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REVIEW ARTICLE

Modulation of Smooth Muscle Cell Phenotype for Translation of Tissue-Engineered Vascular Grafts

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Translation of small-diameter tissue-engineered vascular grafts (TEVGs) for the treatment of coronary artery disease (CAD) remains an unfulfilled promise. This is largely due to the limited integration of TEVGs into the native vascular wall—a process hampered by the insufficient smooth muscle cell (SMC) infiltration and extracellular matrix deposition, and low vasoactivity. These processes can be promoted through the judicious modulation of the SMC toward a synthetic phenotype to promote remodeling and vascular integration; however, the expression of synthetic markers is often accompanied by a decrease in the expression of contractile proteins. Therefore, techniques that can precisely modulate the SMC phenotypical behavior could have the potential to advance the translation of TEVGs. In this review, we describe the phenotypic diversity of SMCs and the different environmental cues that allow the modulation of SMC gene expression. Furthermore, we describe the emerging biomaterial approaches to modulate the SMC phenotype in TEVG design and discuss the limitations of current techniques. In addition, we found that current studies in tissue engineering limit the analysis of the SMC phenotype to a few markers, which are often the characteristic of early differentiation only. This limited scope has reduced the potential of tissue engineering to modulate the SMC toward specific behaviors and applications. Therefore, we recommend using the techniques presented in this review, in addition to modern single-cell proteomics analysis techniques to comprehensively characterize the phenotypic modulation of SMCs. Expanding the holistic potential of SMC modulation presents a great opportunity to advance the translation of living conduits for CAD therapeutics.

Keywords: smooth muscle cell phenotype, scaffolds, growth factors, vascular grafts, coronary artery disease, atherosclerosis

Impact Statement

Tissue-engineered vascular grafts (TEVGs) are a promising approach to improve coronary artery bypass graft procedures. However, current approaches to vascular regeneration lack seamless tissue integration and are prone to stenosis and graft failure. In this review, we aim to present the potential of smooth muscle cell (SMC) modulation for TEVGs, targeting long-term patency and full integration. In particular, we describe the diversity of SMCs, state-of-the art techniques for phenotype modulation, and novel methods for translating TEVGs. Overall, this review can serve as a guide for the development of bioactive materials for vascular regeneration.

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Introduction

Coronary artery disease (CAD) is the leading cause of death in adults worldwide.[1–3] CAD arises from the accumulation of low-density lipoproteins in the coronary endothelium, which triggers an inflammatory response that recruits leukocytes, macrophages and smooth muscle cells (SMCs) to scavenge lipids.[4–6] This subsequently leads to the formation of fibrous atherosclerotic plaques,[5] which reduce the vessel diameter and limit myocardial blood circulation. Coronary artery bypass grafting (CABG) is the preferred therapy for restoring blood flow in severe artery occlusion,[7,8] with several vessel types as autologous grafts, such as left internal thoracic artery or greater saphenous vein.[8–11] Currently, ~200,000 CABG procedures are performed each year;[12,13] however, autologous tissue grafts face critical challenges, including post-implant stenosis, anastomotic compliance mismatch, and a low long-term patency.[14,15] Therefore, there is a growing need for effective non-autologous grafts in CABG surgery.[16,17]

A promising alternative to autologous grafting is vascular tissue-engineered (VTE) conduits. Weinberg and Bell[18] created the first TEVG by assembling layers of endothelial cells (ECs) and SMCs, but with insufficient resistance to pulsatile flow. Since then, the field has developed significantly.[19–25] Nonetheless, TEVG translation to the clinic still faces limitations, such as the lack of an easily implantable TEVG with integrative potential and mechanical properties resembling those of the native vessel and low thrombogenesis.[17,26] These limitations are even accentuated in TEVGs with a smaller vessel diameter (<6 mm) because of intimal hyperplasia or loss of patency.[27]

The vascular wall is a dynamic structure composed of three layers: the *tunica intima*—involved in antithrombogenesis,[28] inflammatory processes,[29] and mechanotransduction; the *tunica media*—the main effector of vascular tone due to the high contractility of SMCs and it contains a series of fenestrated elastin sheets (i.e., lamellae); and the *tunica adventitia*—composed of collagen fibers, myofibroblasts, and fibroblasts (Fig. 1a–d).[30–33] This type of layered structure is particularly difficult to mimic due to the limited infiltration of SMCs into the polymeric and decellularized scaffolds.[34–37] Such a technical limitation is mainly the failure to promote SMC migration and ECM deposition onto TEVGs.[38]

SMCs are involved in the vascular tone, and play a key role in tissue mechanics and remodeling. In particular, their *contractile* behavior is a response to sympathetic and parasympathetic cues.[39,40] In addition, SMCs also exhibit important plasticity and can undergo phenotype modulation processes (e.g., switching to a noncontractile *synthetic* phenotype). In this state, contractile markers are downregulated, while proliferation, migration, and extracellular matrix (ECM) deposition are increased.[41] This unique SMC phenotype switch occurs in pathological conditions such as vessel injury and atherosclerosis.[42,43]

Despite the potential of SMC phenotypical switching to promote cell migration and ECM deposition, this process is often overlooked in TEVG design and fabrication. Thus, this review aims to cover the diversity of SMC origins, various cues and modulators that regulate SMC phenotypes, different VTE strategies for SMC modulation, and their potential implications for translation of TEVGs to the clinic.

