MOLECULAR ASPECTS OF VASCULAR TISSUE ENGINEERING

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1. ABSTRACT

Cardiovascular disease remains the number one cause of death in the United States. Most current surgical procedures to alleviate this disease rely on the availability of suitable small diameter vascular grafts, which are constrained by several limitations. Tissue engineering brings new hope to this field, but still faces many challenges. This review focuses on the molecular aspects of the different components of vascular tissue engineering. The topics addressed include the cell type, extracellular matrix, and physical and biochemical stimulation with respect to their role in the development of a tissue engineered vascular graft.

2. INTRODUCTION

2.1. Clinical need for vascular tissue engineering
2.1.1. Cardiovascular disease

Cardiovascular disease is the leading cause of death in the western world. According to the American Heart Association, almost one million lives are claimed every year in the United States alone, and more than 50% of those are caused by coronary artery disease. (1). Since avaloro's first successful implantation of a saphenous vein graft to the right coronary artery in 1968, this procedure remains the gold standard therapy to alleviate this disease, and the saphenous vein continues to be the most commonly used graft for coronary artery bypass grafting. (2). However, 12% to 27% of vein grafts become occluded in the first year and one half of those occlusions occur within the first month. (3, 4). There is a subsequent annual occlusive rate of 2% to 4%, and only 69% of patients can reach a 10-12 year period free of reoperations or percutaneous transluminal coronary angioplasty. (5, 6). Reoperations account for 10% to 30% of coronary surgery in the United States. (5). Many attempts have been made to increase patency rates of saphenous vein grafts by improving harvest methods, preservation, and early anti-thrombotic therapy. Arterial grafts are the preferred conduits due to improved long term patency. (7). However, they are limited due to their short size, small diameter, and availability, as many patients require multiple grafts. Synthetic grafts have had only limited success, mainly in larger diameter vessels. (8).

2.1.2. End stage renal disease (ESRD)

According to the American Society of Nephrology, more than 300,000 Americans have ESRD and are dependant on artificial dialysis to stay alive. (9). Arteriovenous (AV). fistulae are commonly constructed to create vascular access for hemodialysis. However, access failure is currently one of the leading causes of hospitalization for patients with ESRD. Infection and early thrombosis of synthetic grafts such as those made from expanded polytetrafluoroethylene (ePTFE). and intimal hyperplasia of AV fistulas prevent these procedures from having better success rates. (10). Furthermore, graft availability for AV fistulas is poorer on diabetic patients that account for almost 40% of ESRD population. (9, 11).

2.1.3. Need for a new graft material

The clinical shortcomings in the treatment of vascular disease and of dialysis patients have guided new approaches in the development of alternative bypass and hemodialysis grafts. (12). However, a highly successful alternative has yet to be discovered due to the many properties that a graft must possess to remain patent for an extended period of time. A tissue engineered vascular graft (TEVG). appears as a promising solution that could meet all requirements needed for that purpose. In the past several years, many groups have investigated different approaches to achieve the biological and biomechanical properties that mimic the native vascular tissue. The purpose of this article is to review those components that we feel are essential for vascular tissue engineering, focusing primarily on the molecular aspects involved in them.

2.2. Current issues in vascular tissue engineering

There are certain basic properties of the native vasculature that a successful TEVG must achieve and these can be categorized into mechanical and biological properties. (13). First, the vessel must be of sufficient mechanical strength not to rupture when exposed to arterial blood pressure and flow. This means that any technique used to fabricate the blood vessel must result in a conduit that will function mechanically immediately upon implantation. It is also essential that the TEVG match the mechanical properties of the vessel to which it is being grafted, as compliance mismatch at the anastomosis is thought to be involved in the development of intimal hyperplasia and subsequent graft failure. (14). From a biological standpoint, it is essential that any TEVG be anti-thrombogenic. Finally, another major requirement - and unfortunately a severe limitation in current vascular grafts - is that the vessel must be vasoresponsive in order to function adaptively under changing blood pressure and flow. In order to achieve these properties successfully for a TEVG, several design considerations must be taken into account: cell source, scaffolds, mechanical stimulation and biochemical stimulation.

2.2.1. Cell source

In a recent review, Nerem and Seliktar identified *cell source* as one of the key issues that needs to be addressed in order to achieve a successful vascular tissue engineering approach. (13). In order to be clinically viable, any vascular tissue engineering technique should utilize a method by which *autologous* cells could be easily harvested and used. The cell population should be easy to expand *in vitro* to reduce the time of construction. Optimal combinations and concentrations of cytokines and growth factors are important to regulate smooth muscle cell (SMC). and endothelial cell (EC). proliferation and migration. Finally, the response of the cells to physiologic mechanical stimuli needs to be addressed to predict their behavior in the very active *in vivo* hemodynamic environment. The importance of molecular signaling in various potential cell sources for vascular tissue engineering is discussed further in Sections 5 and 6, below.

2.2.2. Scaffolds

One of the areas of heaviest activity in vascular tissue engineering investigation has been the development of an optimal method for the creation of a tubular construct. Factors that must be considered include the ability to control the inner diameter, wall thickness and length. (13). Although the study by L'Heureux and colleagues demonstrated their ability to generate TEVGs using only sheets of cells, most vascular tissue engineering approaches have relied on some form of scaffold, whether it be natural or synthetic, to form the basis for the graft. (15, 16). Many of the techniques employed in the fabrication of a tissue-engineered blood vessel revolve around the use of a polymer scaffold to provide mechanical integrity to the graft so that it will not rupture upon implantation when faced with arterial pressure and flow. Furthermore, in many vascular tissue engineering applications, mechanical conditioning has been implemented in the fabrication process, and it is the presence of synthetic materials that allows such regimens to be applied without compromising the integrity of the TEVG. The synthetic materials previously used as constructs have varied, ranging from inert biomaterials such as Dacron and ePTFE, to biodegradable synthetic polymers such as polyglycolic acid (PGA)., polylactic acid (PLA). and poly (ester-urethane).urea, to natural biopolymers such as collagen, elastin, and GAGs. (17-23). In most cases, scaffolds are seeded with cells or loaded with growth factors to stimulate cell migration. These interactions involve several molecular events, which are discussed further in this review.

2.2.3. Mechanical stimulation

The vascular system is a highly dynamic environment, where the cells in the blood vessel wall are exposed to pulsatile pressure and flow, cyclic wall deformations and fluid shear stress. Additionally, in the coronary system, blood vessels are exposed to cyclic bending, twisting and stretching due to their attachment to the beating heart. (24-31). As such, constituent smooth muscle and endothelial cells respond to these stresses and strains to maintain homeostasis. (32).

Biomechanical stimulation also has a profound effect on cellular phenotype. It is well documented in the literature that a loss of cyclic strain causes an alteration in SMC from a contractile to a synthetic phenotype and

Table 1. Common smooth muscle cell markers used for characterization in tissue-engineering studies

Marker	Stage of differentiation	Synthetic phenotype	Contractile phenotype
alpha-SMA	Early	Yes	Yes
Calponin	Intermediate	Yes	Yes
Caldesmon	Intermediate	Yes	Yes
Myosin heavy chain (SM1 and SM2 subtypes).	Late	No	Yes

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thereby a loss of associated function within the vessel. (33). Previous studies from our lab have shown that rat bone marrow progenitor cells can differentiate towards a smooth muscle phenotype by means of mechanical stimulation. (34). Furthermore, mechanical forces can lead to a remodeling and strengthening of the tissue structure. (35, 36). The combination of a particular scaffold and specific dynamic culture conditions establishes a physical environment in which the cells will develop. The molecular aspects of these interactions are further reviewed in Section 5.

