INFLUENCE OF MECHANICAL STIMULATION ON THE DIFFERENTIATION OF MESENCHYMAL STEM CELLS ON 3D SUBSTRATES

TECHNICAL NOTE

Importance of 3D cell culture

Three-dimensional (3D) cell culture involves growing cells in a 3D environment, matrix or on a scaffold with 3D architecture as opposed to the flat surface of a conventional two-dimensional (2D) culture vessel. The main advantage of 3D cell cultures over 2D setups is 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.

The main therapeutic application of 3D cell culture is tissue engineering, a multidisciplinary field aimed to replace organs or tissues with functional biological grafts created in the lab using 3D techniques. Moreover, 3D cell culture has become a more and more powerful tool for basic and applied research on cell biology as well as in drug discovery methods.

Challenges in automating 3D cell culture

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.

Figure 1. *TEB1000 Master Unit*

Adding to the challenge is the fact that the requirements involved in tissue engineering experiments do vary significantly among applications in terms of size and shape of the sample, number of samples, flexibility of the material, number of cell types involved, etc. 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.

Further issues concern the automation and standardization of important processes such as cell seeding or the application of mechanical stimulation. With the TEB1000 family of bioreactors, EBERS has developed a fully automated system dedicated for in vitro 3D cell culture applications.

EBERS' TEB1000 family of 3D bioreactors

Within the TEB1000 platform a full system is composed of a Master Unit (**Fig. 1**), 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).

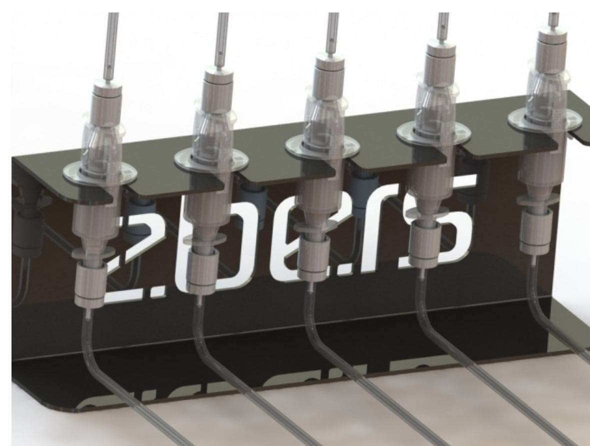

Figure 2. *P3D chambers*

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 flow system can be used to perfuse the samples enhancing mass transport and nutrient exchange, to automatically seed cells on 3D constructs, to automate the exchange of medium or to mechanically stimulate cells in a controlled manner.

The Culture Package includes a variable number of chambers where cells and substrate are accommodated. Several types of chambers (**Fig. 2**), both disposable and reusable, are available, with a validated and optimized design to cover a wide range of applications: perfusion of cylindrical scaffolds, coculture on tubular scaffolds, large organs decellularization, etc.

Differentiation of MSC: Experimental setup and conditions

In order to evaluate the influence of flow-mediated mechanical stimulation on the differentiation of mesenchymal stem cells (MSCs) an experiment was performed in which two groups of scaffolds were first seeded with MSCs and later cultured under different conditions: static and dynamic (perfusion with a flow rate of 100 µL/min). MSCs were derived from Wharton's jelly of human umbilical cord and polymer/bioglass scaffolds were used as substrate. Both groups of scaffolds were grown inside the Master Unit under 37 °C and 5% CO_2.

The dynamic cultures were performed with the pumping system of the TEB1000 Master Unit and P3D disposable perfusion chambers —EBERS' cell culture disposable vessels devoted for perfusion 3D experiments—. For this purpose, two closed circuits were built, each one composed of one reservoir, shared by five chambers. Each circuit was assigned one pumping system of the Master Unit (**Fig. 3**). After 21 days of culture, scaffolds were analyzed by means of Alizarin red and von Kossa staining.

Figure 3. *Main page of the TEB1000 Master Unit software*

Biological results and discussion

Calcium and phosphate deposits, detected by the two types of staining, were significantly increased in perfused scaffolds. Not only the level of mineralization was greater in the group of scaffolds grown under dynamic conditions, but also the spatial distribution of the deposits was more uniform (**Fig. 4**), since the scaffolds kept under static conditions only presented reduced signs of staining near the outer edges of the surface.

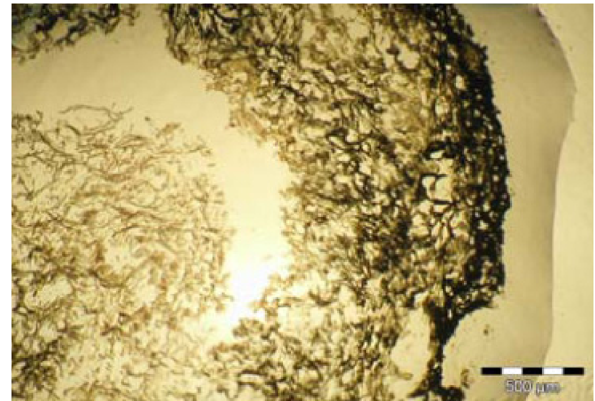

Figure 4. *Von Kossa staining of a dynamically cultured polymeric/bioglass scaffold (by courtesy of the Institute for Technical Chemistry at the Leibniz University of Hannover)*

Conclusion and outlook

These results suggest that an adequate level of mechanical stimulation of MSCs by means of flow-mediated shear stress is able to induce the osteogenic differentiation of MSCs, as detected by the differences in the level of mineralization achieved between scaffolds grown under static and dynamic conditions.

A critical issue in 3D cell cultures under perfusion is to guarantee a uniform distribution of nutrients, what in turn can be obtained only thanks a homogeneous flow of medium across the whole volume of the construct. EBERS' P3D chambers enjoy an optimized fluidic design and reduce scaffold manipulation minimizing the contamination risk.

The Master Unit is the perfect tool to be used with P3D chambers, since it is a convenient fully-equipped incubator for cell culture under flow, which eliminates the need for other equipment and overcomes 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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