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Review article

Adipose-derived stem cell differentiation as a basic tool for vascularized adipose tissue engineering

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ABSTRACT

The development of in vitro adipose tissue constructs is highly desired to cope with the increased demand for substitutes to replace damaged soft tissue after high graded burns, deformities or tumor removal. To achieve clinically relevant dimensions, vascularization of soft tissue constructs becomes inevitable but still poses a challenge. Adipose-derived stem cells (ASCs) represent a promising cell source for the setup of vascularized fatty tissue constructs as they can be differentiated into adipocytes and endothelial cells in vitro and are thereby available in sufficiently high cell numbers.

This review summarizes the currently known characteristics of ASCs and achievements in adipogenic and endothelial differentiation in vitro. Further, the interdependency of adipogenesis and angiogenesis based on the crosstalk of endothelial cells, stem cells and adipocytes is addressed at the molecular level. Finally, achievements and limitations of current co-culture conditions for the construction of vascularized adipose tissue are evaluated.

Contents

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1. Introduction

The treatment of huge adipose tissue defects originated from high-graded burns or lesions all over the body as well as congenital structural abnormalities preferentially in the face area and tissue replacement after tumor removal, e.g. breast tissue still remains a challenge. Here, fatty tissue implants are strongly needed (Patrick, 2001). Adipose tissue engineering is a growing field which addresses the replacement of lost or diseased tissue with artificially engineered substitutes as well as the creation of fatty tissue constructs for use as in vitro test systems (Mizuno et al., 2012).

The transplantation of autologous fatty tissue has already shown good results to replace small amounts of lost or damaged tissue. Briefly, fatty tissue is liposucked at one side and injected at another side of the body (Pu et al., 2008). For the treatment of large adipose tissue defects, tissue engineering strategies are a promising approach. Here, preadipocytes, differentiated into the adipogenic lineage as well as mature adipocytes are used to build up 3D in vitro constructs. For these constructs, the diffusion of oxygen and nutrients is limited, currently restricting them to a thickness of around 2 mm, which are often clinically insignificant dimensions (Griffith et al., 2005). Therefore, adipose tissue exceeding a critical size is at risk to suffer from undersupply and cell necrosis. Some tissues, like cartilage or bone, maintain their physiological functions in great distance to blood vessels, but mainly all other tissues consist of a highly branched vascular system which ensures a sufficient supply to the surrounding cells with nutrients and oxygen (Carmeliet and Jain, 2000; Kannan et al., 2005; Janssen et al., 2006; Portner et al., 2005). Fatty tissue is a highly metabolic active and therefore a highly vascularized organ. It is thought that every adipocyte has contact to one or more capillaries (Christiaens and Lijnen, 2010). Besides the supply with nutrients and oxygen, the vascular system is also important for the transportation of growth factors, hormones and cytokines, which are essential for the survival and maintenance of the adipocytes. Further, circulating stem cells and immune cells can be brought to the tissue. The vascularization system is also highly important to remove waste material from fatty tissue, as well as bring the released energy in form of fatty acids to peripheral tissues. In activated and growing vessels, endothelial cells release growth factors and cytokines which are signals to surrounding adipocytes that the tissue is expanding. Besides that, blood vessels also serve as a cellular store by creating a perivascular niche for preadipocytes and stem cells, which are needed to stabilize the vessel and control fatty tissue mass and function (Cao, 2010).

An approach gaining interest is the use of adipose-derived stem cells (ASCs) which are differentiated into the adipogenic and endothelial lineage for the composition of vascularized adipose tissue in vitro. In this review, we focus on the current knowledge concerning ASC characterization and their differentiation into adipocytes and endothelial cells. Next, the crosstalk between these cells in adipogenesis and angiogenesis in native tissue will be reviewed as a basic knowledge for the composition of vascularized adipose tissue. Further, we discuss the state of the art in co-culturing ASCs and differentiated adipocytes with endothelial cells.

