Adventitia contribution to vascular contraction: Hints provided by tissue-engineered substitutes

François A. Auger a,b,⁎, Pédro D’Orléans-Juste c, Lucie Germain a,b

a Laboratoire d’Organogénèse Expérimentale (LOEX), Hôpital du Saint-Sacrement du CHA, Québec, Canada
b Department of Surgery, Laval University, Québec, Canada
c Institute of Pharmacology, Medical School, Sherbrooke University, Sherbrooke, Québec, Canada

Received 23 January 2007; received in revised form 29 May 2007; accepted 1 June 2007
Available online 8 June 2007
Time for primary review 27 days

Abstract

It is well accepted that the adventitia is much more than a simple elastic membrane which surrounds the media. However, the extent of its contribution to vascular physiology, as well as the mechanisms involved, remains to be clearly established and characterised. Investigation into these topics is hampered by a few technical challenges, like the paucity of available healthy human vascular samples and the variability such samples can display. Another challenge is the isolation and preparation of intact adventitia without contaminating cells from the media. For those reasons, although other models have proved useful to address these questions, data from tissue-engineered vascular substitutes can also provide quite valuable answers. Results from such substitutes indicate that a reconstructed adventitial layer can respond to classic vasoactive agents such as endothelin and sodium nitroprusside.

© 2007 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Adventitia; Tissue engineering; Blood vessel; Vascular tone

1. Introduction

The contribution of the adventitia in vascular tone is a complex yet highly important parameter to consider towards a better understanding of the physiological function of each component of the blood vessel wall. Investigators are even more challenged if these experiments are to be done by utilising human tissues. The difficulties lie in the problematic access to human tissues and blood vessels combined with the significant variance in many parameters between different donors such as age, sex, previous diseases and life habits. It is in such a context that utilisation of tissue-engineered blood vessel (TEBV) may shed some light on some particular and complex issues in cardiovascular pharmacology. Organ constructs created by tissue engineering have the following advantages: 1) obtaining a more consistent culture process resulting in complex 3D reconstructed tissues, 2) achieving an acceptable level of reproducibility, 3) access to human cells, 4) individual tailoring of the various components of the construct can be achieved for special experiments, 5) the nature of the donor can be well defined, such as young, old or hypertensive.

The role of the media in cardiovascular tone control is well established [1]. A lingering question is the evident yet poorly explored significance of the adventitia in that very same function. Many previous studies have used elegant and interesting techniques to investigate specific vascular layers. For example, isolated cells in culture offer a powerful system to investigate many cellular mechanisms, as shown by An et al. to investigate how adventitial fibroblasts could express endothelin-1 in response to angiotensin-II [2]. Other groups have used analytical tools such as immunohistochemistry to unravel tissue-specific and cell-specific mechanisms [3–6], or ingenious devices such
clearly separating the adventitia from the media. Thus, this approach has been hampered by the technical difficulty of clearly separating the adventitia from the media [15]. Current knowledge can be obfuscated by experimental drawbacks. The use of tissue-engineered vascular substitutes should introduce a novel and additional approach that can bring additional insight to the field.

Our research group, amongst others, has been a proponent of tissue-engineered tissues and organs substitutes as in vitro models for more than 14 years [1,16–33]. Thus, we believe such an approach can provide relevant answers to address the various functions of specific vascular wall components: intima, media and adventitia [1,21,22,29,30]. Admittedly, tissue-engineered vascular substitutes lack some of the more complex features found in native vessels, such as vasa vasorum, resident immune cells and nerve endings. However, they offer a useful intermediate level of complexity between monolayer cell cultures and native human blood vessels. Previous publications have shown that our tissue-engineered vascular substitutes display a number of characteristics that are similar or close to those observed in native small diameter blood vessels. These similarities support the physiological relevance of tissue-engineered constructs of this type for investigating vascular components [1,17,22,25].

