

# Tissue engineering on matrix: future of autologous tissue replacement

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**Abstract** Tissue engineering aims at the creation of living neo-tissues identical or close to their native human counterparts. As basis of this approach, temporary biodegradable supporter matrices are fabricated in the shape of a desired construct, which promote tissue strength and provide functionality until sufficient neo-tissue is formed. Besides fully synthetic polymer-based scaffolds, decellularized biological tissue of xenogenic or homogenic origin can be used. In a second step, these scaffolds are seeded with autologous cells attaching to the scaffold microstructure. In order to promote neo-tissue formation and maturation, the seeded scaffolds are exposed to different forms of stimulation. In cardiovascular tissue engineering, this “conditioning” can be achieved via culture media and biomimetic in vitro exposure, e.g., using flow bioreactors. This aims at adequate cellular differentiation, proliferation, and extracellular matrix production to form a living tissue called the *construct*. These living autologous constructs, such as heart valves or vascular grafts, are created in vitro, comprising a viable interstitium with repair and remodeling capabilities already prior to implantation. In situ further in vivo remodeling is intended to recapitulate physiological vascular architecture and function. The remodeling mechanisms were shown to be dominated by monocytic infiltration and chemotactic host-cell attraction leading into a multifaceted inflammatory process and neo-tissue formation. Key mol-

ecules of these processes can be integrated into the scaffold matrix to direct cell and tissue fate in vivo.

**Keywords** Tissue engineering · Cardiovascular · Heart valve · Biocompatibility · Matrix · Remodeling

## Introduction

The ultimate goal of any tissue engineering approach is the creation of autologous living neo-tissues similar in architecture and function to native human structures. Therefore, an accurate understanding of the fundamentals of native tissue—representing the “gold standard”—constitutes a prerequisite to a successful development of native analogous tissue-engineered substitutes. Interestingly, it is the research on tissue engineering of recent years, which has fundamentally stipulated a novel interest in embryology, native tissue architecture, and development. In cardiovascular medicine, the in vitro fabrication of heart valves represents an example of how tissue engineering solutions aim to overcome obvious clinical limitations of currently available treatment options (Fig. 1). Native heart valves are composed of living, dynamic tissue capable of continuous remodeling to adapt to the constantly alternating hemodynamic environment [1]. None of the currently available valvular replacements are capable of fully restoring the native function due to insufficient adaptive capacity. State-of-the-art prostheses in today's clinical use show considerable limitations. These include the lack of growth, repair and remodeling capabilities, once they are implanted into the body. Additionally, mechanical valve substitutes are inherently susceptible to thromboembolic events due to high shear stress, nonphysiological flow profiles, and blood damage necessitating lifelong anticoagulation therapy [2,

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**Fig. 1** Tissue-engineered heart valve. Autologous living tissue-engineered heart valve before implantation after in vitro conditioning in a bioreactor system

3]. Bioprostheses from xenogenic or homogenic origin are inherently prone to structural degeneration, and the associated need for repeat reoperations makes them less suitable for many patients [4, 5]. Tissue engineering of heart valves represents a technology with the potential to overcome these limitations by creating a living autologous valve replacement that prevents an immune response, clotting activation, and valvular degeneration on the one hand, and allows for growth, remodeling, and repair throughout the patient's lifetime on the other hand.

The basis of most tissue engineering approaches is the fabrication of temporary supporter matrices in the shape of a desired construct. These biodegradable matrices promote tissue strength and provide functionality during the engineering process until sufficient neo-tissue is formed to restore adequate physiological function. In cardiovascular tissue engineering, fully synthetic polymer-based scaffolds, as well as decellularized biological tissue of xenogenic or homogenic origin, can be used. After in vitro tissue formation, the living autologous constructs are implanted into the patient, where further in vivo remodeling is intended to recapitulate physiological vascular architecture and function. This step of in situ remodeling represents an essential part of the tissue engineering concept, as it will fundamentally influence the fate and success of the substitute. The mechanisms involved in this remodeling process were shown to be mainly dominated by monocytic infiltration and host-cell attraction to the construct; however, little is known about the actual molecular and cellular pathways involved, which are central for this essential reorganization of bioengineered implants.