2.2.4. Biochemical stimulation

Growth factors and vitamins play a key role in the tissue engineering field since they are able to trigger a large number of cell-related processes in terms of proliferation and differentiation. Loaded within the scaffolds or supplemented in the media, biochemical stimuli are able to drive many desired responses from the target cells. When stem cells are taken into consideration for tissue engineering purposes, the biochemical environment may induce differentiation towards the desired lineage. In this review, several factors known to have influence on the smooth muscle, endothelial, and progenitor cells are discussed to address the question of what adequate biochemical environment should be used for vascular tissue engineering.

3. CELLULAR CONSTITUENTS FOR VASCULAR TISSUE ENGINEERING

SMCs and ECs are the two primary cell types that are needed to build a TEVG that resembles a native artery. SMCs are required for a "media equivalent" to provide mechanical stability of a TEVG. ECs are necessary to line the lumen in order to provide antithrombogenicity and selective permeability. To achieve this goal, many researchers have explored various cell sources, which could be divided into two general types. First, is the terminally differentiated type, where cells with the desired phenotype are isolated, cultured, expanded to the required number, and then used for the TEVG constructs. (16, 18, 37-39). The second type of cell source that has been explored is the stem or progenitor cell which has given a new, challenging approach to address in the field. (34, 40).

3.1. Terminally differentiated cells

The main advantage of using terminally differentiated cells is the possibility of building a TEVG with the same cellular constituents of a native artery and thus getting greater chances of reproducing functionality. However, the use of terminally differentiated cells for the construction of a TEVG presents a number of barriers. Due to the high number of cells required for seeding a TEVG, for example, their isolation may be difficult to accomplish for use in autologous grafts without invasive procedures. Non-autologous grafts, which could be derived from donor cell sources, would require intervention to avoid host rejection, including immunosupression. In this Section the various terminally differentiated cell types used for the construction of a TEVG are considered.

3.1.1. Smooth muscle cells

SMCs have been utilized in several approaches to construct a TEVG. They have been seeded on biodegradable scaffolds, (41). cultured in biological gels, (42). or used as part of cellular-based constructs. (16). Like a native vessel, engineered vessels must possess both a physical and functional component, with the physical component defined by the presence of an organized extracellular matrix (ECM)., and the functional component defined by contractile SMCs. (39). Collagen synthesis and cross-link formation can be stimulated by supplementing the cells with ascorbic acid, copper ion, amino acids, or growth factors. (43, 44). Dynamic culture of SMCs is thought to be required to avoid a phenotypic change from contractile to synthetic, (33). though a synthetic phenotype may be a desired feature during TEVG development since it provides greater proliferation and ECM production. Therefore, modulation of SMC phenotype can be a powerful tool in vascular tissue engineering. (45). Consequentially, it is important to be able to trace the differentiation process that SMCs undergo in different culture conditions (46)., and this can be achieved by evaluating the levels of expression of cytoskeletal proteins such as alpha-SMA, calponin-1, caldesmon-1, and smooth muscle myosin heavy chain (MHC). (Table 1).

One of the main concerns for the use of adult autologous smooth muscle cells in tissue engineering applications is their limited proliferative capability. (47). In order to address that point, McKee *et al.* (48). conducted a series of studies where they extended the lifespan of cultured SMCs, by the ectopic expression of telomerase reverse transcriptase subunit (TERT). This enzyme subunit prevents telomere shortening that occurs after each cell division and allows the cell to overcome the limitations on expansion. (49). In these studies, cells were transfected with retroviruses containing TERT to create a stable population that underwent over 100 population doublings without associated karyotypic alterations. Although an extensive oncological mapping was performed, the feasibility of this approach to develop a TEVG for clinical use might be low since TERT is not constitutively expressed in adult cells and has been shown to be active in most cancer cells. (50).

3.1.2. Endothelial cells

One of the most difficult goals to achieve when developing a TEVG is a high patency rate with low incidence of thrombosis, inflammation and hyperplasia. (16, 39, 51, 52). For this reason, it is thought that a successful TEVG cannot be achieved without the inclusion of an inner cellular layer resembling a native endothelium. Within a blood vessel, the endothelial cell layer functions to prevent the adhesion of platelets and other blood components (e.g., macrophages). by acting not only as a physical barrier, but also through the continued release of inhibitory factors such as NO, thrombomodulin, tPA, and PGI_2. However, expression of these important molecules by ECs is downregulated under culture conditions. (53). In contrast, upregulation of procoagulant and proinflammatory molecules such as VCAM-1 and ICAM-1 occurs under the same conditions due to a variety of factors. (54-56).

Several authors have attempted seeding TEVGs with different types of isolated ECs in order to achieve a functional endothelial layer. Human umbilical vein endothelial cells (HUVECs). are the most commonly utilized cell type, though bovine and porcine aortic ECs have also been used. (48, 51, 57). In the "self-assembly approach" reported by L'Heureux *et al.*, (16). the authors showed that endothelial cells cultured on the inner membrane of their construct were able to maintain thrombomodulin expression in contrast with those cultured on plastic. Furthermore, von Willebrand factor secretion, a known marker for mature ECs, was similar to that of ECs *in vivo*. (57). Newer options are under current investigation and might include the regulated overexpression of thrombomodulin, or other anticoagulant proteins, by gene therapy as stated by Fields *et al.* (51). This group has recently demonstrated the ability to stably infect ECs, laying the groundwork for future adenoviral transfections for tissue engineering applications.

3.1.3. Fibroblasts

The adventitial layer of native arteries surrounds the medial layer and is composed mainly of fibroblasts and collagen. These cells have been used for different tissue engineering approaches for their ability to produce ECM and growth factors. In 1998, L'Heureux and colleagues produced a TEVG that utilized a number of individual sheets of human fibroblasts. The sheets were obtained by culturing fibroblasts and SMCs with ascorbic acid for extended periods of time until a tissue-like layer of cells was achieved. (16). These sheets were then wrapped around a cylindrical mandrel to produce a tubular construct with an inner SMC-based "media" and an outer fibroblast-based "adventitia". Although this approach has demonstrated success in animal models, the construction period is thought to require too long a duration, lacking the off-the-shelf availability feature desired for a TEVG.

Using a different approach, Hoerstrup *et al.* (58). have seeded ovine vascular myofibroblasts in a PGA tubular scaffold that was then cultured in a pulsatile bioreactor, inducing the cells to produce collagen. This resulted in a construct with a high collagen content after 21 days in dynamic culture. In a similar approach, Braddon *et*

al. used a Vycril biodegradable polymer with human dermal fibroblasts to study the interaction of these cells with endothelial cells. They found that the fibroblast populated substrate promoted endothelial arrangement and NO production under shear stress. (59, 60). The fact that fibroblasts can be easily harvested from dermal biopsies is a positive feature for tissue engineering purposes where cell acquisition can often be problematic.