2. Vascular tone control: putting the role of the adventitia in perspective

The role of the adventitia has long been considered mostly limited to its structural and mechanical functions, with some involvement of the perivascular innervation in overall vascular responses [34]. Until the early eighties, the adventitial layer had been known to be involved in the anchoring of the blood vessel to extravascular matrices (e.g. connective tissue). Aside from fibroblasts, other cell types can be found in the adventitia. Endothelial cells are present in the adventitia of blood vessels containing a vasa vasorum. Aside from the possibility that they could provide the adventitia with a close source of vasoactive factors such as nitric oxide (NO), published evidence indicates that the vasa vasorum could be involved in various vascular pathologies such as atherosclerosis [35,36]. Other, experimental results indicate the adventitia can be a source of NO [6,11,13]. This could be related to adventitial resident mastocytes and nerve cells, which display close contacts and have been suggested to be involved in the local release of vasoactive compounds [3,5,37,38]. Furthermore, previous results show that the adventitial fibroblasts themselves can also be involved in the local release of NO [6,10,39].

Pericytes represent another cell type that can be found in the adventitia. Although some evidence points towards their implication in the calcification commonly associated with atherosclerosis [40–42], their role in vascular stability and angiogenesis is also well accepted [43,44]. There are indications that pericytes could also, in addition to their role in the neovascular structures, provide progenitor cells for other lineages in wound repair [45,46]. Finally, although not morphologically part of the adventitia itself, perivascular adipocytes are another anatomically specialised cell type in close contact with the outer region of blood vessels, and some results convincingly point to their contribution towards vascular tone regulation and remodelling [47–49].

2.1. Role of the adventitia in vascular compliance

The involvement of the adventitia has been proposed in angiogenesis and arteriosclerosis [3,36,37,39,50]. Indeed, vascular remodelling occurring in advanced arteriosclerosis causes loss of vascular compliance (i.e. enhanced rigidity) and triggers intimal thickening. The latter pathology may involve adventitial progenitor cell migration towards the more intraluminal compartments of the vasculature [50–53]. These and other results point towards the significant contribution of the adventitia in post-transplant or post-injury vascular remodelling [14,52,54]. The triggering mechanisms for adventitial myofibroblast migration are yet to be fully understood and controlled, albeit part of the answer might be found in the stimulation of toll-like receptors and inflammatory responses [55,56] as well as in the combined role of TGF-β and the Connective Tissue Growth Factor (CTGF) [57]. It has also been proposed that reactive oxygen species generated by adventitial fibroblasts through NAD(P)H oxidase activity can modulate pathological fibroblast proliferation, stimulate medial hypertrophy and affect vascular tone [39]. Finally, it has been shown that balloon injury causes an overall reduction in vascular α-adrenoceptors expression [58]. This reduction is associated with an enhanced growth-factor-like activity of catecholamines on vascular smooth muscle cells and adventitial fibroblasts which leads to vascular wall hypertrophy [58].

2.2. Role of the adventitia in autocrine control of vascular function

The overall contribution of the adventitial layer in the biomechanical characteristics and responsiveness of the blood vessel, under physiological conditions, was not abundantly explored in the literature until recently. Actually, not only does the adventitia respond to hormones such as endothelin-1 [21–23] but adventitial fibroblasts can secrete ET-1 under certain stimuli such as hypoxia or angiotensin-II [2,59]. Furthermore, the vascular outer layer can release vasoactive factors such as the adventitial-derived relaxing factor (ADRF) [60,61]. Calcium entry-dependent activation of tyrosine kinase and protein kinase is the suggested mechanism of action of ADRF. Its physiological role remains, however, largely undocumented in in vivo settings and the readers are referred to the elegant study of Gao et al. for further details [62]. On the other hand, Singhal et al. [63] have successfully correlated the role of adipocyte-derived leptin release as one of the cardinal causes of atherosclerosis. This group demonstrated that in obese subjects...
an overproduction of leptin from perivascular adipocytes was positively correlated with significant changes in vascular distensibility of brachial arteries from healthy adolescent subjects independently of other obesity-related factors such as body weight, mass index or LDL/cholesterol index [63,64]. Another group used leptin-deficient hyperlipidemic mice to provide more evidence that leptin can accelerate atherosclerosis [65]. Considering that perivascular adipocytes are predominantly located close to the adventitial layer of blood vessels, studies support the concept that secretion of factors such as leptin and ADRF from the adventitial layer may greatly influence vascular compliance either acutely (i.e. short term response to biomechanical stimuli) or chronically (i.e. long term angiogenetic, remodelling and/or atherosclerotic and arteriosclerotic processes) [62–64]. Additional concepts, based on some in vitro studies and deductive reasoning, have been suggested regarding the influence of periarteriolar fat adipocytokines, such as tumor necrosis factor alpha and interleukin-6, on local vascular signalling [49]. The authors of this study hypothesised a role for these adipocytokines in obese subjects in the relation between insulin resistance and arteriolar vasoconstriction in skeletal muscles.

