CHAPTER 1

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Tissue engineering and regenerative medicine are a multidisciplinary fields, which combine knowledge of pathophysiology of disease, biomaterials, growth factors, and stem cells to repair, replace or regenerate damaged organs and tissues to restore function [1,2]. Tissue engineering follows the principles of cell transplantation, materials science, medical science and engineering to develop biological substitutes that can restore and maintain normal function. Tissue engineering strategies generally comprise of two main categories: (i) the use of natural matrices, depending on the body’s natural ability to regenerate for the suitable direction of new tissue growth, and (ii) the use of matrices with factors and/or cells e.g. stem cells [3]. The possibility to tailor biomaterials brought new aspects to the field of regenerative medicine. To date, we can fabricate numerous types of biomaterials, e.g. as scaffolds, with a wide range of physical, chemical and biological properties. Recent developments in stem cell science, stem cell-associated growth factors, and regenerative medicine allows the use of stem cells to repair tissue damage and, eventually, to replace organs. Stem cells vary from embryonic stem cells (ESCs) and, induced pluripotent stem cells (iPSCs) to postnatal adult stem cells. In contrast to ESCs, (autologous) adult stem cells are immunocompatible, and there are no ethical concerns related to their use. Multipotent mesenchymal stem cells (MSCs) are non-hematopoietic cells of mesodermal origin that are present in postnatal organs and connective tissues. In the past decades, MSCs, with similar characteristics to bone marrow-derived MSCs, have been isolated from different tissue sources including adipose tissue. The main and common problem for most of the adult stem cells is that both the amount of tissue and the number of cells that can be harvested often is low. Therefore, almost all adult-derived stem/progenitor cells require at least some degree of ex vivo expansion or further manipulation before they can be used. However, adipose tissue-derived stem cells (ADSC) are one of the most promising stem cell population identified thus far, since these are easily harvested in large quantities with low morbidity and discomfort. Therefore, the use of ADSC both for research and as cellular therapeutics is feasible and has been shown to be safe and efficacious in preclinical and clinical studies in which ADSC were used to treat injury and degenerative organ disease [4,5]. Thus, the behavior of stem cells as candidates for tissue restoration could theoretically easily be tailored by adjusting biomaterial properties. However, the adequate and functional design of biomaterial-based scaffolds remains a challenge. A stem cell, by definition is characterized by its ability to self-renew and to differentiate. Several types of stem cells produce and secrete trophic factors that act on their microenvironment, albeit that this is not restricted to stem cells. In this thesis we discuss the isolation, characterization and differentiation of adipose tissue-derived stem cells (ADSC) and the development and use of delivery devices for cardiac regeneration. In the second part of the thesis, we discuss the potential of ADSC for tissue engineering to alleviate vascular problems such as the need for replacement vessels and repair of aneurysmal arteries. Despite considerable advances in therapeutic intervention, cardiovascular disease still is the main cause of death worldwide. The World Health Organization reported about 17.3 million worldwide deaths from CVD in 2008 and it is estimated that by 2030, 23.3 million people will die due to the consequences of CVD. Of all CVD, acute myocardial infarction (aMI) ranks
number one as the cause of death, in which the underlying cause often is of vascular nature i.e. atherosclerosis. Stem cells have the potential to develop into many different cell types in the body. As stated above, stem cells are unspecialized cells capable of renewing themselves through cell division and under certain physiologic or experimental conditions; they can be induced to become tissue or organ-specific cells with special functions.