3.2. Progenitor cells

Progenitor cells show great potential for use in tissue engineering applications and may circumvent many of the shortcomings associated with other solutions to cell sourcing. For example, they are easy to obtain from a patient that requires the procedure and are easy to expand in culture. The bone marrow stroma hosts a large number of multipotent progenitor cells. It has been sorted based upon density and the expression of many different clusters designation (CD). markers and membrane receptors. (61). The main division for bone marrow has been along the marker for CD34, with $CD34^+$ cells being considered hematopoietic stem cells and the $CD34^-$ cells being considered mesenchymal stem cells. Regardless of hematopoietic or mesenchymal designation, BMPCs have been shown to express CD117 and CD90, and have been sorted accordingly using fluorescent activated cell sorting. (62-66). Even with the use of CD34, CD117, and CD90 as lineage markers, researchers are continuing to sort based upon markers such as SH2, SH3, CD45 (62, 67)., and most recently CD133 (a.k.a. AC133)., which has been used as a marker for endothelial progenitor cells. (68-70). The mesenchymal progenitor cells have been characterized as having a high proliferation potential and the ability to differentiate into cells of the mesenchymal lineage. (71). Furthermore, the bone marrow is thought to supply distant mesenchymal tissues with progenitor cells that will be committed for one purpose. (71). The fact that these cells are mobilized from one compartment to another indicates that they are at some point circulating in the blood stream. Therefore, peripheral blood is thought to be another viable source of progenitor cells. (72-74).

First described by Cohnheim in 1867, (75). adult mesenchymal stem cells have been thoroughly studied in attempts to understand the numerous factors that lead these cells to differentiate into specific lineages. It has been shown that these cells can develop into terminally differentiated cells and tissues such as bone, cartilage, fat, muscle, tendon, neural tissue and hematopoietic-supporting stroma. (67, 68, 76-80). An extensive set of markers for characterizing bone marrow progenitor cells (BMPCs). - including specific antigens, adhesion molecules, cytokine receptors and production of ECM - have been utilized for classifying cells that would allow further sorting for specific purposes (Table 2) (72).

With particular attention to vascular tissue engineering, it has recently been shown that progenitor cells can be stimulated towards smooth muscle and endothelial cell lineages. (34, 40, 81). However, the specific factors that contribute to this differentiation are still ill defined. Several cytokines, including the growth factors

Table 2. The specific antigens, cytokine receptors, adhesion molecules, cytokines, and matrix molecules expressed or produced by bone marrow progenitor cells

Marker type	Designation
Specific antigens	SH2, SH3, SH4, STRO-1, alpha-SMA
Cytokines and growth factors	IL- 1, 6, 7, 8,11, 12, 14, and 15, LIF, SCF, Flt-3 ligand, GM-CSF, G-CSF, M-CSF
Cytokine and growth factor receptors	IL-1R, IL-3R, IL-4R, IL-6R, IL-7R, LIFR, SCFR, G-CSFR, IFN-gammaR, TNF-alphaIR, TNF-alphaIIR, TGF-betaIR, TGF-betaIIR, bFGFR, PDGFR, EGFR
Adhesion molecules	*Integrins subunits:* alpha1, alpha2, alpha3, alpha4, alpha5, alphav, beta1, beta3, beta4, beta5 *Surface adhesion molecules:* ICAM-1, ICAM-2, VCAM-1, ALCAM-1, LFA-3, L-selectin, endoglin, CD44
Extracellular matrix	Collagen types I, III, IV, V, and VI, fibronectin, laminin, hyaluronan, proteoglycans

Adapted from 72

TGF-beta, bFGF and VEGF, as well as retinoic acid, are known to play an important role in SMC and EC phenotype expression and have been tested as potential factors in the differentiation process as discussed in Section 6 below. (74, 81-83). Further, it has been shown that the environment where cells are grown plays a key role in their differentiation. (34, 84-88). Progenitor cells have been shown to express adhesive molecules, such as integrins for vascular matrices (74, 86)., P-selectin (89)., and E-selectin (90). which, along with other unknown mechanisms, possibly facilitate the ability of these cells to home or seek out injured tissue and actively participate in remodeling and healing (91, 92). Since the circulatory system is a highly dynamic environment, it is important to consider how mechanical forces affect progenitor cells. Our recent findings showed that bone marrow derived progenitor cells were driven to express smooth muscle markers (alpha-SMA, h1-Calponin). in response to uniaxial cyclic strain in monolayer culture. (34). Furthermore, the cells aligned perpendicular to the direction of strain and ceased proliferating, suggesting a differentiation process. (34, 93). A recent approach to using bone marrow cells for vascular tissue engineering purposes was made by Matsumura and colleagues who implanted a biodegradable scaffold seeded with BMPCs in the inferior vena cava of dogs and found that these cells contributed to the construction of the new components of the vessel wall. (40).

Other sources of progenitor cells apart from the bone marrow are being investigated and may have a great potential in vascular tissue engineering. Human pre-adipocytes were reported to be transdifferentiated to smooth muscle lineage by the overexpression of ACLP, an ECM protein. (94). Furthermore, human adipose progenitor cells have been shown to differentiate towards endothelial lineage in the presence of VEGF. (95). Additionally, skeletal muscle-derived stem cells exhibit multipotentiality and self-renewal capabilities that might be exploited for use in vascular tissue engineering. (96-98). Further work is necessary to understand how progenitor cells might behave in tissue-engineered constructs, but the features mentioned above indicate a promising future for the field.

4. PHYSICAL COMPONENTS OF VASCULAR TISSUE ENGINEERING

The physical environment of a tissue provides specific signals to the constituent cells, and these signals are simultaneously integrated with biochemical cues to regulate cellular proliferation, matrix production, migration, or death. The two main sources of physical stimulation are derived from the ECM (through both the chemical composition of the matrix and the physical constraints the matrix places on the cells). and from the forces transmitted through the matrix to the focal adhesions and mechanoreceptors located on the cellular surface. For the purposes of this review, we will focus on the chemical and physical effects of the matrix separate from the mechanical forces, mainly due to the fact that the use of mechanical forces in vascular tissue engineering has been directly translated from monolayer experiments, and is still being evaluated in three-dimensional (3-D). environments. (26, 27, 99-103).

4.1. Chemical and physical implications of the matrix (21, 104, 105)

The ECM, or scaffold, defines the 3-D and functional environments of a tissue. The gene expression profile of cells grown in 3-D cultures is profoundly different from similar conditions in a monolayer culture, suggesting that the 3-D environment can influence many aspects of cellular behavior. (21). In fact, the ECM provides many types of signals to the cells entrapped within the tissue through surface chemistry, mechanical forces, and insoluble matrix and growth substrates. (21, 104, 106-109). These signals affect the proliferation, migration, differentiation, alignment, and apoptosis of the resident cells through mechanisms including, but not limited to, the MAPK, Akt, PKC, PLC-gamma, and PI3K pathways. (21, 105). Two basic types of matrices, natural and synthetic polymers, are mainly used for vascular tissue engineering. Collagen and GAGs are the most commonly used natural polymers because of their effects on cellular function. (21). Collagen has a particularly good ability to support growth, differentiation, and functionality of many cell types, while GAGs have physical and biochemical properties that have been shown to regulate cell function and play a role in tissue development and repair. (21). Elastin, another natural polymer, has been shown to stabilize smooth muscle phenotype by promoting actin fiber formation and inhibiting proliferation and migration. (110). Synthetic polymers, including PLA and PGA, are often used because their structure and porosity can be controlled. These types of matrices tend to induce cellular proliferation, promote matrix deposition, and provide an excellent environment for a tissue to develop. (12, 104, 107, 111).

