

Short Review

Biochemical and Biomechanical Myogenic Differentiation of Adipose-Derived Stem Cells for Tissue Engineering Applications

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Abstract

Tissue engineering has shown great promise in generating vascular grafts with properties similar to that of native blood vessels. Vascular Smooth Muscle Cells (VSMC) are the main component of the vasculature tunica media. Recreation of this layer represents a major challenge in tissue engineering due to difficulties in harvesting and culturing autologous VSMC. The use of stem cells and their inherent ability to differentiate into diverse cell types, including vascular lineages, have been proposed. Adipose-derived stem cells have attracted great interest in the field since they can be easily harvested using minimally-invasive procedures. VSMC can be differentiated from adipose-derived stem cells by supplementing cultures with different biochemical factors. Under physiological conditions, vascular cells are exposed to biomechanical forces generated by the heart beat and topological cues created by the extracellular matrix. Mimicking these conditions *in vitro* have also been shown to drive the myogenic differentiation of adipose-derived stem cells. Obtaining a fully mature vascular graft based on an autologous source of cells is the ultimate objective in tissue engineering field. Thus, identifying molecular mechanisms and optimizing conditions to generate VSMC is a major step prior to the deployment of this technology into the clinic. In this review, we highlight the recent advances focusing on the biochemical, biomechanical and topological cues that influence the differentiation of adipose-derived stem cells into VSMC.

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Introduction

Tissue engineering, the generation of tissue/organs from cells and supporting biomaterials, is a rapidly evolving research area that aims at providing alternatives to surgical reconstruction and organ transplants for the replacement of lost or severely damaged tissues and organs. Tissue engineering solutions are in great demand and currently expanding to all areas of healthcare including vascular and cardiac tissues, liver, bladder, bone, cornea, skin and cartilage among others. Although significant advances and innovations are being made in this field, multiple technical challenges still need to be overcome to create 'off-the-shelf' tissues for rapid implantation into patients; this includes selection of cell sources, scaffold production and optimization, cell growth under controlled environment in bioreactors and access to suitable animal models for preclinical studies.

Cardiovascular diseases are the leading cause of hospitalization worldwide, often requiring bypass surgery for treatment of ischemic heart and peripheral vascular diseases. Native vessels remain the gold standard in revascularization procedures; however, due to age, disease, or prior usage for bypass surgery, access to autologous arteries or veins may be restricted [1]. Tissue engineering has shown great promise in generating vascular grafts that mimic the native structure of blood vessels. This process requires the identification of suitable matrix materials and specific cell types to fully recreate the biochemical and biomechanical properties of native arteries and to promote integration in the host tissue without immunologic reaction.

Arteries have a tunica intima comprised of Endothelial Cells (EC), involved in regulating coagulation, immune cell trafficking, vascular tone, and angiogenesis, and a tunica media, composed of Vascular Smooth Muscle Cells (VSMC), collagen, elastin, and proteoglycans. VSMC possess a complex cytoskeleton, with structural and functional contractile proteins that play an important role in maintaining homeostasis and mechanical properties of the vasculature. The outermost layer of vessels is the tunica externa or tunica adventitia composed of connective tissue that provides structural support to the vessel.

In this review, we focus on the generation of the VSMC layer for vascular tissue engineering applications. Sources of VSMC include autologous cells obtained from the patient, allogeneic cells obtained from a human donor other than the patient, and xenogeneic cells from animal origin. Allogenic and xenogenic cells are immunogenic and require immunosuppressive therapy after implantation. Autologous cells are the preferable source for tissue engineering, but their isolation requires invasive biopsy that yields cells with low proliferative capacity that undergo a rapid loss of phenotype when cultured *in vitro* [2]. Alternatively, mesenchymal stem cells have shown great potential for regenerating vascular tissue due to their high proliferative capacity and high differentiation potential [3].

Differentiation of VSMC from various stem cell sources, including adipose-derived stem cells (ASCs), can be achieved using a combination of biochemical factors and biomechanical forces [4-6].