#### Strategies in tissue engineering: in vivo or ex vivo?

Tissue engineering is defined as an interdisciplinary field, applying the principles and methods of engineering to the

development of biological substitutes that can restore, maintain, or improve tissue formation [6]. According to this predefinition, two principle strategies have been developed to generate living autologous replacements: The in vitro as well as the in vivo approach. The first approach, requires an ex vivo phase generating the optimal native-like substitute in vitro. This traditional tissue engineering paradigm comprises the isolation and expansion of cells from the patient, subsequent seeding onto an appropriate scaffold material, in vitro tissue formation and finally, implantation into the patient from whom the cells were taken (autologous approach). This paradigm, further referred to as the in vitro tissue engineering approach, being employed as the principal approach for heart valve tissue engineering and is aimed at full development of the tissue substitute ex vivo (see Fig. 1). Several different cell sources serve as the basis for the generation of these constructs, where minimally invasively accessible stem and progenitor cells have shown tremendous potential [7–9].

The second approach of in situ heart valve tissue engineering circumvents the in vitro tissue culture phase by straight implantation of natural tissue-derived heart valve matrices, aiming at potential cell in-growth and remodeling in vivo [10]. In recent years, a further approach has emerged—mainly driven by the advances in stem cell technology and signaling. By seeding the construct with autologous cells, such as progenitor and/or mononuclear cells, using a cell carrier matrix, host-cells can be attracted to the implant site via chemo-attractive paracrine pathways. These attracted immune cells then support a distinct remodeling process, resulting in enhanced extracellular matrix and collagen formation [11].

#### The in vitro fabrication of an autologous construct: steps in cardiovascular tissue engineering

According to the approach of in vitro tissue engineering techniques, such as heart valve tissue engineering, the successful fabrication of autologous living replacements similar to the native benchmark is supported by three main elements: (1) autologous cells that resemble their native counterparts in phenotype and functionality are isolated and expanded using standard cell culture methods. For this purpose, several different sources are available, ranging from mature vascular-derived cells to prenatally harvested fetal progenitor cells. (2) The cells are seeded onto a temporary biodegradable supporter matrix fabricated in the shape of a trileaflet heart valve, termed the *scaffold*, which promotes tissue strength until the produced ECM (extracellular matrix) provides functionality on its own. Several different matrix materials, including synthetic as well as biologic materials, have been assessed for these purposes

[12]. (3) In order to promote tissue formation and maturation, the seeded scaffolds are exposed to mechanical stimulation transmitted via a culture medium (biological stimuli) or via “conditioning” of the tissue in a bioreactor. This bioreactor phase targets at the *in vitro* generation of a matured, high-quality extracellular matrix, having the capacity to grow as well as being able to respond to varying physiological needs and to repair structural injury by remodeling [13, 14; Fig. 2].