2.3. Role of the adventitia in neurogenic control of vascular tone

Vascular innervation of the adventitia and neurogenic control of vascular tone have been well documented in coronary [3,66–68] and cerebral [5,69–72] vasculature for many years. The presence and role of vascular nerve endings have also been investigated in various blood vessels [73–76]. Vascular autonomic and sensory nerves of various types inclusive of cholinergic, adrenergic, peptidergic or nitricergic are found in different proportions and density depending on the specific anatomical site [37,66,71,72,75,77,78]. The variety of nerves which can be found in the adventitia is underlined by the presence of many neuron-related peptides and molecules such as acetylcholine [72], noradrenaline [12,79], neuropeptide Y [67,80], substance P [67,80], calcitonin gene-related peptide [67], neurotensin [81] and vasoactive intestinal peptide [67,72,80]. Nerve stimulation may elicit different responses, either in type or amplitude, in different parts of the vascular system [71,80]. Neurogenic vasocontrol can thus follow different patterns depending on which molecules are released and local reactions they trigger. For example, active molecules released locally from adventitial nerves may diffuse and act directly on the adventitia and the media or act on the endothelium which in turn will release factors such as NO which will then influence the media [37,70,75,76,78,79]. Yet another component of this complex question is the presence of resident mast cells, which can be affected by neurons. Such a link has been proposed by Dimitriadou et al. based on data from temporal arteries of cluster headache patients in which adventitial mast cells showed signs of progressive degranulation compared to normal controls [82]. Another example of possible mast cell involvement has been proposed for atherosclerosis by Laine et al. [3]. Sensitive C-fibers richly innervate the medial-adventitial interface [78] and neuropeptides (such as substance P and Calcitonin Gene Related Peptide) released from C-fibers can interact with resident mast cells. In pathological conditions, Laine et al. [3] demonstrated increased mast cell/C-fiber contacts. They conclude that activated mast cells may release pro-inflammatory molecules such as leukotrienes and histamine leading, perhaps, to exaggerated vasospastic responses of the injured blood vessel wall. The authors do not identify which specific leukotrienes are involved in this process. Substance P, however, has been shown to induce murine mast cells to generate leukotriene c4 in vitro, although it did not also elicit granule release in the given experimental conditions [83].

2.4. Role of the adventitia in overall vascular reactivity to hormones and autacoids

Very few studies have addressed the topic of adventitial reactivity to exogenous, let alone endogenous, hormones, perhaps because of the technical difficulties of dissecting this particular vascular compartment in the overall vascular responsiveness. Surgical layer stripping is one way to address this problem, although much care needs to be exercised to ensure the integrity and purity of the separated layers [13,15]. In one such study, adventitia-denuded rabbit carotid artery rings showed a lower response to noradrenaline than control rings [14]. Other experiments, based on rat aorta, carotid and iliac arteries which also used surgical separation of the vascular layers, have provided more evidence that the adventitia is involved in vascular contraction and relaxation [8–14]. Those results showed differences in the in vitro contractile and relaxation capacity of vascular samples devoid of adventitia and, in some cases, also of endothelium. In a number of experiments using agents such as noradrenaline, forskolin, urotensin-II, LPS, KCl, acetylcholine and sodium nitroprusside (SNP), adventitial control of NO release has been shown to have an important influence on vascular tone [8–14]. In contrast, it should also be noted that superoxide anions generated in the adventitia may reduce the bioavailability of NO and thus counteract the modulation of adventitial tone by the later labile factor [39,84].