Origin and Isolation of Adipose-Derived Stem Cells

Adipose tissue, initially considered an organ for energy storage, has been re-discovered as a rich source of mesenchymal stem cells. Adipose-Derived Stem Cells (ASCs) express surface markers of mesenchymal stem cells such as CD29, CD36, CD73, CD90, CD105, whereas no CD34, CD106, HLA-DR or LIN is normally observed [7,8]. Since being isolated by Zuk et al., [9] from processed lipoaspirate, the therapeutic potential of ADSCs in the clinical setting has gained increasing attention [10,11]. ASCs are considered one of the most promising sources of stem cells for vascular tissue engineering due to the relatively easy accessibility, and their high abundance in the fat tissue [12,13]. For instance, the adipose tissue has a significantly higher stem cell density (~500-fold) when compared to bone marrow, another possible source of mesenchymal stem cells [14]. ASC multi-lineage potential has been demonstrated by their ability to differentiate towards multiple cell types, including as adipocytes, cardiomyocytes, endothelial cells, osteoblasts, chondrocytes and smooth muscle cells [13,14].

Adipose tissue can be divided into white (WAT) and brown (BAT) adipose tissue. Adipose-derived stem cells from WAT and BAT are characterized by a different expression of cell surface markers. For instance, ASCs from BAT express noticeably more TMEM-26, SSEA-4, CD106, CD105, HLA-A,B,C, and CD-137 but less CD86 and LIN, than WAT [15]. Subcutaneous WAT from the abdomen, thighs and arms is the most commonly used for ASCs isolation [16] with those obtained from thighs showing the best viability [17].

Phenotypic and Functional Characterization of Differentiated ASCs into VSMCs

Upon specific biochemical and biomechanical challenges, ASC acquire a VSMC phenotype that significantly differs that from the parental lineage. ASCs generated from fresh human lipoaspirates normally exhibit a fine elongated fibroblast-like morphology [5]. Following differentiation, cells acquire a spindle-like morphology and grow in a “hill and valley” pattern that closely resembles the morphology of VSMCs isolated from blood vessels.

In general, mature VSMCs demonstrate gene and protein expression of a set of characteristic contractile markers that include α -SMA, SM22 α , calponin, caldesmon, Smooth Muscle Myosin Heavy Chain (SMMHC), smoothelin, smooth muscle myosin heavy chain and myocardin [18,19]. These proteins are involved in processes that regulate actin and myosin attachment and smooth muscle contraction. Thus, confirming the expression of these contractile proteins, absent in the ASCs, allows to determine the level of VSMC differentiation of the parental cells. From these markers, α -SMA, SM22 α , calponin and caldesmon are considered early markers of progression towards a VSMC lineage during ASC differentiation [18]. SMMHC and smoothelin, in contrast, can only be identified in mature VSMC and mark the end stage of differentiation towards a contractile phenotype [18,20].

Blood vessel tonus is mainly controlled by the vascular smooth muscle excitation-contraction coupling in response to mechanical, humoral, or neural stimuli [21]. Depolarization of cells increases intracellular calcium concentration leading to activation of myosin light chain kinase by Ca^{2+} /calmodulin and consequent contraction. In VSMC differentiated from ASC, depolarization by hormonal regulation has been studied by single cell patch-clamp method. Electrophysiological analyses of currents mediated by numerous vasoactive

agents, indicated that current amplitudes in differentiated cells are similar to those observed in VSMC directly isolated from native vessels [22].

The contractibility of differentiated cells can also be assessed with the collagen lattice assay. This assay is performed by embedding cells into a collagen gel matrix on the bottom of a well plate. Prior to the contractile challenge, the gel is manually separated from the well bottom to loosen the gel from the plate walls and enable contraction [23]. The contractile forces generated by cells propagate throughout the collagen matrix and arrange collagen fibers to higher density structure leading to decreases in matrix volume. Measuring the decrease in size of a gel matrix by imaging and subsequent analysis provides a direct way to evaluate contractility. Acquisition of a contractile phenotype has been demonstrated in VSMC differentiated from ASC in response to depolarization by KCl, as well as to other vasoactive agents such as carbachol, noradrenaline, and vasopressin [5,24,25]. These results highlight not only the expression of the contractile machinery in differentiated cells but also the membrane expression of receptors involved in the excitation-contraction coupling typical of VSMC.

