Biotechnology Advances 34 (2016) 112–121

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Prevascularization in tissue engineering: Current concepts and future directions

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ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form 16 November 2015
Accepted 4 December 2015
Available online 7 December 2015

Keywords:
Tissue engineering
Angiogenesis
Vascularization
Endothelial cells
Stem cells
Microvascular fragments
Scaffold
Arteriovenous loop
Microfluidic

ABSTRACT

The survival of engineered tissue constructs during the initial phase after their implantation depends on the rapid development of an adequate vascularization. This, in turn, is a major prerequisite for the constructs' long-term function. 'Prevascularization' has emerged as a promising concept in tissue engineering, aiming at the generation of a preformed microvasculature in tissue constructs prior to their implantation. This should shorten the time period during which the constructs are avascular and suffer hypoxic conditions. Herein, we provide an overview of current strategies for the generation of preformed microvascular networks within tissue constructs. *In vitro* approaches use cell seeding, spheroid formation or cell sheet technologies. *In situ* approaches use the body as a natural bioreactor to induce vascularization by angiogenic ingrowth or flap and arteriovenous (AV)-loop techniques. In future, these strategies may be supplemented by the transplantation of adipose tissue-derived microvascular fragments or the *in vitro* generation of highly organized microvascular networks by means of sophisticated microscale technologies and microfluidic systems. The further advancement of these prevascularization concepts and their adaptation to individual therapeutic interventions will markedly contribute to a broad implementation of tissue engineering applications into clinical practice.

Contents

1. Introduction

During the last two decades, tissue engineering has become a rapidly growing field of research in biotechnology and medicine. It is driven by the fascinating idea of generating autologous tissue substitutes for the

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http://dx.doi.org/10.1016/j.biotechadv.2015.12.004
0734-9750/

treatment of tissue defects and organ failure. Consequently, several successful clinical applications have recently been reported, such as the implantation of tissue engineered urethras (Raya-Rivera et al., 2011), vaginas (Raya-Rivera et al., 2014) and tracheas (Elliott et al., 2012; Jungebluth et al., 2011) or the functional restoration of nasal cartilage defects (Fulco et al., 2014). Excellent follow-up results of these interventions indicate that clinical tissue engineering is no longer fiction but has become reality (Gonfiotti et al., 2014). Nonetheless, it is still restricted to a few types of tissue substitutes and has not yet entered clinical routine. A major reason for this is the fact that the initial survival of larger tissue substitutes with a complex 3D arrangement of multiple cell types is crucially dependent on the rapid development of an adequate blood supply after implantation (Laschke et al., 2006; Novosel et al., 2011). Accordingly, the establishment of a functional vascularization represents one of the major challenges to be overcome for the broad implementation of tissue engineering applications into clinical practice.

Classical vascularization approaches in tissue engineering focus on the stimulation of vascular ingrowth into tissue constructs (Laschke and Menger, 2012). This can be achieved by optimizing the material properties of scaffolds (Choi et al., 2013; Joshi et al., 2013; Rücker et al., 2006) or by incorporation of growth factor delivery systems (Laschke et al., 2008a; Rui et al., 2012; Singh et al., 2012). However, these so-called angiogenic approaches face the problem that the average growth rate of newly developing microvessels is only ~5 μm/h (Utzinger et al., 2015). Thus, the complete vascularization of large implants by angiogenesis needs a prolonged time period which is associated with major tissue loss due to hypoxic conditions. To overcome this problem, 'prevascularization' has emerged as a novel, promising concept in tissue engineering. This concept basically aims at the generation of preformed microvascular networks inside tissue constructs prior to their implantation. After implantation, these networks can then be rapidly perfused with blood by inosculation with the surrounding host microvasculature (Laschke et al., 2009; Laschke et al., 2011) or by surgical anastomosis of feeding and draining blood vessels (Beier et al., 2010; Eweida et al., 2011).

In the following we provide an overview of the current possibilities for the generation of preformed microvascular networks. These include both *in vitro* and *in situ* approaches. Moreover, we present novel concepts of prevascularization, which may further contribute to the rapid establishment of an effective blood supply to implanted tissue constructs.