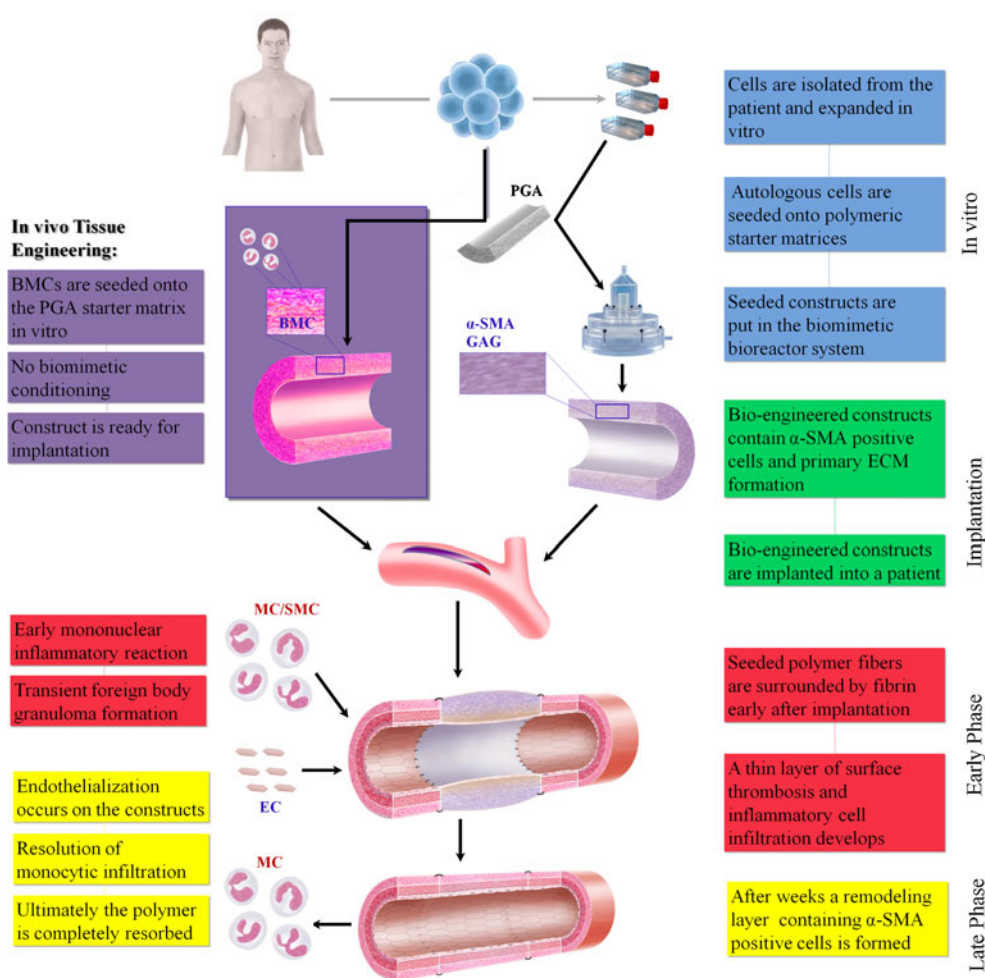
**Cell sources for cardiovascular tissue engineering: from vascular cells to stem cells**

The *in vitro* formation of a durable, well-structured and viable tissue is crucial for the *in vivo* functionality of a tissue-engineered construct. In this context, the choice of the optimal cell source is critical for the quality and long-term success of heart valve tissue engineering [1, 15; Fig. 2]. An established approach for heart valve tissue

engineering uses cells originating from aortic, saphenous vein, or peripheral artery biopsies [15]. Out of these vessels, two cell types can be isolated: Endothelial cells (ECs) with antithrombogenic properties and myofibroblasts capable of ECM development [16–18]. After preliminary studies, mainly in sheep [16–21], the potential of human vascular-derived cells was evaluated by seeding on biodegradable scaffolds and revealed excellent tissue formation [15, 22, 23]. A promising alternative cell source for regenerative medicine is bone marrow-derived stem cells (BMSCs) [3, 24, 25]. BMSCs were successfully used for *in vitro* production of heart valves [24, 26], and have been implanted *in vivo* demonstrating adequate functionality [27].

To improve the functional capacities and to reduce the risk for complications [28] tissue-engineered heart valves are usually covered with autologous human ECs [18]. Differentiated ECs have been isolated from vascular sources exhibiting promising results in heart valve tissue engineering [16, 19, 20, 22]. Furthermore, endothelial

**Fig. 2** Concept of cardiovascular tissue engineering. Autologous cells are harvested from the patient and expanded *in vitro*. When sufficient numbers are reached, cells are seeded onto a biodegradable scaffold. Constructs are either positioned in a bioreactor and conditioned (*in vitro* approach) or directly implanted into the patient (*in vivo* approach). After implantation of the tissue-engineered construct, the proposed mechanism of vascular remodeling comprises an early monocyte recruitment to the scaffold with the release of multiple angiogenic cytokines and growth factors. These factors (i.e., VEGF) cause recruitment of host cells, such as MC, SMCs, and ECs, to the scaffold. The invading host cells originate from circulating progenitors and (trans-anastomotic) migration/in-growth of mature vascular cells from adjacent vessel segments. Incoming ECs and SMCs appropriately organize into a mature blood vessel structure on the luminal surface of the scaffold (with the remaining scaffold in the center of the construct). As the scaffold degrades, early monocytes migrate away, leaving behind a remodeled, completely autologous neo-vessel