Biochemical Factors for Differentiating ASC into VSMC

ASC differentiation into VSMCs can be achieved with various factors and culture conditions. Supplementing growth media with high concentrations of fetal bovine serum (20%) can induce the expression of some molecular markers of VSMC in ASCs [26]. Notably, late markers of VSMC differentiation such as SM-MHC, were absent when cells were kept under these conditions. Other protocols described to obtain VSMC from ASC involve the activation of thromboxane A2-dependent pathways [6] or the treatment of cells with heparin [27]. Early studies also indicated that numerous agents that play a role in the development and physiology of the cardiovascular system such as Sphingosylphosphorylcholine (SPC), angiotensin II and bradykinin were able to induce the expression of VSMC markers in ASC [28-30]. A common denominator of these reports is the major role that autocrine secretion of transforming growth factor- β (TGF- β) played in the differentiation towards a smooth muscle-like phenotype.

TGF- β is a multifunctional cytokine that regulates numerous cell processes including differentiation, proliferation and expression of genes related to the smooth muscle cell function [31]. It has been reported that TGF- β 1 alone at the concentration of 10 ng/ml is capable of inducing the expression of molecular markers of smooth muscle cells in ASC [32]. Lower concentrations of TGF- β 1 are also effective when used in combination with other biochemical factors such as retinoic acid, and bone morphogenic protein 4 (BMP-4) [5,22,24,33]. Cells treated with 5 ng/mL of TGF- β 1 and 1 μ M retinoic acid for 1 week acquired the elongated and spindle-like cell shape that is characteristic of mature VSMC. This was accompanied by gene (α -SMA, SM22 α , calponin, caldesmon, SMMHC, smoothelin) and protein (α -SMA, calponin, SMMHC, smoothelin) expression of VSMC markers and acquired contractility in response to vasoactive agents such as norepinephrine and vasopressin [25]. Similar changes in morphology, expression of early, intermediate and late markers of VSMC differentiation, are observed when ASC are kept in medium supplemented with 5 ng/ml of TGF- β 1 and 2.5 ng/ml of BMP4 [5]. Recently, protocols investigating the long term differentiation effects of TGF β 1 and BMP4 [24], and a mixture of TGF β 1, BMP4, and ascorbic acid [33] have been reported. Whether ASC are continuously kept in differentiation media for up to 17 days [33], or differentiated

for 4 or 7 days and then transferred to regular growth media [24], molecular markers of VMSC are expressed and contractility was present throughout the time course of the studies. These results indicate that the differentiation process is stable and suitable for tissue engineering applications, especially considering the extended timeline required to obtain a fully mature vascular graft.

It should be noted that the use of growth factors is a significant challenge due to high cost and increased potential for contamination. Many of these factors have been shown to play a role in numerous pathological states. For instance, TGF β is strongly associated with cardiovascular diseases such as coronary artery disease and neo-intimal hyperplasia [34,35]. BMP4 induces ectopic bone formation *in vivo* and may be involved in vascular calcification [36]. These observations highlight the need to thoroughly characterize and standardize the differentiation process in order to minimize the exposure to biochemical factors. Moreover, identifying complementary methods that do not rely on the supplementation with exogenous agents may help the translation of these findings into the clinic [37].

Effect of Substrate Topography on ASC Differentiation

In addition to the exogenous supplementation of biochemical factors, a non-traditional strategies based on biomechanical and biomaterial cues that mimic the environment that mature VSMC are subjected to *in vivo*, and attempts to recreate the physiological vascular microenvironment with co-culture of different cell populations have emerged as promising alternatives in the field [5,20,33,38,39].