Table 3. Vascular extracellular matrix-related integrins and their associated ligands

ECM Ligand/Cell Type	Integrin	Reference
Collagen	$alpha_1beta_1$, $alpha_2beta_1$, $alpha_{10}beta_1$, $alpha_{11}beta_1$	104, 245
Laminin	$alpha_1beta_1$, $alpha_2beta_1$, $alpha_6beta_1$, $alpha_7beta_1$, $alpha_6beta_4$, $alpha_vbeta_3$	245
Fibronectin	$alpha_2beta_1$, $alpha_3beta_1$, $alpha_4beta_7$, $alpha_5beta_1$, $alpha_8beta_1$, $alpha_vbeta_1$, $alpha_vbeta_3$, $alpha_vbeta_5$, $alpha_vbeta_6$, $alpha_vbeta_8$, $alpha_{IIb}beta_3$	104, 245
Vitronectin	$alpha_vbeta_5$, $alpha_vbeta_3$, $alpha_vbeta_1$	104,113, 246
Tropoelastin, fibrillin-1 (elastin precursors or associated proteins).	$alpha_vbeta_3$	247, 248
RGD peptide	$alpha_3beta_1$, $alpha_5beta_1$, $alpha_8beta_1$, $alpha_vbeta_1$, $alpha_vbeta_3$, $alpha_vbeta_5$, $alpha_vbeta_6$, $alpha_{IIb}beta_3$	245
YIGSR peptide	$alpha_1beta_1$, $alpha_7beta_1$, $alpha_6beta_4$	249
Endothelial	$alpha_1beta_1$, $alpha_2beta_1$, $alpha_6beta_1$, $alpha_vbeta_3$, $alpha_vbeta_5$	105
Smooth Muscle	$alpha_1beta_1$, $alpha_2beta_1$, $alpha_3beta_1$, $alpha_4beta_1$, $alpha_5beta_1$, $alpha_7beta_1$, $alpha_8beta_1$, $alpha_9beta_1$, $alpha_vbeta_1$, $alpha_vbeta_3$, $alpha_6beta_4$	104, 250

Note: This listing does not include other matricellular proteins such as thrombospondin, osteonectin, and tenascin, which are also ligands for integrins but are outside the scope of this review.
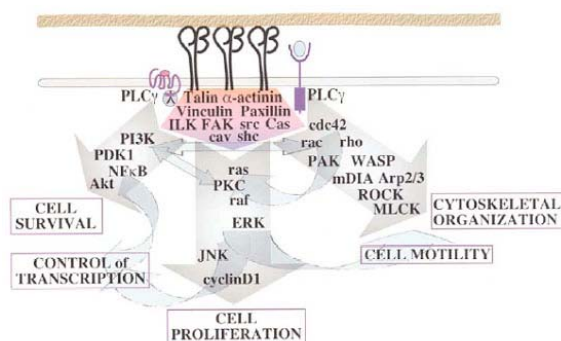
Figure 1. Schematic diagram of the signaling pathways through integrins. Integrin signaling mediates many aspects of cellular function through common secondary pathways. Figure reproduced with permission from Elsevier. (116).

The surface chemistry of the ECM controls which proteins present in serum adsorb to the matrix. (21, 104, 107). It has been well established that these proteins have profound effects on cell survival, DNA transcription, proliferation, motility, and cytoskeletal organization (Figure 1). by signaling through integrins. (112-115). These heterodimeric proteins, consisting of alpha and beta subunits, connect the ECM (Table 3). to the cytoskeletal matrix through several adaptor proteins including ILK and are capable of bi-directional signaling. (116). ILK has been shown to signal towards the nucleus along the Akt pathway and contributes to cell survival (117). while internal signaling can cause activation or inactivation of integrins through enzymes such as calpain or phosphatidylinositol phosphate kinase. (113, 116). Synthetic matrices, such as PLGA, exhibit very different effects on cell function than natural matrices such as collagen and elastin because they do not have natural binding sites, and must adsorb more binding proteins, such as fibronectin and vitronectin, to their surface for integrin binding to occur. (21, 104, 107). Collagen and elastin, because they are natural ligands for integrins, do not bind as many additional proteins to facilitate cellular adhesion. SMCs use the $alpha_5beta_1$,

$alpha_vbeta_5$, and $alpha_2beta_1$ integrins to bind fibronectin, vitronectin, and collagen I, respectively. (104, 107). Because of differences in physical form and surface chemistry, synthetic matrices exhibit more proliferation and elastin deposition than collagen scaffolds, which have higher actin filament formation (109)., and lower proliferation and elastin production. (107). The mechanisms for these changes in cellular function, although not specifically known, may indeed be partially attributed to outside-in signaling through integrins.

Because vascular tissue walls may be greater than 250um in thickness, they may require the development of a vascular supply network to provide nutrients to the intramural cells, and integrins have been shown to play important roles in angiogenesis. (106, 118, 119). Endothelial cells bind to their matrix via beta1 or alphav integrins to activate JAK/STAT pathways, increasing c-fos transcription leading to proliferation, and binding of the alphavbeta3 integrins are required for sustained activation of MAPK/ERK pathways for angiogenesis. (105, 120). Lumen formation during development depends upon alpha2beta1 interaction with collagen and alphavbeta3, and alpha5beta1 interacting with the RGD sequences of fibronectin. (105, 118). In fact, blocking the alpha5beta1 integrins forces the cells to rely more heavily on the alphavbeta integrins, demonstrating redundant mechanisms ensuring survival. Interestingly, a second peptide domain from fibronectin, REDV, is an exclusive ligand to ECs through the alpha4beta1 integrin. (121, 122).

There also appears to be a synergism between the binding of integrins to the matrix and soluble growth factors. (123). Research has shown that fibronectin enhances EC differentiation induced by VEGF (124)., and cell adhesion enhances autophosphorylation of EGF and PDGF. (21). PDGF-BB induces EC migration via $alpha_vbeta_3$ integrins in an RGD- dependent manner on fibronectin, and VEGFR2 signals with $alpha_vbeta_3$. However, both the PDGFR and VEGFR2 receptors associate with the beta-subunit of the integrin without directly causing signaling or activation of their respective tyrosine kinases, thus indicating that the integrins may amplify, or provide secondary signals that amplify, the signal provided by growth factors binding to RTKs. (105).

Integrins can also participate in "inside-out" signaling, whereby growth factors or other cellular processes can change the expression of integrins or their binding ability. It has been shown that $VEGF_{165}$ stimulates $alpha_1beta_1$, $alpha_2beta_1$, and $alpha_vbeta_3$ integrins, that bFGF increases expression of $alpha_vbeta_3$ integrins in microvascular endothelial cells, and that VEGF enhances migration through $alpha_vbeta_3$ as well as activating $alpha_vbeta_5$, $alpha_5beta_2$, and $alpha_2beta_1$ integrins. (105). The ECM also serves as a binding site for soluble or semi-soluble growth factors, such as the VEGF isoforms 165 and 181 and bFGF. The immobilization of these growth factors within the matrix offers a dynamic concentration of free molecules. (21). This dynamic chemical environment can cause differential signaling, resulting in varying cellular function and chemotaxis.

Another factor to consider for matrices is the physical constraints the matrix places on the cell. Research by Ingber *et al.* has demonstrated the importance of cell shape on the proliferation, differentiation, and apoptosis of vascular cells. (106). Decreased adhesion area, with the same total cell-ECM contact area, promotes cell survival, but decreasing the total available surface area for adhesions promotes apoptosis. (125). Constraining cell growth into a linear pattern causes ECs to differentiate and form capillaries. (126). It has also been shown that the shape of progenitor cells can affect their differentiation. (127, 128). By allowing a bone marrow progenitor cell to elongate and contract against its matrix, smooth muscle proteins such as alpha-SMA and calponin are produced in a MAPK-dependent manner, indicating differentiation towards a smooth muscle lineage. (129).

