https://doi.org/10.1016/j.bbadis.2019.165645

The Interplay of Signaling Pathway in Endothelial Cells–Matrix Stiffness Dependency with Targeted-Therapeutic Drugs

Vicki Vania[1], Lu Wang[1], Marco Tjakra [1], Tao Zhang[1], Juhui Qiu[1]*, Youhua Tan[2], Guixue Wang[1]*

[1] Key Laboratory for Biorheological Science and Technology of Ministry of Education, State and Local Joint Engineering Laboratory for Vascular Implants, Bioengineering College of Chongqing University, Chongqing, 400030, China.

[2]The Hong Kong Polytechnic University Shenzhen Research Institute, Shenzhen, China; Department of Biomedical Engineering, The Hong Kong Polytechnic University, Hong Kong, China.

*Correspondence to: Prof. Guixue Wang, Email: wanggx@cqu.edu.cn; Dr. Juhui Qiu, Email: jhqiu@cqu.edu.cn

Abstract

Cardiovascular disease (CVD) has been one of the major causes of human deaths in the world. The study of CVD has focused on cell chemotaxis for decades. With the advances in the field of mechanobiology, accumulating evidence has demonstrated the influence of mechanical stimuli on arterial pathophysiology and endothelial dysfunction that is a hallmark of atherosclerosis development. An increasing number of drugs have been exploited to decrease the stiffness of vascular tissue for CVD therapy. However, the underlying mechanisms have yet to be explored. This review aims to summarize how matrix stiffness mediates atherogenesis through various important signaling pathways in endothelial cells and cellular mechanophenotype, including RhoA/Rho-associated protein kinase (ROCK), mitogen-activated protein kinase (MAPK), and Hippo pathways. We also highlight the roles of putative mechanosensitive non-coding RNAs in matrix stiffness-mediated atherogenesis. Finally, we describe the usage of tunable hydrogel and its future strategy to improve our knowledge underlying matrix stiffness-mediated CVD mechanism.

Keywords: Cardiovascular disease, matrix stiffness, endothelial cell, RhoA/ROCK, MAPK, Hippo pathway

Outline

1. Introduction

Cardiovascular disease (CVD) has become a major cause of human death in almost every region of the world, including atherosclerosis and pulmonary hypertension. There were 422.7 million of diagnosed cases and 17.92 million of patient deaths with CVD in 2015 [1]. Therefore, the development of novel strategies is highly desirable for the effective CVD therapy. Previous research has focused on the exploration of systemic risk factors and molecular mechanisms underlying CVD, including cell chemotaxis in the development of atherosclerosis. Recent evidence has demonstrated strong association between mechanical stimuli and arterial pathophysiology [2].

An artery consists of three layers; tunica intima, media, and adventitia that contain endothelial cells (ECs), smooth muscle cells (SMCs), and fibroblasts in each layer, respectively. These cells have different responses to distinct micro-environmental stimuli, including biological, chemical, and physical stimuli. The influence of biochemical cues on CVD has been extensively investigated [2], while there are few concerning the roles of physical or mechanical cues. Nowadays, increasing evidence has demonstrated that mechanical stimulus should be considered as one of the important factor to arterial diseases [3]. There are mainly two types of mechanical stresses in blood vessels: shear stress mainly on tunica intima and axial stress in both tunica media and adventitia (Figure 1) [4,5]. These mechanical stresses may affect cellular functions and induce pathophysiological phenotypes in arteries [4]. Many findings have demonstrated that disrupted shear stress promotes local lipid accumulation and the progression of stable plaques with high-risk atherosclerotic lesions [6]. Low and/or oscillatory shear stress alters circumferential stress and leads to the increased endothelial permeability and thickened intima, which are the early symptoms of atherosclerosis disease [7,8].

In the past several decades, accumulating evidence shows the roles of matrix stiffness in vascular diseases [3]. In facts, several therapeutic drugs have been designed to reduce vascular stiffness for the treatment of CVD. ECs have been recognized as mechanosensitive cells and play a vital role in the early development of atherogenesis behavior. ECs dysfunction has been reported to alter the homeostasis, thrombosis, vascular tone, and inflammatory responses [9]. However, the molecular mechanisms underlying matrix stiffness-mediated ECs dysfunction are not completely understood. Therefore, a better understanding of the significant roles of matrix stiffness in vascular diseases is necessary for the development of novel

strategies against these diseases. Here, we aim to summarize the key matrix-stiffness-dependent signaling in EC dysfunction and CVD progression.

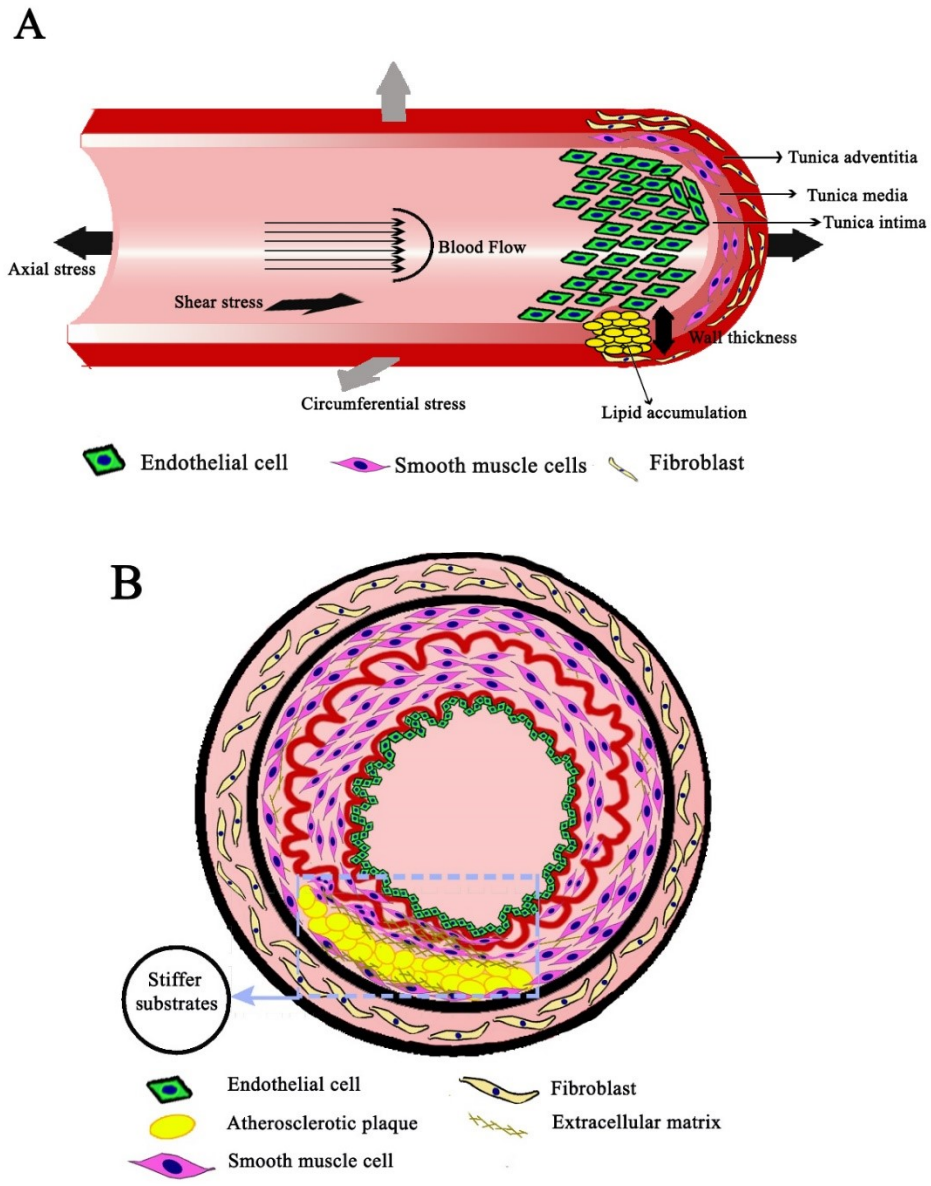

Figure 1 A) Different type of mechanical stress inside blood vessel. There are 3 main types of mechanical forces in blood vessel; shear stress, circumferential stress, and axial stress. The great role of shear stress in tunica intima not only promotes lipid accumulation, but also affects the circumferential stress in tunica media and adventitia as well as axial stress that corresponds to the cell-matrix interaction. B) Pathophysiological condition in layers of blood vessel. The lipid accumulation may become atherosclerosis plaque, which induces ECM remodeling and press the VSMCs in tunica media and the ECs, thus create a stiffer substrate for both VSMCs and ECs.

2.Why vascular stiffness is important?

The aortic stiffness has been reported to increase periodically in patients with hypertension and atherosclerosis. The tight association between vascular stiffness and vascular diseases may

suggest that vascular stiffness could be an important risk factor of CVD [3]. Change of aortic stiffness correlates with aortic blood pressure and blood flow, thus affecting cellular functions [10]. Despite the role of aging in the reduction of vascular elasticity, vascular diseases such as atherosclerosis have been found to associate with arterial stiffness. The large amount of cholesterol in atherosclerotic vessel triggers several pro-inflammatory molecules and induces plaque formation and collagen deposition [11,12]. Arterial stiffness is partially altered by the loss of elastin fibers within collagen deposition in the arterial wall. The change of elastin and collagen ratio induces extracellular matrix (ECM) remodeling that can be sensed by the residing cells [13–15]. Cells have the ability to sense and respond to mechanical cues by converting them into biochemical signals through mechanotransduction. These altered intra- and intercellular signaling may affect many cellular functions, including cell proliferation, migration, morphological shift [14], actin polymerization, and cellular contractility. As a result, blood vessels become thicker and stiffer compared to the healthy condition (Table 1). It has been reported that matrix stiffness has an important reciprocal role in many mechanisms that lead to atherosclerosis, including ECM rearrangement, calcification, and activation of several signaling pathways to pathologic cell fate [16,17].

Table 1 Elasticity value from multiple part of blood vessel in mammals

Source	Tissue types	Condition	Layer type in blood vessel	Young modulus (kPa)	References
Porcine	Aorta	Healthy	Tunica adventitia	25.8	[18]
Human	Artery	Healthy	Tunica Media	10.7 ± 3.3	[19]
		Advance atherosclerosis	Tunica Media	15.5 ± 3.6	
Mice	Aorta	Young	Tunica intima	6.8±0.5	[20]
		Aging	Tunica intima	17±2	
Mice	Artery	Atherosclerosis	Tunica intima	58.3±6.3	[21]
Human	Femoral artery	Healthy	Tunica intima	34.4	[11]
Sheep	Aorta	Healthy	Tunica media	42.9	[22]
		Aging	Tunica media	113.9	[22]
Mice	Pulmonary vessel	Healthy	Tunica adventitia	41.1±8	[23]

	Fibrosis	Tunica adventitia	105.2±13.6	[23]

3. Can therapeutic drugs target vascular stiffening?

ECM stiffness influences the mechanophenotype of ECs that further leads to the progression of CVD disease. Recently, several new drugs were discovered to target the key molecules underlying vascular stiffening and treat CVD progression, many of which have been tested in pre-clinical and clinical trials and some have been approved by FDA [24]. Here, we summarized the development of putative therapeutic drugs to treat CVD-dependent matrix stiffening.

Previously, the systemic factors such as hypercholesterolemia were targeted by decreasing the level of low density lipoprotein cholesterol (LDL-C) in order to treat CVD [25]. Statin (HMG-CoA reductase inhibitor) was initially known as a drug for lowering systematic cholesterol level [24]. Meta-analysis study has shown that short-term statin therapy can prevent and recover arterial stiffness compared to placebo in CVD patients [26]. Human clinical studies have shown the strong effect of statin family (such as simvastatin and atorvastatin) on arterial stiffness in atherosclerosis and coronary artery disease (Table 1). The safety of statin has been reported for any age but its efficacy for elders over 75 years is moderate [27]. It has been shown that statin increases the level of Rac1 expression and inhibits matrix stiffness-mediated RhoA activation [28,29]. Besides, statin can inactivate YAP/TAZ [28]. However, the mechanism underlying the repressive effects of statin on matrix stiffness-mediated atherosclerosis has not been completely understood.

Table 2 The latest update on clinical trials for CVD therapeutic drugs

Drug	Phase	Efficacy assessment	Outcomes	NCT number	Ref.
Losmapimod	3	To cardiovascular outcomes in patients hospitalized with acute myocardial infarction	Losmapimod does not have significant effect to reduce risk major ischemic cardiovascular compared to placebo	NCT02145468	[30]
	2	To cardiovascular outcomes in patients with myocardial infarction Non ST elevation myocardial infarction (NSTEMI)	Losmapimod reduce the infarct size, reduce hsCRP but not troponin I compared to placebo group	NCT02931188	[31]
Anacetrapib	N/A	To vascular function and arterial stiffness	N/A	NCT02931188	
	3	To reduce major coronary events	Lower incidents of major coronary events compared to placebo (atrovastatin)	NCT01252953	[32]

Simvastatin +Ezetimibe	4	To compare the efficacy of Ezetimibe-simvastatin combination with doubling dose of statin for lowering the LDL-C	High efficacy for mitigating LDL-C parameter	NCT00166530	[33]
Simvastatin (Zocor)	4	To compare high dose monotherapy of statins compared to the combination of low dose ezetimibe and statin	High-dose statin monotherapy has greater efficacy to represses ROCK activity and endothelial function.	NCT00560170	[34]
Atorvastatin + Ezetimibe	3	LDL-C level in hypercholesterolemia patients ≥65 years	Combination of Atorvastatin + Ezetimibe has greater efficacy to reduce LDL-C level	NCT00418834	[35]
Fasudil	2	To vascular function in patients with atherosclerosis and hyperlipidemia	N/A	NCT00120718	
Ticagrelor	3	To prevent cardiovascular events in patients with myocardial infarction in patients with prior heart attack	Ticagrelor reduces the MI consistently among subtypes and sizes including large MIs and ST-segment elevation MI	NCT01225562	[36]
	3	To compare Ticagrelor and Clopidogrel for prevention of major adverse cardiovascular events in patients with peripheral artery disease	Ticagrelor associated with lower rate of ischemic and all cause of stroke compared to Clopidogrel	NCT01732822	[37]

Pharmacological prevention of CVD has focused on decreasing LDL level and designing drugs to increase high density lipoprotein (HDL) level. The inhibition of cholesteryl ester transfer protein (CTEP) is known to enhance the level of HDL cholesterol and thus contribute to the attenuation of CVD risk. Anacetrapib has been reported as an inhibitor of CTEP and significantly increases the level of HDL-C [38]. As the result, lowering LDL decreases arterial stiffness and atherosclerotic heart disease phenotype. Together with statin treatment in human clinical trial, Anacetrapib successfully reduces cardiovascular risk and is currently being tested in phase III clinical trial. Administration of Anacetrapib has no detectable side effect in treatment period for over 18 months [39]. Nevertheless, the underlying molecular mechanism remains unclear.

