I S A C B

ESSAYS

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Where to Now...That We Have Tissue Engineered Blood Vessels? Tissue Engineered Blood Vessels! Heavens, What Will They Think of Next !

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My father would have wished me to be more frequently his assistant as he was an active vascular surgeon. We did only " operate " together three times : but these surgeries and my intimate relationship with vascular and cardiothoracic surgeons has instilled into me a good dose of respect for this art. Such surgical prowess has not gone unnoticed in the medical history of the XXth century.

The advent of vascular grafts has saved countless lives and paved the way to audacious surgical heart and limb salvages. Synthetic prosthesis for vascular implantation are one of the most fascinating story in medicine and many great names come to mind : Carrel, Blakemore and Voorhees, Sauvage, DeBaquey. However, as we are now entering into the XXIst century, a critical appraisal of these approaches is unavoidable. These prosthesis have limitations and the most blatant is their complete lack of biological function. This renders them unsuitable for small diameter grafting. The few attempts to transplant vessels less than 5 mm of internal diameter have mostly led to dismal results on a long term basis. Thus, the stage is set for a new approach in this clinical niche, such as tissue engineered blood vessels.

One must never forget that tissue engineering was first introduced as a life saving procedure for burn patients [1]. The successful engraftment of autologous epidermal sheets was the initial and seminal proof of concept of the powerful technology that we know today.

The subsequent efforts in this biotechnological field were developed according to essentially three types of approaches. The first approach consists in the seeding of cells into various gels, which are then reorganized by the incorporated cells.[2-8] Alternatively, a second approach is to deposit cells into a scaffold where they will thrive and secrete an extracellular matrix.[9-11] The scaffold materials are at times bioresorbable over a wide range of time periods depending on their chemical nature.[12-14] A third approach is different since it uses the principle of a tissue template that allows, after implantation, the ingress of cells into the appropriately organized scaffold. Thus, these grafts are acellular and must stimulate the regenerative potential of the tissue, in vivo, wherever they are implanted.[15-17]

However, our group has initiated a different and original method for the reconstruction of soft tissues. It takes full advantage of the various intrinsic properties of cells when appropriately cultured. This entails particular media composition and appropriate mechanical straining of these three-dimensional structures. We call it the **self-assembly approach.**

Our own experience with the culture of autologous epidermal sheets gave us some insight in the properties of cells, in order to recreate in vitro human living tissue substitutes.[26] Furthermore our various adaptations of the gel construct approach has shown that cells could be aligned along with their extracellular matrix if the mechanical forces generated were appropriately harnessed: either passively, by matrix anchorage,[18] or actively, by cyclic traction of these constructs.[6]

The self-assembly approach is a combination of all our previous experiences. Basically, we coax the cells into secreting their own extracellular matrix in a sheet form. Thereafter, we either roll or stack these sheets to create a three-dimensional organ substitutes. Our concept has been applied with impressive results to the reconstruction of blood vessel and skin.[19, 20]

The first attempt to produce a reconstructed blood vessel by tissue-engineering methods appeared in 1986 with the model published by Weinberg and Bell.[3] The method devised by these researchers was based on collagen gels seeded with bovine vascular cells. Such a technique was the basis for subsequent research conducted by other teams.[4,21,22] But the resulting structures were not resistant enough to sustain normal blood pressure[3,4] and some of these prostheses had to be reinforced with a synthetic mesh[3,23] making them hybrid artificial substitutes (composed of living cells in association with a synthetic support) with all the untoward properties associated with such constructions.