Surface topography has been shown to influence pivotal processes that are required for ASC differentiation such as cell adhesion, alignment, and differentiation. Cells grown on polydimethylsiloxane samples with nanometer and micrometer directional topography gradients suggest that a minimal topographic environment is required for optimal differentiation of ASC [39]. It has been determined that TGF β -induced differentiation of ASC was significantly reduced on topographies with a wavelength and amplitude smaller than 784 nm and 209 nm, respectively. Recently, a micro-grooved surface that closely mimics the topography of the tunica media in the vascular wall has been developed [40]. During differentiation, expression of α -actin and calponin was significantly higher in micro-grooved substrates when compared to cells grown on a smooth surface [40]. Not surprisingly, some types of substrates may also have negative impact on differentiation. Whereas a mixture of TGF β 1, BMP4 and SPC strongly induced the expression of molecular markers of VSMC differentiation on tissue culture plastic surface, this effect was significantly reduced when cells were incorporated into a fibrin scaffold [20]. The authors of this study proposed that this inhibitory effect on differentiation is due to reduced matrix-cell interactions involved in myogenic differentiation, highlighting the need to extensively characterize the scaffold of choice for tissue engineering applications.

Mechanical environment in which cells reside, mainly determined by the stiffness of the ECM can also modulate the differentiation of ASC. Upon induction of differentiation, the expression levels of α -actin and calponin are significantly upregulated in the ASCs cultured on stiff substrates when compared with those cultured on softer substrates [41]. It has been proposed that weak cell adhesion strength on soft substrates decreases the formation of stress fibers and expression of SMC markers [42]. Focal adhesions are key players in the mechanotransduction that influences the differentiation of ASC. It has

been shown that reducing the expression and maturation of focal adhesions by modulating the nanotopography of substrates is associated with decreased differentiation of ASC into VSMC [39,43]. Currently, there is a paucity in understanding the exact mechanisms governing the differentiation mediated by substrate topography. Furthermore, synthetic structures are unable to induce complete differentiation of ASC into VSMC. Nonetheless, the design of biomaterial topographies that improve the efficiency of this process is an exciting field in tissue engineering.

Effect of Mechanical Forces on ASC Differentiation

In the cardiovascular system, blood vessels are under a permanent mechanical load due to the pulsatile blood pressure which accounts for proper maturation of VSMC. Cells react to mechanical stimuli by cytoskeleton remodeling, activation of numerous signaling pathways, and modulation of gene and protein expression [44,45]. Tissue maturation of VSMCs is a complex process closely regulated by the interplay between biochemically and mechanically-activated pathways and thus, has a crucial impact on vascular engineering from stem cells [44]. A fully functional engineered vascular graft should form a layer with contractile VSMC phenotype, sufficiently matured to secrete extracellular matrix proteins.

Uniaxial strain has been shown to play a significant role in the differentiation of ASC. Applying uniaxial strain during TGF β 1-induced differentiation of ASC upregulates the expression of molecular markers of VSMC phenotype [46]. Cyclic stretch (1Hz, 10% amplitude) has been shown to increase the expression of early-markers of VSMC differentiation such as α -actin and SM22 α [47]. Moreover, mechanical stimulation not only promotes increased differentiation but also cell alignment, cytoskeletal arrangement and stress fiber generation. This phenotype is critical for obtaining mature VSMC capable of synthesizing ECM proteins with optimal force generation and contractile properties.

Deployment of perfusion bioreactors in tissue engineering of vascular grafts allows for optimal nutrient and oxygen transport to all cells forming the 3D scaffold, as well as for the generation of cyclic tension that mimic physiological conditions. It has become evident that this setup is optimal to achieve full ASC-derived VSMC maturation. The pulsatile perfusion in bioreactor systems results in circumferential alignment of cells and the cell morphology of mechanically stimulated ASC closely relates to that of smooth muscle cells with a contractile phenotype [20]. Collagen-I deposition, an indicator of extracellular matrix production, is only observed in mature VSMC, and is significantly induced by mechanical stimulation. Furthermore, expression of VSMC markers and contractility can be significantly enhanced by the combination of biochemical and biomechanical stimulation [20]. It should be noted that, although mechanical stimulation alone can induce the expression of early- and intermediate markers of VSMC differentiation, late contractile SMC phenotype can only be achieved when mechanical and biochemical stimulation are combined [20]. Therefore, biomechanical forces and biophysical cues can serve as a complement to biochemical induction approaches. These results underline the importance of combined biochemical and biomechanical stimulation for complete myogenic differentiation of ASC (Table 1).

