# DEVELOPMENT OF A TISSUE ENGINEERED VASCULAR GRAFT UNDER DYNAMIC CULTURE CONDITIONS

## 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 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.

A nice example is the development of vascular grafts. Recent advances have looked at developing a completely artificial biological graft engineered from the patient's cells with surface and viscoelastic properties similar to autogenous vessels. The challenges faced by this approach are substantial: providing a conduit that has sufficient strength, a vessel wall that is elastic and can withstand cyclic loading, matching compliance of the graft with the adjacent host vessel, and a lining of the lumen that is antithrombotic, among others.

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.


**Figure 1**. *TEB1000 Master Unit*

In vascular tissue engineering the application of adequate mechanical stimuli is essential. Culturing smooth muscle cells with a pulsatile flow through the lumen of the graft helps mimic nature's vessels. Experiments show that, compared with non-pulsed engineered grafts, pulsed grafts are thicker, have greater suture retention and improve physiological SMC density together with a higher collagen density.

Further issues concern the automation and standardization of important processes such as cell seeding, which determines the initial number of cells in the construct, as well as their spatial distribution throughout the matrix. 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.

Thus, it becomes imperative that tissue engineering bioreactors are able to provide mechanical stimulation of adequate nature and magnitude, as well to automate cell seeding. 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 **(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). 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**. *Culture chamber for vascular applications*

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. A tubular vessel is available for the engineering of tubular grafts (blood vessels, esophagus, trachea) of variable length and diameter **(Fig. 2)**. The chamber is designed for dual circuit flow loops to deliver media separately to both the external and internal surfaces of a scaffold. When assembled with the Master Unit, the system can impart two independent flows: one, typically pulsatile, through the lumen of the graft, and another to renew the medium that bathes the external surface. Several chambers can be operated simultaneously under different conditions with the Master Unit.

## Development of a tissue engineered vascular graft: experimental setup and conditions

In this experiment, a tissue engineered vascular graft was developed by coculturing porcine coronary artery endothelial (PCAEC) and smooth muscle cells (PCASMC) in a tubular scaffold under dynamic conditions. For this purpose, a braided biodegradable polymeric tubular scaffold with an inner diameter of approximately 3 mm was mounted in the EBERS' vascular culture chamber. First, PCASMCs were seeded on the construct lumen by means of oscillatory perfusion making use of the cell seeding module of the Master Unit.

After cell attachment pulsatile inner flow through the lumen was applied progressively up to the physiological rate over 7 days and then maintained afterwards for 7 more days. The inner flow was applied in a closed circuit using one of the pumping systems of the Master Unit capable of regulating flow pulsatility (frequency, flow rate). At the same time the external surface of the scaffold was bathed by culture medium, which was also circulated for gas exchange at a steady flow rate through an independent closed circuit, using the second pumping system of the Master Unit. After 14 days of culture under pulsatile flow, PCAECs were sequentially seeded on the construct lumen. After cell attachment the flow was gradually increased back to physiological values during 14 days. During the whole process the medium was replaced every 2 days and the cultures were maintained inside the Master Unit at 37°C and 5% $CO_2$. Cell proliferation was estimated by quantifying the DNA content. Histology and immunohistochemical analysis were conducted.

## Biological results and discussion

The vascular graft displayed integrity without deformation during the culture period. Full coverage of the luminal surface with PCASMCs was observed on seeded grafts harvested before PCAEC seeding. Histology revealed significant tissue formation as well as the alignment and elongation of PCASMCs. A nice coverage of the luminal surface with PCAECs was observed after sequential seeding. Some cell loss was observed after 14 days of coculture, with PCASMCs still retaining their alignment in the direction of flow.

## Conclusion and outlook

The TEB1000 bioreactor has shown the capacity to develop a tissue engineered vascular graft by coculturing smooth muscle cells and endothelial cells on a tubular scaffold under pulsatile flow conditions.

A critical issue in vascular tissue engineering is the application of flow through the lumen of the graft under physiological conditions, what requires control over the flow rate and the frequency of the pulsatility of the flow. The Master Unit includes this advanced flow regulation capabilities, as well as a cell seeding module, which permits to impart an oscillatory perfusion of a suspension of cells for seeding onto a variety of scaffolds. The integration of all the necessary equipment in a single device with high automation capabilities converts the Master Unit into a very useful and versatile tool for tissue engineers. In this regard, the range of application of the tubular chamber is not restricted to the engineering of vascular grafts, being also adequate for other applications in which coculture of different types of cells in a tubular scaffold is required.


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