

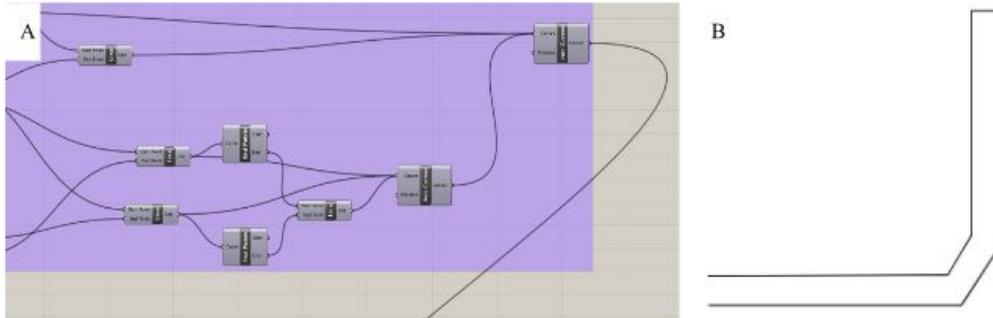


Figure 5. (A) Commands used for the format of the support, (B) Format of the support.

With the shape ready, "Figure 6A" shows the revolution command being used to create the form, while "Figure 6B" shows the result of the previous command.

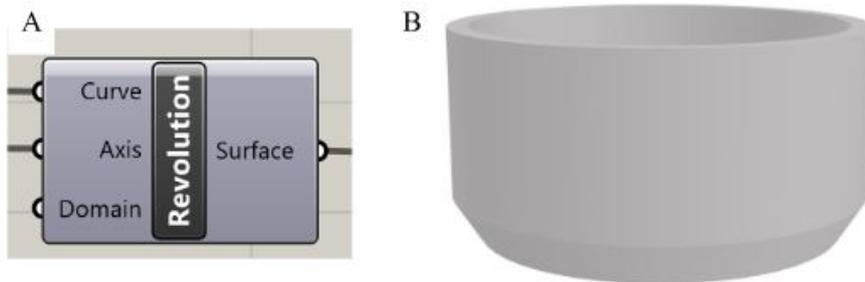


Figure 6. (A) Command used to finish the pattern, (B) Finished pattern.

After all the procedures listed above, there was a need to place the wells on the support and multiply them, so Python was used to create a list of points that would serve as central points for the hexagons where they would be arranged. The main idea was to create a kind of triangular pattern of points, as shown in "Figure 7A," and multiply that pattern throughout the length of the support, again using the polar array command until completing 360°, as illustrated in "Figure 7B." As a result, it was possible to position the hexagons, using these points as references, and thus distribute the micropins throughout the extent of the support and ensure that there is no overlap between them.

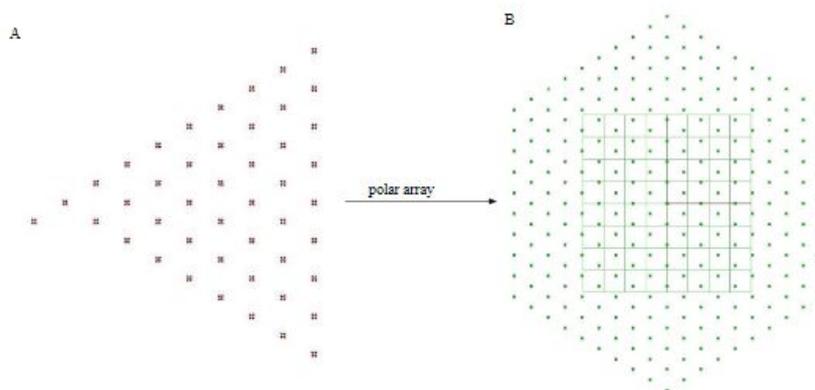


Figure 7. (A) Result of the code, (B) Completion of the distribution of the centers of the hexagons.

3. RESULT AND DISCUSSION

The aim of this study was to develop a parametric model of a micromold using CAD software, with the purpose of fabricating customized devices for in vitro cultivation of cellular spheroids. The parametrization of the micromold was carried out through the Grasshopper extension of Rhinoceros software, allowing for the generation of micropins with varying hemisphere diameters, intended to accommodate the cellular spheroids. Using Python programming, these micropins were distributed to avoid overlapping with each other on the surface of a support plate. “Figure 8” displays the developed micromold with different sizes of micropins.

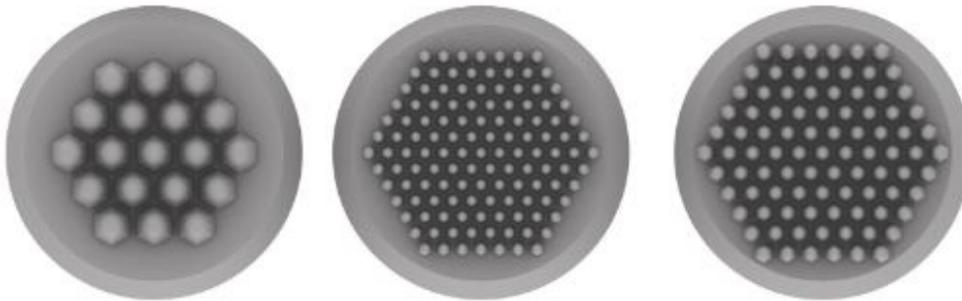


Figure 8. Finished micromold.

The geometry of the micropins was developed to obtain uniform and reproducible cellular spheroids. The diameter of the hemisphere at the lower end of micropins can be customized according to the diameters result in the formation of smaller spheroids, which is advantageous for investigating phenomena such as cell proliferation (Sart *et al.* (2014)). Conversely, larger diameters allow for the generation of larger spheroids, which are used in applications such as assessing the efficacy of anticancer drugs, particularly in the study of cancerous cell aggregates (Däster *et al.* (2017)). The hexagonal base of the micropin in contact with the support plate was designed to vary proportionally to the diameter of the hemisphere.

One additional relevant feature of this device is the flexibility to adjust the number of micropins on the support plate according to the diameter of the hemisphere. This adaptability capability is automated, ensuring a more efficient and speedy modeling of this device.

4. CONCLUSION

The research conducted aimed to parameterize a micromold for use in generating cellular spheroids. The micromold is composed of a support structure where micropins are arranged in a hexagonal pattern at the upper portion and a hemisphere at the lower portion. The parameterization was done using Grasshopper, an extension of Rhinoceros, and the diameter of the hemisphere where the cellular spheroids would be housed was varied. Additionally, the Python programming language was used to distribute all these micropins on the support. Therefore, it can be concluded that the process of customizing the micropins represents an important step in the manufacturing of devices for 3D cell culture.

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6. REFERENCES

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