MINI REVIEW

Ex vivo engineering of blood and lymphatic microvascular networks

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Abstract

Upon implantation, engineered tissues rely on the supply with oxygen and nutrients as well as the drainage of interstitial fluid. This prerequisite still represents one of the current challenges in the engineering and regeneration of tissues. Recently, different vascularization strategies have been developed. Besides technical approaches like 3D printing or laser processing and de-/recelluarization of natural scaffolds, mainly co-cultures of endothelial cells (ECs) with supporting cell types are being used. This mini-review provides a brief overview of different co-culture systems for the engineering of blood and lymphatic microvascular networks.

Key Words

- co-culture
- vascularization
- endothelial cells
- lymphangiogenesis
- scaffolds
- microvasculature
- mesenchymal stem/stromal cells
- fibroblast
- fibrin

Necessity for prevascularization

Tissue engineering and regenerative medicine are emerging disciplines focusing on the repair and regeneration of injured or diseased tissues. Except very few tissues like cartilage, epidermis, the cornea and the lens in the eye, most of the organs in the human body rely on a functional supply with vascular structures to provide the cells with oxygen and nutrients on the one side (blood vessels) and to drain interstitial fluid back into the venous circulation (lymphatic vessels) on the other side. Similar to solid tumors, tissues which grow beyond the diffusion limit of oxygen (100–200 µm) are in need of blood vessels for oxygen and nutrient supply. In the last two decades a plethora of approaches have been developed in order to engineer vascular structures, both of blood vascular and lymphatic nature.

Technical approaches for vascularization

In order to achieve a 'pre-patterned' extracellular matrix (ECM), technological solutions comprise the use of 3D printing in order to establish 'vascular trees' in biocompatible hydrogels (1) or the decellularization of larger vascular structures for instance from the small intestine of the pig (2) or from the human placenta (3) (Fig. 1). The generated grafts or scaffolds can be reseeded with autologous ECs on the inside and supporting cells (fibroblasts, smooth muscle cells, etc.) on the outside of the tubes. Moreover, microfluidic systems have been established integrating a vascular network in pre-fabricated channels, which turns out to be suitable for basic biological studies of cell-cell communication, and further might serve as a model system for drug testing. In addition, more sophisticated models utilize different cell

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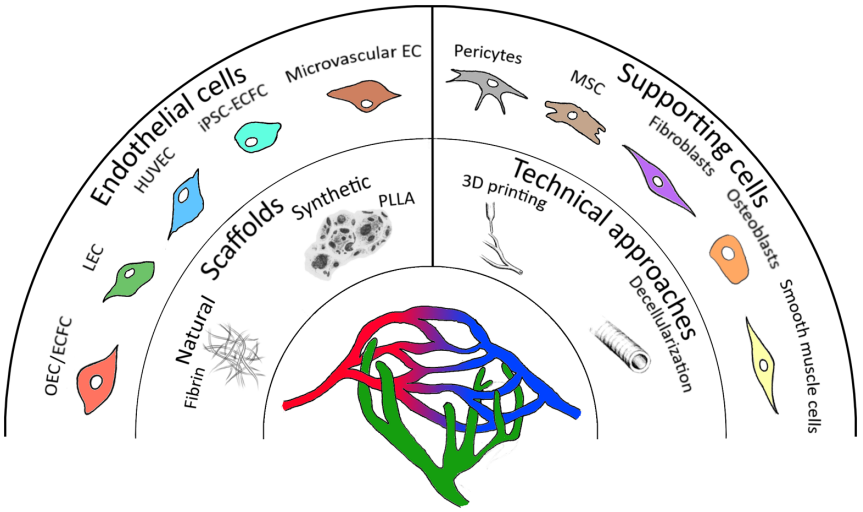

Figure 1
Overview of different vascularization strategies. Functional blood and lymphatic microvasculature can be achieved by co-culturing of endothelial and supporting cells from different origins. Natural or synthetic scaffolds are generated from different materials to provide a 3D structure. Furthermore, technical approaches such as 3D printing or decellularization aid in the fabrication of these structures.

types to create organoids/mini-organs, resulting in organ-on-a-chip systems, which rely on functional vasculature as well (4). As a rather new process 3D bioprinting has also been considered as an approach for successful vascularization with a wide range of applicability (5).

Scaffolds for 3D engineering

Based on the necessity for 3D co-culture to engineer vasculature, different scaffolds have been used to provide the required stability in 3D and allow for angiogenic/vasculogenic remodeling and thus the formation of functional vessels (Fig. 1). For that purpose, both synthetic and natural scaffolds have been described. Synthetic scaffolds include materials such as poly-L-lactic acid (PLLA), poly-lactic-co-glycolic acid (PLGA) or polycaprolactone fumarate (PCLF) (6, 7, 8) as well as self-assembling nanopeptides (9). Their main advantages are accessibility, high reproducibility and an eminently controllable degradation rate; however, low cell adhesion represents the main disadvantage (10). Nevertheless, this difficulty can be mastered by binding of cell recognition motifs in form of small immobilized peptides such as the RGD sequence, which stimulates cell adhesion via integrins (11). The most employed natural scaffolds for engineering vascular networks are collagens (12, 13) or fibrin matrices (14, 15, 16). These types of scaffolds have a high degree of biocompatibility and provide superior adhesion sites leading to improved growth and differentiation capability of the cells (17). Since both types of materials – natural and synthetic – can be fine-tuned with high precision, they are also utilized to deliver different proangiogenic factors such as vascular endothelial growth factor (VEGF) for the recruitment of EC (18, 19). Nevertheless, natural scaffolds represent the predominant type used due to their physiological characteristics resulting in improved cellular functions (20).