One group reported that removal of the adventitia caused a notable proliferation of the media [14]. This raises the question of whether the stripping procedure might trigger rapid modifications in the smooth muscle cells that could influence contractile capacity [85]. The reduction in contractile response of vascular samples from which the adventitia has been stripped might thus not be entirely attributed to the absence of this layer. In order to have a model in which no tissue stripping is necessary, tissue-engineered vascular constructs are an interesting tool to elucidate adventitial involvement in vascular tone. Indeed, 3D vessels constructed with adventitial cells provided by human donors [23] introduce a new approach that has allowed to determine
the overall responsiveness of a reconstructed layer to potent vasoactive agents and to estimate an adventitia versus media intrinsic activity ratio in such constructs. Recent data show that apparent affinities and maximal contractility responses can be estimated in comparative fashion. Furthermore, the adventitial layer may account for about 50% of overall vessel constrictive response to endothelin-1 in engineered adventitia/media vessels [21].

3. The value of tissue-engineered substitutes as in vitro models

Animal cell culture, either in 2D (monolayer) or 3D (engineered tissues), and animal models share many biological functions with human tissues, including blood vessels, and have been of great value in numerous experiments. However, beyond their common characteristics, human and animal cells have some inherent differences which have to be taken into account when selecting models and analysing experimental results [86–90]. The use of tissue-engineered constructs as in vitro models has recently undergone a significant resurgence of interest due to distinct advantages. One important advantage of using human cells is to avoid any inter species differences, subtle or not, that can affect the experimental results. Alternatively, human tissue-engineered constructs may be profitably used to validate results obtained in animal models prior to more expensive and ethically restricted clinical assays. On the other hand, even when generated from human cells, tissue-engineered constructs are still simplified versions of the actual physiological tissues they aim to represent and they may not perfectly reflect all the mechanisms present in vivo. Despite these limitations, tissue-engineered models have advantages worth exploiting in various applications [1,16–18,20–24,26,27,29–33,91,92]. Overall, it is possible to generate tissue-engineered vascular constructs with characteristics which, although not perfectly identical to native tissues, provide enough similarities to become useful experimental models [1,17,21–23,25,29,30,85]. Two recent and interesting reviews of the value of tissue-engineered 3D models can be suggested to the reader [93,94], as this aspect is beyond the scope of the present review.

4. Tissue-engineered vascular constructs: a novel approach to the functional analysis of the vascular wall

4.1. Tissue engineering of vascular constructs

The tissue-engineered vascular models generated by the self-assembly approach are entirely made from human cells and achieve their mechanical properties without the addition of exogenous synthetic biomaterial. As described for a tissue-engineered blood vessel, the cells produce and organize their own extracellular matrix [25]. The development of tissue-engineered vascular models with three different “architectures” generated with this approach has recently been reported [21]. The first vascular construct contained only an adventitial component assembled uniquely with vascular fibroblasts (VF) (4 layers) (TEVA), the second vascular construct contained only a medial component with vascular smooth muscle cells (VSMC) (4 layers) (TEVM) and the third one was a combination of adventitial (2 layers) and medial (2 layers) components (TEVMA) (Fig. 1). This last construct was the one closest to native small diameter human constricting blood vessels. Although the technique has evolved to include an
intima [25] when necessary, its absence is not a drawback when specific comparison of media to adventitia is desired.