The current treatment strategies against vascular diseases are to reduce the systemic factor and inhibit key molecules involved in the disease progression. Fasudil, a Rho signaling inhibitor, has been considered as a promising drug for CVD treatment and has excellent efficacy in several animal models, such as rabbit, porcine, dog, mouse, and rat. These treatments strongly reduce arterial stiffness and endothelial dysfunction [40]. Intraperitoneal administration of Fasudil for 4 weeks prevents pulmonary vascular remodeling in end-stage of rat pulmonary

hypertension due to left heart disease (PH-LHD) model. This efficacy is supported by the reduction of endothelin-1 (ET-1) and endothelin A receptor together with the escalation of NO production [41]. To date, Fasudil has entered phase II human clinical trial for atherosclerosis treatment [40]. It also has been approved for CVD medication in human since it shows minor side effects [42]. Another drug, Losmapimod, has been found to significantly reduce atherosclerosis-dependent key molecule as well as p38 MAPK signaling [43]. Although Losmapimod has shown insignificant results during phase III trial [30], a thorough mechanistic study is needed to evaluate the connection between Losmapimod with other important molecules in p38 MAPK signaling pathway. It is well known that Ticagrelor antagonizes P_2Y_{12} receptor and prevents acute coronary syndrome (ACS) events. It suppresses the ADP-induced platelet aggregation and leads to attenuation of thrombus formation and occlusion of coronary artery [44]. Recently, the efficacy of Ticagrelor in human has been evaluated, which reduces vascular stiffness within 24 hours, followed by suppression of atherosclerosis symptoms [45]. However, the underlying mechanism remains unclear. Currently, Ticagrelor has entered clinical trial phase III to reduce myocardial infarction in CVD patients [36].

The molecular mechanisms underlying matrix stiffness-mediated vascular disease have been widely investigated, including several putative target drugs against YAP. Verteporfin has been found to inhibit YAP-TEAD interaction with miR-130/301 and interfere mouse pulmonary hypertension [46]. However, the relevant clinical trial to attenuate CVD risk has not been completed yet. The combination of LOX inhibitor with YAP/TAZ-targeting drugs has been proposed to prevent pulmonary hypertension [47]. LOX inhibitor, such as β-aminopropionitrile (BAPN), is also considered as a promising drug for CVD treatment. LOX is able to crosslink ECM such as collagen and elastin followed by induction of vascular remodeling into pathological condition. BAPN blocks LOX and LOXL type irreversibly and represses collagen deposition and ECM remodeling, which is one of CVD phenotypes in human cells [48,49]. BAPN inhibits the crosslinking of collagen fibers, reduces arterial stiffness, as well as attenuates atherosclerotic lesion area by ~50% and macrophage adhesion in ApoE null mice [50]. However, the high toxicity and low efficacy of BAPN administration should be considered, such as lathyrism as the side effect [24,51].

Meanwhile, ncRNAs have the potential as prospective drugs against CVD diseases. Many studies have demonstrated promising efficacy of therapeutic drugs targeting mechanosensitive miRNAs in preclinical and clinical trials [52,53]. However, the clinical trials against mechanosensitive miRNA for atherosclerosis treatment have not been conducted yet. Several

ncRNAs drugs that target miR-21, miR-145, and miR-181 have been tested in animal models. Ma *et al.* (2016) has demonstrated the potential of miR-181b to reduce the potency of atherosclerosis. E-selectin nanoparticles coated with miR-181b can be delivered directly to atherogenic lesions and reduce atherosclerotic plaque size [54]. miRNA inhibitor/antagomir have been utilized by adding synthetic RNA that complements the targeted miRNA and modifies with 2'-O-methyl (2'-OMe) to enhance cellular uptake. Antagomir against miR-21 has been found to reduce cardiac fibrosis in mice with transverse aortic constriction (TAC) and suppress neointima formation in rat with balloon-carotid injury model [55].

Other pharmacological approaches involve nitric oxide (NO)-dependent pathways, antioxidants, RAAS inhibitor, TGF-B inhibitor, and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA)-reductase inhibitor. RAAS activation is induced by escalation of AT2 synthesis, leading to NO reduction, which can be reversed by RAAS inhibitor with improved efficacy [56]. Taken together, accumulating evidence in matrix stiffness-mediated CVD has discovered several putative mechanosensitive molecules as promising targets. Therefore, it is necessary to strengthen our understanding to the molecular mechanisms underlying matrix stiffness-mediated CVD.

4. How does matrix stiffness influence molecular signaling and cell phenotype?

It is known that matrix stiffness regulates EC proliferation, migration, and angiogenesis that are closely related to atherogenesis [56]. At the molecular level, the change of mechanical properties alters the interaction between ECM and their receptor [57]. It is indispensable to elucidate the mechanisms underlying the alterations in matrix stiffness, including intra- and inter-cellular dependent signaling in EC. Cells sense and respond to micro-environmental mechanics through mechanosensitive proteins on cell membranes, including integrin [25]. As the result of the myriad mechanisms, cells modulate the contractility and transmit the force sensing from ECM by outside-in signaling [57]. However, the regulation of matrix stiffness-mediated cellular activity shift is still limited.

4.1. The role of integrin in matrix stiffness sensing

Generally, integrin is a heterodimer consisting of α and β subunits located on different chromosomes. In human, integrin family comprises of 24 αβ heterodimeric members that mainly mediate cell-matrix interactions. Both α and β subunits are transmembrane proteins with multiple domains that act in bidirectional signaling and mediate cytoskeletal interactions. Different types of integrin have different affinity against different types of ECM ligands [25].

Only 8 out of 24 integrins are able to recognize RGD motif, a general integrin-binding motif/ligand that is also found in most of ECM ligands [58].

As a transmembrane protein, integrin is able to transduce the extracellular signals across cell membrane and then into cell interior, which is referred as outside-in signaling. Upon the attachment of a cell to the substrate, integrins are stimulated with ECM ligands and clustered in response to micro-environmental stiffness [59]. The cytoplasmic domain of integrin binds cytoskeleton through focal adhesion molecules, such as talin, vinculin, paxillin, etc [25]. This binding cascade thus promotes the translocation of talin and kindlin to β-integrin at cytoplasmic tails, leading to integrin activation and increases its' affinity to bind with extracellular ligands [60,61]. These events are termed as inside-out signaling [61]. In addition, outside-in signaling is released in the condition with high affinity integrin. Together with cytoskeleton, focal adhesion (FA) molecules are accumulated and influence intracellular signaling pathways [60,62]. As a result, tyrosine kinase-mediated molecules, such as FAK and Src, are activated. FAK becomes phosphorylated at Tyr397 residues that expose the binding site for Src-homology 2 (SH2) domain [24,63]. Together with vinculin and talin, FAK/Src also mediates p130cas activation with the adaptor protein Crk [64]. At the same time, FAK/Src complexes also phosphorylate nascent-adhesion adaptor protein paxillin, paxillin kinase linker (PKL), and Pak-interacting exchange factor-beta (β-PIX). These complexes secrete Crk to activate Dock180 -motility 1 and ELMO1 that are Rac-specific GEF to promote the formation of nascent focal complexes. β-PIX also produces Cdc42 [65]. Taken together, integrin-mediated mechanotransduction signaling involves RhoA/ROCK pathway as described below.

4.2. RhoA/ROCK pathway

RhoA/ROCK pathway acts as a transducer of mechanical signals in integrin-mediated FAK/Src activation to regulate cellular functions, including cell migration, stress fiber formation, contractility, and membrane protrusion that lead to CVD progression [66]. The major molecules in RhoA/ROCK pathway are Rho GTPase family proteins, such as Rho, Rac1, and Cdc42, which have pivotal roles in cellular process. In general, RhoA and Rac1 antagonize each other and their activities should be in balance to achieve homeostasis [67]. However, the phosphorylation of RhoA is increased in the pathological condition with stiffened microenvironment until the abundance of RhoA surpasses Rac1. RhoA activates the effector protein Rho-associated coiled-coil-containing kinase (ROCK). Furthermore, ROCK promotes myosin II activity by activating myosin light chain kinase (MLCK) that corresponds to actin contractility and stress fiber formation[68]. RhoA also plays a role in FilGAP phosphorylation

at Ser402 residues and phosphatase and tensin homolog (PTEN) that enables actin polymerization [65]. The matrix stiffness-activated myosin II restricts migration and branch initiation in 2D and 3D conditions. Meanwhile, ROCK phosphorylates FilGAP and then activates MAP kinase that contributes to ERK and JNK signaling pathway [69,70].

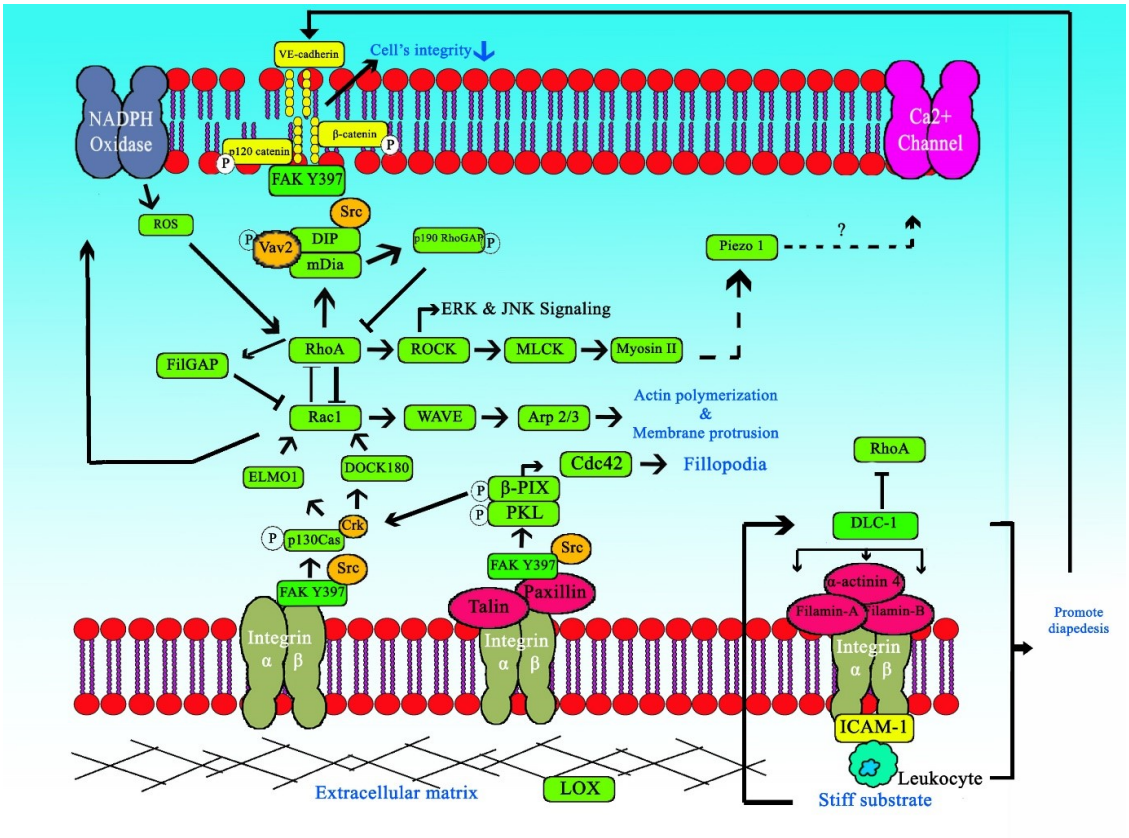

Figure 2 Overview of RhoA/ROCK Signaling Pathway. The changes of matrix stiffness that has been sensed by integrin triggers RhoA/ROCK signaling pathway. When the abundance of RhoA passed Rac1 expression, actomyosin contractility is stimulated through ROCK activation. Rac1 and Cdc42 correspond to fillopodia formation and membrane protrusion. The occurrence of DLC-1-dependent substrate stiffness causes inhibition of RhoA as well as ICAM-1 stabilization and leukocyte adherence.

ECs have the ability to suppress RhoA activation and prevent matrix stiffness-mediated CVD. Rac1 in association with NAD(P)H oxidase forms reactive oxygen species (ROS) to inactivate Rho [71], while RhoA/mDia signaling regulates cytoskeletal remodeling. However, mDia has inhibitory effect on ROCK, leading the activation of p190RhoGAP to inhibit RhoA and ROCK expression [72]. Src phosphorylates mDia interacting protein (DIP) to inhibit RhoA and cell motility through p190RhoGAP. DIP also binds to Vav2 for Rac1 stimulation [73]. When substrate stiffness increases, the abundance of p190RhoGAP-A is decreased, which then increases actomyosin contractility [74]. Another RhoGAP protein, DLC-1 has been involved

in vascular stiffening. In ECs, DLC-1 has been known to repress RhoA activity and lead to cell protrusion like p190RhoGAP-A [75,76].