In this thesis, the future application of ADSC-loaded microspheres for treatment of the consequences of acute myocardial infarction (aMI) is specifically addressed. Myocardial infarction is caused by the obstruction of a coronary artery which deprives the downstream tissue of oxygen. This sets off a process of wound healing and inflammation. Rapidly after aMI the inflammation develops, which is initiated by the high level of reactive oxygen species (ROS) and necrotic cellular debris. Following ROS production and necrosis, leukocytes exit the circulation and infiltrate to infarcted and non-infarcted tissue as a response to aMI. Upon entering the damaged tissue, these leukocytes release various pro-inflammatory cytokines, proteolytic enzymes and participate in phagocytosis of cellular debris and adverse remodeling. Inflammation usually reaches the peak at 1 or 2 weeks post-aMI and onset of chronic inflammation at 3 or 4 weeks. Resident cells, which survived the first phase of inflammation, like cardiomyocytes, endothelial cells and smooth muscle cells are at risk of apoptosis. In response to inflammation, they attract neutrophils and later on monocytes/macrophages to the damaged side, which contributes to inflammation and adverse remodeling of cardiac tissue. When cardiac damage occurs such as after myocardial infarction, stem cells migrate from the bone marrow and in some cases from the heart itself to facilitate wound healing. Much of the influence is mediated through paracrine signaling, however, occasionally stem cells may differentiate into cardiac cells [6-8]. However, after myocardial damage, the potency of endogenous cardiac progenitors to yield sufficient new cardiomyocytes is under par, while extra-cardiac stem cells have virtually no cardiomyogenic potential, while post-natal cardiomyocytes lost mitogenic capacity. The post-infarction wound healing therefore, ensues in the generation of a strong, yet rigid, intramyocardial scar. Its rigidity together with the loss of contractile cells, causes cardiomyocyte hypertrophy, cardiac fibrosis which may result in heart failure. Over the past decade, stem cell therapy was investigated as a potentially promising treatment modality for cardiac disease. The assumption is that stem cells that originate from other organs such as bone marrow or adipose tissue can engraft into the injured tissue and facilitate functional tissue repair. Integration of stem cells ideally means differentiation to cardiomyocytes, vascular cells, modulation of inflammation and augmented tissue remodeling. In that way stem cells contribute to improving regeneration and function of the target tissue. However, clinical reality showed a low retention and integration rate of stem cells into the host tissues, i.e. myocardium most probably due to the unfavorable microenvironment [9,10]. This raises questions such as what are the ideal stem cells and which is the best delivery technique. Bone marrow and adipose tissue are adequate sources of mesenchymal stem cells. As discussed below, adipose-derived stem cells are multipotent and hold promise for a range of therapeutic applications.
Adipose tissue-derived stromal cells

A variety of terms has been used to describe the plastic adherent cell population isolated from collagenase digests of adipose tissue. For instance, Adipose (tissue)-derived Stromal Cells (ADSCs); Adipose (tissue)-Derived Adult Stem (ADAS) Cells, Adipose Derived Adult Stromal Cells, Adipose Derived Stromal Cells (ADSC), Adipose Stem Cells (ASC) and Adipose Stromal Cells (ASC). These have all been used to identify the same adipose tissue cell population. Adipose tissue-derived stromal cells are promising in the field of cardiovascular tissue engineering and regenerative medicine. ADSCs are present in white adipose tissue throughout the body, but usually ADSCs are isolated from subcutaneous adipose tissue around the waist and legs with liposuction. ADSCs are plastic adherent cells that reside in the stromal vascular fraction (SVF), which is isolated from lipoaspirate white adipose tissue by enzymatic treatment and centrifugation. The SVF consist of different cell types i.e. ADSCs, endothelial cells, pericytes, smooth muscle cells, fibroblasts and circulating cells such as leukocytes. Tissue culture plastic-adhesion (TCPS) considered as the main criteria for ADSCs. Besides plastic adhesion, the expression of a combination of surface marker CD49d, CD44, CD90, CD105, CD13, and CD73 is used to characterize ADSCs. Whereas these cells should not express CD106, CD34, CD45, and CD31 to discriminate these from immune cells and endothelial cells respectively [11]. ADSCs are multipotent and can differentiate into mesodermal lineages such as osteoblasts, chondrocytes, adipocytes and smooth muscle cells. Evidence that ADSCs harbor the capacity to differentiate into two major cellular constituents of the heart, i.e. cardiomyocytes and endothelial cells, is contradictory, though few publications exist: [6]. However, these findings could not be replicated in vivo [12,13].