Obviously, our vascular constructs are devoid of nerves, vasa vasorum, mast cells and perivascular adipose layer. Although elastin has been detected in the reconstructed adventitia, the constructs did not display elastic laminae. The absence of these components, however, did not seem to compromise the similarity of the pharmacological responses to those elicited in native human vessels. Accumulated observations of the various vascular constructs from histological, immunohistochemical and ultrastructural analyses have shown that, although notable differences can be seen, they show interesting similarities to native human arteries of small diameter [1,17,21–23,25,29,92]. Histological analysis revealed cells embedded in a self-generated extracellular matrix (ECM). In a TEBV, VSMC expressing \( \alpha \)-smooth muscle actin (\( \alpha \)SMA) and desmin were seen as elongated cells in an orientation resembling that in human media [25]. Vascular VSMC density, although high for an in vitro model, was still lower than in a normal vascular media [25]. However, when treated with classic agents such as KCl, SNP, endothelin, histamine and bradykinin the constructs nonetheless displayed vasomotor responses similar to normal human blood vessels [1,17,21–23,30]. TEVM contained a high percentage of cells expressing \( \alpha \)SMA. In contrast, only a low number of \( \alpha \)SMA-expressing cells were detected in TEVA, reflecting that the differences between VF and VSMC were preserved in the reconstructed vascular tissues (Fig. 2) [21]. As studied in the TEBV, fibroblasts synthesized high amounts of elastin assembled in small fibers, which were organized in large circular arrays [25]. Immunostaining also indicated that the ECM contained type I, III, and IV collagens as well as laminin, fibronectin, and chondroitin sulfates [25,95].

### 4.2. Pharmacodynamic characterisation of tissue-engineered vascular constructs

#### 4.2.1. Pharmacological responses of the TEVA to the endothelin peptides and precursors

The tissue-engineered adventitial vessel (TEVA) responds to isoforms of the endothelin peptides, with the notable exception of ET-3 and its precursor big-ET-3 (Table 1). Even if both ET-1 and ET-2 have similar apparent affinities (i.e. \( pD_2 \) and EC50) in the nanomolar range, the former peptide is slightly more potent that the latter one, while both peptides have similar intrinsic activities (i.e. \( \alpha \)). The lack of response of the TEVA to ET-3 suggests that this particular engineered vessel does not possess functional ETB receptors. RT-PCR demonstrated the presence and absence, respectively, of mRNA for ETA and ETB receptors in TEVA [21]. This observation correlates with results from other groups, where ETB was mostly present in the media and absent from the adventitia [4,96]. The TEVA response to the precursors of ET-1 and ET-2, namely big-ET-1 and -2, demonstrates the presence of a functional endothelin-converting enzyme.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>( pD_2 )</th>
<th>EC50 (M)</th>
<th>R.A. (%)</th>
<th>( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1</td>
<td>9.85</td>
<td>1.4 \times 10^{-9} M</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>ET-2</td>
<td>9.72</td>
<td>1.9 \times 10^{-9} M</td>
<td>73.4</td>
<td>1.2</td>
</tr>
<tr>
<td>ET-3</td>
<td>In.</td>
<td>In.</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Big-ET-1</td>
<td>8.66</td>
<td>2.1 \times 10^{-8} M</td>
<td>6.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Big-ET-2</td>
<td>7.77</td>
<td>1.7 \times 10^{-7} M</td>
<td>0.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Big-ET-3</td>
<td>In.</td>
<td>In.</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

\( pD_2 := \log (EC50); \) R.A.: relative affinity expressed in percentage of the apparent affinity of ET-1; \( \alpha \): intrinsic activity calculated for each peptide considering the maximal response to ET-1 as unitary value. In.: Inactive at concentrations up to 10 \( \mu \)M.
(ECE) in these engineered vessels. If the presence of such an enzyme in the adventitia of normal blood vessels can be molecularly confirmed, it would be interesting to explore how it is compatible with the observations that ET-1 can be produced by adventitial fibroblasts [259], thus suggesting a possible paracrine role for this molecule in the vascular wall. It is, on the other hand, important to point out that we were unable to confirm the capacity of the ECE to convert big-ET-3 to ET-3 because of the lack of intrinsic activity of the later peptides in the TEVA. Thus, a vessel constructed from cultured cells of a particular vascular tunic, such as the adventitia, possesses functional integrity, in terms of receptors and maturing enzymes, in a similar fashion as to what has been shown in intact human blood vessels [21–23].