2. Current prevascularization concepts

2.1. In vitro approaches

2.1.1. Cell seeding

The most widely applied *in vitro* prevascularization approach is the seeding of vessel-forming cells onto scaffolds, which are of synthetic origin or consist of natural decellularized matrix. The latter ones bear the major advantage that they already exhibit an intact 3D anatomical architecture of the microvascular system, serving as an ideal template for the seeding process (Ott et al., 2008; Song and Ott, 2011). Originally, endothelial cells have been used as cell source (Schechner et al., 2000). After seeding on different biomaterials, these cells rapidly assemble into immature microvessels, which become blood perfused within the first 10 days after implantation (Chen et al., 2014; Nör et al., 2001; Peters et al., 2002; Tremblay et al., 2005). However, endothelial cells bear the major disadvantage that they cannot be easily harvested in large quantities under clinical conditions and that they do not exhibit a high proliferative activity during cultivation. Moreover, endothelial cells originating from different types of blood vessels and different organ tissues markedly differ in terms of their homeostasis, molecular permeability, vascular tone, immune tolerance and angiogenic potential (Baiguera and Ribatti, 2013; Baldwin et al., 2014; Chi et al., 2003). Accordingly, endothelial progenitor cells (EPCs) have been suggested as a promising alternative for tissue engineering approaches (Duttenhoefer et al., 2013; Guerrero et al., 2013; Herrmann et al., 2014; Sasagawa et al. in press; Wu et al., 2004). These cells can be harvested minimal-invasively from bone marrow or peripheral blood. Although the amounts of EPCs obtained in this way may be low in the adult, the cells can be rapidly expanded in culture and can undergo >1000 population doublings (Lin et al., 2000; Wu et al., 2004). Distinct subtypes of EPCs can be achieved, which markedly differ in morphology and function. They can be assigned to early and late outgrowth EPCs dependent on their time of appearance during *in vitro* cultivation (Cheng et al., 2013; Hur et al., 2004; Minami et al., 2015). Early EPCs indirectly contribute to vessel formation in a paracrine fashion by secreting angiogenic growth factors. In contrast, late EPCs differentiate into endothelial cells and form capillary-like tubes (Hur et al., 2004). Hence, the latter ones are of particular interest for the *in vitro* generation of microvascular networks within tissue constructs.

Besides late EPCs other cell types may also be suitable for the vascularization of tissue constructs. These include pluripotent mesenchymal stem cells (MSCs) from bone marrow (Liu et al., 2014; Pill et al., 2015) or adipose tissue (Klar et al., 2014; Miranville et al., 2004; Pill et al., 2015; Scherberich et al., 2007; Zuk et al., 2001), amniotic fluid-derived stem cells (Benavides et al., 2015), induced pluripotent stem cell-derived endothelial cells (Clayton et al., 2015) and glandular-derived stem cells (Kruse et al., 2006). Of interest, glandular-derived stem cells have been shown to markedly improve the formation of new microvessels in implanted dermal matrices (Danner et al., 2012; Egaña et al., 2009), which are so far the most frequently studied scaffold types to establish novel vascularization strategies in tissue engineering research.

Cell-based prevascularization approaches are usually associated with complex and time-consuming cell isolation and cultivation procedures. Their safety and success is dependent on the quality of the cell isolates, the applied seeding strategy and the number of seeded cells. Paik et al. (2015) recently reported an optimum ratio between vascular cells and tissue-specific cells within a construct. In fact, the incorporation of stromal vascular fraction cells into fat substitutes improves their vascularity after implantation. In contrast, the addition of too many vessel-forming cells significantly decreases the vascularization of the grafts and induces their regression. This may be best explained by a higher metabolic load and, thus, supercritical hypoxic levels within the grafted tissue (Paik et al., 2015). These results indicate that the simplified concept 'the more, the better' may not be appropriate for distinct cell seeding approaches.

In addition, functionality and long-term survival of *in vitro* generated immature microvessels markedly differ from that of native blood vessels (Jain, 2003; Koike et al., 2004). To overcome this problem, survival and proliferation of the used vascular cells may be improved by gene transfection (Schechner et al., 2000; Yang et al., 2001). However, this genetic manipulation bears an oncogenic risk. Alternatively, endothelial cells can be co-cultivated with mural cells (Fig. 1A), which fulfill several important functions during the development and maintenance of preformed microvascular networks. They are essential for the stabilization, maturation and long-term survival of newly formed microvessels (Erber et al., 2004; Levenberg et al., 2005). In a seminal approach of Koike et al. (2004) co-cultivation of human umbilical-vein endothelial cells (HUVECs) with mural precursor cells resulted in stable microvascular networks, which survived for one year *in vivo*, whereas microvessels solely engineered with HUVECs rapidly regressed over time. Mural cells have further been shown to regulate vascular remodeling (Bodnar et al., 2013; Simonavicius et al., 2012), thus contributing to the optimal adaptation of an engineered microvasculature to the specific metabolic needs of a tissue construct. They are also crucially involved in the regulation of vascular permeability, contractile function, coagulation and immunomodulation (Gökçinar-Yagci et al., 2015; Kim et al., 2015; Koike et al., 2004). Taken together, these findings indicate