Biomaterials for tissue engineering and regenerative medicine

Biomaterials are designed to repair damaged or lost tissue through their physical and chemical properties e.g. as scaffolds, drug and cell delivery among others. A biomaterial is a natural or synthetic material that fulfills predetermined biological criteria for medical use, including biocompatibility, suitable surface topography, physical and chemical properties. Poly-L-lactic acid (PLLA), polytrimethylene carbonate (PTMC) and polyglycolic acid (PGA) are examples of degradable synthetic materials, which can be used for cell delivery [14]. Natural biomaterials are usually derived from natural polymers often isolated from native tissue of autologous, allogeneic, or xenogeneic sources [15]. Natural polymers can be divided into three main categories: polysaccharides such as chitin, proteoglycans and hyaluronic acid, proteins such elastin, collagen and its denatured product gelatin, and polyesters, e.g. of microbial origin, such as the polyhydroxalkanoate polybutyrate [16]. Irrespective of the promising outcome of natural materials in repair and replacement of damaged tissues, these are missing functional capabilities of natural tissues, such as degradation time, unless they are chemically cross-linked, batch-to-batch variations and risk of contamination. The main drawback of allogeneic and xenogeneic materials is host’s immune response, which has particular concerns such as using immunosuppressive drugs, even though the tissue maybe rejected. Although the autogenic tissues, which called autograft, are the suitable choice for tissue replacement, however, they have limited supply and could not answer the requirements of patients.
Biomaterials for cardiac cell delivery

Researchers used different types of biomaterials for cardiac regeneration and repair purposes with or without cells in animal models. For instance, in cardiac repair different types of formulation of biomaterials such as microspheres (MS), sheets and 3D porous scaffolds have been used. Injectable MS, degradation, cell-material interaction and mechanical properties are the important variables. Alginate, collagen, hydrogel and hyaluronic acids are the most commonly used materials for cardiac regeneration [17]. In part I, we discuss a new way of fabrication of recombinant gelatin based microspheres as delivery tools in chapter 2. Secondly, in chapter 3 and 4 we describe how the stem cell-loaded microspheres attenuate the arrhythmogenic effect of stem cells on monolayer-cultured cardiomyocytes. Further on, in chapter 5, we describe and discuss the foreign body reaction (FBR) against different types of recombinant collagen-based microspheres.