Challenges

Despite great advances in the preclinical testing of cell-based tissue-engineered vascular grafts, few approaches have reached clinical

| Biochemical factors | Differentiation time | Biomechanical forces | Substrate topography | Markers of VSMC differentiation | Reference |
|--|----------------------|----------------------|--------------------------------|---|-----------|
| TGFβ1 (5 ng/mL) + BMP4 (2.5 ng/mL) | 7d | – | – | α-SMA, SM22a, calponin, and SM-MHC | [20] |
| TGFβ1 (5 ng/mL) + BMP4 (2.5 ng/mL) | 7d | – | – | α-SMA, SM22α, calponin and SM-MHC, electrophysiology | [22] |
| TGF-β1 (5 ng/mL) ± BMP4 (2.5 ng/mL) ± AngII (1 μM) | 7d | – | – | α-SMA, calponin, caldesmon, SM-MHC | [7] |
| TGFβ1 (5 ng/mL) + BMP4 (2.5 ng/mL) | 4d, 7d | – | – | α-SMA, SM22α, caldesmon, contraction | [24] |
| TGF-β1 (5 ng/mL) + Retinoic acid (1 μM) | 7d | – | – | α-SMA, SM22α, calponin, caldesmon, SM-MHC, smoothelin | [25] |
| TGF-β1 (10 ng/mL) | 7d | – | TCPS, PTMC, glass surface | α-SMA, SM22α and calponin, contraction | [32] |
| TGF-β1 (1 ng/mL) | 7d | – | Topography gradients | αSMA, SM22α | [39] |
| TGF-β1 (5 ng/mL) | 2, 6d | – | Micro-grooved | α-SMA, calponin and gelsolin | [40] |
| TGFβ1 (2.5 ng/mL) + BMP4 (2.5 ng/mL) + AA (150 μM) | Up to 17d | – | Modified poly(L-lactide) foils | αSMA, calponin, SM-MHC | [33] |
| – | 24h | Cyclic stretch | – | αSMA, SM22α | [47] |
| TGF-β1 (10 ng/mL) | Up to 24h | Cyclic stretch | – | α-SMA, SM22α, caldesmon and calponin | [46] |
| TSB | 8d | Pulsatile bioreactor | – | α-SMA, calponin and smoothelin, contraction | [20] |

Table 1: Summary of latest studies investigating the VSMC differentiation from ASC in the presence of biochemical factors, biomechanical forces and/or different substrate topographies.

AA (ascorbic acid), α-SMA (α-smooth muscle actin), TCPS (tissue culture polystyrene), TSB (TGF-β1 + Sphingosylphosphorylcholine + BMP-4), PTMC (poly(1,3-trimethylene carbonate)), SM-MHC (smooth muscle myosin heavy chain).

trials. A number of barriers still hinder the translation of this technology from bench to bedside. The necessity for *in vitro* culture under GLP conditions requires dedicated facility and trained personnel and is associated with high costs. The use of stem cells could lead to potential cellular transformation or contamination. Moreover, it has been shown that biochemical differentiation of ASC may be donor dependent [48], and that cryopreservation may have an impact on the differentiation of ASCs towards VSMC-like cells [25]. To achieve clinical safety, a rigorous *ex-vivo* characterization and refinement of ASC subgroups and optimization of cell characteristics, culture processes and differentiation protocols must be performed. Finally, pulsatile bioreactor perfusion systems are costly and sophisticated and the excess patient waiting time due to lengthy fabrication process needs to be addressed (Table 1).