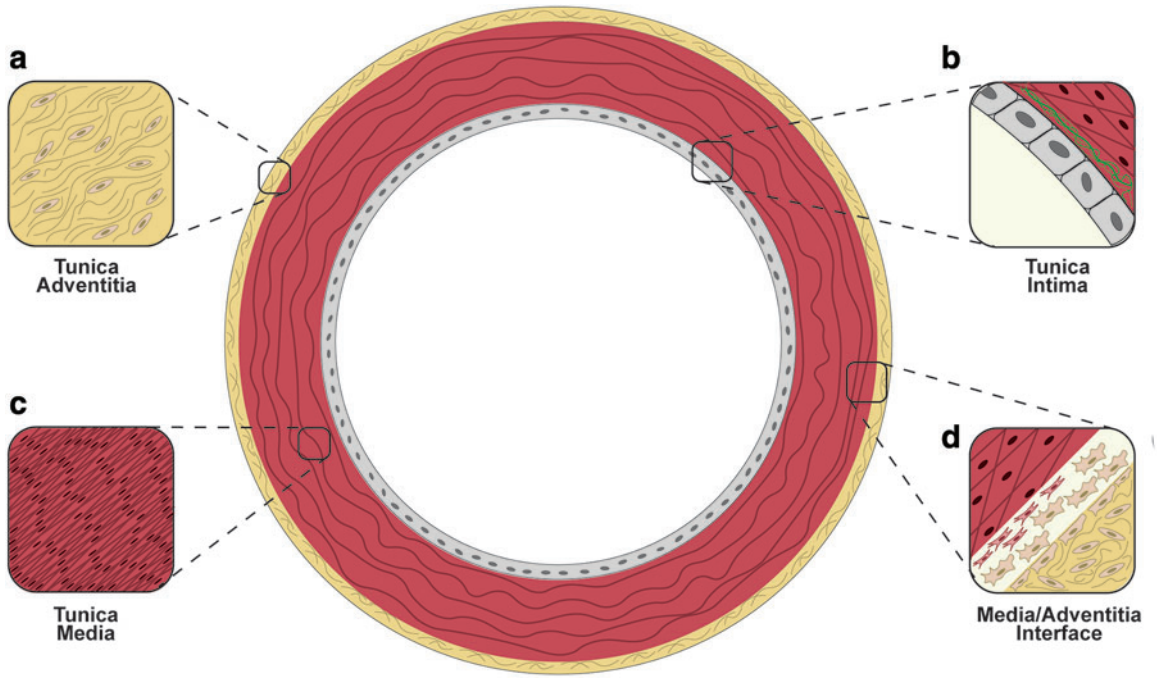

FIG. 1. Layered structure of the coronary arterial wall: **(a)** the tunica adventitia and its fibrous microstructure, **(b)** monolayer of endothelial cells and their contact with the elastic lamina (in *green*) separating the intima and media, **(c)** highly aligned organization of smooth muscle cells in their contractile phenotype *in vivo*, and **(d)** the contact between the tunica media and the adventitia where myofibroblasts can migrate to repopulate the media. Color images are available online.

Embryonic origin of the SMC diversity

SMCs in different regions of the systemic vasculature do not share a unique embryological origin. For example, coronary SMCs arise from proepicardial cells, which are developed at the proepicardium and are precursors of the complete coronary wall.[44,45] SMC differentiation involves the transformation of proepicardial cells into mesenchymal cells by FOG-2 protein signaling.[45,46] Then, the stimulation of proepicardium-derived mesenchymal cells with the platelet-derived growth factor (PDGF) and the epidermal growth factor results in the differentiated coronary SMCs.[47–49] This embryological origin of coronary SMCs is described as an indicator of the characteristic behavior of coronary artery tissues in response to mechanical stimuli, environmental cues, and pathologies.[50,51] Besides this contractile/proliferative duality, SMCs also possess in a wide spectrum of genetic expressions.[38,52] Therefore, a systematic understanding of the behaviors of SMC phenotype and diversity is crucial for TEVG design.

SMC phenotype modulation

The SMC lineage is diverse and difficult to identify under pathological conditions. Efforts have been made to establish the markers for SMC identification in atherosclerotic lesions and inflammatory processes.[41,53] For example, lineage tracking has been developed to characterize the fate of this cell line,[43] thereby allowing the detection of various SMC phenotypes (Fig. 2). The key SMC phenotypes and phenotype switching will be described below.

Contractile phenotype. The SMC contractile phenotype is the differentiated version of the cell line characterized by the expression of alpha-smooth muscle actin (α-SMA), smooth muscle myosin heavy chain (SM-MHC), SM22α, and SM-calponin, and alpha-tropo-myosin.[54] Contractile markers enable the SMCs to exert mechanical forces to control the vascular tone.[55] Maintenance of this contractile phenotype can be regulated by the transforming growth factor (TGF),[56–58] insulin-like growth factor,[59,60] circumferential stress,[61] and shear stress.[59,60,62]

Synthetic phenotype. Alterations in environmental cues and ECM microstructure can lead to a decreased expression of SMC contractile markers, an increased proliferation, and an upregulation of ECM deposition (e.g., type I collagen, elastin, laminin, fibronectin).[63,64] Switching from a contractile phenotype to a synthetic/proliferative one is