$\alpha$ -SMA: Alpha Smooth-Muscle-Actin; GAG: Glycosaminoglycans BMC: Bone Marrow Mononuclear Cells MC: Monocytes; EC: Endothelial Cells; SMC: Smooth Muscle Cells

progenitor cells (EPCs), discovered in human blood [29], have been established as source of ECs [30, 31]. Since they are easily accessible, current research aims at their trans-differentiation into myofibroblast-like cells to establish blood as single cell source for heart valve tissue engineering.

Another interesting cell source is the umbilical cord, containing several cell types that can be used for heart valve tissue engineering: (1) umbilical cord vein-derived and artery-derived cells, (2) Wharton's Jelly-derived MSCs, and (3) umbilical cord blood-derived EPCs. These cells demonstrated excellent growth properties and tissue formation [32–35]. Vascular neo-tissues were produced using human umbilical cord blood-derived EPCs seeded on vascular scaffolds [36]. By using umbilical cord-derived cells several different cardiovascular replacements could be generated [37–40].

The ideal pediatric tissue engineering paradigm comprises a prenatal fetal cell harvest allowing for tissue engineering processes during pregnancy followed by the implantation of the autologous tissue-engineered construct directly after birth. A new concept, using human prenatal progenitor cells derived from chorionic villi and umbilical cord blood for the production of autologous heart valve leaflets, has been introduced by Schmidt et al. [9]. Furthermore, human amniotic fluid-derived cells, as an easily accessible cell source, have been used as a sole cell source for the fabrication of living autologous heart valves prior to birth [8, 41].

Human adipose tissue contains mesenchymal stem cells with the potential to differentiate into various phenotypes *in vitro* [42, 43] and *in vivo* [44]. Due to the high availability and the ease of harvest, adipose-derived stem cells (ADSCs) represent a potential alternative stem cell source to BMSCs [45].

### **Biocompatible starter matrices: the optimal scaffold for cardiovascular tissue engineering**

The development of scaffolds for heart valve tissue engineering has proceeded along two fronts: a biological matrix material and a fully synthetic scaffold [46]. Regardless of the material of the scaffold matrix, the design of a scaffold capable of supporting cellular growth and of withstanding mechanically complex cardiovascular environment is critical to the success of the tissue-engineered construct. In addition to meeting all the standard design criteria of traditional tissue valves, in which durability and biocompatibility are effectively passive attributes of the underlying materials, and selecting the best scaffold material, it requires consideration of the active behavior of the cells in the regulation of tissue growth, remodeling, and

homeostasis. These matrices must be able to support cell growth and cell-to-cell interaction guiding tissue formation into a functional organ with organotypic ECM. The surfaces of these starter vehicles must be biocompatible, allowing cellular ingrowth and the formation of antithrombogenic cell linings, and biodegradable, providing an optimized degradation rate for cellular expansion [13]. These specific requirements entailed the development of various approaches to identify the optimal scaffold material, including the creation of synthetic [12] and biological scaffold materials [47]. These can be further subdivided into native tissue-derived ECM scaffolds [48], polymeric scaffolds [49–53], biological-polymeric hybrid scaffolds [54–56], and collagen or fibrin gel scaffolds [57–60]. Although significant advances have been made in all these approaches, the polymeric scaffolds have, to date, received most attention regarding heart valve tissue engineering applications.

