L.I.A.C Latinorum Investigatorum de Arteriis Colloquium
The Latin Society for Vascular Research

*XXXII LIAC Meeting, Ustica, September 14-17, 2016*

# Decellularized Saphena: Biologic Scaffold for 3D Cellular Growth

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**Keywords:** Cell Culture Models, Blood Vessels, Tissue Engineering, Regenerative Medicine, Bioreactor

Cardiovascular diseases are leading causes of mortality in Western society and countries adopting an Occidental Life style (1). Restoration of the circulation via bypass surgical treatment is regarded as the gold standard treatment of peripheral vascular diseases, and requires (vascular/tissue/circulatory/blood vessel - delete as appropriate) grafts. Normally the great saphena vein is used as (a/the) "donor" blood vessel. It is collected from its superficial location on the leg and used in case of auto-transplantation in coronary artery bypass operations, and also for peripheral arterial bypass operations. Autologous great saphenous veins are often not available, and synthetic grafts have their limitations. Therefore novel techniques to produce alternative grafts have been developed, including tissue engineering which is a promising alternative to provide biocompatible grafts. Our aim is to reconstruct the endothelium layer of decellularized vein scaffolds using mesenchymal stem cells (MSCs) and growth factors.

The use of allo/xenogenic material as a scaffold for donors has previosuly been embraced by the scientific community (2), and standardised procedures have been developed that consists of a decellularization phase of the donor element, a re-seeding phase of the decellularized scaffold and an implant phase on the host (3). In particular the opportunity to use a decellularized blood vessel as a scaffold for vascular tissue engineering, and the saphena vein specifically, is something well known (4-5). Our project concerns the development of blood vessel substitutes to be used mainly in arteriovenous fistulae surgery. We propose to develop a procedure that automates the initial phases (decellularization and re-seeding) to reduce the manipulation of the vein and to promote a more efficient and natural cell seeding.

We have developed a specific bioreactor linked with a rotary infusion pump to create a closed circuit in which the donated saphena vein was integrated during the decellularization phase. We have enabled a flow of SDS (0.1% in sterile PBS) for 24h inside the circuit, and consequentially inside the saphena. After 24h the saphena was removed, decellularized from the circuit and samples were prepared for analysis. Observations were performed using an optical microscope and a transmission electric microscope to evaluate the condition of connective scaffold and the potential presence of cells. The total removal of cellular elements and the upkeep of the connective architecture, essential conditions for the subsequent seeding phase, were observed,.

Subsequently, we replaced the SDS solution inside the reservoir of the bioreactor with a specific stromal medium (alpha-MEM, supplemented with 20% FBS, 1% L-glutamine and 1% penicillin/streptomycin) containing adipose-derived mesenchymal stem cells obtained from rats in order to obtain the re-seeding of the scaffold. Scaffolds were kept under these conditions for 7 days in order to obtain a proper vascular equivalent. After this period, samples

were prepared for analysis. Observations with a light microscope confirmed the presence of properly differentiated endothelial-like cells that however were too few to form a continuous layer.

In conclusion, our model represents a valid system to obtain natural scaffolds to be implemented in the creation of vascular equivalents, however, more experiments are needed in order to validate the results obtained so far.

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