RhoA/ROCK pathway has been suggested as the major mechanotransduction signaling for leukocyte transendothelial migration (TEM)/diapedesis with ECs [77]. Cytoskeletal tension and actomyosin contractility generated from matrix stiffness-mediated RhoA/ROCK signaling are known to promote ICAM1-mediated leukocyte adhesion on ECs and diapedesis [78]. Recent studies show that matrix stiffness-mediated DLC-1 expression stabilizes ICAM-1 adhesome by recruiting actin adaptor proteins filamin A, filamin B, and α-actinin 4 and thus leads to leukocyte diapedesis [79]. In fact, α-actinin 4 has been found to increase matrix stiffness-mediated ICAM-1 clustering. The activation of ICAM-1 also activates RhoA/ROCK signaling pathway [78]. During diapedesis, the increased expressions of pro-inflammatory genes such as TNF-α might affect other signaling pathways [80].

The increase of substrate stiffness impairs vascular endothelial growth factor receptor (VEGFR) and disrupts endothelial barrier function. The increased permeability in endothelium allows the accumulation of leukocytes and lipids in the tunica intima, a hallmark of atherosclerosis [81]. RhoA/ROCK-mediated ICAM-1 also induces the activation of VEGFR, resulting in the phosphorylation of p120 catenin and β-catenin that bind to VE-cadherin through Vav2-Rac-PAK cascade [82]. The FAK/Src-Tyr397 activities have been reported to associate with vascular permeability [83]. VE-cadherin is a crucial cell-cell adhesion molecule and mainly influenced by actin cytoskeleton. The phosphorylation activities contribute to VE-cadherin internalization and degradation. Hence, impaired VE-cadherin function induces vessel leakiness [82].

Although matrix stiffness-mediated RhoA/ROCK pathway has been intensively investigated, the roles of other mechanosensitive molecules such as Piezo1 in CVD remain unclear. It has been suggested that matrix stiffness-mediated myosin II activity in ECs leads to Piezo1 activation. Piezo1 responds to matrix stiffness in neural progenitor cells [84]. Shear stress enhances Piezo1 activity in ECs and leads to Ca^{2+} entry. *In vivo* study shows that inhibiting Piezo1 alters the development of vasculature and causes embryonic lethality [85]. More rigorous studies are required to elucidate the relationship between matrix stiffness and Piezo1 expression in ECs in the future.

4.3. Mitogen-activated protein kinase (MAPK) Pathway

MAPKs are a family of Ser/Thr kinases that play vital roles in cellular functions in response to extracellular stimuli. MAPKs are known to involve in cell proliferation, inflammation, apoptosis, and vascular tissue remodeling. There is evidence showing the synergic relationship between RhoA/ROCK and MAPK pathways. MAPK signaling mainly includes extracellular signal-regulated kinase (ERK)1/2, c-Jun N terminal kinase (JNK) 1-3, p38, and ERK5. At least 3 cascades are involved in the mechanotransduction of ECs, including p38 MAPK, ERKs, JNKs [86,87].

P38α kinase is a member of MAP kinases and becomes activated in the presence of pathogenic stimuli, including mechanical cues. Its activity increases during integrin engagement with FAK and contributes to cytoskeletal re-organization [88]. Mechanotransduction-mediated ICAM-1 ligation or neutrophil adherence also induces p38α activation [89]. p38α MAPK activates small heat shock protein (HspB1 or Hsp25/27) to regulate actin remodeling in response to mechanical stress. The phosphorylation of HspB1 suppresses cell migration [88,90]. Together with T-cell infiltration, p38α MAPK is also responsible for collagen deposition in tunica externa, leading to aortic stiffening and hypertension [91]. p38α also plays a central role in foam cell formation, the development of atherosclerosis plaque, along with generation of reactive oxygen species (ROS) and IL-6, a main feature of vascular inflammation [92].

Mechanical cues, including matrix stiffness, have profound influence on ERK signaling. RhoA/ROCK pathway enhances actomyosin contractility, which is required for ERK activation. Previous study shows that matrix stiffness regulates the co-localization of pERK with stress fibers and actomyosin bundles [93]. FAK/Src binding also involves in ERK activation. The binding of FAK/Src in RhoA/ROCK pathway induces Ras activation. In turn, phosphorylation of FAK generates a positive feedback to activate downstream signaling pathway, such as ERK and PI3-K/Akt [94]. On the other hand, the binding of FAK with c-Src on SH2 domain containing Grb2-SOS (GEF molecule) facilitates Ras activation. The activated Ras dephosphorylates FAK at 397 residues and thus mediates Raf-Fgd1-Cdc42-PAK1-MEK-ERK signaling cascade [94,95]. Furthermore, matrix stiffness-mediated ERK signaling regulates transcriptions through c-fos. Prolyl isomerase Pin1**,** a regulator of key transcription factors by S/TP motif phosphorylation, has been known to regulate the phosphorylation of MEK1/2-ERK1/2 [96] followed by c-Fos activation. As a result, Pin1 regulates dimeric activator protein-1 (AP-1) [97].

Similar to ERKs-MAPK signaling, JNKs-MAPK pathway can also be activated by mechanical stimuli and further contributes to CVD. Mechanosensitive JNKs-MAPK molecules have been known to upregulate inflammatory genes, consequently promote atherogenesis. [98]. JNKs activate c-Jun for AP1 regulation, which upregulates several gene expressions, such as MMPs, interleukin (IL)-8, IL-6, proliferating cell nuclear antigen (PCNA), and TGF-β crucial in neointima formation. The activation of ERK2 and JNK1 in arteries have been found to enhance blood pressure and smooth muscle hypertension [99]. Despite the involvement in CVD as mechanosensitive molecules, how JNKs-MAPK pathway in EC and matrix stiffness in pathological conditions are related and induce the disease progression requires further studies.

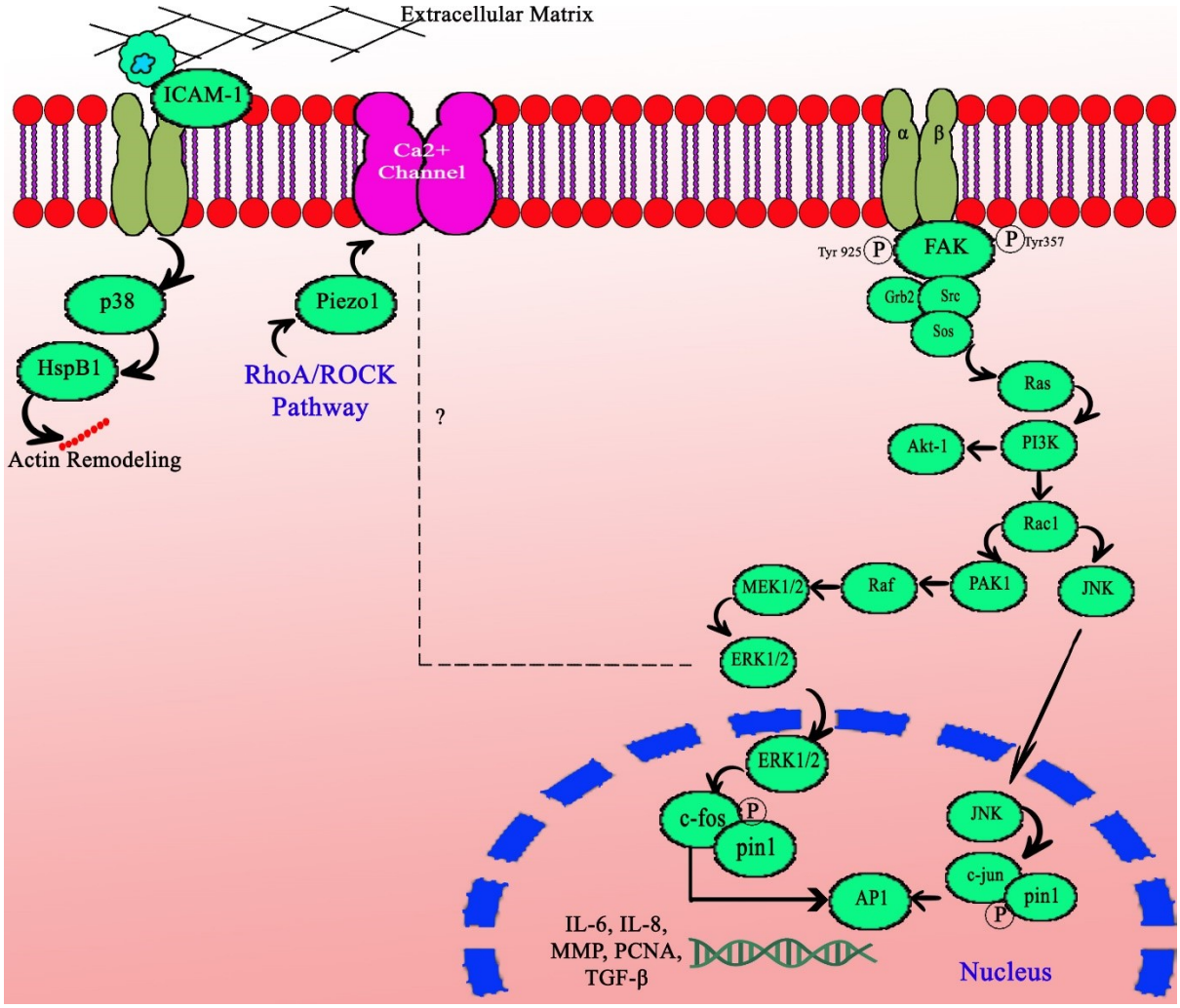

Figure 3 An Overview of MAPK pathway during High Stiffness Substrate. There are 3 common cascades related to mechanotransduction-mediated ECs such as p38 MAPK, ERKs, and JNKs. P38 MAPK corresponds to actin remodeling through HspB1 activation. Meanwhile, the binding of FAK with c-Src on domain SH2 that contains Grb2-SOS facilitate Ras activation and responsible to ERKs and JNKs activation followed by c-fos and c-jun activation to regulate AP1 transcription factor. As the result, several downstream signaling genes from MAPK pathway such as IL-6, IL-8, MMP, PCNA, and TGF-β were arisen.

Taken together, matrix stiffness-mediated MAPK pathway plays an important role in the development of CVD. However, more thorough studies are necessary to elucidate matrix stiffness-mediated MAPK in ECs. For instance, previous study has reported the role of Piezo1-mediated ERK1 activation. RhoA/ROCK-induced myosin II increases Ca^{2+} and ERK1 signaling to regulate the transition from G2 phase to M phase and promote cell division and proliferation. However, the detailed mechanism remains not fully understood [100].

4.4. Hippo Pathway

Hippo signaling pathway has an important role in controlling the organ size through proliferation and apoptosis [101]. The key factor of Hippo signaling is the transcriptional coactivators YAP (Yes-associated protein 1) with TAZ (transcriptional coactivator with PDZ-binding motif). YAP/TAZ has been recognized as a critical factor in mechanotransduction and vascular homeostasis [102,103]. Canonical Hippo pathway mainly includes two kinases: mammalian ste20-like kinases (MST1/2) with salvador (SAV) as its cofactor and large tumor suppressor 1/2 (LATS 1/2), which is independent of YAP/TAZ-mediated mechanotransduction signaling. Phosphorylated YAP interacts with 14-3-3 protein that leads to YAP/TAZ cytoplasmic retention, indicating that the canonical hippo pathway negatively regulates YAP/TAZ signaling [104]. MST1/2 is not able to act alone in LATS1/2-mediated YAP/TAZ activation. Phosphorylated LATS1/2 together with MAP4Ks (including MAP4K4) in a parallel pathway induces YAP phosphorylation at serine 127 residues [105,106]. However, the mechanisms of how MAP4K interacts with MST1/2 to activate LATS1/2 are still not clear. It has been shown that MAP4K is an upstream of JNK signaling [107] while another study unveils the dispensable role of MAP4K4 in MAPK activation [108,109]. Still, it has been proposed that MAP4K plays a role in atherosclerosis by enhancing permeability and inflammation [109].

Mechanotransduction-mediated YAP/TAZ signaling can be activated through Hippo-independent pathway, including Rho-GTPases/actomyosin-dependent pathway [103]. RhoA/ROCK-mediated cytoskeletal tension and F-actin polymerization will activate YAP/TAZ and translocate it to the nucleus [17,110,111]. Note that the activation of YAP is not caused directly by F-actin, but via RhoA/ROCK activation [102]. Meanwhile, the binding of FAK/Src with integrin triggers PI3K and PDK1 pathway, consequently activates YAP signaling [112]. Previous study has shown the influence of cell density on YAP/TAZ activity [113]. When growing on stiff substrates, the cells tend to spread and have high RhoA activity

to trigger YAP/TAZ activation, while on soft substrates they will have low cellular tension which leads to YAP/TAZ inactivation and cell apoptosis [102,113].

Mechanical stresses enhance ROCK/myosin II-dependent actomyosin contractility and strongly inhibit ADF/cofilin activity [114,115]. The loss of cofilin and capping actin protein of muscle Z-Line (CapZ, an actin-depolymerizing factor) has been reported to release YAP/TAZ nuclear localization in epithelial cells and affect transcriptional activity [116]. When YAP/TAZ are translocated into the nucleus, they bind TEADs and promote the transcription of their downstream genes [117]. Nevertheless, the transcription factor AP1 is also reported to bind with nuclear YAP/TAZ and then regulate the expressions of target genes, such as connective tissue growth factor (CTGF) and ankyrin repeat domain 1 (ANKRD1), that implicate the involvement of ERK pathway in YAP/TAZ signaling [118,119]. Another downstream gene cysteine-rich angiogenic inducer 61 (CYR61) has been identified as a marker of the early stage of atherosclerosis [120].