The effect of the ECM on production of matrix components by vascular cells has received some attention because alterations in composition in the arterial wall can indicate pathologic states such as arteriosclerosis or atherosclerosis. (130). Additionally, the production of ECM is an important factor that requires control in tissue engineering. If a biodegradable matrix is employed in the construction of a TEVG, the cells must produce enough ECM before the initial scaffold material is degraded, but they must not produce too much ECM so as to inhibit the functionality of the tissue. It has been established that bioactive materials can control cell-material interactions. For example, Mann *et al.* (121). demonstrated that specific peptides can control the proliferation and production of matrix components by ECs and SMCs. RGD, the major adhesive peptide in fibronectin, has been shown to induce spreading of ECs (131, 132)., and this increased spreading has been correlated to decreases in ECM production over time. (121, 133-135). YIGSR, an adhesive peptide in laminin, has also shown increased spreading and proliferation in ECs (121, 132). with similar decreases in ECM production over time compared to RGD. (134, 135). Laminin has been shown to cause tubule formation in HUVECs through binding with the $beta_1$ integrin. (136). This relationship is not only dependent on the actual coating used, but also on the physical characteristic of the substrate, with gels, not rigid surfaces, allowing for tubule formation. (136). The relationship between peptide

sequences and matrix production seems to depend on the "adhesiveness" of the cell-ECM interaction. Mann *et al.* (121, 133). have shown in SMCs that the type of peptide and its density are correlated to the strength of adherence, and that ECM production decreases with increasing strength of adherence. There also appears to be a correlation between SMC proliferation and ECM production, where increasing ligand density inhibits proliferation. Fibrillar collagen has been shown to inhibit SMC proliferation through cyclin dependent kinase-2 inhibition. (21). Mann *et al.* (133). have found that a peptide density of 2.8-7umol/mL is optimal for promoting cell attachment, spreading, migration, proliferation, and ECM production. This suggests that integrin-binding during the adhesion process initiates secondary signaling to control ECM production. This might occur through saturation mechanisms whereby when a particular type or family of integrins becomes saturated by ligands, their collective secondary signaling overwhelms the promoters for ECM production.

Proteoglycans are another important component of the ECM in the arterial wall, with SMCs, ECs, and macrophages each able to produce these molecules. The synthesis of proteoglycans by SMCs can be affected by the presence of other types of matrix materials. For example, fibronectin enriched matrices promote conversion of SMCs from contractile to synthetic phenotypes, while laminin maintains their contractile phenotype. (137, 138). These changes in SMC phenotype were shown to be related to the proteoglycan heparin sulfate secreted by neighboring cells. Phenotypic changes in SMCs were also demonstrated by Figueroa *et al*, (139). who stimulated proteoglycan synthesis and increased proliferation in SMCs by removing proteoglycans, collagen, or elastin from the matrix. Removal of heparin sulfate proteoglycans has recently been shown to inhibit FGF-induced proliferation in injured arterial SMCs. (140). It has also been demonstrated that during development, SMCs participating in vessel maturation exhibit a synthetic phenotype (141). Synthetic SMCs appear to revert back towards the fetal state, tending to produce more ECM, proliferate, and migrate more readily than contractile SMCs. (142). Therefore, it stands to reason that a matrix not containing the fundamental proteins found in mature vessels instead resembles a developing vessel, thus causing SMCs to exhibit their synthetic phenotype.

4.2. Mechanical stimulation (143-145)

Vascular tissues exist in a highly dynamic environment. Vascular cells within a tissue experience three basic forces: shear stress, stretch, and pressure. These forces are part of the constant signaling inputs that vascular smooth muscle and endothelial cells experience and can affect their proliferation, differentiation, and ECM production. Many genes are activated by mechanical forces and are differentially controlled by various signaling pathways, both temporally and spatially, and this has fundamental implications to vascular tissue engineering and vascular biology. (143, 144).

Mechanical forces are transmitted through the ECM and then across the cell membrane by several
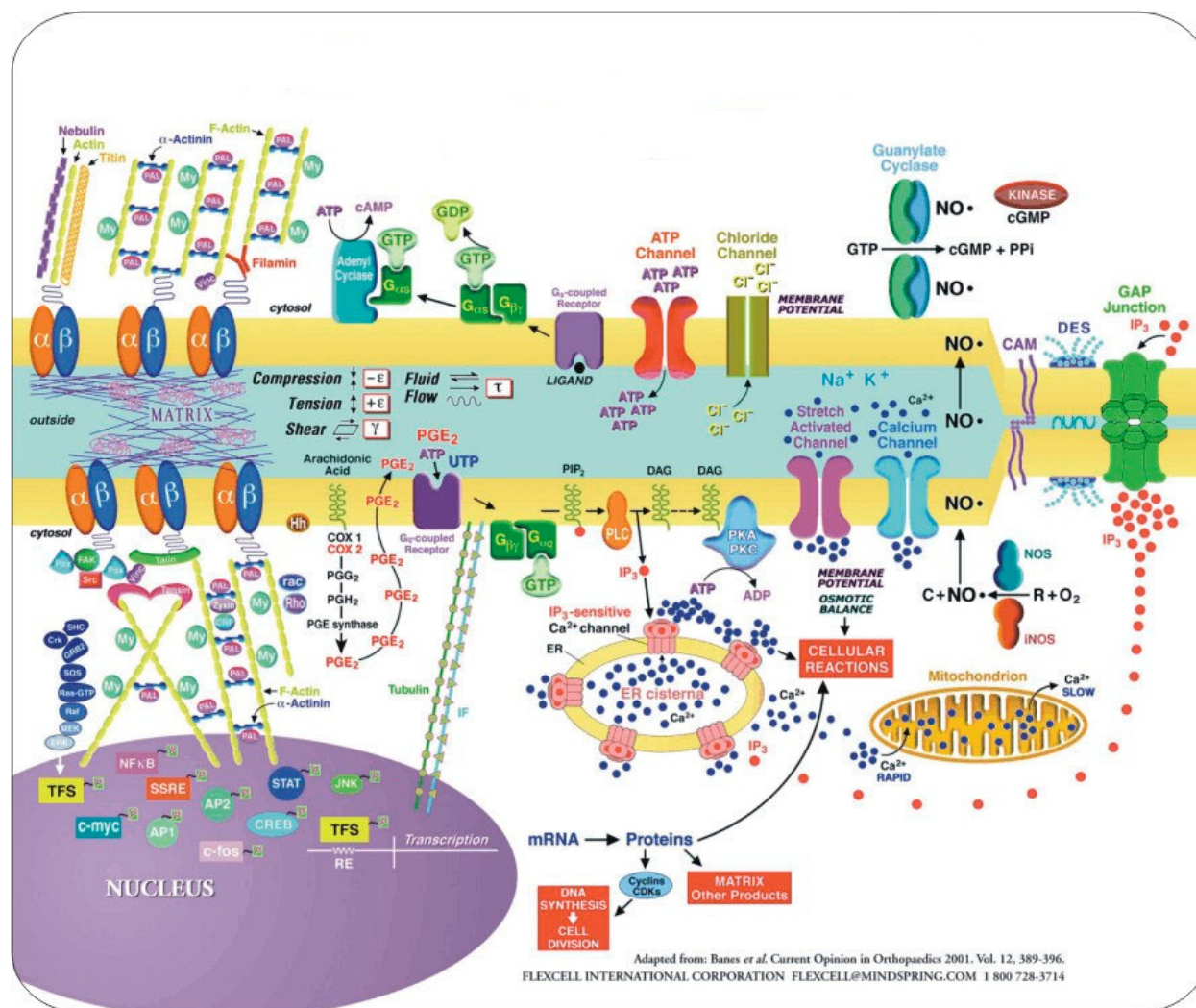
Figure 2. Schematic of cellular mechanisms for sensing mechanical signals which are relevant to vascular tissue engineering. Cells within a tissue can sense mechanical stimuli, such as compression, tension and shear stress, through various mechanisms including stretch activated ion channels, GTP-coupled proteins, integrins, and RTKs (not depicted here). These sensors trigger secondary signaling that can include prostacyclin, cAMP, changes in membrane potential, Src, MAPK, phospholipases, and PKC. The secondary pathways converge upon the nucleus, and depending upon other environmental factors, initiate gene transcription leading to alterations in proliferation, survival, and protein synthesis. Additionally, the spatial and temporal gradients of the magnitude and frequency for a given mechanical stimulus within a tissue can influence the secondary signaling, ultimately impacting the final cellular response. Figure reproduced with permission from Flexcell International Corporation. (251).

