AUTOMATED DYNAMIC CELL SEEDING OF CERAMIC SCAFFOLDS FOR TISSUE ENGINEERING

TECHNICAL NOTE

Tissue engineering and 3D cell culture

The main goal of tissue engineering is to create artificial constructs that could repair or simply replace lost or damaged osseous tissue. Common tissue engineering strategies involve the extraction of cells from a small biopsy of tissue for in vitro expansion. This culture can be carried out in a three dimensional (3D) scaffold that allows and induces the formation of new tissue after implantation. Furthermore, the *in vitro* creation of an efficient construct can be improved by applying certain stimuli that can elicit specific responses to the cells.

In addition to tissue engineering applications, 3D cell culture has become a more powerful tool for basic and applied research on cell biology and drug discovery methods. The potential of 3D cell cultures over 2D setups relies on the ability to provide more physiological cues and to replicate complex tissue structures and in vivo-like morphology. This permits to better mirror the environment experienced by normal cells in the body as well as to better reflect normal differentiation, polarization, cell behavior and intercellular interactions.

Challenges in automating tissue engineering cultures

3D cell culture poses specific problems that are hard to face with conventional techniques and equipment. Long-term 3D cultures typically end with poor results when performed under static conditions. Thus, one critical aspect is to obtain adequate distributions of nutrients and oxygen across the whole volume of the sample. Adding to the challenge is the fact that the requirements involved in tissue engineering experiments do vary significantly among applications (size and shape of the sample, rigidity, number of cell types involved). In terms of automation, this results in the demand for very versatile equipments with a modular architecture, that permits to work with multiple samples in parallel in a number of different applications. In many tissue engineering applications, bioreactors are also used to impart certain forces that imitate different mechanical stimuli occurring in the body, thereby enhancing the formation of an extracellular matrix (ECM) similar to the in vivo matrix.

However, it is essential to remark that the first step to culturing cells in a three dimensional environment is the seeding of scaffolds with cells appropriate for the desired application. Scaffold seeding determines the initial number of cells in the construct, as well as their spatial distribution throughout the matrix. Therefore, proliferation, migration and the specific phenotypic expression of the engineered tissue will be affected by the utilized seeding technique. Static seeding has been the most widely used method of cell seeding; however, poor cellular distribution and a low seeding efficiency are obtained with this technique.

Figure 1. *Seeding rack with pre-built circuits and P3D chambers*

Thus, it becomes imperative that tissue engineering bioreactors are able to automate the cell seeding phase, (i) with a high seeding efficiency and (ii) inducing a cell distribution throughout the scaffolds that is as homogeneous as possible in order to generate a uniformly distributed ECM. With the TEB1000 family of bioreactors, EBERS has developed a fully automated system dedicated for tissue engineering applications, capable of automating cell seeding and culture in a variety of scaffolds.

EBERS' TEB1000 family of 3D bioreactors

Within the TEB1000 platform a full system is composed of a Master Unit, common element providing basic control and regulation capabilities, and a Culture Package, where cells and substrate are housed and thus adapts to particular requirements. The Master Unit integrates the functions of a CO_2/O_2 incubator and an advanced pumping system, providing the necessary monitoring and control over basic process parameters (temperature, gas composition, flow rate, customized flow profiles). Moreover, monitoring of dissolved oxygen, pH and other metabolic variables can be integrated with the unit if needed. The flow system operates two independent multichannel pumps controlling flow rate, pulsatility and

sense of flow and permits to introduce complex user-defined flow profiles. Up to 20 independent channels can be used simultaneously under different conditions.

The Master Unit includes a specific module for cell seeding based on oscillating perfusion. This methods seeds cells in the construct by repeated filtering of the suspension of cells through the scaffold, using for this purpose the oscillating flow capabilities of the cell seeding module of the Master Unit. This module permits to select the volume of suspension to perfuse in each cycle as well as the flow rate per channel.

