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Research review paper

Spheroids as vascularization units: From angiogenesis research to tissue engineering applications

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ABSTRACT

Multi-cellular spheroids mimic the complex three-dimensional environment of natural tissues. Accordingly, they are also used as vascularization units in angiogenesis research and regenerative medicine. Spheroid sprouting assays are versatile in vitro models for the analysis of molecular and cellular determinants of blood vessel development, including different endothelial cell phenotypes, pro- and anti-angiogenic factors as well as cell-matrix and cell-cell interactions. In tissue engineering, spheroids serve in vivo as paracrine stimulators of angiogenesis and building blocks for the generation of prevascularized microtissues and branched vascular trees in macrotissues. Rapid progress in the automatized high-throughput production of spheroids currently provides the conditions for a widespread use of these applications in future drug discovery and bioengineering of functional organ substitutes.

1. Introduction

Natural tissues and organs exhibit a three-dimensional architecture with direct cell-cell and cell-matrix contacts. Moreover, they contain highly organized vascular and lymphatic vessel networks. This complex environment guarantees cell survival and crucially determines fundamental cellular functions, such as gene expression, proliferation, differentiation and metabolic activity (Costa et al., 2016; Fitzgerald et al., 2015). Moreover, it is associated with a heterogeneous spatial distribution pattern of oxygen, metabolites and signaling molecules that cannot be mimicked in conventional two-dimensional cell cultures (Benien and Swami, 2014; Kinney et al., 2014). Hence, three-dimensional cultures, including scaffold-free multi-cellular spheroids or scaffold-based culture systems, are increasingly used in basic research to study cell biology and physiology under more realistic conditions (Knight and Przyborski, 2015). In addition, they have become important tools for drug and toxicity testing (Damania et al., 2014; Mehta et al., 2012; Pampaloni and Stelzer, 2010) and provide the basis for the generation of artificial tissue constructs in the field of biotechnology and tissue engineering (Fennema et al., 2013; Laschke and Menger, 2017).

Multi-cellular spheroids are the most common three-dimensional cell culture system. They can be generated from one cell type or as co-culture spheroids by combining different cell types (Gu et al., 2013; Wittig et al., 2013). For this purpose, several fabrication techniques have been established, as reviewed in detail elsewhere (Achilli et al., 2012). These include cell suspension cultures (Yoon et al., 2012), hanging drop cultures (Zhang et al., 2016), liquid overlay (Metzger et al., 2011) or microfluidics devices (Sabhachandani et al., 2016). Some of these techniques have recently been adapted to automatized high-throughput production (Fonoudi et al., 2015; Neto et al., 2015; Tung et al., 2011; Zhao et al., 2014). This opens the door to the broad use of spheroids in drug discovery and bioprinting of tissue substitutes.

Angiogenesis, i.e. the development of new blood vessels from pre-existing ones, is a major prerequisite for the physiological function of the female reproductive system (Shimizu et al., 2012) as well as for wound healing and tissue regeneration (DiPietro, 2016; Laschke and Menger, 2016; Verrier et al., 2016). On the other hand, it determines many pathological conditions, such as cancer (Folkman, 2002), rheumatoid arthritis (Elshabrawy et al., 2015), endometriosis (Laschke and Menger, 2012a) and diabetic retinopathy (Wang et al., 2012). Accordingly, angiogenesis represents an important target for the development of new therapeutic strategies. To achieve this, it is necessary to unravel the regulatory mechanisms of blood vessel formation and to test the efficiency of novel pro- and anti-angiogenic compounds in a reproducible and cost-efficient preclinical setting. For this purpose, three-dimensional angiogenesis assays on the basis of gel-embedded endothelial cell spheroids have been established during the last years. Due to their unique angiogenic and vasculogenic properties, spheroids containing endothelial cells or stem cells are also increasingly used as

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building blocks for scaffold-based and -free tissue engineering. In this review article, we provide an up-to-date overview of these in vitro and in vivo approaches, which focus on the application of spheroids as vascularization units in angiogenesis research and regenerative medicine.

2. Spheroids in angiogenesis research

The development of new blood vessels is a dynamic process that underlies the strict regulation by pro- and anti-angiogenic factors and is dependent on the close interaction of endothelial cells with perivascular cells and extracellular matrix (ECM) compounds (Ribatti and Crivellato, 2012). Briefly, angiogenesis is initiated by pro-angiogenic growth factors, such as vascular endothelial growth factor (VEGF). These stimulate the transformation of endothelial cells from a resting stage into activated tip cells under the control of Notch signaling. The tip cells degrade the extracellular matrix by releasing matrix metalloproteinases (MMPs) and migrate into the surrounding tissue. They are followed by proliferating, endothelial stalk cells, resulting in the formation of capillary buds and sprouts, which progressively grow toward the angiogenic stimulus. Finally, the sprouts form a lumen and interconnect with each other to new blood-perfused microvessels, which are stabilized by the coverage with pericytes and smooth muscle cells (SMCs).