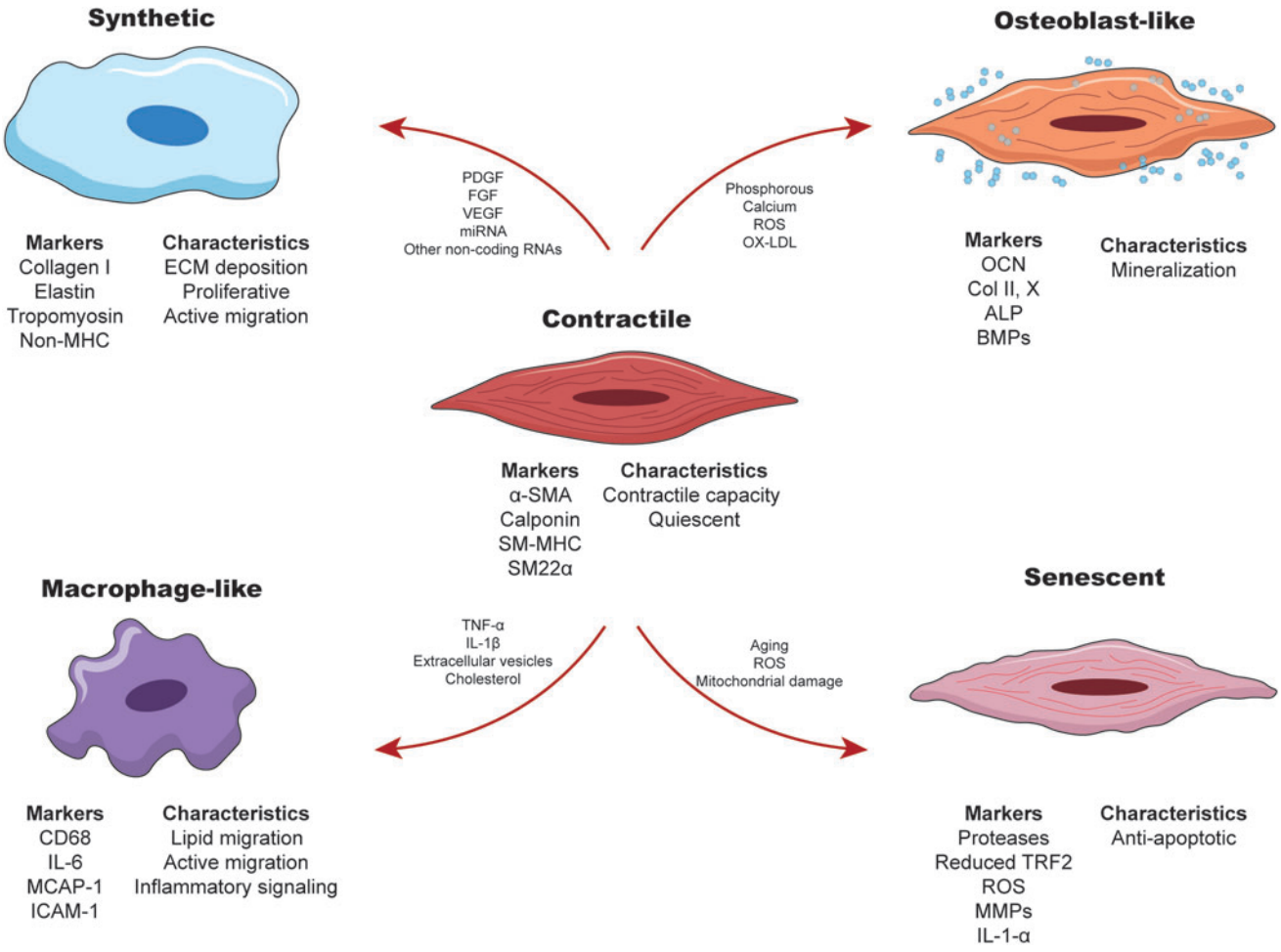

FIG. 2. Schematics representing the various SMC phenotypes, their respective markers, and the main characteristics. *Arrows* indicate the modulators described to induce modulation of the contractile SMC toward each phenotype. For abbreviations, please see Table 1. Color images are available online.

facilitated by a cross talk of signals.[63,64] For example, the PDGF has been shown to play an antagonistic role in SMC differentiation[65,66] through phosphorylation of Elk-1 that competes with myocardin for the docking site on the serum response factor (SRF).[67] Furthermore, the fibroblast growth factors (FGFs) are another group of potent mitogens in the SMCs that promote activation of the Ras/MAPK signaling pathway associated with SMC proliferation and migration.[68–70] Interestingly, FGFs can also inhibit the activity of TGF-β in the upregulation of contractile markers.[57] Vascular endothelial growth factor (VEGF), S100A4, micro-RNAs, and other noncoding RNAs are found to also promote synthetic phenotype switching.[71–75]

Other SMC phenotypes. In addition to the canonical duality, the SMCs can undergo modulation to other phenotypes. For example, a *proinflammatory* SMC exhibits downregulation of traditional contractile markers along with a large increase in the expression of inflammatory markers (e.g., MCP-1, MMP-3, MMP-9, VCAM-1, CCL5, CCL20, CXCL6, CXCR1,[76–79] see Table 1 for the definition of these abbreviations). The inflammatory phenotype promotes SMC migration and proliferation during early stages of atherosclerosis and plays a key role in cytokine release to promote inflammatory conditions.[80,81] This inflammatory behavior can be induced by different inflammatory signals, such as the tumor necrosis factor-alpha (TNF-α),[77] interleukin-1 beta (IL-1β),[79] extracellular vesicles[78] and cholesterol.[76,79]

The SMC may also exhibit a macrophage-like phenotype that shows upregulation of scavenger markers and CD68, without expression of α-SMA.[6,76,82–85] In this scavenging process, lipid phagocytosis by SMCs is driven by the ATP-binding cassette transporter A1(ABCA1)—a protein that enables cholesterol influx. However, ABCA1 expression in SMCs is lower than in myeloid macrophages,[6,86,87] leading to lipid accumulation and the formation of SMC foam cells.[6,86] Interestingly, this macrophage-like transformation is transient and reversible, allowing SMCs to revert to a contractile phenotype, suggesting an atheroprotective role for SMCs in the early stages of CAD.[88]

Another dynamic fibroblast-like phenotype has also been described for SMCs. As mentioned previously, proepicardial cells can become cardiac fibroblasts or SMCs. These precursor cells express the transcription factor (TCF21) is maintained in cardiac fibroblasts, but downregulated in the SMCs.[89,90] However, in atherosclerosis this marker is *upregulated* in the SMCs.[91,92] For example, Wirka et al.[92] found that the SMCs in Apoe$^{-/-}$ atherosclerotic mice exhibited a decrease in contractile markers and an upregulation in fibroblast markers. They also observed that these SMCs did not transition into a macrophage-like phenotype, but became more distant to the monocyte-derived macrophages.[93]

Furthermore, SMCs have been described as a key driver of vascular wall calcification.[94,95] This occurs when the SMC is modulated into an osteoblast-like phenotype associated with the downregulation of contractile markers.[96,97] This osteogenic phenotype is characterized by the formation and deposition of calcifying vesicles that are mineralization components made of phosphatidylserine and are involved in atherosclerosis.[98–100] Modulation to an osteoblast-like phenotype, and hence calcification, is mediated by *RUNX2,* a transcription factor that drives the differentiation of mineralizing cells.[101–103]

Finally, the SMC can also undergo *senescence* due to aging or atherogenic factors (e.g., oxidative stress, inflammation, etc.). Senescence is associated with telomere dysfunction due to reduced expression of telomeric repeat-binding factor-2 (TRF2).[104] During atherosclerosis,

