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3D cultivation of mesenchymal stem cells on polylactide scaffolds

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Abstract: The aim of this article optimizes the methodology for three-dimensional (3D) culture of human chorionic mesenchymal stem cells (hCMSCs) on polylactide (PLA) scaffolds, with a focus on the preparation and sterilization of the scaffolds. A significant problem when using PLA scaffolds is ensuring effective sterilization while maintaining the structural integrity of the material sensitive to elevated temperatures. A combined sterilization protocol using thermal and chemical decontamination was applied in the work. Scaffolds with a lattice architecture and 50% filling were prepared by 3D printing technology and subsequently seeded with hCMSCs. During the preparation of the scaffolds, technical complications related to their porous structure were observed, which required adjustment of the cultivation procedure before cell seeding. Preliminary results confirmed the preservation of sterility during cultivation without visible degradation of the scaffolds and at the same time demonstrated satisfactory cell viability. The obtained knowledge points to the potential of a combined sterilization approach in the preparation of 3D printed PLA scaffolds for tissue engineering applications.

1 Introduction

Traditional in vitro stem cell cultivation in biomedical research has long been carried out in two-dimensional (2D) monolayer systems on plastic surfaces, which are optimized for easy manipulation and efficient cell expansion, but only partially mimic natural physiological conditions in vivo [1,2]. In the human body, cells are located in so-called niches, which create a complex three-dimensional microenvironment that controls their behavior [1,3]. This fact has led to growing interest in the transition to three-dimensional (3D) culture systems, which allow for more realistic modeling of tissue architecture and the mechanical properties of the extracellular matrix. In a 3D environment, processes such as cell adhesion, proliferation, and cell-material interaction are simulated more naturally, which fundamentally alters the biological response of cells compared to flat substrates. For the field of tissue engineering [1], the 3D configuration is crucial because it supports the maintenance of the stem cell phenotype and stimulates the secretion of bioactive factors with high therapeutic potential, which are often suppressed in conventional 2D cultures [2].

Mesenchymal stem cells (MSCs) represent a population of multipotent non-hematopoietic cells capable of self-renewal and differentiation into various cell lineages, making them a fundamental tool in regenerative medicine. MSCs are functionally defined by their ability to adhere to plastic and exhibit a specific immunophenotype characterized by the expression of CD73, CD90, and CD105 markers, while not expressing hematopoietic markers such as CD34 or CD45. Although bone marrow has historically been the best-known source of MSCs, their clinical use is hindered by the invasive nature of collection and the low number of cells obtained from adult donors. In recent years, the human chorion, obtained as perinatal waste after childbirth, has emerged as a promising and ethically acceptable source. Human chorionic mesenchymal stem cells (hCMSCs) possess high proliferative potential, low immunogenicity, and significant secretory activity, making them ideal for the development of innovative cell-based and cell-free therapies [4].

Polylactide (PLA), a biocompatible and biodegradable polymer, plays a significant role in the design of 3D scaffolds for cell culture; thanks to its properties, it is an ideal material for FDM (Fused Deposition Modeling) 3D printing

technology. This technology enables the rapid production of scaffolds with a precisely defined internal architecture that simulates the structural integrity of natural tissues. Key design parameters include the lattice infill and the overall porosity of the scaffold; the 50% infill used in this study ensures sufficient permeability for the free diffusion of nutrients and the efficient removal of metabolites [5]. Furthermore, the properly designed architecture of the PLA scaffold provides mechanical stimuli for cell attachment and their spatial organization along the print lines, thereby actively influencing the resulting morphology of the tissue-engineered construct [5].