Biomaterials for small diameter vascular tissue engineering

Vascular disease, mainly coronary artery occlusive disease, is a leading causes of death worldwide. Arterial lesions can affect both the cardiovascular and peripheral vascular beds; however, the characteristics of plaques differ according to the location and type of arterial vessel, including size, elasticity, and bifurcations. In case of defects in large arteries (Ø>5mm), autologous grafts are no option. Instead synthetic biomaterials grafts made of materials such as Dacron (polyethylene terephthalate) or expanded polytetrafluoroethylene (ePTFE) are used. Unfortunately, these are non-degradable polymers and may cause complications on long time implantation such as fibrosis. In contrast to large vessels, these polymers cannot be used to replace small blood vessels (Ø<5mm) due to rapid coagulation. This is due to the almost negligible adhesion capacity of ePTFE for the physiological anticoagulatory cellular layer i.e. the endothelium. To date, autologous replacement vessels are used such as the saphenous vein or the arteria mammaris. Current failures of small diameter blood vessel tissue engineering are primarily due to lack of an antithrombogenic layer caused by incomplete or too slow endothelialization, similar as for ePTFE [18,19]. Yet, tissue engineering is a promising approach for development of small-diameter vascular grafts. Biodegradable synthetic polymers are suitable candidates for vessel tissue engineering; however, the main objective is to generate biological replacements of small-diameter arterial tubes with functional characteristics of native vessels with cellular components. The main challenge is the development of functional grafts with high patency as well as the antithrombotic properties. A polymer-based construct approach for vessel tissue engineering proved that blood vessel substitues could be made exhibiting adequate characteristics for arterial implantation [20]. Within the plethora of available materials Poly-(1,3-trimethylene carbonate) (PTMC) has been investigated for potential biomedical applications in soft tissue engineering and in drug delivery systems and holds suitable potential for tissue engineering purposes. PTMC is a linear, amorphous polymer with a glass transition temperature of approximately -15°C. The polymer is relatively stable towards hydrolysis, although it degrades in vivo. High molecular weight PTMC is a flexible material that cross-links during sterilization by gamma-irradiation. Upon cross-linking, an elastic network is obtained that can effectively resist creep that is frequently observed in other polymeric materials after long-term cyclic
deflections, like cell culturing under dynamic flow conditions. This renders PTMC an interesting material for the preparation of scaffolds for vascular tissue engineering. Current strategies for vascular tissue engineering use arterial wall cells such as endothelial cells (EC), endothelial progenitor cells (EPC), smooth muscle cells (SMC) or mesenchymal stem cells (MSC) with or without a biodegradable scaffold [21]. Bioreactor technology and bioprocess engineering principles, which can impart physiologically similar biochemical and mechanical stimuli to engineered grafts, are well accepted to facilitate the aseptic growth and maturation of functional grafts. Since vascular cells like EC and SMC are significantly influenced by arterial dynamics such as fluid shear stress and cyclic strain, those physiologically related stimuli are necessary to fabricate and mature functional vascular grafts in a bioreactor. ADSC became an interesting cell source for various tissue engineering purposes including vascular tissue engineering due to their differentiation capacity to vascular cell types. It has been shown that ADSC can differentiate to smooth muscle cells (SMC) [22-27]. In this thesis, we show that TGFβ-1 induces SMC differentiation of ADSC. Differentiation toward SMC was confirmed with various techniques and further investigated in a bioreactor culture system. Differentiated and non-differentiated ADSC were seeded into the highly porous tubular PTMC scaffold. Our finding, described in chapter 6, suggested that mechanical stimulation has additional value for pre-differentiated ADSC and should be considered for TEVG.

ADSC application for abdominal aortic aneurysm

Aneurysm is the dilation of the arterial vessel wall for more than 50%. Abdominal aortic aneurysm (AAA) is the dilation and weakening of all three layers of the abdominal aorta, which mostly occurs infrarenally. The population aged above 50 years is at risk of AAA development, while a familiar history doubles the risk. Progression of AAA can cause imminent rupture of the vascular wall and has a high mortality and morbidity risk. Additional risk factors for AAA development include male gender, smoking and dyslipidemia. In general, pathological features of AAA include inflammation, degradation of the extracellular matrix (ECM) and smooth muscle cells apoptosis. The main pathophysiology of AAA development is still unknown. Besides available treatment modalities for large AAA, which associate with a high mortality risk effective alternative and safer treatments are required, preferably already at an early stage of AAA. In the chapter 7, we will highlight the main pathobiology of AAA and introduce ADSC as a new promising therapeutic source for small AAA. As a proof, we employed an existing rat AAA model as described previously with small modification. Recombinant collagen based patches were fabricated (by Fuji film) and used as a novel stem cell delivery technique. Our findings, described in chapter 8, revealed that used biomaterials are safe for in vivo application and ADSC loaded biomaterial prevented the AAA formation and progression.

In summary, this thesis aims to present and discuss our results of stem cell therapy in cardiovascular tissue engineering and regenerative medicine. In chapter 2, we describe the method to develop suitable delivery tools for stem cells. Moreover, the paracrine effects of ADSC on myocardial conduction in monolayers of cardiomyocytes will be discussed in chapter 3 and 4. In chapter 5, the foreign body reaction and stem cells function will be covered in preclinical setup. Chapter 6 will explain how we can use differential capacity of stem cells
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toward small diameter tissue engineered blood vessels. In chapters 7 and 8, we discuss the pathobiology of abdominal aortic aneurysm and the potential effect of stem cells in the treatment of small AAA. In chapter 9 we will summarize our findings and in chapter 10 we discuss future perspectives that should be taken in account for stem cell therapy.
References