Since the previous approaches did not seem to be conducive to an appropriate clinical result, we developed a tissue-engineered blood vessel (TEBV) based exclusively on the use of human cells in the absence of any synthetic or

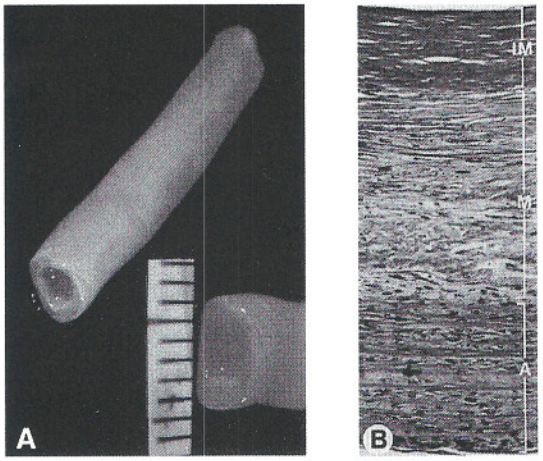

FIGURE 1

Macroscopic (A) and microscopic (B) views of the mature TEBV.

(A) When removed from the tubular mandrel, the TEBV is self-supporting with an open lumen (3 mm internal diameter).

(B) Paraffin cross section of the TEBV wall stained with Masson's Trichrome. Collagen fibers are stained in blue-green and cells in dark purple. Inner membrane (IM)=125 mm, media (M)=320 mm and adventitia (A)=235 mm. Note the endothelium covering the luminal surface of the TEBV.

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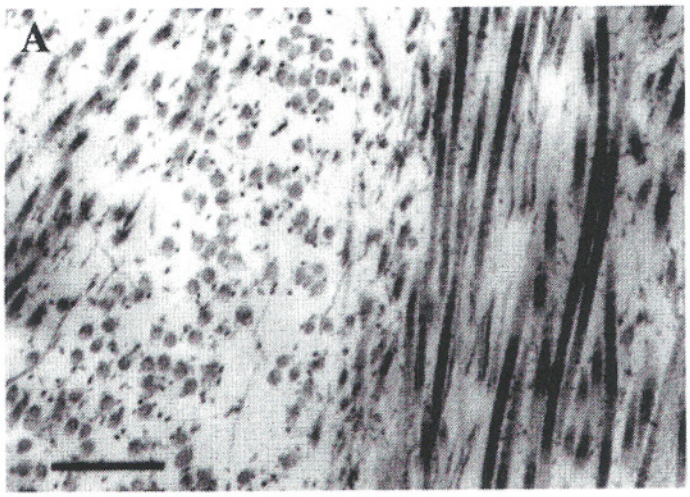
exogenous material[19] (figure 1). This prosthesis was shown to have a supra-physiological blood pressure resistance (figure 2) and a histological organization comparable to that of a native artery (figure 1).

The cells used for such a TEBV were endothelial cells (EC), smooth muscle cells (SMC) isolated from human umbilical cord vein using an enzymatic method for EC[24] and the method of Ross for SMC isolation.[25] The fibroblasts were provided by the enzymatic treatment of a small biopsy of human skin [(26)]. Each layer of the vascular wall was thus reconstructed : the intima (composed of EC), the media and the adventitia. In order to obtain an abundant extracellular matrix production, fibroblasts and SMC were cultured in media supplemented with ascorbic acid until they self-assembled into sheet that could be detached from the culture support and then be wrapped around a tubular mandrel.

The mechanical, histological and physiological properties of the TEBV were very interesting. Although completely biologic, this reconstructed blood vessel was highly resistant with a burst strength of over 2500 mm Hg (figure 2B). This resistance is significantly higher than that of the human saphenous vein, considered to be the best biological material for lower limb vascular reconstruction.[27] This impressive resistance is due to the well-organized extracellular matrix composed of collagen fibrils that were oriented in perpendicular directions to



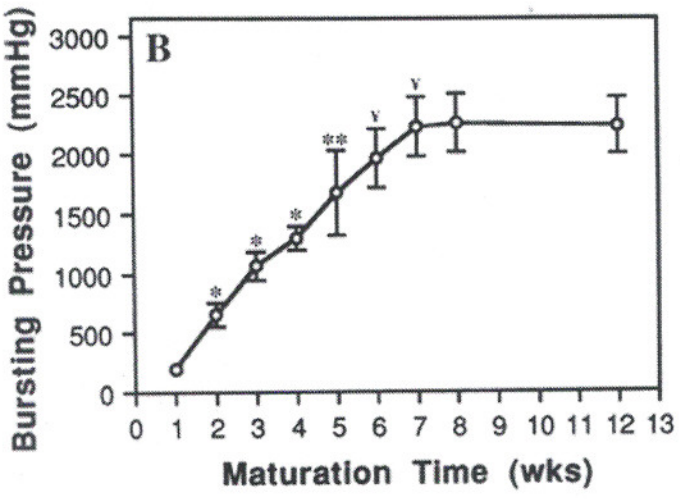


FIGURE 2

(A) Adventitial extracellular matrix ultrastructure observed by transmission electron microscopy. Uranyl acetate and lead citrate staining (scale bar=500 nm).