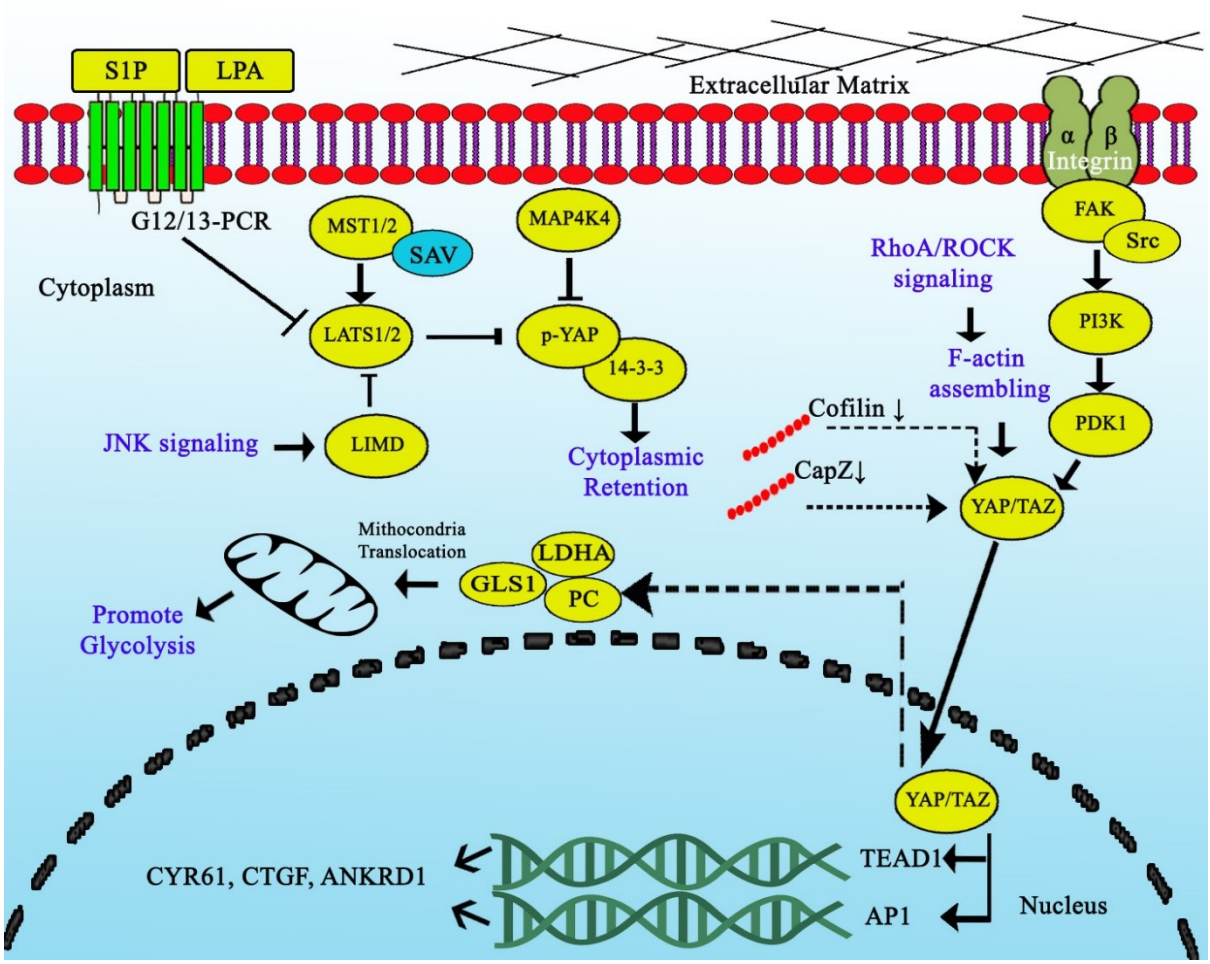

Figure 4 An overview of Canonical and Non-Canonical Hippo Pathway influenced by matrix stiffness. The starting point of canonical hippo pathway is begun from MST1/2 that binds to SAV cofactor to activate LATS1/2

and together with MAP4K4 act to phosphorylate YAP, thus binding to 14-3-3 protein and being sequestered in cytoplasm. Meanwhile, the matrix stiffness-regulated RhoA/ROCK pathway also has a role in YAP activation and nucleus translocation. The nucleus-YAP/TAZ activation thus bind with TEAD1 and AP1 transcription factor that corresponds to several downstream signaling expression such as CYR61, CTGF, ANKRD1. Separately, YAP/TAZ activation promotes lactate dehydrogenase A (LDHA) and ATP-dependent pyruvate carboxylase (PC) and promote glycolysis metabolism.

ECM stiffening activates YAP/TAZ and GLS1 that is necessary to regulate glutaminolysis and glycolysis. GLS1 replenishes the amount of aspartate that promotes cell proliferation and thereby associates with metabolic reprogramming and dysregulation of vascular metabolism. Besides, ECM stiffening-activated YAP/TAZ also promote lactate dehydrogenase A (LDHA) and ATP-dependent pyruvate carboxylase (PC) for supporting glycolysis and anaplerosis. As a result, high level of aspartate is produced to support respiratory function and cell proliferation, hence plays a role in pulmonary hypertension [46,47].

YAP/ TAZ signaling has suppressive effects on Notch pathway. It has been shown that the upregulation of YAP/TAZ suppresses numerous Notch target genes, such as LFNG, DLL4, and HES1, while knockdown of YAP/TAZ leads to the increase of Notch reporter activity in ECs [121] and epidermal stem cells [122]. However, the information about the interplay between Notch and YAP/TAZ signaling is still lacking and should be elucidated in the future. Taken together, mechanotransduction-activated YAP/TAZ signaling interacts with many other signaling pathways, which should be further defined and elucidated.

4.5. Matrix stiffness-mediated intercellular signaling

Despite the influence on intracellular signaling, matrix stiffness also affects the intercellular signaling for communication between cells, including Notch signaling and WNT/β-catenin, which have been reported as mechanotransduction-dependent signaling. Notch pathway is known to promote cell-cell communication through juxtacrine signaling and is involved in the response to mechanical stimuli, including matrix stiffness. Dsl ligand Jagged 1 or JAG1, JAG2, Delta-like1 or DLL1, DLL3, and DLL4 from the donor cell can bind Notch receptor (NOTCH1-4) [123]. This binding is cleaved either by ADAM metalloprotease family at S2 site or/and by γ-secretase complex at S3 site in plasma membrane, resulting in the release of Notch intercellular domain (NICD). This fragment is translocated to the nucleus and binds the transcription factor CBF1 or CSL or other nuclear effectors, such as Mastermind-like (MAML), co-activators, and p300 protein. Hence, the activation of Notch signaling corresponds to several downstream targets, such as Het- and Hes- like genes. In the absence of NICD, CSL binds with co-repressors and is unable to initiate Notch-responsive genes [124].

One characteristic of pulmonary hypertension is vascular remodeling and neointimal thickening. Notch-1-3 have been found to be upregulated in pulmonary hypertension, leading to the activation of Notch signaling. This activation promotes EC apoptosis due to the regulation of MAPK pathway through p21 which further contributes to neointimal hyperplasia. Meanwhile, Notch-4 serves as an athero-protective receptor that could inhibit apoptosis through JNK pathway [125]. Previous study has shown that activated YAP/TAZ signaling can induce the expression of Notch receptors and/or Notch ligands [110]. Notch1 acts as a mechanosensor in ECs and has atheroprotective functions by maintaining cell proliferation, reducing inflammatory genes, stabilizing cell-cell junction, and preventing the escalation of calcium signaling. The suppression of Notch 1 contributes to cell proliferation and Ca^{2+} signaling. Deletion of Notch 1 in mice endothelium leads to discontinuity of cell-cell junctions, reduction in ECs elongation, and increase in cell proliferation [126].

Wnt/β-catenin signaling has been demonstrated to be mechanosensitive and plays important roles in endothelial to mesenchymal transition (EndMT) that enables the differentiation of endothelial cells into mesenchymal cells [127]. *In vivo* study in porcine with diseased aortic valves shows the co-localization of WNT3A, β-catenin, and TGF-β1 in myofibroblasts [128]. EndMT-induced β-catenin nuclear translocation is also supported by MALAT1 in response to ox-LDL, but how MALAT1 increases the activity of β-catenin remains unclear [129]. Since WNT/β-catenin signaling is involved in the intercellular signaling, more studies are required to dissect the complicated cell-cell communication network. Moreover, the crosstalk between Notch and WNT/ β-catenin signaling has been proposed to contribute to EndMT that is mainly induced by mechanical cues including matrix stiffness [130]. It is shown that β-catenin upregulates Dll4 transcription in ECs and alters their differentiation, accordingly affecting vascular development [131].

4.6. ncRNA in mechanotransduction

The advance in high-throughput genomic technologies such as microarrays and next-generation sequencing enables the identification of non-coding RNAs (ncRNAs) in many cellular processes. ncRNAs include short RNAs (miRNAs with 18-25 nucleotides) and long non coding RNA (lncRNA) with more than 200 nucleotides in length. It is known that dysregulation of miRNA and lncRNA is implicated in many diseases, including cardiac diseases [53,132]. Here, we review several mechanosensitive-ncRNAs and their responses to matrix stiffness.

Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is initially known as a tumor-associated lncRNA, which has been recognized as a mechanosensitive lncRNA that regulates proliferation and migration in SMCs and ECs, hence contributes to atherogenesis progression [133,134]. MALAT1 targets miR-503 and prevents cell proliferation in ECs [135]. However, the mechanisms of how mechanical cues influence MALAT1 are still lacking. HOX transcription antisense RNA (HOTAIR) is a long intergenic non coding RNA (lincRNA) that is initially found as a predictor of cancer invasiveness by re-targeting Polycomb repressive complex 2 (PRC2) and altering histone H3 lysine 27 methylation (H3K27me). Recent study has shown that HOTAIR is also a mechanosensitive lncRNA and acts as a repressor of calcification gene. HOTAIR is associated with WNT/β-catenin during high vascular calcification due to matrix stiffness-mediated Ca^{2+} deposition [136,137]. High calcification increases β-catenin expression and suppresses HOTAIR expression, leading to higher expression of two osteogenic genes, such as alkaline phosphatase (ALPL) and bone morphogenetic protein 2 (BMP2) in aortic valves interstitial cells (AVICs) [137,138]. ox-LD-triggered HOTAIR is reported to induce atherosclerosis events by sponging miR-330-5p in THP-1 cells [139]. Nevertheless, how this lncRNA changes cell fate and induces atherosclerosis needs to be explored in the future.

Furthermore, several CVD-associated lncRNAs have been identified, such as lncRNA p-21, H-19, MEG2, TUG1, N1LR, ANRIL, SNHG14, and SENCR [140,141]. Despite the emerging study of mechanotransduction-mediated lncRNA expressions, the mechanisms underlying the regulation of lncRNAs by matrix stiffness have not been well understood. For instance, antisense noncoding RNA in the INK4 Locus (ANRIL) is the first exon located in the ARF promoter which overlaps with two exons of CDKN2B, and it has been proposed as prime candidate of coronary artery disease (CAD) markers. ANRIL is increased in CAD patients over 60-years old with symptoms of hypertension and hyperlipidemia [139] which strongly correlates with the increase of arterial stiffness [141]. ANRIL has been found to increase along the high hydrogel stiffness and mediate JNK phosphorylation, followed by downregulation of connexin 43. Hence, this cascade impacts the contractility and myofibril organization in cardiomyocyte, resulting in a higher possibility of heart failure [142]. However, study regarding the effects of matrix stiffness on lncRNAs is still lacking.

Many studies have identified several mechanosensitive miRNAs, such as miR-181b, miR-145, miR-203, miR-765, miR-29, miR-29, miR-21, miR-599, miR-223, and miR-130 [143]. Previous study has reported that ECM stiffness promotes YAP/TAZ-OCT4-miR-130/301 and

PPARγ-ApoE-LRP8 axis, initiating collagen deposition and LOX-dependent remodeling, which serve as a feedback loop to further activate YAP/TAZ in pulmonary artery ECs (PAEC) and PASMC [46]. miR-130/131 in ECs is regulated by matrix stiffness-dependent genes, such as KCNK3, ATP13A3, and BMPR2 [144]. miR-181 is known to be athero-protective in vascular stiffening and hypertension by suppressing the inflammation through NF-kB and TGF-B signaling [145,146]. miR-21 also plays a role in several pathological phenotypes related to CVD. It can induce neointima formation [147] and influence fibrosis as a positive regulator of ECM deposition. miR-21 targets PTEN and thus activates ERK and Akt signaling to prevent apoptosis in ECs. To stimulate inflammation, miR-21 also regulates ICAM-1 and VCAM-1 expression and the transcription factor AP-1 [143,148] Furthermore, miR-29 is involved in mechanotransduction and identified as a pro-atherogenesis agent that regulates ECM protein content such as COL1A and COL3A and increases atherosclerotic lesion size [149]. Its expression is higher in aging mice aorta compared to young mice aorta [150].

miR-765 is known to regulate EC functions. Its overexpression suppresses apelin (APLN), a potent vasodilator, by binding G-protein-coupled receptor APJ [151]. This overexpression is followed by inhibition of eNOS phosphorylation and ERK/Akt/AMPK signaling that leads to reduction of arterial stiffness. On the other hand, miR-765 overexpression also increases MMP2/9, consequently also increases arterial stiffness. It has been proposed that miR-765 potentially influences several target genes, such as NADPH oxidase, LDL receptor-related protein 4 (LRP4), and LDL receptor-related protein (LRP6) [152].