different mechanisms that can be cell and/or force dependent. Some proposed "sensors" have included integrins, (32, 146). ion channels, (147). G-coupled proteins, (148). and growth factor receptors. (32). Many researchers believe that all of these proposed sensors transmit information simultaneously across the cell membrane, resulting in a complex converging signaling cascade (Figure 2). (21, 33, 106, 108). This theory is supported by findings that many of the cellular responses to mechanical signals are also dependent upon the type of ECM (33, 145, 149). For instance, SMCs exposed to cyclic strain on fibronectin or vitronectin, but not laminin or elastin substrates, exhibit increased proliferation. While such mechanical stimulation on a laminin substrate did not

increase proliferation, it did increase the expression of SM-1, a contractile marker for SMCs, and this increased expression was not found when the strain was applied on a fibronectin substrate. Therefore, one might conclude that fibronectin supports growth and laminin supports a differentiated phenotype under similar mechanical stimulation. (33, 149).

The main focus of mechanical stimulation in vascular tissue engineering has been on improving the strength and functionality of TEVG constructs. This has been accomplished by stimulating the cells within the tissue, typically with cyclic strain, causing them to produce more ECM (13, 39, 130, 150-152)., remodel their matrix

with proteases like MMP-2 (26, 38, 153-155)., proliferate (149, 156, 157)., and express stable, differentiated phenotypes. (33, 158, 159). Most of the information on the molecular mechanisms of mechanical stimulation has been performed in monolayer culture, but the importance of signaling in a 3-D environment is quickly gaining attention. (108, 144, 160, 161). However, regardless of monolayer or 3-D culture, the secondary signaling cascades transducing the mechanical signal from the ECM towards the nucleus include the MAPKs, phospholipases C, D, and A_2, as well as PKC, Src, cAMP, FAK, and cytochrome p450. (33, 106, 144). While most of these cascades had been previously demonstrated for cardiomyocytes, similar responses are being seen in vascular SMCs. (33). Second messenger activity has also been shown to be dependent on the ECM, implicating integrin involvement. For example, integrins may act through src to activate Ras, which can then activate MAPK leading to protein synthesis. (145).

Like the synergism between integrins and soluble growth factors, there also appear to be autocrine growth factor loops that are generated by exposing vascular tissue to mechanical forces. It has been shown that shear stress transiently increases the expression of potent mitogens, PDGF-B, bFGF, and heparin bound EGF by endothelial cells. (143). Furthermore, two loops have been identified as involving both PDGF and angiotensin II in SMCs exposed to cyclic strain. That is, angiotensin II and PDGF are produced by SMCs under such stimulation, and these then interact with their respective receptors in an autocrine or paracrine manner, which can lead to more secondary signaling along the MAPK pathway, which can lead to protein synthesis and cellular replication. (33, 130, 156, 157). The PDGF receptor itself is also activated by mechanical stimulation in SMCs, further suggesting a role for this autocrine/paracrine signaling loop. (162).

Another important design issue in vascular tissue engineering is the incorporation of a non-thrombogenic blood-contacting surface. The key to success in this area relies on generating a functional endothelial layer to separate the underlying ECM and SMCs from the blood. Like SMCs, mechanical stimulation plays a role in the function of ECs. For example, both shear stress and cyclic strain have been shown to be modulated in ECs by K^+ channels (163, 164)., intracellular Ca^{2+} (165)., G-proteins (148)., cAMP (166)., cGMP (164, 167)., inositol trisphosphate (168)., PKC (167)., MAPKs (169, 170)., small GTPases (170)., and PTKs (171, 172). (both growth factor receptors such as PDGF and VEGF, and non-receptors such as FAK and src).

The response of ECs to mechanical stimulation is dependent on magnitude and temporal application and generally behaves in a dose-dependent manner. (143, 144). For instance, sustained laminar shear stress increases mRNA and protein levels of NOS (173, 174)., TGF-beta (175, 176)., cyclooxygenase-2 (177-179)., tissue factor pathway inhibitor (180)., and thrombomodulin (181, 182)., production of NO (183)., and sustained fibrinolytic activity through tPA expression, (184, 185). while such stimulation decreases ET-1 (143)., PAR-1 (186)., and TNF-alpha

induced MCP-1 (187). mRNA levels. The decrease in ET-1 mRNA only occurs for arterial levels of shear stress (i.e. 5-30 dynes/cm^2). In fact, ET-1 mRNA levels increase, compared to static controls, for low levels of shear stress. (167). Therefore, shear stress at physiologic arterial levels sustains the expression of vasodilators (NOS, NO)., SMC proliferation inhibitors (TGF-beta)., and anti-thrombogenic factors (cyclooxengase-2, thrombomodulin, TFPI)., and it depresses expression of vasoconstrictors (ET-1)., pro-coagulation proteins (PAR-1)., and inflammatory proteins (MCP-1). The mechanisms for the increase of NOS gene expression and depression of ET-1 protein have been shown to result from differential signaling. The NOS gene expression is linked to membrane signaling while integrin/cytoskeletal signaling regulates ET-1. (188). It has also been shown that transcription of immediate early genes (ERK, JNK). takes only a few minutes and peaks by one hour followed by downregulation. In contrast, vasoactive genes (NOS, ET-1). and anti-thrombogenic genes (cyclooxygenase-2, thrombomodulin). are persistently controlled by longer-term applied forces. Therefore, it would seem that immediate early genes are needed for a short-term response to injury while the vasoactive, anti-thrombogenic genes are maintained for homeostasis. (143).

Mechanical forces may also play an important role in the use of progenitor cells in vascular tissue engineering. Several researchers have demonstrated this in orthopedic and musculoskeletal tissues (189-192)., but the role of mechanical forces on progenitor cell differentiation for vascular tissue development is still in its infancy. In a recent study, our laboratory has demonstrated that mechanical stimulation of bone marrow progenitor cells can induce the expression of smooth muscle proteins (Figure 3). (34). This finding may have significant implications for many areas of vascular biology because it established that bone marrow progenitor cells are load responsive and can activate SMC-specific genes in response to cyclic strain. The potential of using defined biomechanical and biochemical stimuli to produce a source of fully functional SMCs from BMPCs might have applications in tissue engineering, as well as help to improve biocompatibility of synthetic grafts and stents.