(B) Burst strength of the adventitia over time of maturation in vitro.

Significantly different than the precedent point ($p<0.001$, **$p<0.005$, ¥$p<0.05$) with the Student's t test (n=8 to 13).

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one another in the concentric layers of the adventitia (figure 2A). Moreover, a constant gelatinase activity was measured starting at 2 weeks of adventitia maturation.

Histological and immunohistological analyses of the TEBV showed cells surrounded by a dense extracellular matrix composed of collagen and elastin. Interestingly, desmin, a protein component of the cellular intermediate filaments, known to be lost in culture, is reexpressed in SMC of the TEBV. This result indicates how close to its physiological counterpart our model is. Only quiescent SMC can produce such a molecule.

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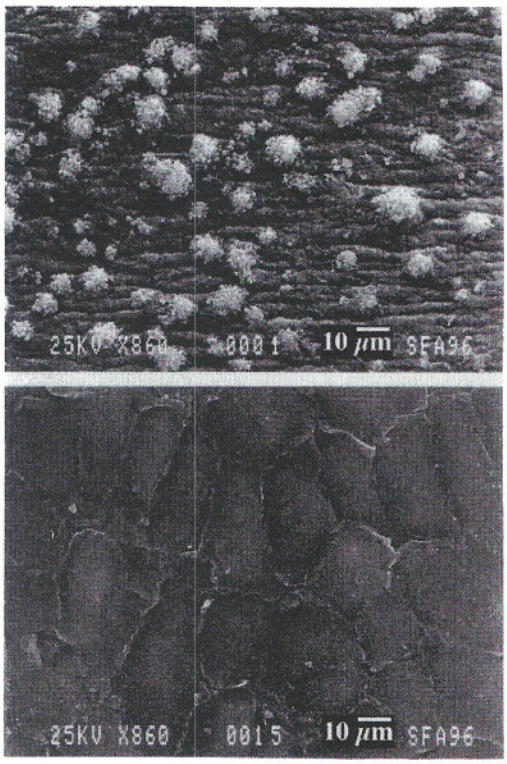

FIGURE 3

Inhibition of platelet adhesion by the endothelium. Scanning electron micrographs of unendothelialized IM (A) promoted platelet adhesion and activation whereas endothelialized IM (B) almost completely inhibited the process.

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The EC seeded on the inner membrane formed a confluent monolayer (figure 3) expressing von Willebrand factor and the cells were able to incorporate acetylated low-density lipoproteins. This endothelium inhibited platelet adhesion in contrast to the non-endothelialized acellular membrane (figure 3). Thus, this endothelium was functional and provided an anti-thrombotic surface.[19]

These human TEBVs were implanted for one week in femoro-femoral interposition in the dog. Because of the xenogeneic situation, the prostheses did not contain EC. The implanted prostheses demonstrated that they could be easily handled and sutured by the use of conventional surgical techniques. A patency level of fifty percent was obtained after one week of implantation and the patent implants were exempt of early tearing or dilatation.[19]

We are convinced in our research group, the LOEX, that this self-assembly approach may offer great opportunities in the field of soft tissue reconstruction. Thus, the next generation of small diameter blood vessel should be of a tissue engineered nature.

We feel that we presently have the most advanced technology in the field of cardiovascular reconstruction. The auto-assembly approach could be easily translated into other areas of this field in order to produce cardiac valves, cardiac muscle, etc. The future of cardiovascular surgery could then be brighter for clinician and patients alike.

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