HIGH DENSITY CULTURE SYSTEM BASED ON ALGINATE-AGAROSE SPHERES FOR THE COLLECTION AND CHARACTERIZATION OF THE SECRETOME OF MESENCHYMAL CELLS

Lucía Bravo (1,2)*, Mauro Llop-Miguel (1), Irene Monleón-Guinot (1,2), María Sancho-Tello (1,2), José Javier Martín de Llano (1,2), Carmen Carda (1,2,3), Lara Milián (1,2) and Manuel Mata (1,2,3)

1. Department of Pathology, Faculty of Medicine and Dentistry, Universitat de València, 46010 Valencia, Spain; 2. INCLIVA Biomedical Research Institute, 46010 Valencia, Spain; 3. Biomedical Research Networking Center of Respiratory Diseases (CIBERES), 28029 Madrid, Spain.

E-mail (corresponding author): brabalu@uv.es

Introduction

The use of the secretome of mesenchymal stem cells (MSCs) as a substitute for cell therapy in the regeneration of articular cartilage has recently gained importance due to the potential problems involved in the use of cells in regenerative therapies. This hypothesis is based on the fact that the effects observed with the use of MSCs could be due to the substances released rather than to the introduction of the cells themselves [1]. Our aim is to develop a secretome, a conditioned medium enriched with the essential factors for the regeneration of hyaline cartilage, while avoiding the need to introduce stem cells into the body and the potential associated risks.

Methods

We developed a three-dimensional (3D) culture platform to achieve our goal. We used MSCs, specifically human dental pulp stem cells (hDPSCs). Cells were encapsulated in microspheres of a hydrogel composed of alginate and agarose, two polymers that, in combination, allowed the culture. To carry out this encapsulation, we generated a suspension of both polymers and cells, which were generated in a spherical shape thanks to the use of a peristaltic pump (Figure 1). Once generated, we kept them in a bioreactor for long periods, which was made possible by the continuous addition and removal of culture medium. Additionally, we controlled critical parameters such as temperature, humidity, and the percentage of CO_2 to which the cells were exposed. After a defined proliferation period, we induced hDPSCs differentiation with a fetal bovine serum (FBS)-free differentiation medium [2], a relevant feature for potential clinical translation. Once the microspheres were generated and cultured, we evaluated their elastic modulus in the presence and absence of cells using a Zwick Roell elastomer. We measured the dimensions of the microspheres to verify their homogeneity. We collected the conditioned medium to perform various experiments to determine whether the factors released by the cells were suitable to achieve the desired tissue regeneration.

Results

Our method for generating microspheres guarantees a consistent size and optimal cell density, obtaining microspheres of 2.4 ± 0.5 mm in diameter. More than

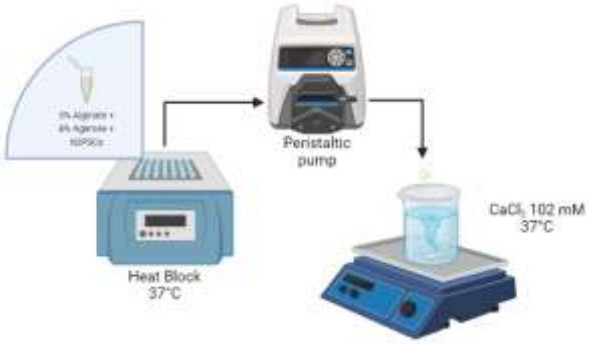

Figure 1. Manufacturing of alginate-agarose microspheres.

90% cell viability was observed after 6 weeks of culture. The elastic modulus measurement exhibited a significant increase in hydrogel stiffness in the presence of cells, showing a 2.3-fold increase in differentiation media and a 1.5-fold increase in proliferation. Additionally, the use of a bioreactor for culture allowed us to establish a constant rate of medium turnover, further supporting sustained cellular activity.

Discussion

Our findings indicate that the culture system designed preserved the structural integrity of the microspheres, and the stiffness of the hydrogel even showed an increase in the presence of cells, which suggested a potential secretion of specific components by the cells. Moreover, the sustained cell viability suggests that the hydrogel does not hinder nutrient entry or gas exchange, further supporting its suitability for our purposes.

References

1. Chang et al, Adv. Healthc. Mater. 10(7), e2001689, 2021.
2. Monaco et al, Front. Bioeng. Biotechnol. 8, 243, 2020.