### **Polymeric starter matrices for cardiovascular tissue engineering: the future of autologous tissue replacement**

The use of polymeric scaffold materials for different tissue engineering approaches has already been broadly demonstrated [12]. The ideal scaffold matrix for heart valve tissue engineering has to be at least 90% porous [61], and comprises an interconnected pore network, as this is essential for cell growth, nutrient supply, and removal of metabolic waste products. Besides being biodegradable, biocompatible, and reproducible, the scaffold material should also display a cell-favorable surface chemistry and match the bio-mechanical properties of the native heart valve tissue [12]. In addition, the rate of matrix degradation should be controllable and commensurate with the rate of novel tissue formation in order to provide a sufficient but reducing mechanical stability of the construct over time [1, 62]. Several synthetic biodegradable polymers have been investigated as potential starter matrices for heart valve tissue engineering that vary in their manufacturing possibilities and degradation rates (Table 1).

Aliphatic polyesters, including polyglactin (PG), polyglycolic acid (PGA), and polylactic acid (PLA), degrade by cleavage of the polymer chains due to hydrolysis of their ester bonds. The resulting monomer is either excreted via urinal secretion or enters the tricarboxylic acid cycle [61]. In order to fabricate single heart valve leaflets, the creation of scaffolds was initially based on combinations of aliphatic polyesters, including PG non-woven PGA meshes with layers of PGLA and non-woven PGA meshes. The major limitations of aliphatic polyesters, when used as a sole material, are their thickness, initial stiffness, and non-pliability, making the fabrication of trileaflet heart valves a difficult process.



**Table 1** Examples of polymeric starter matrices used for cardiovascular tissue engineering

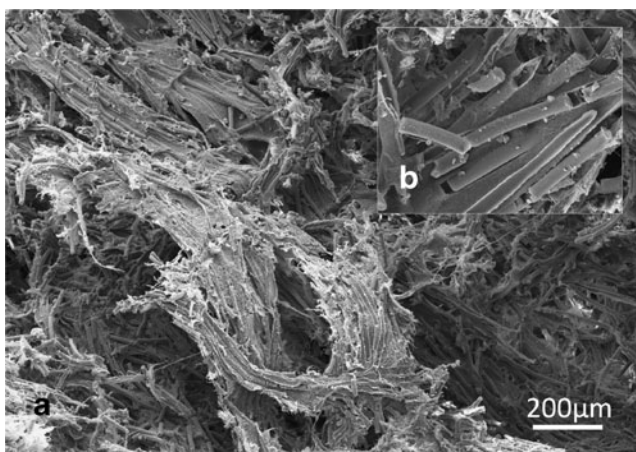
Scaffold	Construct	Reference
Lactide acid and P-caprolactone and PGA/PLLA	Vascular autograft	16, 17, 20, 99
PEUU and PEEUU	Vascular patches	100
PGA	Vascular patches/graft	18, 21, 66
P4HB	Vascular graft	66
PHA	Vascular graft	66
PHO	TEHV	22
PGA/P4HB	THEV	8, 9, 19, 27, 68, 69

*PGA* polyglycolic acid; *PHA* polyhydroxyalkanoate; *PHO* polyhydroxyoctanoate; *PEUU* poly(ester–urethane)urea; *PEEUU* poly(ether–ester–urethane)urea; *PLLA* polylactic acid; *P4HB* poly-4-hydroxybutyrate; *TEHV* tissue-engineered heart valve

A further group of widely used polymers is the polyhydroxylalkanoates (PHA) family, which is composed of polyesters built up from hydroxyacids that are produced as intracellular granules by various bacteria [63]. PHAs, as well as poly-4-hydroxybutyrate, have been used to create trileaflet heart valve conduits [22, 64]. These materials possess thermoplastic properties and can be molded into any desired shape using stereolithography [22, 65]. A limitation of PHAs can be found in their slow degradation. Combinations of aliphatic polyesters and PHAs have also been tested as alternative composite materials [19, 66]. Particularly, the use of PGA coated with P4HB (Fig. 3), combining the thermoplastic properties of P4HB and the high porosity of PGA, for the fabrication of complete trileaflet heart valves revealed promising results in a rapidly growing sheep model [8, 19, 26, 66, 67].