An essential prerequisite for successful in vitro cultivation is ensuring the absolute sterility of the materials used; however, this poses a significant methodological challenge in the case of 3D-printed PLA scaffolds. Polylactide is a thermolabile polymer with a Young's modulus of approximately 5 GPa, which is extremely sensitive to high temperatures [1,6]. Conventional sterilization methods, such as autoclaving, are unsuitable for this type of material because they lead to irreversible thermal deformation of the lattice structure and changes in the mechanical properties of the scaffold. The available literature often focuses on final biological outcomes but rarely analyzes in detail the practical complications associated with the effective sterilization of porous polymers and the need to eliminate air bubbles from the carrier's microstructure, which prevent uniform wetting of the surface by the medium. It is precisely this lack of detailed practical protocols for working with 3D-printed PLA in cell laboratories that underscores the need for methodologically oriented research.

The aim of this study was to optimize and validate a methodology for the preparation and sterilization of 3D-printed PLA scaffolds intended for the long-term culture of human chorionic mesenchymal stem cells (hCMSCs). The experiment focused on verifying the effectiveness of a combined sterilization procedure, preserving the structural integrity of the scaffolds, and subsequently assessing cell viability during a 35-day culture. The results provide an important methodological basis for integrating 3D-printed polymeric structures into tissue engineering and regenerative medicine processes, with a focus on the stability and biocompatibility of the system.

2 Materials and methods

2.1 Preparation of PLA scaffolds

The experimental scaffolds were manufactured using FDM (Fused Deposition Modeling) technology from pure polylactide (PLA) (Figure 1). The scaffolds were designed with a regular circular lattice architecture and a 50% infill density, which ensures the necessary porosity for cell colonization. The dimensions of the scaffolds were adapted for cultivation in 12-well plates [6].

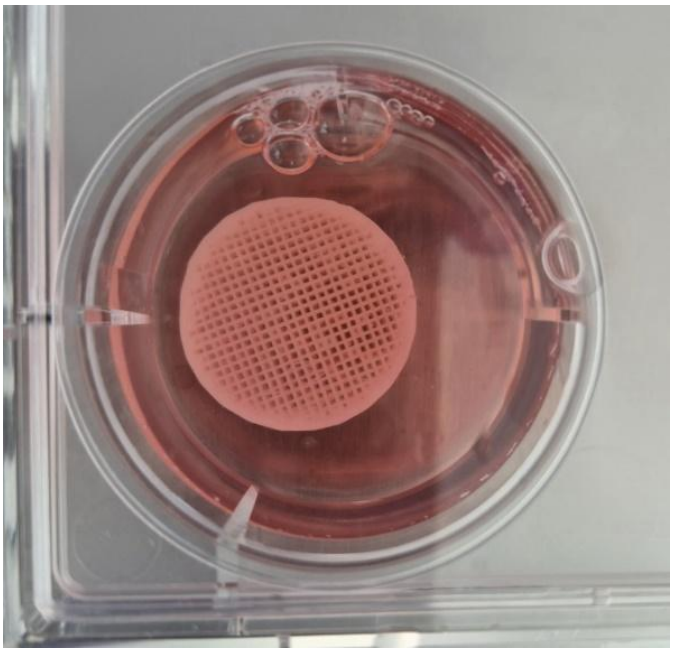

Figure 1 A PLA scaffold placed in a culture dish containing culture medium prior to cell seeding [source: author]

2.2 Sterilization Protocol

Given the low glass transition temperature of PLA, a combined aseptic protocol was used to protect the material from deformation. Heat sterilization was performed in a sterilizer at 50 °C for 1 hour. This was followed by chemical

sterilization by immersion in 70% ethanol for 24 hours. The final step was physical sterilization using germicidal UV radiation, which was performed in a laminar flow hood for 2 hours on each side of the sample.

2.3 *Conditioning of scaffolds*

Prior to cell seeding, the scaffolds underwent a preconditioning process to eliminate air bubbles in the pores. The material was left submerged in complete culture medium for 1 hour, ensuring complete wetting of the hydrophobic PLA surface. This step is crucial for establishing optimal contact between the material and the cell suspension [6].

2.4 Isolation and culture of hCMSCs

Human chorionic mesenchymal stem cells (hCMSCs) were isolated from fetal membranes obtained via cesarean section. The process involved mechanical separation of the chorion followed by two-step enzymatic dissociation using dispase (2.4 U/ml) and type II collagenase (1.0 mg/ml). The cells were cultured in alpha-MEM medium with 2% FBS at 37 °C and 5% CO_2 [7].