4.2.2. Contribution of the adventitial layer in the overall vasoactive response to endothelins

To the best of our knowledge, Laflamme et al. [21–23] are the first to report data generated with adventitia alone, showing measurable contractile properties which may contribute to the overall vascular compliance in response to endogenous hormones and autacoids. Overall, the adventitia contributes to a lesser extent than the media to the contractile response of the tissue to ET-1, in our vascular substitutes. Indeed, potency of ET-1 is markedly enhanced in TEVM, in terms of maximal response, when compared to vessels composed of adventitial as well as adventitial/medial cells.

The presence of an ETB-dependent contraction in both TEVM and TEVMA engineered vessels when compared to TEVA constructs is another striking result (Table 2). Indeed, whereas ET-3 is inactive in TEVA, this particular peptide possesses a weaker relative affinity (40 to 50 times less potent than ET-1), yet shows a similar intrinsic activity in both TEVM and TEVMA. This ETB-dependent component has been described in more detail by Laflamme et al., both at the functional (via the use of selective ETA and ETB receptor antagonists and ETB agonist) as well as at the molecular level, either with a system expressing both ETA and ETB [23] or ETA alone [22]. Finally, although ET-3 contracts both TEVM and TEVMA, its precursor big-ET-3 is inactive (Table 2). It is therefore possible to conclude that the above-mentioned vessels allow the study of the specificity of action of the endothelin-converting enzyme. In these vessels, ECE discriminates between the precursors of ET-1 and ET-3, is sensitive to the ECE inhibitor phosphoramidon [21] and therefore possesses the selective proteolytic characteristics of the ECE-1 isoform extensively detailed in the literature [97–99]). This is relevant to the in vivo situation for two reasons. Firstly, the endothelium which possesses the ETB receptor type will respond equally well to ET-1 or ET-3 since it possesses the same affinity for both peptides. Secondly, is that it has been previously shown that the ECE possesses substrate specificity for big-ET-1 and big-ET-2 but does not convert big-ET-3 [100]. This suggests that the engineered vessels express a functional ECE of the ECE-1 type, as indicated by the fact that big-ET-3 is inactive in all three constructs. By analogy, the lack of hemodynamic properties of big-ET-3, as opposed to ET-3, would also suggest that in vivo the predominant ECE isoform is of the ECE-1 type [100].

4.2.3. Does the adventitia respond to endothelium-dependent or independent vasoactive factors?

L’Heureux et al. [25] were the first to demonstrate the presence of a functional endothelium in engineered vessels. Indeed, this particular study showed that the addition of an endothelial layer in TEBVs contributed to an inhibition of platelet deposition on the vascular wall. On the other hand, little is known about the vasoactive functionality of the endothelium seeded on the luminal portion of engineered vessels. One of the first steps towards measurement of this important function of the endothelium was to assess whether the underlying smooth muscle cells of engineered vessels would respond to activators of guanylate cyclase, the main mechanism involved in the vasodilatory response of endothelial derived relaxant factor, nitric oxide (NO). Indeed, all three engineered vessels, namely TEVA, TEVM and TEVMA, dilate in a concentration-dependent fashion to the guanylate cyclase activator, SNP [1,21].

These results demonstrate that the adventitia, similarly to the media, may be able to respond to endothelium-dependent vasoactive factors such as nitric oxide. Since NO is extremely labile, we suggest that the luminaly-released autacoid may not be the major contributor in the relaxation of the distantly localized adventitial layers, in conductance or resistance vessels. In contrast, Somoto et al. [101] have shown an important contribution of the adventitia to the production of vasoconstrictive superoxide ions in animal vessels mechanically denuded for the above-mentioned layer. Whether tissue-engineered vessels composed of adventitial cells generate superoxide ions as well remains to be determined. At the current stage, our group also has yet to identify if the adventitia, or even the media for that matter, responds to stimulators of adenylate cyclase such as the guanylate cyclase activator, SNP [1,21].