5. BIOCHEMICAL FACTORS IN VASCULAR TISSUE ENGINEERING

Bioactive chemicals provide yet another signal to vascular cells *in vivo* that requires consideration when fabricating a TEVG. These signals can be provided by growth factors such as VEGF, PDGF, bFGF, IGF, and TGF-beta. Other biochemicals, such as ascorbic acid (vitamin C). and Cu^{2+} function in a cofactor role to help produce ECM. (43, 44, 193). Most vascular cells have receptors for these growth factors, with the exception of ECs, which are the only cells that have VEGF receptors and lack PDGF receptors in their quiescent state. (194). As a result, each of these growth factors causes overlapping secondary signaling to occur within the cell. In addition, research has demonstrated biphasic responses for different growth factors. These responses are not only dependent
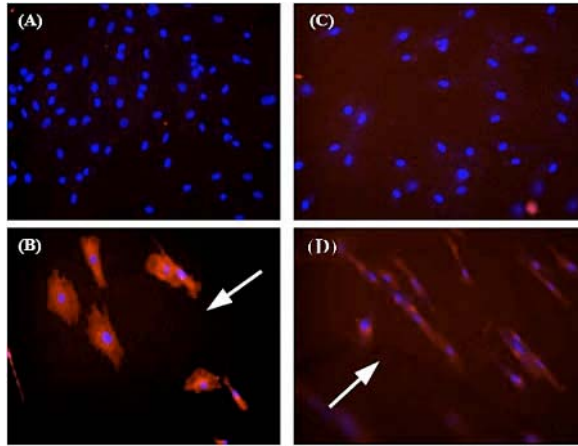
Figure 3. Immunohistochemical images of BMPCs stained with alpha-SMA or h1-calponin. Detection of alpha-SMA in (**A**). unstrained and (**B**). 10% strained cultures and h1-calponin in (**C**). unstrained and (**D**). 10% strained cultures. Note only strained cultures are positively stained for alpha-SMA and h1-calponin after 7 days of culture. Arrow indicates direction of strain in (**B**). and (**D**). Nuclei are labeled blue and stain is red. Original magnification (**A–D**). x200. Figure reproduced with permission from Mary Ann Liebert Inc (34).

upon the cell type involved, but also the ECM chemistry and binding regions, thus creating dynamic concentrations of free molecules. (21). For the purposes of this review, we discuss each of the relevant growth factors individually below.

5.1. Basic fibroblastic growth factor (194)

bFGF is a strong mitogen and morphogen for most cell types, including SMCs and ECs. (194, 195). This growth factor is localized to HSPG molecules on the cellular surface and the ECM, which protects it from proteolytic degradation. (196). However, there is no evidence that bFGF is actively secreted so its presence in the ECM remains unexplained. bFGF also plays a significant role in wound healing and angiogenesis by inducing the proliferation of SMCs and ECs around an injured tissue. In early development, bFGF plays a role in differentiation of the splanchnopleuric mesoderm into the vasculature, but postnatally, it appears to only have a mitogenic effect on vascular cells. (197). The mechanism for bFGF-induced mitosis has been traced through PKC to MAPK phosphorylation and the subsequent phosphorylation and translocation of c-myc to the nucleus, resulting in DNA synthesis. (82). The effects of bFGF in concert with other factors, such as ascorbic acid, may prove useful in tissue engineering because their concerted effects induce proliferation *and* matrix production in SMCs. (198). The matrix materials produced by the SMCs included collagen I, III, and IV as well as fibronectin. However, bFGF alone inhibits collagen I and III production in vascular SMCs. (199).

5.2. Platelet derived growth factor (194, 200)

PDGF is a mitogen for SMCs, EC progenitors, and microvascular ECs, but not quiescent ECs. PDGF

induces proliferation by allowing cells to pass through G_0 into G_1 phase (201)., and also has an effect on SMC phenotype. (200). Several researchers have reported that a specific isoforms of PDGF, PDGF-BB, not only induces SMC proliferation, but also suppresses the expression of their contractile machinery. (45, 200). Corjay *et al.* have reported that the effect of PDGF-BB does not work to inhibit the transcription of contractile proteins, such as alpha-SMA and MHC, but rather destabilizes the mRNA as it leaves the nucleus. (202, 203). While these studies were carried out in a monolayer culture model, Stegemann *et al.* have provided further evidence that this process is conserved in 3-D collagen gels, and that PDGF inhibits the response of SMCs to mechanical stimulation in these gels. (45). Kinner *et al.* have shown that this effect of PDGF is applicable to BMPCs, where it increases proliferation and inhibits the expression of alpha-SMA, and they hypothesize that the decrease in contractile machinery is related to proliferation rather than phenotype. (204).

5.3. Transforming Growth Factor Beta (194, 205, 206)

The TGF-beta family plays a significant role in mitogenesis and morphogenesis throughout the body in both development and adult tissue remodeling. With respect to vascular tissue, TGF-beta modulates EC and SMC proliferation over a wide range of concentrations (10pg/ml-10ug/ml). (205). and helps to maintain their differentiated states. (194). However, during angiogenesis, TGF-beta appears to stimulate growth through mechanisms that may be related to the cellular phenotype and ECM composition. (207). TGF-beta is responsible for increases in matrix production by SMCs and helps to maintain the vessel wall integrity by increasing integrin and matricellular protein content. (205). TGF-beta also increases MMP expression in SMCs and ECs and is induced by hypertension *in vivo* or mechanical strain *in vitro*. (130, 208, 209). Taken together, the increase in matrix production and MMP expression seem to indicate a role for TGF-beta in cell migration and remodeling. It is also notable that TGF-beta acts in conjunction with PDGF in a biphasic manner. Low concentrations of TFG-beta (1-2 fg/ml). stimulate PDGF-AA and PDGFR production, while higher concentrations that inhibit proliferation (10 pg/ml-10 ug/ml). do so by downregulating PDGFR protein synthesis. (205, 210). Exposure to TGF-beta can also cause SMCs to act in an opposing manner to PDGF with respect to their expression of alpha-SMA. Stegemann *et al.* have shown that TGF-beta increases alpha-SMA and matrix production in SMCs in static and mechanically stimulated environments in collagen gels (45)., while Kinner *et al.* have shown it induces the expression of alpha-SMA in adult BMPCs. (204). This contrasts with increases in proliferation and inhibition of alpha-SMA when BMPCs are exposed to PDGF as mentioned above. (204). TGF-beta has also been shown to induce alpha-SMA in non-muscle cells such as fibroblasts (converting them to myofibroblasts). and endothelial cells. (211). TGF-beta, therefore, plays an important role in determining the phenotype of vascular cells, and the use of this growth factor in vascular tissue engineering will likely increase, with reports that tethering it to polymer matrices increases cell adhesion and matrix production. (212).

5.4. Insulin-like Growth Factor (213)

IGF is a protein found throughout the body during prenatal and postnatal development and functions within cardiovascular tissue to control the progression of cell division through G_1 into the S phase of mitosis. Its role in regulating proliferation of vascular SMCs has been highlighted by Clemmons *et al.* who used IGF-1 antibodies to halt proliferation induced by PDGF. (214). Similar results have been demonstrated with angiotensin II stimulated proliferation, (215). indicating that IGF is required to propagate the effects of mitogens on SMCs. There is, however, no evidence to support similar effects on macrovascular ECs. (213). Control over IGF mRNA has been demonstrated by angiotensin II, (215). thrombin, (213, 216). and mechanical stimulation. (217). Thrombin downregulates IGF mRNA while angiotensin II and hypertension demonstrate an increase in IGF mRNA. This indicates that IGF is important in the wound healing process, which is further supported by findings of increased IGF expression in balloon injury models. (213). The effects of IGF are transduced through G-coupled protein receptors and carried to the nucleus via PKC-gamma pathways. (218, 219). IGF receptors are upregulated by angiotensin II, PDGF, FGF, and thrombin signaling. (220). Because several growth factors upregulate IGF receptors, it demonstrates that IGF receptor density may be critical for their mitogenic effects.