Table 1. List of Abbreviations Used in This Article and Their Definitions

Abbreviation	*Definition*
α-SMA	Alpha-smooth muscle actin
ABCA1	ATP-binding cassette transporter A1
Apoe	Apolipoprotein E
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CCL20	Chemokine ligand-20 (CC motif)
CCL5	Chemokine ligand-5 (CC motif)
CD31	Cluster of differentiation 31
CD44	Cluster of differentiation 44
CD68	Cluster of differentiation 68
CXCL6	Chemokind ligand-6 (CXC motif)
CXCR1	Chemokind ligand-1 receptor (CXC motif)
EC	Endothelial cell
ECM	Extracellular matrix
Elk-1	E26 transformation-specific-like 1
EPL4	Elastin-like polypeptide-4
FGF	Fibroblast growth factor
FOG-2	Friend of GATA-2
ICAM-1	Intercellular adhesion molecule-1
IL-1-α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
MCAP-1	Mitotic chromosome-associated protein-1
MCP-1	Monocyte chemoattractant protein-1
MMP-3	Matrix metalloproteinase-3
MMP-9	Matrix metalloproteinase-9
MPAK	Mitogen-activated protein kinases
PAH-PAA	Poly(allylamine hydrochloride)/ poly(acrylic acid)
PCL	Polycaprolactone
PDGF	Platelet-derived growth factor
PELA	poly(DL-lactide)–poly(ethylene glycol)
PLLA-PCL	Poly(L-lactide-co-caprolactone)/ poly(L-lactic acid)
RADA	RADARADARADARADA (Aminoacid sequence)
Ras	Rat sarcoma protein
RNA	Ribonucleic acid
RT-PCR	Reverse-transcription polymerase chain reaction
SM-MHC	Smooth muscle myosin heavy chain
SM22α	Smooth muscle protein 22-alpha
SMC	Smooth muscle cell
SRF	Serum response factor
TCF21	Transcription factor 21
TEVG	Tissue-engineered vascular graft
TGF	Transforming growth factor
TGF-β	Transforming growth factor-beta
TNF-α	Tumor necrosis factor-alpha
TRF2	Telomeric repeat-binding factor-2
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VTE	Vascular tissue engineering

senescent vascular SMCs may only be present in the intima,[105] where they may contribute to plaque instability due to protease release and decreased collagen production.[106] The senescent SMCs may also induce proinflammatory behaviors in the neighboring SMCs and ECs,[107,108] resulting in the atherogenic behavior of the vascular wall.

Overall, the SMC exhibits a broad spectrum of phenotypical expressions highly dependent on environmental cues. Due to this environment-sensitive nature, the use of SMCs in the production of TEVGs requires a thorough understanding of their behavior when cultured on biomaterials to prevent unwanted marker expressions and adverse effects on the neighboring cells. This advanced knowledge can ultimately promote their integration into the native coronary tissue. In the next section, we will describe the state-of-the-art research on SMC phenotype modulation in the context of TEVG development.

SMC phenotype modulation for vascular tissue engineering

Over the past few decades, VTE has aimed to produce TEVGs, beginning with the work of Weinberg and Bell[18] to the recent success of acellular vessels for the access of hemodialysis.[109] However, these developments still suffer from the limitations with SMC infiltration into polymeric substrates and the incomplete integration of TEVGs into the native tissue.[17] Ideally, modulation of the SMC phenotype through careful biomaterial design to achieve specific SMC behaviors offers a promising approach to advance the development of TEVGs. In the following section, we will summarize different approaches to modulate SMC phenotype with biomaterials (Fig. 3).

Tuning of scaffold topography. Modifying scaffold topography is an effective method to modulate SMC phenotypes. The topography of the substrate on which the SMCs are seeded has a strong influence on factors such as cell morphology. Zhu et al.[110] characterized the behavior of SMCs when cultured in fibrin-coated polycaprolactone (PCL) electrospun scaffolds and showed that the SMCs were distributed along the fibers and exhibited a similar morphology to the native contractile phenotype. Conversely, random fibers resulted in heterogenous SMC shapes that resembled the synthetic phenotype morphology. Their findings were confirmed by various studies using electrospun and meltspun PCL fibers in conjunction with other polymers,[111,112] where it was reported that aligned fibers sustained the expression of α-SMA, calponin, and smoothelin.[110–114]

Tijore et al.[115] used micropatterned gelatin scaffolds for topographic modulation and found that highly aligned cell organization could mimic the native state of the contractile SMCs (Fig. 4a), as evidenced by the upregulation of α-SMA and SM-MHC expressions in the patterned constructs (Fig. 4b). However, the studies on topographical cues in the literature seem to be conflicting: Zeng et al.[113] observed increased proliferation on scaffolds with aligned topographies, while Tijore et al.[115] found higher proliferation in flat, nonstructured gels.[115] In general, aligned fibers and patterned structures promote higher expressions of contractile markers. However, the expression of synthetic and inflammatory markers in different types of topographies has not been systematically characterized.

Another important parameter in the topography of fibrous structures is fiber diameter. Han et al.[116] found that thinner fibers (0.5–1 μm) resulted in higher proliferation than thicker ones (5–10 μm). In contrast, cell infiltration was higher (nearly complete) for scaffolds with thicker fibers. To better understand these phenomena, they further characterized the expression of contractile and synthetic markers. Interestingly, they found that α-SMA was similar across all scaffolds, while non-MHC increased over time in scaffolds with thicker fibers, suggesting potential SMC modulation toward a synthetic phenotype.

Bioactive molecules as stimuli. Biochemical cues are fundamental for the modulation of SMC phenotype *in vivo* and *in vitro*. Thus, bioactive molecules can be used to