### Decellularized tissue-derived matrices: the biologic counterpart

In principal, donor heart valves (homografts) or animal-derived heart valves (xenografts) are among the most



**Fig. 3** The matrix for cardiovascular tissue engineering. SEM images of the PGA mesh coated with P4HB at low (a) and high (b) magnification

obvious choices for scaffold materials. They are fixed and depleted of cellular antigens, which makes them less immunogenic, and/or thus eligible to be used as a scaffold material in tissue engineering. The removal of cellular components results in a template composed of extracellular matrix proteins that serve as an intrinsic medium for subsequent cell attachment. Nevertheless, they still possess a native-like geometry and architecture with bio-mechanical and hemodynamic properties similar to their native counterpart [8, 13, 67].

Various decellularization techniques have been extensively investigated in order to minimize the residual immunologic potential of biological matrices. Although it is essential to remove all cellular components, the decellularization treatment should avoid any harm or alteration of the ECM properties. This preservation of matrix integrity, as well as the efficiency of cell removal is highly dependent on the method used for decellularization [68]. Several different decellularization methodologies for heart valve scaffold fabrication have been reported, including trypsin/EDTA [68–71], freeze drying [72], osmotic gradients [73], non-enzymatic detergent treatment [68, 74], and multistep enzymatic procedures [75]. The use of non-enzymatic detergent-based techniques has been shown to result in a much more efficient cell removal, while preserving the overall matrix integrity of the scaffold, when compared to other more aggressive decellularization methods such as trypsin/EDTA [68, 76, 77]. In order to avoid this impairment of the matrix integrity and function due to tissue-derived protease activation, the use of suitable protease inhibitors has been recommended [78]. Accessorily, nuclease digestion steps should be embedded into the decellularization procedure to remove any residual RNA or DNA within the scaffold.

Several in vivo studies have proven the feasibility to use decellularized scaffolds as starter matrix for cardiovascular tissue engineering [79, 80]. Moreover, first clinical trials have been initiated [81]. However, using xenogenic materials, serious complications have been reported due

to residual  $\alpha$ -Gal-mediated immunogenicity [81, 82]. Furthermore, the use of xenografts principally involves the risk of zoonoses and prionic diseases, in terms of human diseases caused by animal-derived infectious agents, which has given rise to widespread concern [83, 84]. When the matrix material is from homogenic origin, the limited availability of donor valves and associated ethical concerns represent a considerable shortcoming. Moreover, the lack of evidence of growth and remodeling capacities of valve replacements when using decellularized scaffolds seems to be a further drawback, especially with regard to the pediatric field [9]. These drawbacks and uncertainties raise a common concern associated with the use of decellularized starter matrices, also fuelling the search for synthetic scaffold alternatives.

### **Implant-mediated inflammation: the key to optimal tissue remodeling?**

Healing of a tissue-engineered constructs *in vivo* has to be seen as a continuous but multifaceted process. Following the initial blood-material interactions, inflammatory processes occur around the implanted construct. The extent of this response depends upon the degree of maturation of the tissue-engineered construct and the extent of surgical injury, which directs a physiological healing reaction, consisting first of an acute inflammation, followed by repair processes. Immediately after implantation, phagocytic cells (predominantly neutrophils and monocytes) migrate from the microcirculation to the interface between the implant surface and the injured tissue. The inflammatory phase, (which lasts up to several weeks in humans) consists of phagocytic removal of debris due to trauma, and then, provides the appropriate signals for the shift from inflammation to repair and remodeling of the tissue. Taken as a whole, two main processes seem to be indispensable for a successful remodeling of tissue-engineered constructs *in vivo*: (1) The formation of an atypical vascular response to injury at the luminal surface, including intimal thickening, pannus formation, and neointima development, and (2) deep tissue biomaterial-associated effects of foreign body reaction, granulation, tissue formation, and fibrosis forming a media-like structure [85].