EC for vascular tissue engineering

Due to their ease in isolation and availability, ECs isolated from the human umbilical cord (HUVEC) have become the 'gold standard' in several areas of vascular biology including vascular tissue engineering (21). In addition to HUVEC, ECs from microvascular origin (brain, dermis) have been successfully employed in 3D co-culture models (Table 1). However, these cells cannot be translated into clinical settings, making autologous tissue sources like fat or peripheral blood more interesting for the use of ECs in prevascularization strategies. Thus, cells like endothelial colony-forming cells (ECFCs, also described as outgrowth endothelial cells (OECs)), induced pluripotent stem cell (iPSC)-derived ECs will be able to account for organotypic vascular beds (21). Another possibility is the direct reprogramming of differentiated human cells, such as fibroblast (22, 23) or mature amniotic cells (24) making these cells attractive for tissue-specific vascular bioengineering.

Different sources of supporting cell types

Initially, fibroblasts were utilized as supporting cell types for capillary formation in co-culture with ECs (16, 25). Later, also mesenchymal stromal/stem cells (MSCs) mainly from bone marrow (14, 26) and adipose tissue

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Table 1 Cell types used in co-culture models for microvascular network formation.

Endothelial cell type	Supporting cell type	Reference
ECFC	MSC (from different sources)	(12, 15, 35)
	Fibroblast	(36)
HUVEC	Fibroblast	(8, 16, 37, 38)
	MSC (from different sources)	(4, 8, 14, 26, 27, 37)
	Human embryonic stem cells/iPSC-fibroblast	(25)
	Osteoblast	(13, 39)
	Smooth muscle cells	(40, 41)
	Human brain vascular pericytes	(41)
	Human embryonic stem cell-derived pericytes	(42)
iPSC-EC	Fibroblast	(16, 43)
iPSC-EC, cardiac tissue EC, pulmonary artery EC	MSC (adipose derived)	(27)
LEC	Fibroblast	(32, 44)
LEC, BEC	ASC	(33)
Microvascular EC	Fibroblast	(32, 45, 46)
	Dental pulp stem cells	(45)
Outgrowth EC	Osteoblast	(39, 47)
	MSC	(17, 48, 49)

Endothelial cells and supporting cell types from different tissue sources mediate the formation of vascular structures.

(14, 17, 27) were used to provide ECs with the cues for vascular network formation (Table 1). These microcapillaries show characteristics of mature vessels, such as pericyte coverage or cell-cell junctions and are capable of blood perfusion when implanted subcutaneously in animal models (12, 28), therefore suggesting functionality of these tissue-engineered constructs. Moreover, a number of studies investigated the complex interplay with the ECM. Different proteases like plasmin or matrix metalloproteinases (16) have been shown to be key players in the morphogenesis and the remodeling of their 3D environment. In addition, these microcapillary structures can produce their own ECM consisting of perlecan, collagen IV and laminin (14). Interestingly, the analysis of biomechanical properties of the ECM revealed local stiffness to be quite heterogeneous (29).

The advent of lymphatic networks

Despite its presence and importance in nearly all organs with blood vasculature, the lymphatic system only recently became the research focus in vascular tissue engineering. Due to this neglect, lymphatic vascular markers like VEGFR3, PODOPLANIN, LYVE-1 and PROX-1 have only been cloned and functionally characterized years after respective markers on blood vascular cells (30). Consequently, the engineering of these structures lags behind. The group of Melody Swartz was among the first who took up this topic and integrated lymphatic ECs in 3D matrices to build lymphatic capillaries (31). Later on the Reichmann group (32) used ECs from the human dermis (comprising both, blood and lymphatic ECs – BEC/LEC) and integrated them in a fibrin matrix together with supporting cells (fibroblasts). Interestingly, the results show separate vascular network formation. Moreover, these vascular structures turned out to be biologically functional, as evidenced in a mouse skin model (32). In addition, our group has shown lymphatic and blood capillary morphogenesis in fibrin, when MSCs from fat tissue were co-integrated in the 3D matrix (33). Recently, the group of Anja Boos described vascular tube formation of LECs when cultured in conditions, where the MSC secretome, but not the MSC themselves were in contact with the LECs (34). Taken together, the importance of engineering of lymphatic microcapillaries is increasingly recognized, but still at the beginning.

Future directions of co-cultures and outlook

Based on the current knowledge on co-cultures for *ex vivo* vascular tissue engineering, many other aspects are currently discussed. For example, the spatio-temporal distribution of gradients which are necessary for vascular network formation can be monitored by microfluidic approaches (50). Furthermore, vascularization of multi-organ-chips is studied among others by the groups of Donald Ingber (51), Ali Khademhosseini (52) and Uwe Marx (4, 53). Moreover, the emerging role of extracellular vesicles (comprising ecto- and exosomes) in the cell-cell

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communication of ECs and supporting cell types will become of interest in the future. Our understanding of microcapillary morphogenesis in engineered vascular networks has constantly increased over the last two decades. *Ex vivo* engineered blood and lymphatic microcapillary structures will be of utmost importance in nearly every tissue engineering approach to provide larger constructs with the necessary oxygen and nutrient supply on the one hand, but also the lymphatic drainage system on the other hand. Integrating organotypic vessels into tissue-specific organoids will further pave the way to transplantable tissues suitable for tissue repair and regeneration.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Author contribution statement

J S, M P and W H designed the outline and wrote the manuscript.

Acknowledgments

The authors thank Severin Mühleder and Johannes Oesterreicher for critical comments on the manuscript.

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Received in final form 2 April 2019
Accepted 8 April 2019
Accepted Preprint published online 10 April 2019