| Table 2: Comparison of relative affinities and intrinsic activities of ET-1, ET-3 and precursors in engineered vessels |
|---------------------------------|------------------|------------------|------------------|
| R.A. (%) | aE | R.A. (%) | aE | R.A. (%) | aE |
| TEVA | TEVM | TEVMA |
| ET-1 | 100 | 1.0 | 82 | 2.2 | 107 | 1.8 |
| ET-3 | – | – | 2 | 2.2 | 1.9 | 1.8 |
| Big-ET-1 | 6.9 | 1.1 | 5 | 2.3 | 4 | 1.7 |
| Big-ET-3 | – | – | – | In. | – | In. |

R.A.: Relative affinity expressed in percentage of the apparent affinity of ET-1; aE: intrinsic activity calculated for each peptide considering the maximal response to ET-1 as unitary value. In: Inactive at concentrations up to 10 μM. R.A. and aE for ET-3 and big-ET-3 is calculated as ratio of the apparent affinity and maximal response to ET-1 in TEVA used as base units (please refer to Table 1 for absolute values).
distant portion of the medial area, and by adventitial cells themselves [6,11,13], may be involved in the vasodilatation of the adventitial layer of vasculatures.

5. Perspectives and conclusions

Two major additions can be envisioned to improve the value of tissue-engineered vascular substitutes as experimental models (and for transplantation). Firstly, intrinsic neuronal networks are crucially involved in the control of vascular compliance in response to factors which influence hemodynamic parameters either acutely (MAP variation, hypovolemia, hypothermia, ischemia/reperfusion episodes) or chronically (arteriosclerosis, atherosclerosis, heart failure, cardiac hypertrophy). The ideal TEBVs should have built in, similarly to normal blood vessels, a neural network composed of sympathetic and parasympathetic as well as non-adrenergic non-cholinergic fibers. To address the difficulty of growing mature nerve cells within the TEBV, the strategy of using stem cell technology may be attractive. Among these lines, Klein et al. [102] have recently reported the use of embryonic hippocampal neuronal cells in post-surgical regeneration of trauma. Furthermore, Ikeda et al. [103] have developed a strategy using MASH-1 transfected mouse embryonic stem cells towards reconstitution of damaged neuronal networks in the mouse central nervous system. Finally, Berthod et al. have also reported new approaches towards nerve regeneration in skin constructs [104,105]. Strategies such as those put forward by Ikeda et al. and Berthod et al. [103–105] could be adapted using human adult stem cells in the engineered blood vessels. This latter concept emphasizes what one may consider as another advantage of engineered blood vessels as it becomes therefore feasible to apply gene transfer technologies to selective cell types and at optimal stages of cellular culture. These experimental conditions are obviously difficult to attain in intact blood vessels undergoing gene therapy.

Secondly, NO released by vessels of the vasa vasorum, located in the distant portion of the medial area, may be important in the overall reactivity of the adventitial layer of blood vessels. The inclusion of a transmural vasa vasorum network within the TEBV remains to be performed. However, our own experience in creating endothelialized skin substitutes may prove useful to achieve this goal [16,33,106]. On the other hand, the optimisation of an intima located within the lumen of the TEBV and in which the endothelial layer presents vasodilatory properties is certainly achievable and will be included in future experiments.

In conclusion, the present review strives to summarize and interpret the most recent observations derived from tissue engineering strategies towards the development and optimisation of functional blood vessels. Clearly, several important aspects still need to be addressed. Nonetheless, tissue-engineered vascular substitutes have and will hopefully continue to play a significant role in the experimental (in vitro) and also the clinical (in vivo) settings.

Acknowledgements

We wish to extend our appreciation to Alexandre Descham-beault for his work on Fig. 1 as well as to Dr Dan Lacroix for editing the manuscript.

References


[59] Davie NJ, Gerasimovskaya EV, Hofmeister SE, Richman AP, Jones PL, Reeves JT, et al. Pulmonary artery adventitial fibroblasts cooperate with vasa vasonum endothelial cells to regulate vasa