The putative roles of ECM stiffness-dependent ncRNA have been reported to be either negative or positive in cardiac disease. Several possible mechanisms related to mechanical stiffness-related lncRNA have been reported within their target of protein-coding genes [133]. Nevertheless, the investigation of more mechanotransduction-related mechanisms will provide new knowledge and insight.

5.How can we mimic the tissue mechanics?

5.1. Materials imitating with tissue mechanics

In order to evaluate the influence of mechanical forces to cell function, a proper *in vitro* model was required to imitate the physiological and pathophysiological condition of tissue. Mechanical properties and biochemical properties must be considered to achieve a proper design of material for tissue-engineering, specifically for mechanobiology application. It has been suggested that a change of matrix stiffness from 2.5 kPa to 10 kPa is enough to alter

contact between cells, endothelium permeability augmentation, and leukocyte transmigration which shows hallmarks of atherosclerosis progression [153].

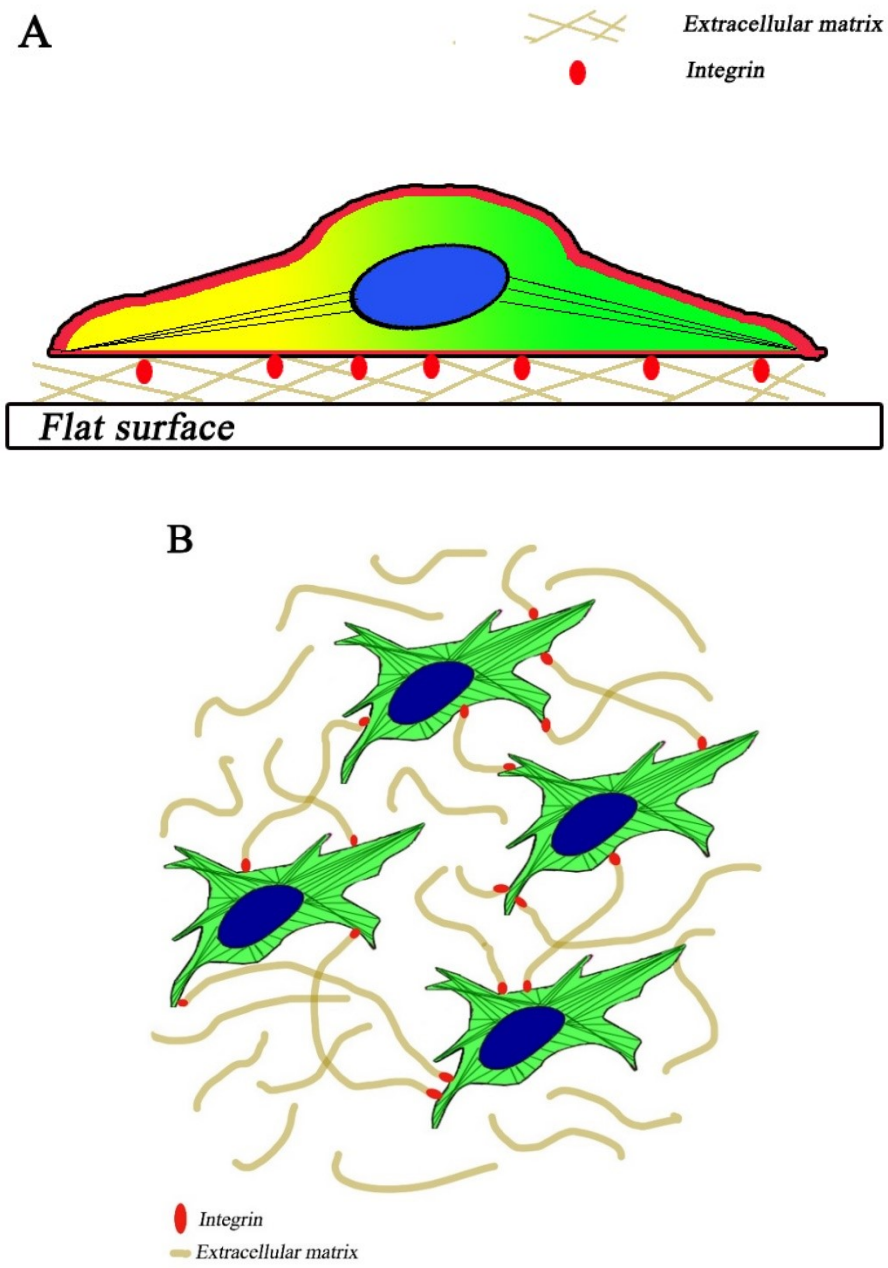

Figure 5 The Differences between 2D cell culture and 3D cell culture system. A) 2D cell culture is performed using a flat substrate that has been coated with ECM such as collagen, fibronectin, laminin, etc. Cell grows as a monolayer cell that only provide 1 sides of integrin binding to the ECM, makes 2D cell culture unable to mimic the native microenvironment. B) 3D cell culture provides a meshwork of fibrillary ECM proteins thus fulfilled the most of intracellular spaces that involve the integrin from all of side of cell to bind which close similar to the native microenvironment.

In applied mechanobiology, the hydrogel can be generated with three dimensional structure of 3 dimension (3D) or 2 dimension (2D) structure. Briefly, 2D cell culture is

conducted on 2D substrates/flat substrates such as plastic, membranes, while 3D cell culture tried to create an analog of cell's native environment. It can be generated by adding meshwork of fibrillary ECM proteins thus fulfilled most of the intercellular spaces [154,155]. The 3D cell culture systems can be performed by growing the cells on the matrix or embedded inside the matrix (Figure 2). As the result, 3D cell culture provides more information regarding the cell-matrix interaction that mimic *in vivo* condition while 2D cell culture unable to show it. However, 3D cell culture is more complex than 2D cell culture, leaving room for further study and result interpretation [155,156].

Material properties which need to be considered are as follows; biocompatible, inert, highly reproducible, tunable, and scalable synthesize [154,157]. In consideration of these requirements, synthetic polymer gels are mainly chosen to generate the force of stiffness against the cells. Several studies have demonstrated many types of common hydrogels to create substrate elasticity together with the effect against cell behavior. Those hydrogels include polyacrylamide (PAAm), polydimethylsiloxane (PDMS), alginate, poly (ethylene glycol), agarose (Figure 2) [157,158].

From all of the hydrogel described in this review, PAAm gel has been recognized as the most versatile hydrogel for studying cell response against the substrate stiffness. It has been well characterized and relatively easy to be produced. Characteristically, it has a hydrophilic surface, inert properties, and low swollen percentage [159]. The elasticity can be tuned by varying the composition of bis-acrylamide and acrylamide monomer followed by initiation of polymerization with adding ammonium persulfate (APS) and N,N,N′,N′-Tetramethylethylenediamine (TEMED) [160]. Previous study has shown the percentage of bis-acrylamide concentration has a positive correlation with Young modulus [160–162].

The functional PAAm hydrogel can be completed by crosslinking via UV activation by adding bi-functional linkers such as sulfosuccinimidyl-6-[4'-azido-2'-nitrophenyl amido] (sulfo-SANPAH) that contain NHS-ester to actively react with primary amine in ECM protein [2,161]. However, PAAm gel elasticity can be tuned by other factors such as temperature, besides the bis-acrylamide/acrylamide ratio. Since Ammonium Persulfate (APS) is temperature-sensitive, the polymerization process will be disrupted along with the rise of temperature [162]. In the other hand, based on this discovery, we can adjust these 2 factors to get the "ideal" hydrogel elasticity.

Another type of hydrogel that has been widely adopted is PDMS/Silicone gel due to its inert properties, non-toxic, and optically-transparent. Unlike PAAm hydrogel, PDMS gel has

a hydrophobic surface caused by gradual coverage of silica structure, thus sulfo-SANPAH is not required and PDMS gel can directly bind to the ECM [163]. The stiffness of PDMS is ranging from 0.1 kPa to MPa, it can be discerned by adjusting the base/curing agent ratio followed by curing for several times in certain temperature [164]. Meanwhile, several studies have shown that the Young modulus can be influenced by variating curing temperature together with curing duration. Like PAAm hydrogel, the curing temperature has a positive correlation with the Young modulus of PDMS hydrogel [165] and prolonged curing duration will cause the Young modulus to decline [166]. Moreover, there is a possibility of hydrogel shrinkage due to total volume deflation [167]. In the other hand, PDMS hydrophobic property makes the adsorption of cell-adhesive molecule become inefficient [168]. This problem might be solved by UV-ozone or oxygen plasma treatment to recover the hydrophilic surface [169,170]. Layer-by-layer assembly also can be an alternative method to modify the hydrogels [171].

PEG gel has a permissible, biocompatible, non-toxic, and inert properties [172]. Since it has a hydrophilic characteristic, the cross-linking can be performed by adding sulfo-NHS under photo-irradiation to create reactive site for binding the primary amine of protein/peptides [173]. The tunable elasticity of PEG is controlled by variating the ratio between acrylate compound groups such as PEG-diacrylate (PEGDA) or PEG-dimethacrylate (PEGDMA) chemical combined with non-acrylated PEG. Previous study has shown that the increase of total weight polymerization with a high concentration of acrylate compound on PEG leads to the increase of Young modulus and to diminish the percentage of swelling behavior [174–176]. Nevertheless, in high concentration of acrylate compound, the optical density of PEG is high, consequently it may disrupt the microscopy observation [174,177]. The promising solution for this problem is achieved by combining PEG-Acrylate with phosphoryl choline (PC) compound [174].

Alginate is also considered as an ideal matrix for mechanobiology study. It is derived from seaweed that contains a linear polysaccharide with repeating units of β-D-mannuronic acid and α-L-guluronic acid. Alginate has a hydrophilic surface, lack of immune-response, non-degradable in mammals, and biocompatible properties [178]. The gelation process can be achieved through reversible reaction using divalent cations such as Ca^{2+}, Ba^{2+}, Mg^{2+}, and Sr^{2+} that will bind to guluronic residues or through irreversible reactions by adding sulfo-NHS or 1-ethyl-(dimethylaminopropyl) carbodiimide (EDC) agent [179,180]. Stiffness of alginate hydrogel can be controlled by varying the concentration of alginate [178]. Escalation of guluronic acid increase the rigidity of hydrogel [181,182]. However, previous study has reported a time-dependent inefficient crosslinking of divalent cation to alginate, resulting an

unstable alginate hydrogel [180,183]. This limitation can be solved by modification of methacrylates in alginate side groups following by photo-crosslinking using -[4-(2-Hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-pro-pane-1-one (Iragacure®-2959) [184].

Another hydrogel derivative from seaweed family is agarose. Agarose consists of D-galactose-3,6-anhydro-L-galactopyranose repeat unit, that has been applied in tissue engineering due to its biocompatibility, non-immunogenic attribute, and low swelling ratio [185,186]. The elasticity of agarose can be altered by modifying the ratio of agarose molecular weight. Increasement of agarose concentration leads to the rise of hydrogel rigidity [186]. The gelation process is thermo-reversible, where the agarose was melted in >80ºC and cooled down to desirable temperature ~40ºC [187]. Crosslinking of adhesive molecule can be done by using chemical crosslinker, such as sulfo-NHS reaction or EDC reaction.

Meanwhile, agarose hydrogel carry several constraint: 1) The difficulty to control mechanical attribute due to the α-helices structure of agarose that is sensitive to the temperature [188]. Different temperature and time in melting and cooling process create different helix aggregate which affects the rigidity of hydrogel; 2) Native agarose gel shows a turbid appearance that will disrupt the observation under microscope [178,189]. In this case, recent studies have shown the effect of carboxylation on agarose that is called physical-crosslinking. This modification switches the α-helix structure to β-sheet secondary structure that is more steady, thus generates more transparent gels [185]. More importantly, by varying the degree of carboxylation, Young modulus can be controlled ranging from 0.1-100 kPa [190].

Gelatin is derived from collagen processed by hydrolytic degradation, so it has a similar chemical composition to the collagen and granted with native arginine-glycine-aspartic acid (RGD) peptide that usually should be added on other hydrogels to propagate adhesive property [191,192]. Besides, it provides a relatively good biocompatibility, low cost and hydrophilic surface. Theoretically, the stiffness of hydrogel can be controlled by varying the concentration of gelatin. However, since this technique also change the concentration of RGD peptide, it will be complicated to determine whether the change comes from the stiffness or the amine concentration [191]. Meanwhile, gelatin is thermo-responsive compound that could melt at 30ºC, which affects its' stability [192,193]. Different approach combined the gelatin hydrogel with methacrylate family such as methacryloyl chloride or methacrylic anhydrate followed by photo-crosslinking using Iragacure®2959. By adjusting methacrylate concentration, the stiffness can be tuned. Chemical crosslinking using glutaraldehyde can be performed in gelatin hydrogel, but it may cause unspecific crosslinking [194,195].

Every hydrogel has their own characteristics with their advantages and limitation. Which hydrogel should be used is depending on the research purpose. In 3D cell culture, materials derived from natural sources such as alginate, gelatin, agarose might be useful, since they have less immunoreactivity from the body. Synthetic gels that widely used for 3D cell culture is PEG hydrogels that has a fine reproducibility, but it lacks of endogenous factor to promote cell behavior [154,196]. Due to its toxicity, the usage of PAAm gel as an implant should be reevaluated although PAAm gel has been widely used *in vitro* studies. The availability of each material should also be considered.