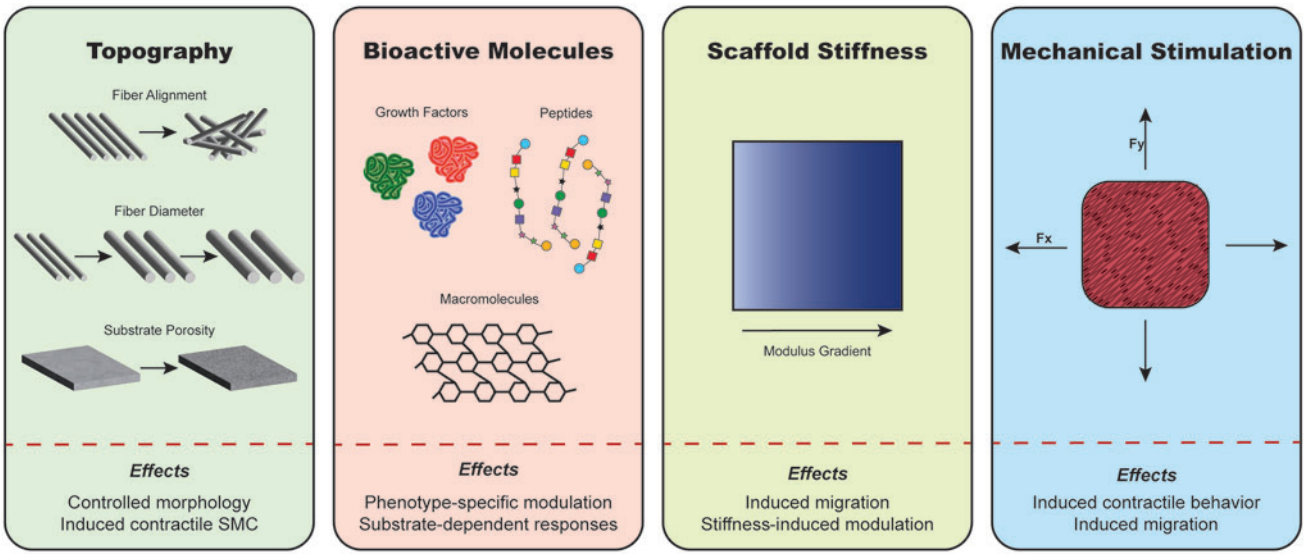

FIG. 3. Graphical representation of the most prominent methods for modulating SMC phenotype using biomaterial properties and mechanical stimulation, and their most representative effects. Color images are available online.

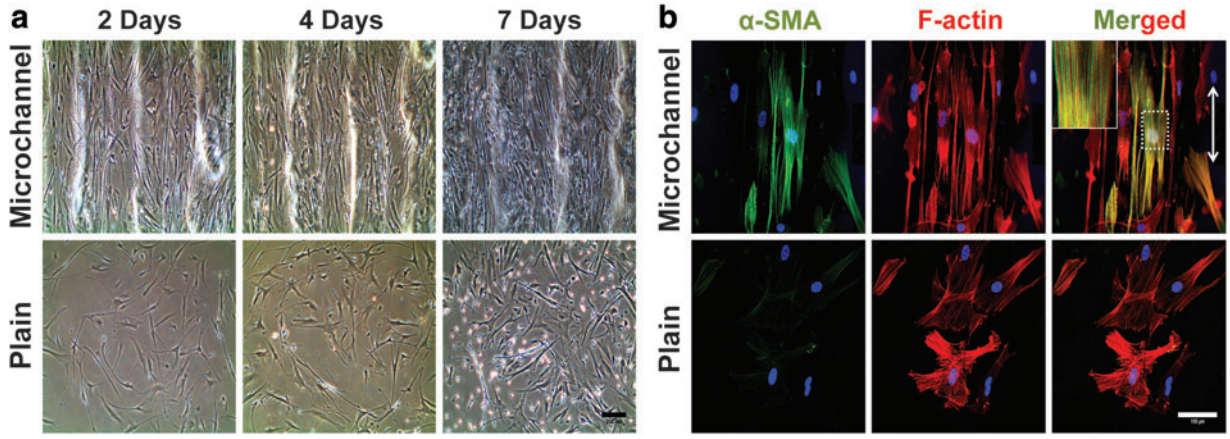

FIG. 4. **(a)** Microscopic images of vascular smooth muscle cells grown on microchanneled and plain gelatin substrates: controlled topography induces elongated and aligned SMCs on microchannels and plain substrate allows random elongation of actin fibrils. (Scale bar = 200 μm). **(b)** Alpha smooth muscle actin (α-SMA, *green*), F-actin (*red*), DAPI (nuclei, *blue*), and merged actin expression in SMCs cultured in the microchanneled and plain substrate after 7 days (*white arrow* indicates fiber orientation; scale bar = 100 μm). Images by Tijore et al.[115] with permission from Springer. Color images are available online.

modulate the SMC phenotype in cultured scaffolds. For example, Liu et al.[117] stimulated SMCs using VEGF-encoding plasmids distributed in a poly(DL-lactide)–poly(ethylene glycol) (PELA) solution to fabricate electrospun scaffolds. They found that when cells were cultured in plasmid-containing scaffolds, SMCs were elongated accompanied by a greater proliferative capacity and increased expressions of α-SMA and type I collagen, suggesting a switch to a synthetic phenotype and an early contractile behavior. This approach takes the advantage of nanotechnology to produce the growth factors essential for SMC proliferation; however, it was unclear whether the SMC phenotype switching could target a specific phenotype, as the advanced contractile markers were not characterized in their work.

In addition, Ardila et al.[118] used electrospun scaffolds with different gelatin/fibrinogen mass ratios and stimulated seeded SMCs with TGF-β2. First, they found that all scaffolds exhibited a similar level of cell migration through the entire scaffold depth. Second, the SMC proliferation was found to be higher at a mass ratio of 80:20 (gelatin:fibrinogen), but lower on a pure gelatin scaffold, indicating an important role played by the ECM structures. When the cultured scaffolds were exposed to lower concentrations of TGF-β2 (≤1 ng/mL), it was found that both cell proliferation and migration were promoted, while higher concentrations of TGF-β2 stopped cell replication and reduced migration. These findings correlated with the behavior of SMCs at different TGF-β2 concentrations,[53,57,119–121] demonstrating a straightforward method for switching the SMC phenotype in cultured scaffolds.

An alternative approach to biochemically stimulate SMCs is the use of peptides. Peptides are short amino acid chains that can mimic the functionality of whole proteins and can be conjugated to polymeric matrices for tissue engineering applications.[122] For example, elastin-like polypeptide-4 (EPL4), with a tropoelastin-like structure and function, has been used to functionalize electrospun polyurethane scaffolds to promote the proliferation and expression of contractile markers (α-SMA and SM-MHC).[123] Another example is the use of the RADA peptide to promote angiogenesis and myocardial infarction lesions *in vivo*.[124] Other angiogenic peptides also exhibit the efficacy in EC recruitment to angiogenesis: for example, QK and PAB2–1c can mimic the activities of VEGF and PDGF, respectively.[125–127] However, their potential as conjugates to polymeric scaffolds to modulate SMC phenotype has not been fully explored.

Finally, the incorporation of ECM macromolecules into scaffolds was also studied. For example, heparin was covalently linked to electrospun fibers,[34] and SMCs were found to be able to infiltrate scaffolds and exhibit higher expression of α-SMA, calponin, and SM-MHC. Later, Geng et al.[128] used a heparin/PCL scaffold with a hydrogel precursor for SMC modulation. The hydrogel-enriched scaffold exhibited an increased tensile strength/burst pressure and a higher suture strength than the pure PCL scaffold, but resulted in decreased SMC proliferation. Moreover, these scaffolds exhibited an excellent patency 6 months after implantation in Wistar rats. Additionally, histology confirmed complete SMC infiltration, collagen and elastin deposition, and mild calcification, while immunohistochemistry showed a potential SMC contractile phenotype. The co-localization of von Willebrand factor (EC specific) and α-SMA, as shown in fluorescence microscopy, further suggested that the cells populating the scaffold were of different origins or from distinct differentiation pathways.