Upon resolution of the acute inflammatory reaction, monocytes are observed within the implanted construct. Indeed, it has been demonstrated that monocyte chemoattractant protein (MCP-1), a potent monocyte-attracting cytokine, is expressed by activated neutrophils at the implant site, and thus, seems to play a central role within the early remodeling phase, which is mainly characterized by chemotactic immune cell infiltration [86]. While the exact

roles recruited monocytes play in the implant transformation remains largely conjectural, their role in tissue repair is better understood. Recent findings suggest that monocytes remain within the implant scaffold until it fully degrades, and therefore, may play a role in the entire remodeling process. At 100-week follow-up, diffuse mononuclear inflammation, particularly in areas of residual polymer fibers, has been observed [87]. In implanted tissue-engineered vascular grafts, recent evidence suggests that monocytes produce important cytokines (i.e., MCP-1/CCL-2, IL-6, IP-10), growth factors, and proteases necessary for vascular cell proliferation/migration and appropriate vascular remodeling [85, 88, 89].

Monocytes/macrophages at the site of long-term implantation express ECM remodeling proteases (i.e., MMPs), cytokines characteristic of the innate immune response (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, and TNF $\alpha$ ) and cell adhesion (ICAM-1, VCAM-1). Moreover, monocytes involved in a pro-fibrotic foreign body response have been shown mainly properties of classically activated macrophages (IL-1 $\beta$ , IL-6, TNF, Ccl20, Cxcl10), and to a lesser extent, wound-healing (factor XIII-A) and regulatory macrophages (IL-10; [90, 91]). While these monocyte properties may affect implant integration (foreign body response vs. tissue integration) via paracrine signaling, the precise role of monocytes/macrophages in tissue remodeling is not understood. However, expression of angiogenic cytokines (VEGF) from implant-associated monocytes/macrophages has lead to a more descriptive understanding of their role in implant neovascularization.

*In vivo* animal studies have revealed that arteriogenesis depends on circulating monocytes and macrophage accumulation. This finding has been confirmed by recent studies, which demonstrate that monocyte recruitment is central to postnatal blood vessel formation [92–94]. This mechanism has, in part, been attributed to the critical role of VEGF in adult neovascularization, and prevention of neovessel regression [95–97]. Indeed, monocyte/macrophage infiltration associates with VEGF expression with the tissue-engineered constructs [98]. In addition to VEGF, recruited monocytes likely release multiple cytokines, which then orchestrate the proper vascular neovascularization of biodegradable implants.

Besides the involvement of MCP-1 for monocytic attraction and VEGF expression, the molecular pathways of these remodeling phenomena remain largely unknown.

The discovery of these underlying pathways seems indispensable for the development of strategies to modulate early inflammatory reactions and enhance remodeling [99]. First success has been achieved by attaching MCP-1-releasing biodegradable microparticles to the scaffold matrix in order to mimic the chemo-attractive properties of seeded cells *in vivo* [98].

## Conclusions

Cardiovascular tissue engineering is a promising approach aiming at the creation of living functional autologous replacements and holds exciting potential for improving therapy of many diseases. However, before clinical application of the tissue engineering concept becomes routine, numerous steps must be overcome in the laboratory. Primary amongst these is the limited knowledge about the mechanisms involved in the *in vivo* remodeling process of tissue-engineered constructs after implantation. Although having first indications as to the influence of cytokine-mediated monocyte-attraction and neovascularization, the underlying exact molecular pathways remain unknown. Another important consideration concerns the definition of the ideal matrix material for engineering, providing a template for directing new tissue growth and organization, as well as for regulating cellular adhesion, migration, and differentiation. The mechanism involved in neo-vascular formation displays many parallels to natural neovascularization and may provide further insights into the biology of these processes. However, although similarities to natural processes exist, the development of a tissue-engineered construct does appear to be a distinct process of vascular formation in itself. A better understanding of how the different steps of *in vivo* remodeling start and are controlled will lead to a new class of implants. Moreover, the identification and attachment of important regulatory molecules to the scaffold matrices, such as VEGF, may provide engineers with the key to enhance the body's innate ability to regenerate implanted constructs. Eventually, this could allow for the fabrication of “*intelligent*” scaffold materials incorporating specific signaling molecules that direct tissue fate within the implanted constructs.

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