2.5 Viability Assessment

Cell viability and metabolic activity on the scaffolds were determined using the MTT assay (Table 1). Measurements were taken at 3, 9, 14, 20, 27, and 35 days. Absorbance was measured spectrophotometrically, and the results were expressed as a percentage of viability.

Table 1 Viability gradually increases until the 20th day, when it reaches its peak, and then declines slightly, while the variability of the measurements remains stable [source: author]

Day	Viability (%)	SD (%)
3	62.3	4.8
9	68.7	5.5
14	74.2	6.1
20	78.5	6.8
27	75.9	6.3
35	70.4	5.9

3 Results and discussion

3.1 Structural integrity and sterility

The combined sterilization protocol (50 °C, ethanol, and UV irradiation) proved to be highly effective, as no microbial contamination was detected throughout the entire 35-day experiment. The use of low-temperature sterilization and UV exposure ensured the preservation of the grid's geometric accuracy without visible hydrolytic degradation or thermal deformation. Preliminary incubation in the medium successfully removed air pockets in the grid [8].

3.2 Cell viability profile

The analysis showed that the viability of hCMSCs on PLA scaffolds ranged from 62.3% to 78.5%. The initially lower value on day 3 (62.3%) reflects the cells' adaptation phase to the new surface. Maximum metabolic activity was reached on day 20 (78.5%), indicating successful proliferation and colonization of the 3D space. The subsequent slight decrease to 70.4% on day 35 is attributed to limited nutrient diffusion in the deeper layers of the 50% infill [5].

3.3 Cell-material interaction

Microscopic observations confirmed that cells adhere to PLA filaments and change their morphology to an adherent form. The phenomenon of contact guidance was observed, in which cells organized themselves spatially along the lines of the 3D print. Although pure PLA does not exhibit significant bioactivity, its topography provides sufficient mechanical stimulation for the organization of the tissue construct [9].

3.4 Discussion and Practical Limitations

The viability values obtained are slightly lower than those reported in the literature for immortalized cell lines, which is due to the greater sensitivity of primary hCMSCs to the microenvironment [2]. The slow degradation of PLA ensured the stability of the substrate; however, for further development, surface modification with hydroxyapatite is a promising approach for improving nanomechanical homogeneity [10]. The study confirmed that the proposed methodological approach is suitable for the long-term study of MSC paracrine activity under 3D conditions.

4 Conclusions

Based on the experimental procedures carried out, it can be concluded that we have successfully developed and validated a fully functional 3D culture model of human chorionic mesenchymal stem cells (hCMSCs) on polylactide scaffolds. The proposed combined sterilization protocol, integrating thermal treatment, chemical decontamination, and intense UV irradiation, successfully maintained the sterility of the environment throughout the entire 35-day experiment without any occurrence of microbial contamination. At the same time, it was confirmed that this low-temperature approach can preserve the structural integrity and geometric accuracy of PLA scaffolds, thereby preventing undesirable thermal deformation of the material.

The results of the viability analysis showed that the hCMSCs remained viable and metabolically active throughout the study, with maximum viability (78.5%) reached on day 20 of cultivation. Experimental validation confirmed that 3D-printed scaffolds with 50% lattice infill are suitable for the cultivation of primary stem cells, as they provide sufficient mechanical support and space for their spatial organization along the print lines.

The practical significance of this work lies primarily in its methodological contribution to the processing of hydrophobic 3D scaffolds, particularly in the introduction of a preconditioning step that eliminates air pockets within the scaffold pores. These findings on the interaction between hCMSCs and the PLA matrix provide a relevant scientific basis for application in tissue engineering in the development of innovative therapeutic constructs [2].

Future research requires further optimization of the bioactivity of the scaffold surface and a detailed comparison of various sterilization methods in order to quantify their impact on nanomechanical properties and subsequent stem cell differentiation.

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