According to the description above, ECM is indispensable together with the mechanical characteristic because of its importance to initialize cell adhesion. It has been known that one of the distinct trait between 3D and 2D cell culture is the addition of ECM meshwork to stimulate cell-matrix interaction [157]. ECM is not only supporting mechanical environment for cell to anchor but also can stimulate both biochemical and mechanotransduction pathway, thus providing signal against extracellular environment [197]. Collagen, fibronectin, laminin, proteoglycan, and glycosaminoglycan are the ECM that frequently involved in mechanotransduction. Commonly occurred specific peptide such as RGD peptide in many cell can be added to the gel for supporting cell-gel adhesion [198].

Table 3 The comparison of hydrogel material characteristic to imitate tissue mechanics

	PAAm	PDMS	Alginate	Gelatin	Agarose	PEG
Inert properties	Yes	Yes	Yes	Yes	Yes	Yes
Natural sources	No	No	Yes	Yes	Yes	No
Toxicity	Yes	No	No	No	No	No
Surface behavior	Hydrophilic	Hydrophobic	Hydrophilic	Hydrophilic	Hydrophilic	Hydrophilic
Optical density	Low	Low	Low	Low	High	High
Young modulus tuning factor	Ratio of bis-acrylamide and acrylamide	Ratio of curing/base agent	Alginate concentration	Gelatin concentration	Molecular weight of agarose	Ratio of acrylate and PEG

Advantages	It has been widely characterized	The ability to bind with ECM directly	Less immunoreactivity in mammals	Contains native RGD peptide	Extensively used in wound-dressing application	Highly capable for cell encapsulation
Limitation	Thermo-sensitive and toxic	Ineffectively adsorb the adhesion molecule (Can be solved by UV-ozone/oxygen plasma treatment)	Inefficiency of ionic crosslinking (Can be solved with photo-crosslinking)	The ambiguity factor: the increase of RGD peptide along with the stiffness (Can be solved by combining gelatin with acrylate family)	Turbidity (Can be solved with carboxylate modification)	Turbidity correspond with addition of acrylate (Can be solved by combining the PEG hydrogel with phosphoryl choline)

Different type of ECM showed co-regulating activity with specific cell behavior [199]. One of the common cases is collagen and elastin ratio shift in blood vessel may induce different manners. It has been known that integrin has a key role to communicate with ECM, contribute to advance molecular signaling in tissue-specific gene [200,201]. Taken together, one type of ECM cannot accurately explain all of the tissue specific behavior of the cell, thus extensive study is required to analyze it.

Table 4 Overview of materials and main technique in imitating matrix stiffness

Substrate type	Fabrication process	Substrate modulus	Reported cell	Description	Reference
PAAM	Crosslinked with fibronectin/collagen I	2.5-10	EC	Combination of bis-acrylamide and acrylamide percentage	[153,202]
PAAM	Photopolymerization with microfluidic technique	4-30	VSMC	Bis concentration is ranging from 0.04% - 0.48%	[203]
PDMS	Crosslinked with layer by layer technique	48-1783	Fibroblast		[204]
PDMS	Crosslinked with laminin/fibronectin	5-1720	SMC		[205]
PEG-DA	Nanolitography technique	3.9-6000	Fibroblast	Modification of PEGDA macromers	[173]
Alginate	With the addition of RGD peptide	1.79-5.81	M28, M6, and M6c		[178]

Alginate	Conjugated with cathecol with different concentration	0.34-6	EC, Huh-7, Neuro-2a	2% dan 4% w/v alginate-cathecol concentration	[180]
Agarose	Soft carboxylated agarose in combination with RGD peptide addition	0.5-5	EC	28% and 60% carboxylation percentage	[206]
PEGDMA: GelLMA	Nanopattern technique	400-1500	EC	Combination of PEGDMA and GelMA concentration	[207]

5.2. Overview of gradient hydrogel and blood vessel-on-chip

Study of matrix stiffness-mediated cell behaviors has been being extensively started and many studies focused on a uniform matrix with different type of stiffness. According to the ability of cells to sense chemotaxis by following gradient concentration of chemical solution, the importance of gradient stiffness has been assessed to mimic tissue microenvironment. Durotaxis refers to the ability of cells to sense the substrate rigidity in the process of cell migration that might be explained using gradient stiffness material [208,209]. The preference of each type of cells to live on the stiffer or softer is different and this behavior will be difficult to be studied with uniform gel [210]. Mostly, PDMS, PAAm and PEG hydrogel was chosen for gradient stiffness substrates [211,212] and the proper protocol are starting to be developed to get a comprehensive manner of cell's signaling-dependent durotaxis.

There are several approaches to obtain gradient concentration stiffness substrates such as microfluidic, photolithography, and micro-pattern technique [213]. Generally, microfluidic technique involves the flowing of two different types of monomer solutions into a gradient generator thus create gradient stiffness along the length of the hydrogel. The flow can be obtained from 4 main methods such as diffusion source-sink, gradient generator, dynamic mixing, and convection [209]. This method is more expensive compared to photolithography method that conducts a photomask overlying the hydrogel to create stiffness gradient that might have less-reproducibility [211].

Unlike Microfluidic, photolithography techniques are able to create gradient stiffness using one type of polymer that mostly is PDMS. This technique employs UV exposure to the gradient-grayscale masks on the polymer precursor in order to generate gradient stiffness [213,214]. The master mold was generated with desired patterns and put under the PDMS mixture to let the PDMS mixture being cured and shaped**.** To facilitate the release, the master

mold was silanized using allyl groups [215]. Light wavelength plays a functional part as the light diffraction limits pattern resolution [216]. Based on photolithography technique, previous study has shown micro-pattern technique using a high resolution photo-patterned KMPR (epoxy) resin as the mold to pattern the ECM with the addition of PDMS layer. This technique successfully decoupled control of ECM pattern and local stiffness against the cell, resulting the cytoskeleton organization to match the underlying substrates [212].

Nowadays, many reports shows the combination of microfluidic, micro-pattern, and photolithography to get the ideal type of compliance-gradient substrates [203,215,216]. This idea has lead the development of blood vessel-on-chip to mimic the functions and interaction of specific organ on miniature devices. Thereby, the development of organ on a chip is expected to provide *in vivo*-like cellular microenvironment [217]. PDMS has been widely used as the polymer to make the vessel tube due to the easy polymerization, tunable elasticity, gas permeability, and its biocompatibility [218,219]. As the blood vessel has been build, microfluidic technique enables the flow of the medium and cell that supposed to attach the imitation vessel. With this technology, the organ on a chip are able to provide a host for heterotypic cells co-cultures as well as the living tissue or biopsy samples that will be useful for studying diseases and test the efficacy of several treatments per individual [220]. Therefore, the technology of organ-on-chip, specifically for blood vessel-on-chip is very promising while several limitations still remains such as improving the usability and reproducibility of organ-on-chip [217].

6.Conclusion and future perspective

Study about matrix stiffness-mediated cell behavior has been emerging and keep developing. The development of uniform stiffness study has led to gradient stiffness that can explain more about durotaxis behavior in cells. Interplay between matrix stiffness-influenced pathways has been progressively elucidated in ECs. However, roles s of ncRNA to regulate mechanogenetic and mechanophenomic in ECs needs further elucidation. An understanding for this mechanism will be beneficial for therapeutic approaches since it can be a promising strategy to prevent or reverse matrix stiffness-mediated CVD with its clinical potential. Meanwhile, the complete mechanism still remains like puzzle pieces, and should be reconfirmed in a model that can mimic the native microvasculature for better understanding of vascular disease regulation. The development of blood vessel on a chip is expected to be an ideal model to demonstrate the

functionality of cells in their real microenvironment. Therefore, interfering by targeting key factors of mechanogenetic is a promising strategy.

Acknowledgements

This study was partially supported by grants from the National Natural Science Foundation of China (11572064, 31701275, 11672255), the National Key Technology R&D Program of China (2016YFC1102305) and Shenzhen Science and Technology Innovation Commission (JCYJ20170303160515987). We are also thankful for the support from the Chongqing Engineering Laboratory in Vascular Implants and the Public Experiment Centre of State Bioindustrial Base (Chongqing) and the National "111 plan" (B06023).

Conflict of interests

The author(s) declare there is no conflict of interest

7.Reference

[1] G.A. Roth, C. Johnson, A. Abajobir, Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015., J. Am. Coll. Cardiol. 70 (2017) 1–25. doi:10.1016/j.jacc.2017.04.052.

[2] J.Y. Wong, J.B. Leach, X.Q. Brown, Balance of chemistry, topography, and mechanics at the cell-biomaterial interface: Issues and challenges for assessing the role of substrate mechanics on cell response, Surf. Sci. 570 (2004) 119–133. doi:10.1016/j.susc.2004.06.186.

[3] P. Boutouyrie, R.M. Bruno, The Clinical Significance and Application of Vascular Stiffness Measurements, Am. J. Hypertens. 32 (2019) 4–11. doi:10.1093/ajh/hpy145.

[4] J.D. Humphrey, J.F. Eberth, W.W. Dye, R.L. Gleason, Fundamental role of axial stress in compensatory adaptations by arteries, J. Biomech. 42 (2009) 1–8. doi:10.1016/j.jbiomech.2008.11.011.

[5] D. Lu, G.S. Kassab, Role of shear stress and stretch in vascular mechanobiology, J. R. Soc. Interface. 8 (2011) 1379–1385. doi:10.1098/rsif.2011.0177.

[6] X. Li, Q. Yang, Z. Wang, D. Wei, Shear Stress in Atherosclerotic Plaque Determination, DNA Cell Biol. 33 (2014) 830–838. doi:10.1089/dna.2014.2480.

[7] Y. Wang, J. Qiu, S. Luo, X. Xie, Y. Zheng, K. Zhang, Z. Ye, W. Liu, H. Gregersen, G. Wang, High shear stress induces atherosclerotic vulnerable plaque formation through angiogenesis, Regen. Biomater. 3 (2016) 257–267. doi:10.1093/rb/rbw021.

[8] J. Qiu, D. Lei, J. Hu, T. Yin, K. Zhang, D. Yu, G. Wang, Effect of intraplaque angiogenesis to atherosclerotic rupture-prone plaque induced by high shear stress in rabbit model, Regen. Biomater. 4 (2017) 215–222. doi:10.1093/rb/rbx007.

[9] M.E. Widlansky, N. Gokce, J.F. Keaney, J.A. Vita, The clinical implications of endothelial dysfunction, J. Am. Coll. Cardiol. 42 (2003) 1149–1160. doi:10.1016/S0735-1097(03)00994-X.

[10] T.J. Anderson, Arterial stiffness or endothelial dysfunction as a surrogate marker of vascular risk., Can. J. Cardiol. 22 Suppl B (2006) 72B-80B. doi:10.1016/S0828-282X(06)70990-4.

[11] J.C. Kohn, M.C. Lampi, C.A. Reinhart-King, Age-related vascular stiffening: Causes and consequences, Front. Genet. 6 (2015) 1–18. doi:10.3389/fgene.2015.00112.

[12] P.S. Timashev, S.L. Kotova, G. V. Belkova, E. V. Gubar'Kova, L.B. Timofeeva, N.D. Gladkova, A.B. Solovieva, Atomic Force Microscopy Study of Atherosclerosis Progression in Arterial Walls, Microsc. Microanal. 22 (2016) 311–325. doi:10.1017/S1431927616000039.

[13] Y.S. Oh, Arterial stiffness and hypertension, Clin. Hypertens. 24 (2018) 18–20. doi:10.1186/s40885-018-0102-8.

[14] K.J. Haley, J.G. Fewell, B.M. Wertheim, A.O. Samokhin, L.-M. Yung, D. Khanna, W.M. Oldham, G.A. Alba, J.A. Leopold, S.O. Vargas, L.E. Fredenburgh, E. Arons, P.B. Dieffenbach, S.M. Marino, I.O. Rosas, M. Matar, S. Seo, J. Loscalzo, R. Kumar, A.B. Waxman, M. Brown, B.B. Graham, P.B. Yu, R.-S. Wang, B.A. Maron, T. Stephens, M. Cao, F.P. Bowman, V.N. Gladyshev, NEDD9 targets COL3A1 to promote endothelial fibrosis and pulmonary arterial hypertension , Sci. Transl. Med. 10 (2018) eaap7294. doi:10.1126/scitranslmed.aap7294.

[15] T. Zhou, Y. Zheng, L. Sun, S.R. Badea, Y. Jin, Y. Liu, A.J. Rolfe, H. Sun, X. Wang, Z. Cheng, Z. Huang, N. Zhao, X. Sun, J. Li, J. Fan, C. Lee, T.L. Megraw, W. Wu, G. Wang, Y. Ren, Microvascular endothelial cells engulf myelin debris and promote macrophage recruitment and fibrosis after neural injury, Nat. Neurosci. 22 (2019) 421–435. doi:10.1038/s41593-018-0324-9.

[16] L. Liu, Z. You, H. Yu, L. Zhou, H. Zhao, X. Yan, D. Li, B. Wang, L. Zhu, Y. Xu, T. Xia, Y. Shi, C. Huang, W. Hou, Y. Du, Mechanotransduction-modulated fibrotic microniches reveal the contribution of angiogenesis in liver fibrosis, Nat. Mater. 16 (2017) 1252–1261. doi:10.1038/nmat5024.

[17] Y. Xiao, J. Leach, J. Wang, J.F. Martin, Hippo / Yap Signaling in Cardiac Development and Regeneration, Curr. Treat. Options Cardiovasc. Med. (2016) 1–9. doi:10.1007/s11936-016-0461-y.

[18] C.A. Grant, P.C. Twigg, Pseudostatic and dynamic nanomechanics of the tunica adventitia in elastic arteries using atomic force microscopy, ACS Nano. 7 (2013) 456–464. doi:10.1021/nn304508x.