Tuning of scaffold stiffness. SMCs are inherently mechanosensitive to the expression of several molecules, such as membrane receptors, integrins and ion channels.[129] The mechanical properties of biomaterials are fundamental to the modulation of SMC phenotypes. For example, Vatankhah et al.[130] studied how the stiffness of electrospun tecophilic/gelatin fibers influenced the phenotypical behavior of human aortic SMCs. This study showed that SMC phenotypic behavior is highly dependent on scaffold stiffness: lower stiffness led to a significant reduction in SMC proliferation than the control (i.e., tissue culture plate, no scaffold), while the stiffest scaffold (30/70 tecophilic/gelatin) achieved the same proliferation rate as the control.

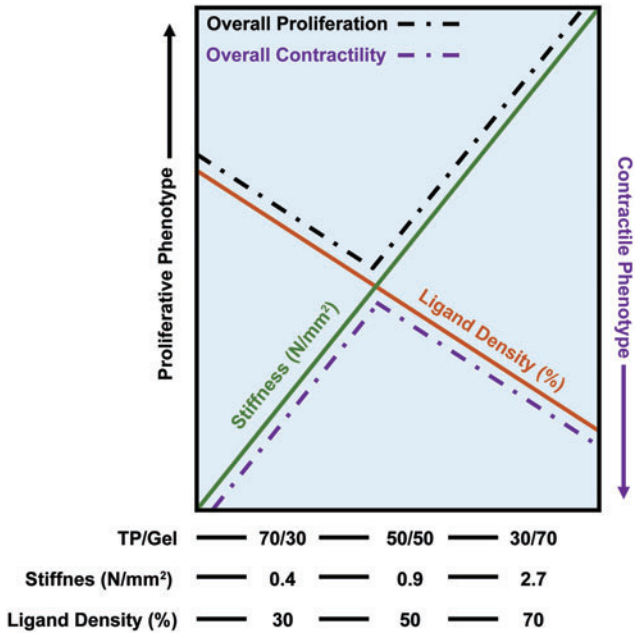

FIG. 5. Diagram of the interaction between ligand density and stiffness of the culture substrate observed in the tecophilic/gelatin electrospun scaffolds. Reprinted (adapted) from Vatankhah et al.[130] with permission from the American Chemical Society. Color images are available online.

Furthermore, contractile phenotype markers (α-SMA and SM-MHC) were more highly expressed in the stiffest scaffold, while the expression of synthetic markers (e.g., type I collagen deposition) was highest in the most compliant scaffold (70/30 tecophilic/gelatin; Fig. 5). They also coated the compliant scaffold with a gelatin layer, resulting in a more contractile phenotype, together with a reduction in cell proliferation.

On the other hand, Moussallem et al.[131] used poly (allylamine hydrochloride)/poly(acrylic acid) (PAH-PAA) multilayers to modulate the SMC phenotype. Their RT-PCR results showed that the expression of contractile markers was enhanced in the cross-linked scaffold. Consequently, the noncross-linked material had a higher expression of synthetic markers than its cross-linked counterpart, indicating a contractile phenotype with a stiffer substrate.

In addition, Yi et al.[132] examined the effects of electrospun fiber stiffness on SMC modulation using a poly(L-lactide-co-caprolactone)/poly(L-lactic acid) (PLLA-PCL) scaffold. They found that scaffold stiffness during electrospinning was directly proportional to the flow rate of the polymeric solution: that is, stiffer scaffolds promoted a higher rate of cell migration, which was confirmed by an increased expression of CD44.[133] Moreover, they showed that α-SMA expression was homogenous among all scaffold groups, but SM-MHC expression was reduced with increasing stiffness, indicating that the transition to a fully contractile phenotype was halted.

