CellPress

Adipose tissue-derived microvascular fragments: natural vascularization units for regenerative medicine

Matthias W. Laschke and Michael D. Menger

Institute for Clinical and Experimental Surgery, Saarland University, 66421 Homburg/Saar, Germany

The establishment of effective vascularization is a key challenge in regenerative medicine. To achieve this, the transplantation of native microvascular fragments has emerged as a promising novel concept. Microvascular fragments can be isolated in large amounts from fat tissue, exhibit a high angiogenic activity, and represent a rich source of mesenchymal stem cells. Originally, microvascular fragments have been used in angiogenesis research for the isolation of capillary endothelium and for functional sprouting assays. More recent studies have demonstrated that they rapidly develop into microvascular networks after transfer into tissue defects. Moreover, they are suitable for the generation of prevascularized tissue constructs. Hence, a wide range of future medical applications may benefit from the use of these natural vascularization units.

Vascularization in regenerative medicine

Tissue regeneration crucially depends on adequate vascularization (see Glossary), which is essential to sustain cell metabolism and to facilitate the clearance of cell debris. Moreover, it enables the recruitment of circulating stem cells, which contribute to distinct steps of the healing cascade [1]. Rapid vascularization further guarantees the survival and function of cells within implanted tissue constructs, which cannot solely survive by oxygen diffusion from the surrounding microvasculature [2,3]. Accordingly, different vascularization strategies have been developed in the field of regenerative medicine and tissue engineering. They can be assigned to two basic concepts: angiogenesis and inosculation [4]. Stimulation of angiogenesis results in the ingrowth of blood vessels into a tissue defect. However, the development of new microvessels is a complex multistep process [5], which requires a substantial amount of time for the establishment of adequate vascularization. The consequence is insufficient healing and death of the hypoxic parenchymal cells within implanted tissue constructs. To overcome this problem, preformed microvascular networks may be generated in tissue constructs prior to their implantation. The preformed microvessels are then rapidly blood perfused after implantation by interconnection with the host microvasculature via inosculation [6].

The *in vitro* generation of preformed microvascular networks can be achieved by seeding scaffolds with endothelial cells or stem cells, which assemble to vessel-like structures under appropriate culture conditions [7,8]. However, the successful implementation of such cell-based procedures requires complex bioreactor systems, which makes their transfer into clinical practice difficult due to logistic and regulatory hurdles. Alternatively, the body may be used as a natural bioreactor for the *in situ* generation of prevascularized tissue constructs by implanting scaffolds into a well-vascularized host tissue [9] or by combining them with an arteriovenous loop [10]. However, the development of new microvessels inside the implants also requires a critical time period. Moreover, *in situ* prevascularized scaffolds finally have to be transferred to the defect site. Hence, this approach involves repetitive surgical interventions with all the associated risks for the patient. Taken together, these facts support the view that there is still an urgent need for the establishment of a more effective and clinically realizable prevascularization strategy. In the following, we present a promising vascularization concept, which combines basic principles of *in vitro* and *in situ* procedures, while avoiding several of the aforementioned fundamental problems. This concept is based on the isolation and transplantation of adipose tissue-derived microvascular fragments. A growing number of studies

Glossary

Angiogenesis: development of new blood vessels from pre-existing ones.
Bioreactor: device or system for the in vitro growth of cells or tissues.
Inosculation: interconnection of preformed microvessels within an implant with microvessels of the host tissue.
Mesenchymal stem cells: multipotent stromal cells that can differentiate into a variety of cell types.
Microvasculature: the part of the circulatory system composed of the smallest vessels, including arterioles, capillaries and venules.
Scaffold: natural or synthetic material serving as a three-dimensional matrix for tissue formation.
Stem cell niche: microenvironment, which contains stem cells and regulates their cell fate.
Tissue construct: artificially generated tissue substitute.
Tissue engineering: combination of cells, engineering and materials to improve or replace biological functions.
Vascular remodeling: maturation and adaptation of a microvascular network to its environment.
Vascularization: formation of blood vessels in living tissue, resulting in a red blood cell-perfused microvascular network.

Corresponding author: Laschke, M.W. (matthias.laschke@uks.eu).
Keywords: microvascular fragments; vascularization; angiogenesis; tissue engineering; mesenchymal stem cells; scaffolds.

0167-7799/
 http://dx.doi.org/10.1016/j.tibtech.2015.06.001

have demonstrated that these fragments rapidly develop into blood-perfused microvascular networks after their transfer into tissue defects.

Isolation of microvascular fragments

Under experimental conditions, microvascular fragments are isolated from epididymal fat pads of donor rats [11,12] or mice [13,14] (Box 1). However, the isolation procedure also applies to human fat tissue. Shepherd *et al*. [15] isolated human microvascular fragments from discarded abdominoplasty fat and showed that they exhibit characteristics similar to those of rat vessel fragments. Microvascular fragments can also be isolated from other tissues [16]. Nonetheless, adipose tissue represents the most attractive source for clinical applications, because it can easily be harvested in large quantities by minimally invasive liposuction with low donor-site morbidity [17]. Hence, it is conceivable that in the future microvascular fragments are rapidly isolated from fat tissue samples and retransferred into a tissue defect in an intraoperative one-step procedure without marked *in vitro* manipulation. The procedure may be performed with closed-system devices, which are already introduced in clinical practice for the automated intraoperative generation of stem cell or platelet-rich plasma preparations [18,19].