[19] A. Rezvani-Sharif, M. Tafazzoli-Shadpour, A. Avolio, Progressive changes of elastic moduli of arterial wall and atherosclerotic plaque components during plaque development in human coronary arteries, Med. Biol. Eng. Comput. 57 (2019) 731–740. doi:10.1007/s11517-018-1910-4.

[20] E. Le Master, I.S. Fancher, J. Lee, I. Levitan, Comparative analysis of endothelial cell and sub-endothelial cell elastic moduli in young and aged mice: Role of CD36, J. Biomech. 76 (2018) 263–268. doi:10.1016/j.jbiomech.2018.06.007.

[21] P. Tracqui, A. Broisat, J. Toczek, N. Mesnier, J. Ohayon, L. Riou, Mapping elasticity moduli of atherosclerotic plaque in situ via atomic force microscopy, J. Struct. Biol. 174 (2011) 115–123. doi:10.1016/j.jsb.2011.01.010.

[22] R. Akhtar, H.K. Graham, B. Derby, M.J. Sherratt, A.W. Trafford, R.S. Chadwick, N. Gavara, Frequency-modulated atomic force microscopy localises viscoelastic remodelling in the ageing sheep aorta, J. Mech. Behav. Biomed. Mater. 64 (2016) 10–17. doi:10.1016/j.jmbbm.2016.07.018.

[23] E. Melo, N. Cárdenes, E. Garreta, T. Luque, M. Rojas, D. Navajas, R. Farré, Inhomogeneity of local stiffness in the extracellular matrix scaffold of fibrotic mouse lungs, J. Mech. Behav. Biomed. Mater. 37 (2014) 186–195. doi:10.1016/j.jmbbm.2014.05.019.

[24] M.C. Lampi, C.A. Reinhart-King, Targeting extracellular matrix stiffness to attenuate disease: From molecular mechanisms to clinical trials, Sci. Transl. Med. 10 (2018) eaao0475. doi:10.1126/scitranslmed.aao0475.

[25] A.C. Finney, K.Y. Stokes, C.B. Pattillo, A.W. Orr, Integrin signaling in atherosclerosis, Cell. Mol. Life Sci. 74 (2017) 2263–2282. doi:10.1007/s00018-017-2490-4.

[26] S. Upala, K. Wirunsawanya, V. Jaruvongvanich, A. Sanguankeo, Effects of statin therapy on arterial stiffness : A systematic review and meta-analysis of randomized controlled trial, Int. J. Cardiol. 227 (2017) 338–341. doi:10.1016/j.ijcard.2016.11.073.

[27] J. Armitage, C. Baigent, E. Barnes, D.J. Betteridge, L. Blackwell, M. Blazing, et al, Efficacy and safety of statin therapy in older people: a meta-analysis of individual participant data from 28 randomised controlled trials, Lancet. 393 (2019) 407–415. doi:10.1016/S0140-6736(18)31942-1.

[28] M.C. Lampi, C.J. Faber, J. Huynh, F. Bordeleau, R. Zanotelli, C.A. Reinhart-king, Simvastatin Ameliorates Matrix Stiffness- Mediated Endothelial Monolayer Disruption, (2016) 1–20. doi:10.1371/journal.pone.0147033.

[29] Z. Mohri, A. Del Rio Hernandez, R. Krams, The emerging role of YAP/TAZ in mechanotransduction, J. Thorac. Dis. 9 (2017) E507–E509. doi:10.21037/jtd.2017.03.179.

[30] M.L. O'Donoghue, C. Glaser, R., A. M. A., B. P. E., M. P., …, A. Budaj, Effect of Losmapimod on Cardiovascular Outcomes in Patients Hospitalized With Acute Myocardial Infarction A Randomized Clinical Trial, JAMA. 315 (2016) 1591–1519. doi:10.1001/jama.2016.3609.

[31] L.K. Newby, M.S. Marber, C. Melloni, L. Sarov-Blat, L.H. Aberle, P.E. Aylward, G. Cai, R.J. De Winter, C.W. Hamm, J.F. Heitner, R. Kim, A. Lerman, M.R. Patel, J.F. Tanguay, J.J. Lepore, H.R. Al-Khalidi, D.L. Sprecher, C.B.I. Granger, Losmapimod, a novel p38 mitogen-activated protein kinase inhibitor, in non-ST-segment elevation myocardial infarction: A randomised phase 2 trial, Lancet. 384 (2014) 1187–1195. doi:10.1016/S0140-6736(14)60417-7.

[32] L. Bowman, J.C. Hopewell, F. Chen, K. Wallendszus, W. Stevens, Effects of Anacetrapib in Patients With Atherosclerotic Vascular Disease, J. Vasc. Surg. 67 (2017) 356. doi:10.1016/j.jvs.2017.11.029.

[33] H.W.O. Roeters van Lennep, A.H. Liem, P.H.J.M. Dunselman, G.M. Dallinga-Thie, A.H. Zwinderman, J. Wouter Jukema, The efficacy of statin monotherapy uptitration versus switching to ezetimibe/simvastatin: results of the EASEGO study, Curr. Med. Res. Opin. 24 (2008) 685–694. doi:10.1185/030079908x273273.

[34] P.Y. Liu, Y.W. Liu, L.J. Lin, J.H. Chen, J.K. Liao, Evidence for statin pleiotropy in humans: Differential effects of statins and ezetimibe on Rho-associated coiled-coil containing protein kinase activity, endothelial function, and inflammation, Circulation. 119 (2009) 131–138. doi:10.1161/CIRCULATIONAHA.108.813311.

[35] F. Zieve, N.K. Wenger, O. Ben-Yehuda, C. Constance, S. Bird, R. Lee, M.E. Hanson, C. Jones-Burton, A.M. Tershakovec, Safety and Efficacy of Ezetimibe Added to Atorvastatin Versus Up Titration of Atorvastatin to 40 mg in Patients ≥65 Years of Age (from the ZETia in the ELDerly [ZETELD] Study), Am. J. Cardiol. 105 (2010) 656–663. doi:10.1016/j.amjcard.2009.10.029.

[36] M.P. Bonaca, S.D. Wiviott, D.A. Morrow, P.G. Steg, C. Hamm, D.L. Bhatt, R.F. Storey, M. Cohen, J. Kuder, K. Im, G. Magnani, A. Budaj, J.C. Nicolau, A. Parkhomenko, J.L. Sendón, M. Dellborg, R. Diaz, F. Van De Werf, R. Corbalán, A. Goudev, E.C. Jensen, P. Johanson, E. Braunwald, M.S. Sabatine, Reduction in subtypes and sizes of myocardial infarction with ticagrelor in PEGASUS-TIMI 54, J. Am. Heart Assoc. 7 (2018). doi:10.1161/JAHA.118.009260.

[37] B.J. Kolls, S. Sapp, F.W. Rockhold, J.D. Jordan, K.E. Dombrowski, F.G.R. Fowkes, K.W. Mahaffey, J.S. Berger, B.G. Katona, J.I. Blomster, L. Norgren, B.L. Abramson, J.L. Leiva-Pons, J.C. Prieto, G. Sokurenko, W.R. Hiatt, W.S. Jones, M.R. Patel, Stroke in Patients With Peripheral Artery Disease, Stroke. (2019) 1–8. doi:10.1161/strokeaha.118.023534.

[38] D.E. Gutstein, R. Krishna, D. Johns, H.K. Surks, H.M. Dansky, S. Shah, Y.B. Mitchel, J. Arena, J.A. Wagner, Anacetrapib, a novel CETP inhibitor: Pursuing a new approach to cardiovascular risk reduction, Clin. Pharmacol. Ther. 91 (2012) 109–122. doi:10.1038/clpt.2011.271.

[39] J. Armitage, M. V. Holmes, D. Preiss, Cholesteryl Ester Transfer Protein Inhibition for Preventing Cardiovascular Events: JACC Review Topic of the Week, J. Am. Coll. Cardiol. 73 (2019) 477–487. doi:10.1016/j.jacc.2018.10.072.

[40] D. Strassheim, E. Gerasimovskaya, D. Irwin, E.C. Dempsey, K. Stenmark, V. Karoor, RhoGTPase in Vascular Disease, MDPI Cells. 8 (2019) 551–571. doi:10.3390/cells8060551.

[41] R. Zhuang, J. Wu, F. Lin, L. Han, X. Liang, Q. Meng, Y. Jiang, Z. Wang, A. Yue, Y. Gu, H. Fan, X. Zhou, Z. Liu, Fasudil preserves lung endothelial function and reduces pulmonary vascular remodeling in a rat model of end-stage pulmonary hypertension with left heart disease, Int. J. Mol. Med. 42 (2018) 1341–1352. doi:10.3892/ijmm.2018.3728.

[42] T. Hata, C. Goto, J. Soga, T. Hidaka, Y. Fujii, N. Idei, N. Fujimura, T. Maruhashi, S. Mikami, Y. Kihara, K. Chayama, K. Noma, J.K. Liao, Y. Higashi, Measurement of Rho-associated kinase (ROCK) activity in humans: Validity of leukocyte p-MBS/t-MBS in comparison with vascular response to fasudil, Atherosclerosis. 214 (2011) 117–121. doi:10.1016/j.atherosclerosis.2010.10.005.

[43] C. Melloni, D.L. Sprecher, L. Sarov-Blat, M.R. Patel, J.F. Heitner, C.W. Hamm, P. Aylward, J.F. Tanguay, R.J. Dewinter, M.S. Marber, A. Lerman, V. Hasselblad, C.B. Granger, L.K. Newby, The Study of LoSmapimod treatment on inflammation and InfarCtSizE (SOLSTICE): Design and rationale, Am. Heart J. 164 (2012) 646-653.e3. doi:10.1016/j.ahj.2012.07.030.

[44] N. Sinha, Review article Ticagrelor : molecular discovery to clinical evidence Ticagrelor : a novel antiplatelet agent, Indian Heart J. 64 (2012) 497–502. doi:10.1016/j.ihj.2012.09.002.

[45] C. Vlachopoulos, C. Georgakopoulos, P. Pietri, N. Ioakeimidis, M. Koutouzis, S. Vaina, K. Aznaouridis, K. Toutouzas, G. Latsios, D. Terentes-Printzios, A. Rigatou, D. Tousoulis, Effect of Ticagrelor Versus Clopidogrel on Aortic Stiffness in Patients With Coronary Artery Disease, J. Am. Heart Assoc. 8 (2019).

doi:10.1161/JAHA.119.012521.

[46] T. Bertero, K.A. Cottrill, Y. Lu, C.M. Haeger, P. Dieffenbach, S. Annis, A. Hale, B. Bhat, V. Kaimal, Y.Y. Zhang, B.B. Graham, R. Kumar, R. Saggar, R. Saggar, W.D. Wallace, D.J. Ross, S.M. Black, S. Fratz, J.R. Fineman, S.O. Vargas, K.J. Haley, A.B. Waxman, B.N. Chau, L.E. Fredenburgh, S.Y. Chan, Matrix Remodeling Promotes Pulmonary Hypertension through Feedback Mechanoactivation of the YAP/TAZ-miR-130/301 Circuit, Cell Rep. 13 (2015) 1016–1032. doi:10.1016/j.celrep.2015.09.049.

[47] T. Bertero, J. Fessel, S.Y. Chan, T. Bertero, W.M. Oldham, K.A. Cottrill, S. Pisano, R.R. Vanderpool, Q. Yu, J. Zhao, Y. Tai, Y. Tang, Y. Zhang, S. Rehman, M. Sugahara, Z. Qi, J.G. Iii, S.O. Vargas, T. De Marco, P.Y. Hsue, A. Morris, M.A. Simon, K.A. Norris, C. Gaggioli, J. Loscalzo, Vascular stiffness mechanoactivates YAP / TAZ- dependent glutaminolysis to drive pulmonary hypertension Find the latest version : Vascular stiffness mechanoactivates YAP / TAZ-dependent glutaminolysis to drive pulmonary hypertension, 126 (2016) 3313–3335. doi:10.1172/JCI86387.to.

[48] D.H. Craighead, H. Wang, L. Santhanam, L.M. Alexander, Acute lysyl oxidase inhibition alters microvascular function in normotensive but not hypertensive men and women, Am. J. Physiol. Circ. Physiol. 314 (2017) H424–H433. doi:10.1152/ajpheart.00521.2017.

[49] J.H. Hutchinson, M.W. Rowbottom, D. Lonergan, J. Darlington, P. Prodanovich, C.D. King, J.F. Evans, G. Bain, identification of an inhibitor selective for LOXL2 over LOX . Small molecule lysyl oxidase-like 2 (LOXL2) inhibitors : the identifi- cation of an inhibitor selective for LOXL2 over LOX ., ACS Med. Chem. Lett. 8 (2017) 423–427. doi:10.1021/acsmedchemlett.7b00014.

[50] D. Kothapalli, S.L. Liu, Y.H. Bae, J. Monslow, T. Xu, E.A. Hawthorne, F.J. Byfield, P. Castagnino, S. Rao, D.J. Rader, E. Puré, M.C. Phillips, S. Lund-Katz, P.A. Janmey, R.K. Assoian, Cardiovascular Protection by ApoE and ApoE-HDL Linked to Suppression of ECM Gene Expression and Arterial Stiffening, Cell Rep. 2 (2012) 1259–1271. doi:10.1016/j.celrep.2012.09.018.

[51] A. González, B. López, S. Ravassa, G. San, The complex dynamics of myocardial interstitial fibrosis in heart failure. Focus on collagen cross-linking, BBA - Mol. Cell Res. (2019). doi:10.1016/j.bbamcr.2019.06.001.

[52] M. Akodad, M. Mericskay, F. Roubille, Micro-RNAs as promising biomarkers in cardiac diseases, Ann. Transl. Med. 4 (2017) 551–551. doi:10.21037/atm.2016.12.38.