2. Adipose-derived stem cells

ASCs are a population of mesenchymal stem cells (MSCs) found in adipose tissue. MSCs are defined by the Minimal Criteria of the International Society for Cellular Therapy whereafter these cells have to be plastic adherent, show trilineage differentiation potential and express cluster of differentiation (CD) 73, CD90 and CD105 (Dominici et al., 2006). One gram of adipose tissue yields in 5×104 up to 2×105 stem cells, which is about up to 2500-fold more than the frequency in bone marrow (Baer and Geiger, 2012; Pittenger et al., 1999). Additionally, adipose tissue harvest shows little donor site morbidity, is cheaper, safer and less invasive compared to bone marrow aspiration (Baer and Geiger, 2012). It is known that ASCs possess an even higher potential to proliferate and differentiate compared to bone marrow-derived (BM)-MSCs (Peng et al., 2008). Compared to embryonic stem cells (ESCs) ASCs are free of ethical concerns (Mazo et al., 2011). Recently, some evidence emerged whereby ASCs might exacerbate the tumorigenic potential of cancer cells or increase the recurrence frequency, however, no direct carcinogenic potential of ASCs was reported (Eterno et al., 2014; Bertolini et al., 2015). In contrast to induced ESCs, ASCs do not express human leukocyte antigen-DR (HLA-DR) and therefore show immune compatibility. This compatibility allows allogenic transfer, at least of undifferentiated cells (Mizuno et al., 2012; Puissant et al., 2005).

ASCs are thought to reside within the vessel wall in vivo, however, the certain bed is still not clearly defined (Baer and Geiger, 2012; Kovacic and Boehm, 2009; Klein et al., 2010; Crisan et al., 2008; Tallone et al., 2011). A highly traded candidate is the vasculogenic zone, a region between media and adventitia where endothelial progenitor cells (EPCs) and hematopoietic stem cells (HSCs) are expected to reside (Tallone et al., 2011). Other studies propose that ASCs are located in the perivascular niche maybe as a subset of pericytes or vascular stem/progenitor cells (Lin et al., 2008). ASC position may also depend on size and maturation stage of the vessel as well as the differentiation stage of the stem cell.

2.1. Isolation and culture of adipose-derived stem cells

Up to date, a vast spectrum of slightly different methods for ASC isolation exists (reviewed in the literature (Gimble and Guilak, 2003; Locke et al., 2009; Zuk et al., 2001)). They mainly differ in the purification of the stromal vascular fraction (SVF). Some authors separate the ASCs from endothelial and hematopoietic cells by fluorescent-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS) and exclusion of CD31 and CD45-positive cells (Zimmerlin et al., 2010; Fischer et al., 2009). Most researchers directly plate the SVF assuming the target cells to be enriched by plastic adherence (6 h) and following culture (Planat-Benard et al., 2004; Arya et al., 2014; Cao et al., 2005; Policha et al., 2002; Zuk et al., 2002).

ASCs or BM-MSCs are normally kept in standard medium like Dulbecco's Modified Eagle Medium (DMEM) mostly supplemented with fetal calf serum (FCS). The use of human serum (HS) may replace calf serum thereby enabling a xenofree cell culture which is important for the intended clinical use of the construct as an implant. HS is consistently linked to higher proliferation rates and sometimes as well as alternating multipotency (Kocaoemer et al., 2007; Lindroos et al., 2009; Mirabet et al., 2008; Rajala et al., 2010). However, Müller et al. could show a higher proliferation rate of cultured ASCs at a similar ability to undergo multipotent differentiation by the addition of human platelets, human frozen plasma, heparin sulfate and L-glutamine (Muller et al., 2006). Unfortunately, HS shows significant lot-to-lot variability which may as well affect the reproducibility of the results (Shi et al., 2010). Therefore, the development of a completely defined medium is highly desirable.

2.2. Adipose-derived stem cell characterization

Defining MSCs in adipose tissue still remains a challenge. It is thought that ASCs include different subpopulations linked to their different functions, e.g. as precursors of adipocytes or as vascular support. Therefore, agreeing on a defined and independent