Figure 2. *P3D chamber*

The Culture Package includes a variable number of chambers where cells and substrate are accommodated. Several types of chamber, both disposable and reusable, are available, with a validated and optimized design to cover a wide range of applications. For cell seeding of cylindrical scaffolds, chambers, pre-built circuits and a holding rack are available and constitute a complete solution **(Fig. 1)**. P3D chambers are disposable vessels with an optimized fluidic design for cell seeding and culture under perfusion **(Fig. 2)**. They can be used with pre-built circuits that permit to perform both cell seeding and the following culture phase without any manipulation, thus reducing the risk of contamination and the intervention of the user. Finally, a rack is also provided, in order to house up to 5 circuits (=10 chambers).

Seeding of mesenchymal stem cells: experimental setup and conditions

A critical parameter that conditions the efficiency of oscillatory perfusion cell seeding is the fluid flow velocity. In order to evaluate the influence of this parameter an experiment was designed in which hydroxyapatite scaffolds (disk-shaped, 10mm x 5mm) were seeded with human bone marrow mesenchymal stem cells following three different procedures: (i) static seeding; (ii) oscillatory perfusion, fluid flow velocity 1 mm/s; (iii) oscillatory perfusion, fluid flow velocity 10 mm/s. 2 million cells were inoculated per scaffold.

Static seeding was done by slowly distributing a cell suspension of 200 µL on top of each scaffold. After cell deposit, the samples were left in complete medium at 37°C for 4 h to allow cell adhesion. Dynamic seeding was performed with the seeding module of the Master Unit and the P3D chambers, pre-built circuits and seeding rack, working with 5 circuits (=10 scaffolds) simultaneously. Cells were suspended in 10 mL of culture medium per circuit and a volume of 3 mL was perfused in each direction during 240 cycles. After cell seeding, the samples were left at 37°C for 4 h in the Master Unit to allow cell adhesion. Two dynamic experiments were conducted at 1 and 10 mm/s. The cell number present in the scaffolds was measured by means a DNA assay.

Biological results and discussion

The efficiency of seeding (percentage ratio of the scaffold cellularity to the number of cells initially suspended) is shown in **Fig. 3** for the different seeding techniques. As a general trend, it was observed that seeding efficiency was higher with the oscillatory perfusion technique than with the static method. Nonetheless, the magnitude of the fluid flow velocity had an influence on the process. Dynamic seeding yielded the highest efficiency (79 ± 8%) at a flow velocity of 1 mm/s.

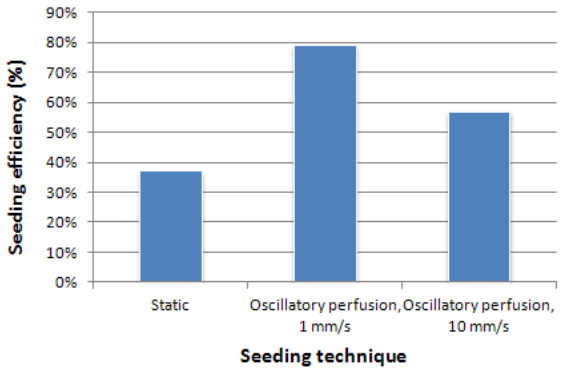

***Figure 3**. Seeding efficiency with different techniques*

Conclusion and outlook

These results permit to conclude that oscillatory perfusion at an adequate level of flow velocity is an adequate technique to automate cell seeding in tissue engineering bioreactors, as detected by the differences in the efficiency between static and dynamic experiments.

A critical issue in dynamic cell seeding is to guarantee a uniform flow of medium across the whole volume of the construct, minimizing recirculation and low velocity regions. EBERS' P3D chambers enjoy an optimized fluidic design and the pre-built circuits allow the user to conduct cell seeding and culture assays without any manipulation between the two phases of the experiment.

The cell seeding module of the Master Unit makes oscillatory perfusion experiments much easier, eliminating the need for other equipment and overcoming the many obstacles and limitations faced by home-made systems. Furthermore, with the Master Unit a large number of experiments can be carried out simultaneously and under different conditions, also reducing manual labor.

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