In addition to its role in propagating proliferative effects of other growth factors, IGF plays a role in phenotypic modulation of vascular cells. IGF stimulates elastin and fibronectin production by aortic SMCs. (221, 222). It also has been shown to play a role in vasodilation by mediating the production of NO. Specifically, IGF stimulates NO production from cultured endothelial cells, but inhibits cytokine-induced NO production from SMCs. (213). Thus, IGF appears to play a significant role in the proliferation and phenotype of vascular cells, and lies at a focal point of pathways induced by other stimulatory factors.

5.5. Vascular Endothelial Growth Factor (223)

VEGF is one of the most studied and characterized vascular growth factors because of its importance during fetal development, oncogenesis, lymphangiogenesis, and angiogenesis. VEGF is a specific mitogen for ECs and progenitor cells that have VEGF receptors (194)., and the stimulation of $CD34^+$ progenitor cells with VEGF is enough to induce differentiation to ECs. (70, 224). This ability to differentiate can be enhanced in a matrix-dependent manner. (124). The VEGF-A family has several isoforms that display different binding affinities for heparin sulfate. The $VEGF_{120}$, $VEGF_{165}$, and $VEGF_{180}$ isoforms are freely soluble, show some heparin sulfate binding affinity, and are totally bound to heparin sulfate, respectively. (223, 225). This differential binding characteristic of VEGF sets up signaling gradients that can cause differentiation, proliferation, or migration depending upon other signals received by an EC, thus making it important for patterning in both tissue engineering and development. VEGF is a powerful mitogen, morphogen, and motogen for ECs, usually through the VEGFR2 (flk-1/KDR). receptor and its coreceptor, neuropilin-1. (226). The VEGFR1 receptor, although it can bind VEGF, may not provide biologically meaningful signals to the cell, and may in fact act as an antagonist to prevent over saturation of the VEGFR2 receptors. (227). VEGFR2 signaling progresses through MAPK, PKC, and PI3K/Akt to promote proliferation, survival (through production of Bcl-2). or migration. (223). The migratory stimulation by VEGF is believed to cause FAK phosphorylation and release MMPs to allow ECs to move freely throughout the matrix and establish new adhesions. (228, 229). VEGF also stimulates the production of vasoprotective molecules such as NO (via upregulation of eNOS mRNA). and PGI2. (230, 231). In addition to their function as vasodilators, the release of NO and PGI2 maintains SMCs in a quiescent state, prevents platelet aggregation and leukocyte adhesion, and has been implicated in podokinesis (small movements with no prescribed direction). (223). Although NO and PGI2 cause podokinesis, adding a VEGF gradient can transform the podokinetic motion into a directed, chemotactic one. (232). VEGF protein and gene transfer has been shown to accelerate re-endothelialization and reduce intimal thickening and thrombus formation following vascular injury. (223, 233, 234). The importance of VEGF-induced NO and PGI2 production is demonstrated through studies that show loss of NO and PGI2 production may have a role in pathologic states of hyperplasia. (235, 236). VEGF's role in angiogenesis has not only been to induce proliferation and migration of ECs within an ischemic tissue, but it can also start the process by disrupting the VE-cadherin cell-cell junctions and by increasing vascular permeability. (225, 237). Several growth factors, such as IGF (238)., bFGF (239)., IL-1beta (240)., and PDGF (241)., and environmental conditions, such as hypoxia (239, 242). or increased shear stress (243)., can stimulate the expression of VEGF in both endothelial and smooth muscle cells.

6. CONCLUSIONS

One needs to control all aspects of a TEVG - including its cellular, physical, and biochemical environments - in order to produce a viable, functional vascular tissue-engineered construct. The cellular component of the TEVG dynamically affects the way the tissue develops and behaves, producing and breaking down matrix and producing molecular factors that can influence cellular behavior in both autocrine and paracrine manners. The cells incorporate information from their environment - consisting of physical and chemical signals - and adapt to maintain homeostasis. The physical signals can be either mechanical stimulation or a physical constraint, caused by ECM fibers or pores, which cause cellular processes to occur once a secondary message is carried through the cell. The biochemical nature of the matrix provides ligands for receptors and binding proteins, which add to the communication occurring within each cell. There are also soluble biochemical signals that produce similar effects within the cells. Therefore, each of these environmental cues results in a complex combination of secondary signals, transcription factors, and posttranscriptional modification that converge into commands for the cell to divide, differentiate, migrate, or die.

A viable TEVG needs to be functional, both mechanically and biologically, in order to be clinically efficacious. To date, there exist limitations in each of the components of vascular tissue engineering that we have outlined. Although great progress has been made in the past 5 years, manipulating the overlapping molecular pathways that govern the components of a functional TEVG addressed in this paper remains challenging. The complexities of vascular tissue engineering, therefore, require multidisciplinary collaboration between engineers, molecular biologists, materials scientists, surgeons, and developmental biologists in order to address vascular tissue engineering from different perspectives, thus increasing the potential impact of the results. A new vascular graft is largely needed and future progress in tissue engineering will likely provide a solution once all the key factors discussed in this review become at least partially controlled.

7. ACKNOWLEDGEMENTS

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Abbreviations: alpha-SMA= smooth muscle alpha actin, ALCAM= activated leukocyte cell adhesion molecule, bFGF= basic fibroblast growth factor, cAMP= cyclic adenosine monophosphate, cGMP= cyclic guanosine monophosphate, eNOS= endothelial nitric oxide synthase, EGF= epidermal growth factor, ERK= extracellular signal-regulated kinase, ET-1= endothelin 1, FAK= focal adhesion kinase, GAGs= glycosaminoglycans, G-CSF= granulocyte colony stimulating factor, GM-CSF= granulocyte macrophage colony stimulating factor, GTP= guanosine triphosphate, ICAM-1= intercellular adhesion molecule 1, IFN= interferon, IGF= insulin-like growth factor, IL= interleukin, ILK= integrin-linked kinase, JNK= c-Jun N-terminal kinase, JAK= janus kinase, LFA= leukocyte function associated antigen, LIF= leukemia inhibitory factor, MAPK= mitogen activated protein kinase, MCP-1= monocyte chemotactic protein 1, M-CSF=macrophage colony stimulating factor, NO= nitric oxide, NOS= nitric oxide synthase, PAR-1= protease activated receptor 1, PDGF= platelet derived growth factor, PGI_2= prostacyclin, PI3K= phosphatidylinositol 3-kinase, PKC= protein kinase C, PLC-gamma= phospholipase C gamma, PTK= protein tyrosine kinase, RTK= receptor tyrosine kinase, SCF=stem cell factor, STAT= signal transducer and activator of transcription, TGF-beta= transforming growth factor beta, TNF-alpha= tissue necrosis factor alpha, tPA= tissue plasminogen activator, VCAM-1= vascular cell adhesion molecule 1, VEGF= vascular endothelial growth factor, VEGFR2= vascular endothelial growth factor receptor 2

Key Words: Vascular Tissue Engineering, Stem Cells, Molecular Biology, Integrins, Growth Factors, Extracellular Matrix, Endothelial, Smooth Muscle, Mechanobiology, Review

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