Conclusion

Due to their significant role in vascular homeostasis, a viable source of autologous VSMCs that can be used in small diameter vascular graft engineering is an important step in enabling clinical translation of this technology. Autologous VSMCs are not easily obtainable and their *in vitro* expansion is limited. Differentiation of vascular smooth muscle cells from adipose-derived stem cells may overcome this problem. ASC are easily obtained and expanded, and their differentiation towards VSMC can be induced not only by biochemical factors, but also by biomechanical cues from the grafts themselves. The bench-to-bedside concept in autologous cell-based therapies has been gaining momentum and the recent insights into the mechanism involved in the differentiation of VSMC indicate that ASC are a potential source cells for the generation of tissue engineered vascular grafts.

References

- McKee JA, Banik SS, Boyer MJ, Hamad NM, Lawson JH, et al. (2003) Human arteries engineered *in vitro*. *EMBO Rep* 4: 633–638.
- Chang S, Song S, Lee J, Yoon J, Park J, et al. (2014) Phenotypic Modulation of Primary Vascular Smooth Muscle Cells by Short-Term Culture on Micropatterned Substrate. *PLoS ONE* 9: e88089.
- Buehrer BM, Cheatham B (2013) Isolation and Characterization of Human Adipose-Derived Stem Cells for Use in Tissue Engineering. *Methods Mol Biol* 1001: 1-11.
- Wang A, Tang Z, Li X, Jiang Y, Tsou DA, et al. (2011) Derivation of smooth muscle cells with neural crest origin from human induced pluripotent stem cells. *Cells Tissues Organs* 195: 5-14.
- Wang C, Yin S, Cen L, Liu Q, Liu W, et al. (2010) Differentiation of adipose-derived stem cells into contractile smooth muscle cells induced by transforming growth factor-beta1 and bone morphogenetic protein-4. *Tissue Eng. Part A* 16: 1201-1213.
- Kim MR, Jeon ES, Kim YM, Lee JS, Kim JH (2009) Thromboxane a(2) induces differentiation of human mesenchymal stem cells to smooth muscle-like cells. *Stem Cells* 27: 191-199.
- Elçin AE, Parmaksiz M, Dogan A, Seker S, Durkut S, et al. (2017) Differential gene expression profiling of human adipose stem cells differentiating into smooth muscle-like cells by TGFβ1/BMP4. *Exp Cell Res Mar* 352: 207-217.
- Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, et al. (2013) Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 15: 641-648.
- Zuk PA, ZhuM, Ashjian P, De Ugarte DA, Huang JI, et al. (2002) Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 13: 4279-4295.