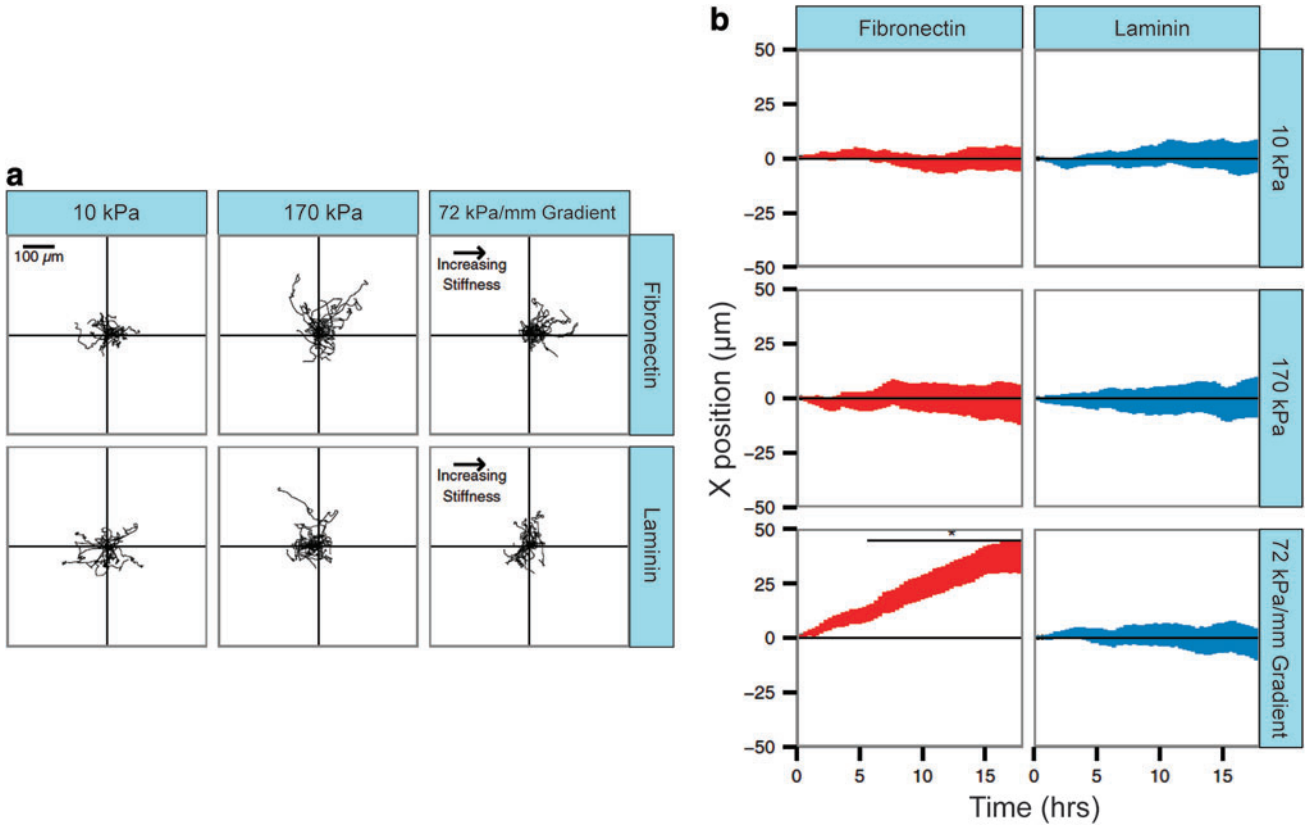

FIG. 6. **(a)** Migration tracks of vSMCs on two different substrates with constant (10 and 170 kPa) and varying (72 kPa/mm) stiffness. VSMCs show a movement toward increased stiffness gradients on fibronectin substrates, but not on laminin. **(b)** Average position of vascular SMCs for different substrates (fibronectin: *red* and laminin: *blue*) and stiffnesses. Reprinted (adapted) from Hartman et al.[141] with permission from Proceedings of the National Academy of Sciences. vSMCs, vascular smooth muscle cells. Color images are available online.

They also observed that osteopontin was upregulated in the stiffer scaffolds, indicating a switch of SMCs to a proinflammatory phenotype.[134,135] Other markers were also affected by the increased scaffold stiffness: (i) the reduction of desmin—a key structural component in the SMC microstructure[136] and (ii) the upregulation of interleukin 6 (IL-6), monocyte chemoattractant protein-1 (MCAP-1), and intercellular adhesion molecule 1 (ICAM-1)—markers observed in the macrophage-like SMC phenotype.[136–139]

As demonstrated in these studies, SMCs can undergo a migration process depending on stiffness gradients (a process known as *durotaxis*).[140] This process was described by Hartman et al.[141] as a substrate-dependent process. They cultured SMCs on polyacrylamide with stiffness gradients coated with different ECM components: fibronectin and laminin (Fig. 6a, b). First, they observed that stiffer gels without specific directionality or preferential ECM coating provoked increased migration velocities. However, when gradient-stiffness gels were prepared, the fibronectin-coated gels exhibited a marked directionality in cell migration.

These observations indicate not only that SMCs are uniquely sensitive to stiffness gradients but also that their migration processes are selective as a function of the ECM substrate. This group also showed that this ECM-specific durotactic behavior can affect the SMC phenotype: increasing laminin stiffness resulted in a more contractile phenotype (increased myosin light chain expression), while increased stiffness in fibronectin substrates promoted a