Characteristics of microvascular fragments

Microvascular fragments are a randomized mixture of arteriolar, capillary, and venular vessel segments with all their morphological characteristics [11]. The majority of fragments exhibits a stabilizing α-smooth-muscle-actin-positive perivascular cell coverage directly after isolation [11,14,15] (Figure 1A–C). This coverage progressively decreases when culturing the fragments in a 3D collagen gel, resulting in a collection of morphologically homogeneous, dedifferentiated vessels [15]. Once implanted and reperfused, these vessels finally redifferentiate back into the specific elements of a mature microvasculature [15].

The length of microvascular fragments ranges between 40 and 180 μm [20]. Under culture conditions, they release vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [14,20]. This indicates that microvascular fragments are not only simple vessel segments, but are also a rich source of proangiogenic factors, which stimulate angiogenesis in a paracrine manner. In addition, microvascular fragments contain stem cells antigen (Sca)-1/VEGFR-2-positive endothelial progenitor cells and mesenchymal stem cells expressing common markers, such as CD44, CD73, CD90, and CD117 [14,21] (Figure 1D–F). It may be speculated that the high vascularization potential of microvascular fragments is, at least in part, caused by these stem cell populations. McDaniel *et al*. [21] compared the regenerative properties of conventionally isolated adipose-derived stem cells and multipotent cells derived from an explant culture of microvascular fragments. They found that the latter ones exhibit a higher proliferation rate, an increased expression of genes involved in differentiation, and an improved ability to form capillary-like structures. In line with the concept of the 'stem cell niche' [22], these findings indicate that compared to single cell isolates, microvascular fragments provide a more physiological environment for mesenchymal stem cells, maximizing their regenerative activity.

Transplanted microvascular fragments rapidly interconnect with each other and the surrounding microvasculature to form blood-perfused microvascular networks [15,23]. This process is characterized by three morphologically and transcriptionally defined vascular phenotypes [13]. Initially, angiogenic sprouts grow out of the

Box 1. Isolation of microvascular fragments

Under experimental conditions, microvascular fragments are isolated from epididymal fat pads of donor mice (Figure IA). These fat pads can be easily harvested and contain a dense microvascular network [55]. In a first step, the fat pads are finely minced with a scissors. Subsequently, they are enzymatically digested for 5–10 min incubation in collagenase at 37 °C with vigorous stirring, which results in the destruction of the fat cells. The collagenase is then neutralized with fetal calf serum to prevent further damage of the microvascular fraction and to maintain vessel integrity. After gentle centrifugation or sedimentation of this fraction, the fat supernatant is decanted. The remaining suspension contains functional microvascular fragments with a lumen (Figure IB,C). Optionally, they can be stepwise purified from large debris and single cells by additional filtration using 500 and 20–30 μm filters. The entire procedure does not take longer than 2 h. The isolated microvascular fragments do not contain relevant numbers of apoptotic or necrotic cells, indicating that the isolation process does not affect cell viability [14].

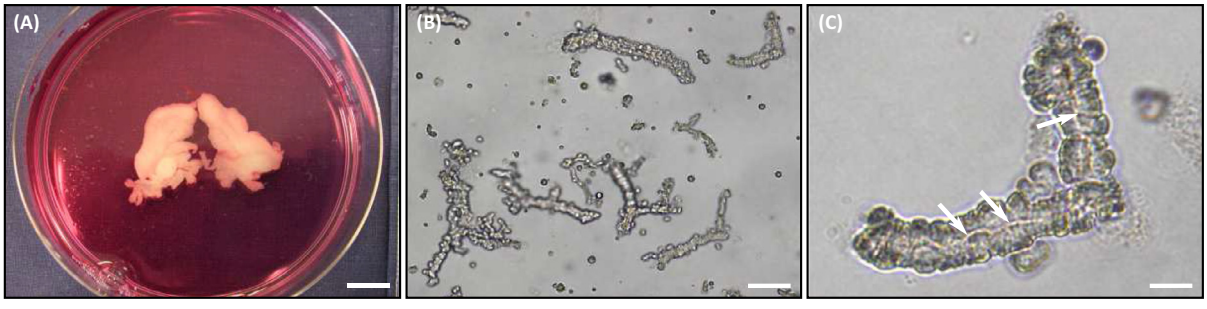

Figure I. Isolation of microvascular fragments. **(A)** Isolated epididymal fat pads of a C57BL/6 donor mouse. **(B, C)** Microscopic images of microvascular fragments directly after the isolation from the fat pads. Higher magnification reveals that the fragments exhibit a functional vessel morphology with a central lumen (C, arrows). Scale bars: A = 14 mm; B = 70 μm; C = 15 μm.