10. Houtgraaf JH, den Dekker WK, van Dalen BM, Springeling T, de Jong R, et al. (2012) First experience in humans using adipose tissue-derived regenerative cells in the treatment of patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol* 59: 539-540.
11. Henry TD, Pepine CJ, Lambert CR, Traverse JH, Schatz R, et al. (2017) The Athena trials: Autologous adipose-derived regenerative cells for refractory chronic myocardial ischemia with left ventricular dysfunction. *Catheter Cardiovasc Interv* 89: 169-177.
12. Travnickova M, Pajorova J, Zarubova J, Krocilova N, Molitor M, et al. (2020) The Influence of Negative Pressure and of the Harvesting Site on the Characteristics of Human Adipose Tissue-Derived Stromal Cells from Lipoaspirates. *Stem Cells Int* 2020: 1016231.
13. Gimble J, Guilak F (2003) Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. *Cytotherapy* 5: 362-369.
14. Fraser JK, Wulur I, Alfonso Z, Hedrick MH (2006) Fat tissue: an underappreciated source of stem cells for biotechnology. *Trends Biotechnol* 24: 150-154.
15. Silva FJ, Holt DJ, Vargas V, Yockman J, Boudina S, et al. (2014) Metabolically Active Human Brown Adipose Tissue Derived Stem Cells. *Stem Cells* 32: 572-581.
16. Si Z, Wang X, Sun C, Kang Y, Xu J, et al. (2019) Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. *Biomed Pharmacother* 114: 108765.
17. Tsekouras A, Mantas D, Tsilimigras ID, Moris D, Kontos M, et al. (2017) Comparison of the Viability and Yield of Adipose-Derived Stem Cells (ASCs) from Different Donor Areas. *In Vivo* 31: 1229-1234.
18. Owens GK, Kumar MS, Wamhoff BR (2004) Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* 84: 767-801.
19. Fisher SA (2010) Vascular smooth muscle phenotypic diversity and function. *Physiol Genomics* 42: 169-187.
20. Helms F, Lau S, Klingenberg M, Aper T, Haverich A, et al. (2020) Complete Myogenic Differentiation of Adipogenic Stem Cells Requires Both Biochemical and Mechanical Stimulation. *Ann Biomed Eng* 48: 913-926.
21. Webb RC (2003) Smooth muscle contraction and relaxation. *Adv Physiol Educ* 27(1-4): 201-206.
22. Zhou R, Zhu L, Fu S, Qian Y, Wang D, et al. (2016) Small Diameter Blood Vessels Bioengineered From Human Adipose-derived Stem Cells. *Sci Rep* 6: 35422.
23. Ngo P, Ramalingam P, Phillips JA, Furuta GT (2006) Collagen gel contraction assay. *Methods Mol Biol* 341: 103-109.
24. Yogi A, Rukhlova M, Charlebois C, Tian G, Stanimirovic DB, et al. (2021) Differentiation of Adipose-Derived Stem Cells into Vascular Smooth Muscle Cells for Tissue Engineering Applications. *Biomedicines* Jul 9: 797.
25. Zhang X, Simmons CA, Santerre JP (2018) Alterations of MEK1/2-ERK1/2, IFN γ and Smad2/3 associated Signalling pathways during cryopreservation of ASCs affect their differentiation towards VSMC-like cells. *Stem Cell Res* 32: 115-125.
26. Heydarkhan-Hagvall S, Schenke-Layland K, Yang JQ, Heydarkhan S, Xu Y, et al. (2008) Human adipose stem cells: a potential cell source for cardiovascular tissue engineering. *Cells Tissues Organs* 187: 263-274.
27. Rodríguez LV, Alfonso Z, Zhang R, Leung J, Wu B (2006) Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. *Proc Natl Acad Sci U S A* 103:12167-12172.
28. Jeon ES, Moon HJ, Lee MJ, Song HY, Kim YM, et al. (2006) Sphingosylphosphorylcholine induces differentiation of human mesenchymal stem cells into smooth-muscle-like cells through a TGF-beta-dependent mechanism. *J Cell Sci* 119(Pt 23): 4994-5005.
29. Kim YM, Jeon ES, Kim MR, Jho SK, Ryu SW, et al. (2008) Angiotensin II-induced differentiation of adipose tissue-derived mesenchymal stem cells to smooth muscle-like cells. *Int J Biochem Cell Biol* 40: 2482-2491.
30. Kim YM, Jeon ES, Kim MR, Lee JS, Kim JH (2008) Bradykinin-induced expression of alpha-smooth muscle actin in human mesenchymal stem cells. *Cell Signal* 20: 1882-1889.