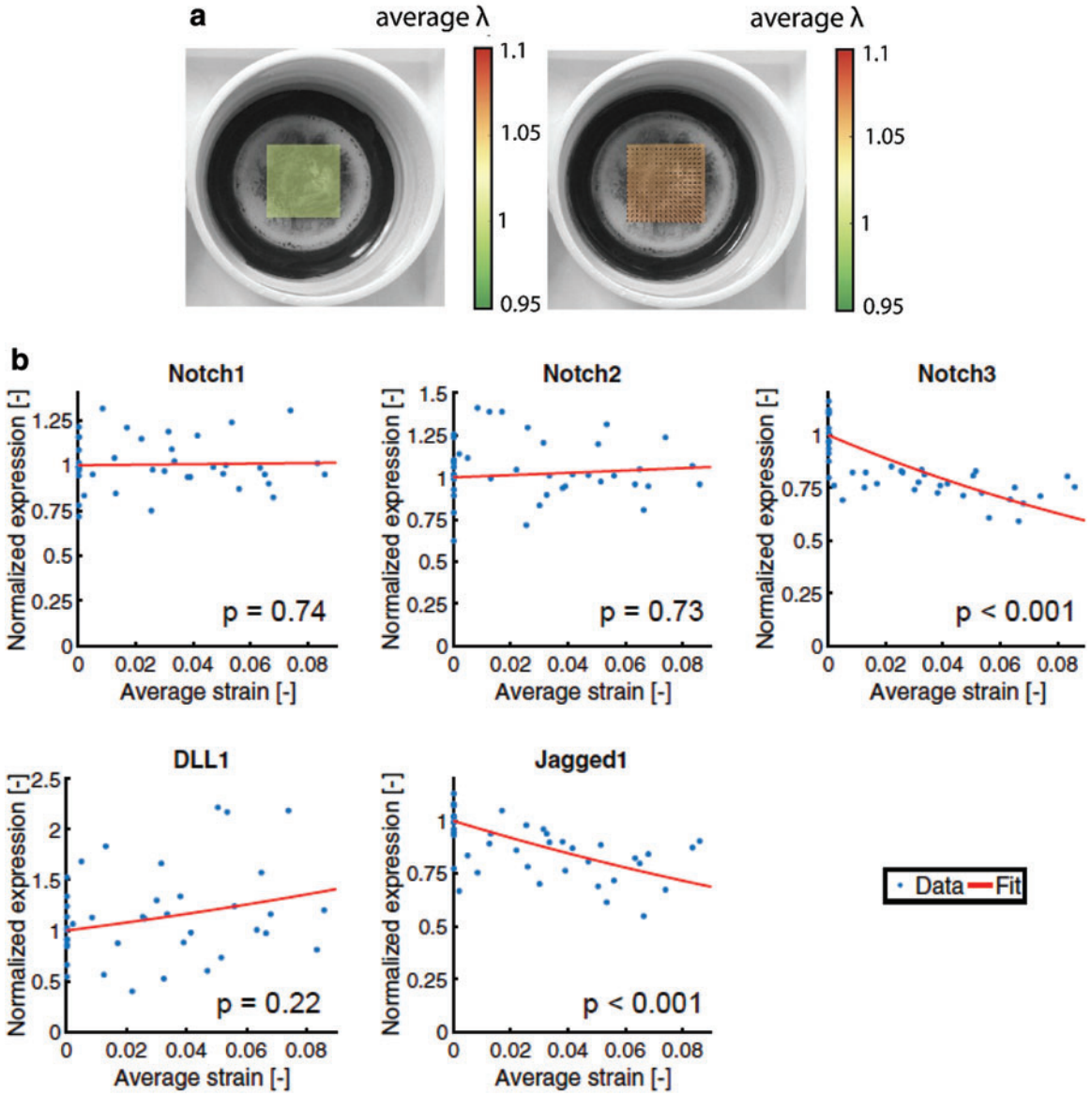

FIG. 7. **(a)** Digital image correlation to quantify stretch λ applied to elastic culture membranes on which the smooth muscle cells have been cultured. **(b)** Changes in Notch receptor expression and DLL1 and Jagged1 ligands as a function of average strain applied. Reprinted (adapted) from Loerakker et al.[151] with permission from Proceedings of the National Academy of Sciences. Color images are available online.

synthetic phenotype.[142] The observed behaviors may explain the increased rate of SMC migration and phenotype modulation during atherosclerosis *in vivo*.[143]

Mechanical stimuli through bioreactors. The SMC is subjected to different mechanical stimuli *in vivo*, including pulsatile pressure and shear stress.[55,144] These stimuli are transmitted between cells[145,146] and can alter gene expression.[147] Therefore, several VTE studies have aimed to modulate the SMC phenotype through mechanical stimuli. An important historical contribution was made by Niklason et al.,[23] where they used bioreactors to emulate physiological pulsatile conditions to stimulate cultured scaffolds. Since then, countless studies have emerged to understand the effects of mechanical stimuli on SMC phenotype expression. For example, Sharifpoor et al.[148] assessed SMC markers during culture on a polyurethane scaffold and reported that calponin expression increased under cyclic strain, accompanied by proliferation, and increased DNA concentration.

These results were ''*confounding*'' because these behaviors could indicate conflicting phenotypes. They later conducted longer-term studies that observed similar behaviors.[149] However, it is not uncommon to observe contradictory phenotypical behaviors *in vivo*: Pan et al.[150] described an *intermediate* SMC phenotype with inflammatory and synthetic behaviors in single-cell genomics and lineage tracing of mice and human atherosclerotic plaques.

Loerakker et al.[151] performed another interesting study of the effects of mechanical strain on SMCs, in which SMCs were cultured on flexible culture plates and subjected to mechanical strain (Fig. 7a). They focused on the Notch signaling pathway, which is crucial for the expression of contractile SMC markers.[56] They observed that mechanical strain ranging from 1% to 9% resulted in a decrease in the Notch 3 receptor (which facilitates PDGF-induced proliferation *in vivo*[152]) and Jagged1 ligand (promotes signaling to neighboring SMCs[152–155]), indicating that mechanical strain promotes the quiescent contractile phenotype (Fig. 7b).

In addition, Gong and Niklason[156] investigated the combined effects of mechanical strain and ECM components on SMC marker expression. They found that fibronectin, elastin, and type I collagen had a significant effect on reducing proliferation under cyclic strain. Furthermore, they observed that α-SMA expression decreased when cells were cultured on fibronectin, but increased when cultured on type I collagen, suggesting that the phenotypic response to mechanical stimuli is substrate dependent, similar to the findings of Hartman et al.[141] on the migratory behaviors of SMC. This proliferative behavior after short-term mechanical stimulation could be explained by the observations of Sun et al.,[157] where SMCs switched to a pro-inflammatory behavior upon shear stress. Nonetheless, there is currently no consensus on how mechanical stimuli affect the SMC phenotype in cultured scaffolds. See Jensen et al.[158] for a more comprehensive review on the mechanical stimulation for SMCs.

Effects of ECM substrates. In the previous sections, we discussed the modulation of SMC phenotype using different techniques (Fig. 3). These studies we reviewed also showed that SMC modulation depended on the presence/absence or concentration of ECM substrates. For example, the study by Geng et al.[128] demonstrated the use of heparin-based enrichment in PCL scaffolds; Ardila et al.[118] observed the effects of different gelatin:fibrinogen mass ratios on TGF-β2-dependent SMC modulation, and Hartman et al.[141] observed substrate- and stiffness-dependent cell migration. Therefore, it is also imperative that SMC modulation is not addressed as a ''one-modulator-only'' phenomenon, but as a complex adaptive mechanism of SMCs.

Conclusion

The modulation of the SMC phenotype is a complex and controversial topic. The implications of SMC modulation in atherosclerosis and vascular remodeling are still an emerging research topic. In this review, we have discussed various SMC phenotypes described in the literature, the modulators that can induce each phenotype, and the prevailing techniques in VTE for SMC modulation. VTE of small-diameter vessels still faces major challenges that correlate with the phenotypic expression of SMCs in the TEVGs. From this review, we concluded that the development of TEVGs must incorporate a more thorough assessment of the SMC markers and their maintenance/modulation. In particular, studies of the effect of material properties on the SMC phenotype can benefit from the modern lineage tracing and single-cell analysis techniques available today.[52,150]

These techniques can eventually help inform the design of biomaterial-based modulation methods for the SMC phenotype. Few studies have characterized the full spectrum of the SMC phenotype in response to their proposed stimuli for modulation. Therefore, we recommend that research focus on studying SMC modulation through a holistic combination of the methods presented in this review and benefit from their overall effects on SMC phenotype. For example, the combined use of biomolecules and topographical cues can help obtain dense SMC populations in TEVGs before contractility is induced and, thus, more desirable mechanical properties can be produced.

In particular, the use of peptides or growth factors can promote modulation toward a synthetic phenotype for full cell infiltration and high proliferation across scaffolds. Once these biochemical cues are depleted through degradation or loss of function, topographical cues (e.g., using highly aligned electrospun fibers) can cause the SMC to switch back to a contractile phenotype. With a thorough understanding of the SMC phenotype in response to these stimuli, translation of TEVGs may achieve the long-awaited promise of providing nonautologous grafts for coronary bypass.

Authors' Contributions

S.A.P.-C. contributed to conceptualization, literature search, writing the original draft, and design of in-house figures. H.A. contributed to conceptualization. M.S.D. and G.A.H. contributed to review and editing. C.-H.L. contributed to conceptualization, supervision, and funding acquisition. All authors read and approved the final article.

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