To analyze this angiogenic process in vitro, endothelial cell spheroids provide a unique three-dimensional approach. They have been originally introduced by Korff and Augustin, 1998. In contrast to two-dimensional endothelial cell cultures, endothelial cells on the surface of spheroids exhibit a quiescent phenotype, which is sensitive to angiogenic stimulation and differentiation (Korff and Augustin, 1998). After embedding in collagen gels, cultured endothelial cell spheroids further show capillary sprout formation, which can be visualized by time-lapse microscopy and quantitatively assessed by measuring the length, branch points and number of sprouts (Heiss et al., 2015). As outlined in the following, this in vitro system has been used to study various factors determining the angiogenic process, including different endothelial cell phenotypes, pro- and anti-angiogenic stimuli as well as cell-matrix and cell-cell interactions (Fig. 1). In addition, endothelial cell spheroid-containing matrices have been injected into mice to study their angiogenic properties under in vivo conditions (Fig. 1) (Alajati et al., 2008).

2.1. Impact of endothelial cell phenotype on spheroid sprouting

Spheroids for angiogenesis sprouting assays have been generated from different cell sources, including murine glomerular and heart microvascular endothelial cells (Chen et al., 2006; Haspel et al., 2002; Weidemann et al., 2013), porcine coronary artery endothelial cells (Chen et al. 2004; Chen et al., 2007), bovine aortic endothelial cells (Mizrahi et al., 2007) as well as endothelial progenitor cells (EPCs) (Finkenzeller et al., 2009), microvascular (Dakouane-Giudicelli et al., 2015; Ng et al., 2015; Thuringer et al., 2013) and macrovascular endothelial cells (Heiss et al., 2015) of human origin.

The most common cell sources are human umbilical vascular endothelial cells (HUVECs), because of their broad availability, easy handling and rapid growth under culture conditions. Heiss et al. (2015) recently demonstrated that spheroids from these cells develop elongated lumenized sprouts with tip cells of typical morphology and filopodia-like extensions. Moreover, they are more sensitive to pro-angiogenic stimuli when compared to endothelial cells derived from adult blood vessels (Heiss et al., 2015). To reduce inter-experimental variations, spheroids may also be generated from immortalized HUVECs. However, these develop more frequently interrupted sprouts (Heiss et al., 2015). This indicates that the results from spheroid sprouting assays are crucially dependent on the utilized endothelial phenotype. While this may hamper the direct comparability of individual studies, it also offers the possibility to study the specific angiogenic sprouting capacity of distinct types of naturally occurring or transfected endothelial cells.

Philippova et al. (2006) generated spheroids composed of adenoviral-infected T-cadherin (T-cad) overexpressing or T-cad siRNA-transfected HUVECs. T-cad is an atypical plasma membrane receptor of the cadherin superfamily, which is highly expressed during tumor angiogenesis (Wyder et al., 2000). Accordingly, they detected a significantly increased or decreased endothelial cell sprouting in response to T-cad up- or downregulation. In line with these in vitro findings, they further showed in an in vivo model of myoblast-mediated gene transfer to murine skeletal muscle that T-cad potentiates VEGF effects on angiogenesis, resulting in the formation of large caliber vessels. Kaluza et al. (2011) reported that overexpression of histone deacetylase (HDAC)-6 promotes spheroid sprout formation, which is mediated by

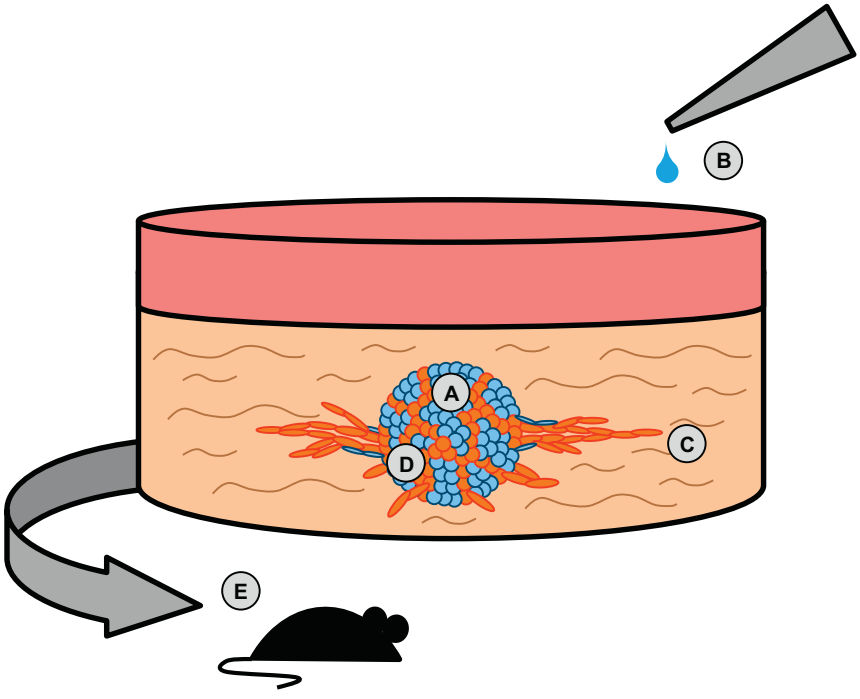

Fig. 1. Schematic drawing showing the versatility of the angiogenesis sprouting assay. This in vitro system can be used to study various factors determining the angiogenic process, including different endothelial cell phenotypes (A), pro- and anti-angiogenic stimuli (B) as well as cell-matrix (C) and cell-cell interactions (D). Endothelial cell spheroid-containing matrices can be further injected into mice to study their angiogenic properties under in vivo conditions (E).