31. Mack CP (2011) Signaling mechanisms that regulate smooth muscle cell differentiation. *Arterioscler Thromb Vasc Biol* 31: 1495-1505.
32. Chen R, Dean D (2017) Mechanical properties of stem cells from different sources during vascular smooth muscle cell differentiation. *Mol Cell Biomech* 14: 153-169.
33. Travnickova M, Kasalkova NS, Sedlar A, Molitor M, Musilkova J, et al. (2021) Differentiation of adipose tissue-derived stem cells towards vascular smooth muscle cells on modified poly(L-lactide) foils. *Biomed Mater* 16: 025016.
34. Low EL, Baker AH, Bradshaw AC (2019) TGF β , smooth muscle cells and coronary artery disease: a review. *Cell Signal* 53: 90-101.
35. Tsai S, Hollenbeck ST, Ryer EJ, Edlin R, Yamanouchi D, et al. (2009) TGF-beta through Smad3 signaling stimulates vascular smooth muscle cell proliferation and neointimal formation. *Am J Physiol Heart Circ Physiol* 297: H540-H549.
36. Yang P, Troncone L, Augur ZM, Kim SSS, McNeil ME, et al. (2020) The role of bone morphogenetic protein signaling in vascular calcification. *Bone* 141:115542.
37. Wang C, Cen L, Yin S, Liu Q, Liu W, et al. (2010) A small diameter elastic blood vessel wall prepared under pulsatile conditions from polyglycolic acid mesh and smooth muscle cells differentiated from adipose-derived stem cells. *Biomaterials* 31: 621-630.
38. Lee MJ, Kim MY, Heo SC, Kwon YW, Kim YM, et al. (2012) Macrophages regulate smooth muscle differentiation of mesenchymal stem cells via a prostaglandin F $_{2\alpha}$ -mediated paracrine mechanism. *Arterioscler Thromb Vasc Biol* 32: 2733-2740.
39. Liguori GR, Zhou Q, Liguori TTA, Barros GG, Kühn PT, et al. (2019) Directional Topography Influences Adipose Mesenchymal Stromal Cell Plasticity: Prospects for Tissue Engineering and Fibrosis. *Stem Cells Int* 2019: 5387850.
40. Abolhasani S, Rajabibazl M, Khani MM, Parandakh A, Hoseinpoor R (2020) The cooperative effects of micro-grooved topography and TGF- β 1 on the vascular smooth muscle cell contractile protein expression of the mesenchymal stem cells. *Differentiation* 115: 22-29.
41. Parandakh A, Anbarlou A, Tafazzoli-Shadpour M, Ardeshiryajimi A, Khani MM (2019) Substrate topography interacts with substrate stiffness and culture time to regulate mechanical properties and smooth muscle differentiation of mesenchymal stem cells. *Colloids Surf B Biointerfaces* 173: 194-201.
42. Park JS, Chu JS, Tsou AD, Diop R, Tang Z, et al. (2011) The effect of matrix stiffness on the differentiation of mesenchymal stem cells in response to TGF- β . *Biomaterials* 32: 3921-3930.
43. Teo BK, Wong ST, Lim CK, Kung TY, Yap CH, et al. (2013) Nanotopography modulates mechanotransduction of stem cells and induces differentiation through focal adhesion kinase. *ACS Nano* 7: 4785-4798.
44. Xing F, Li L, Zhou C, Long C, Wu L, et al (2019) Regulation and Directing Stem Cell Fate by Tissue Engineering Functional Microenvironments: Scaffold Physical and Chemical Cues. *Stem Cells Int* 2019: 2180925.
45. Qi YX, Han Y, Jiang ZL (2018) Mechanobiology and Vascular Remodeling: From Membrane to Nucleus. *Adv Exp Med Biol* 1097: 69-82.
46. Rashidi N, Tafazzoli-Shadpour M, Haghhighipour N, Khani MM (2018) Morphology and contractile gene expression of adipose-derived mesenchymal stem cells in response to short-term cyclic uniaxial strain and TGF- β 1. *Biomed Tech (Berl)* 63: 317-326.

47. Rabbani M, Tafazzoli-Shadpour M, Shokrgozar MA, Janmaleki M, Teymouri M (2017) Cyclic Stretch Effects on Adipose-Derived Stem Cell Stiffness, Morphology and Smooth Muscle Cell Gene Expression. *Tissue Eng Regen Med* 14: 279-286.
48. Lau S, Klingenberg M, Mrugalla A, Helms F, Sedding D, et al. (2019) Biochemical Myogenic Differentiation of Adipogenic Stem Cells Is Donor Dependent and Requires Sound Characterization. *Tissue Eng Part A* 25: 936-948.



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