[53] F. Wahid, A. Shehzad, T. Khan, Y. Young, Biochimica et Biophysica Acta MicroRNAs : Synthesis , mechanism , function , and recent clinical trials, BBA - Mol. Cell Res. 1803 (2010) 1231–1243. doi:10.1016/j.bbamcr.2010.06.013.

[54] S. Ma, X.Y. Tian, Y. Zhang, C. Mu, H. Shen, J. Bismuth, H.J. Pownall, Y. Huang, W.T. Wong, E-selectin-targeting delivery of microRNAs by microparticles ameliorates endothelial inflammation and atherosclerosis, Sci. Rep. 6 (2016) 1–11. doi:10.1038/srep22910.

[55] S. Dangwal, T. Thum, microRNA Therapeutics in Cardiovascular Disease Models, Annu. Rev. Pharmacol. Toxicol. 54 (2013) 185–203. doi:10.1146/annurev-pharmtox-011613-135957.

[56] S.J. Zieman, V. Melenovsky, D.A. Kass, Mechanisms, pathophysiology, and therapy of arterial stiffness, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 932–943. doi:10.1161/01.ATV.0000160548.78317.29.

[57] H. Qiu, Y. Zhu, Z. Sun, J.P. Trzeciakowski, M. Gansner, C. Depre, R.R.G. Resuello, F.F. Natividad, W.C. Hunter, G.M. Genin, E.L. Elson, D.E. Vatner, G.A. Meininger, S.F. Vatner, Short communication: Vascular smooth muscle cell stiffness as a mechanism for increased aortic stiffness with aging, Circ. Res. 107 (2010) 615–619. doi:10.1161/CIRCRESAHA.110.221846.

[58] Z. Sun, M. Costell, R. Fässler, Integrin activation by talin, kindlin and mechanical forces, Nat. Cell Biol. 21 (2019) 25–31. doi:10.1038/s41556-018-0234-9.

[59] Y. Yeh, J. Ling, W. Chen, H. Lin, M. Tang, Mechanotransduction of matrix stiffness in regulation of focal adhesion size and number : reciprocal regulation of caveolin-1 and β 1 integrin, Sci. Rep. (2017) 1–14. doi:10.1038/s41598-017-14932-6.

[60] K.R. Legate, S.A. Wickstro, R. Fässler, Genetic and cell biological analysis of integrin outside-in signaling, Genes Dev. 23 (2009) 397–418. doi:10.1101/gad.1758709.elegans.

[61] T.N. Durrant, M.T. van den Bosch, I. Hers, Integrin α IIb β 3 outside-in signaling , Blood. (2017) blood-2017-03-773614. doi:10.1182/blood-2017-03-773614.

[62] Z. Sun, S.S. Guo, R. Fässler, Integrin-mediated mechanotransduction, J. Cell Biol. 215 (2016) 445–456. doi:10.1083/jcb.201609037.

[63] P.P. Provenzano, P.J. Keely, Mechanical signaling through the cytoskeleton regulates cell proliferation by coordinated focal adhesion and Rho GTPase signaling, J. Cell Sci. 124 (2011) 1195–1205. doi:10.1242/jcs.067009.

[64] Y. Sawada, M. Tamada, B.J. Dubin-thaler, O. Cherniavskaya, R. Sakai, S. Tanaka, M.P. Sheetz, Force Sensing by Mechanical Extension of the Src Family Kinase Substrate p130Cas, (2006) 1015–1026. doi:10.1016/j.cell.2006.09.044.

[65] S. Huveneers, E.H.J. Danen, Adhesion signaling - crosstalk between integrins, Src and Rho, J. Cell Sci. 122 (2009) 1059–1069. doi:10.1242/jcs.039446.

[66] A. Schaefer, P.L. Hordijk, Cell-stiffness-induced mechanosignaling - a key driver of leukocyte transendothelial migration, J. Cell Sci. 128 (2015) 2221–2230. doi:10.1242/jcs.163055.

[67] S. Alimperti, T. Mirabella, V. Bajaj, W. Polacheck, D.M. Pirone, J. Duffield, J. Eyckmans, R.K. Assoian, C.S. Chen, Three-dimensional biomimetic vascular model reveals a RhoA, Rac1, and N -cadherin balance in mural cell–endothelial cell-regulated barrier function, Proc. Natl. Acad. Sci. 114 (2017) 8758–8763. doi:10.1073/pnas.1618333114.

[68] N. Mandriota, C. Friedsam, J.A. Jones-Molina, K. V. Tatem, D.E. Ingber, O. Sahin, Cellular nanoscale stiffness patterns governed by intracellular forces, Nat. Mater. (2019). doi:10.1038/s41563-019-0391-7.

[69] Y. Ohta, J.H. Hartwig, T.P. Stossel, FilGAP, a Rho- and ROCK-regulated GAP for Rac binds filamin A to control actin remodelling, Nat. Cell Biol. 8 (2006) 803–814. doi:10.1038/ncb1437.

[70] R.S. Fischer, M. Gardel, X. Ma, R.S. Adelstein, C.M. Waterman, Report Local Cortical Tension by Myosin II Guides 3D Endothelial Cell Branching, Curr. Biol. 19 (2009) 260–265. doi:10.1016/j.cub.2008.12.045.

[71] L. Moldovan, K. Mythreye, P.J. Goldschmidt-Clermont, L.L. Satterwhite, Reactive oxygen species in vascular endothelial cell motility. Roles of NAD(P)H oxidase and Rac1, Cardiovasc. Res. 71 (2006) 236–246. doi:10.1016/j.cardiores.2006.05.003.

[72] A. Mammoto, S. Huang, K. Moore, P. Oh, D.E. Ingber, Role of RhoA, mDia, and ROCK in cell shape-dependent control of the Skp2-p27kip-1 pathway and the G1/S transition, J. Biol. Chem. 279 (2004) 26323–26330. doi:10.1074/jbc.M402725200.

[73] W. Meng, M. Numazaki, K. Takeuchi, Y. Uchibori, Y. Ando-Akatsuka, M. Tominaga, T. Tominaga, DIP (mDia interacting protein) is a key molecule regulating Rho and Rac in a Src-dependent manner, EMBO J. 23 (2004) 760–771. doi:10.1038/sj.emboj.7600095.

[74] Kshitiz, M.E. Hubbi, E.H. Ahn, J. Downey, J. Afzal, Kim, Matrix Rigidity Controls Endothelial Differentiation and Morphogenesis of Cardiac Precursors, Sci. Signal. 5 (2012) 1–15. doi:10.1126/scisignal.2003002.

[75] T.Y. Kim, D. Vigil, R.L. Juliano, Role of DLC-1 , a tumor suppressor protein with RhoGAP activity , in regulation of the cytoskeleton and cell motility, (2009) 77–83. doi:10.1007/s10555-008-9167-2.

[76] R.-P. Scholz, J. Regner, A. Theil, P. Erlmann, G. Holeiter, R. Jahne, S. Schmid, A. Hausser, M.A. Olayioye, DLC1 interacts with 14-3-3 proteins to inhibit RhoGAP activity and block nucleocytoplasmic shuttling, J. Cell Sci. 122 (2008) 92–102. doi:10.1242/jcs.036251.

[77] P. Karki, A.A. Birukova, Substrate stiffness-dependent exacerbation of endothelial permeability and inflammation: mechanisms and potential implications in ALI and PH (2017 Grover Conference Series), Pulm. Circ. 8 (2018). doi:10.1177/2045894018773044.

[78] H.A. Scott, B. Quach, X. Yang, S. Ardekani, A.P. Cabrera, R. Wilson, …, K. Ghosh, Matrix stiffness exerts biphasic control over monocyte–endothelial adhesion via Rho-mediated ICAM-1 clustering, Integr. Biol. 8 (2016) 869–878. doi:10.1039/C6IB00084C.

[79] L. Schimmel, M. Van Der Stoel, C. Rianna, V. De Waard, S. Huveneers, J.D. Van Buul, S.E. Dlc-, L. Schimmel, M. Van Der Stoel, C. Rianna, A. Van Stalborch, A. De Ligt, Stiffness-Induced Endothelial DLC-1 Expression Forces Leukocyte Spreading through Stabilization of Report Expression Forces Leukocyte Spreading through Stabilization of the ICAM-1 Adhesome, CellReports. 24 (2018) 3115–3124. doi:10.1016/j.celrep.2018.08.045.

[80] M. Schnoor, P. Alcaide, M.-B. Voisin, J.D. van Buul, Crossing the Vascular Wall: Common and Unique Mechanisms Exploited by Different Leukocyte Subsets during Extravasation, Mediators Inflamm. (2015) 1–23. doi:10.1155/2015/946509.

[81] C. Souilhol, M.C. Harmsen, P.C. Evans, G. Krenning, Endothelial-Mesenchymal Transition in Atherosclerosis. Celine, Cardiovasc. Res. 52 (2018) 1–5. doi:10.1093/cvr/cvx253/4788364.

[82] S. Rho, K. Ando, S. Fukuhara, Dynamic Regulation of Vascular Permeability by Vascular Endothelial Cadherin-Mediated Endothelial Cell-Cell Junctions, J. Nippon Med. Sch. 84 (2017) 148–159. doi:10.1272/jnms.84.148.

[83] W. Wang, E.M. Lollis, F. Bordeleau, C.A. Reinhart-King, Matrix stiffness regulates vascular integrity through focal adhesion kinase activity, FASEB J. 33 (2019) 1199–1208. doi:10.1096/fj.201800841R.

[84] J. Wu, A.H. Lewis, J. Grandl, Touch, Tension, and Transduction – The Function and Regulation of Piezo Ion Channels, Trends Biochem. Sci. 42 (2017) 57–71. doi:10.1016/j.tibs.2016.09.004.

[85] J. Li, B. Hou, S. Tumova, K. Muraki, A. Bruns, M.J. Ludlow, A. Sedo, A.J. Hyman, L. McKeown, R.S. Young, N.Y. Yuldasheva, Y. Majeed, L.A. Wilson, B. Rode, M.A. Bailey, H.R. Kim, Z. Fu, D.A.L. Carter, J. Bilton, H. Imrie, P. Ajuh, T.N. Dear, R.M. Cubbon, M.T. Kearney, K.R. Prasad, P.C. Evans, J.F.X. Ainscough, D.J. Beech, Piezo1 integration of vascular architecture with physiological force, Nature. 515 (2014) 279–282. doi:10.1038/nature13701.

[86] J.A. Nick, S.K. Young, K.K. Brown, N.J. Avdi, P.G. Arndt, B.T. Suratt, M.S. Janes, P.M. Henson, G.S. Worthen, Role of p38 Mitogen-Activated Protein Kinase in a Murine Model of Pulmonary Inflammation, J. Immunol. 164 (2014) 2151–2159. doi:10.4049/jimmunol.164.4.2151.

[87] R.A. Scott, P.M. Kharkar, K.L. Kiick, R.E. Akins, Aortic adventitial fibroblast sensitivity to mitogen activated protein kinase inhibitors depends on substrate stiffness, Biomaterials. 137 (2017) 1–10. doi:10.1016/j.biomaterials.2017.05.010.

[88] L. Hoffman, C.C. Jensen, M. Yoshigi, M. Beckerle, V.M. Weaver, Mechanical signals activate p38 MAPK pathway-dependent reinforcement of actin via mechanosensitive HspB1, Mol. Biol. Cell. (2016) 1–35. doi:10.1091/mbc.E17-02-0087.

[89] Q. Wang, C.M. Doerschuk, The p38 Mitogen-Activated Protein Kinase Mediates Cytoskeletal Remodeling in Pulmonary Microvascular Endothelial Cells Upon Intracellular Adhesion Molecule-1 Ligation, J. Immunol. 166 (2014) 6877–6884. doi:10.4049/jimmunol.166.11.6877.

[90] N. Azuma, N. Akasaka, H. Kito, M. Ikeda, V. Gahtan, T. Sasajima, B.E. Sumpio, N. Akasaka, M. Ikeda, V. Gahtan, T. Sasa-, B.E. Sumpio, Role of p38 MAP kinase in endothelial cell alignment induced by fluid shear stress, (2001) 189–197. doi:10.1152/ajpheart.2001.280.1.h189.

[91] J. Wu, S.R. Thabet, A. Kirabo, D.W. Trott, M.A. Saleh, L. Xiao, M.S. Madhur, W. Chen, D.G. Harrison, Integrative Physiology Inflammation and Mechanical Stretch Promote Aortic Stiffening in Hypertension Through Activation of p38, Circ. Res. 114 (2015) 616–625. doi:10.1161/CIRCRESAHA.114.302157.

[92] M. Fisk, P.R. Gajendragadkar, K.M. Mäki-Petäjä, I.B. Wilkinson, J. Cheriyan, Therapeutic potential of p38 MAP kinase inhibition in the management of cardiovascular disease, Am. J. Cardiovasc. Drugs. 14 (2014) 155–165. doi:10.1007/s40256-014-0063-6.

[93] H. Hirata, M. Gupta, S.R.K. Vedula, C.T. Lim, B. Ladoux, M. Sokabe, Actomyosin bundles serve as a tension sensor and a platform for ERK activation, EMBO Rep. 16 (2015) 250–257. doi:10.15252/embr.201439140.

[94] Y. Zheng, Y. Xia, D. Hawke, M. Halle, M.L. Tremblay, X. Gao, X.Z. Zhou, K. Aldape, M.H. Cobb, K. Xie, J. He, Z. Lu, Article FAK Phosphorylation by ERK Primes Ras-Induced Tyrosine Dephosphorylation of FAK Mediated by PIN1 and PTP-PEST, Mol. Cell. 35 (2009) 11–25. doi:10.1016/j.molcel.2009.06.013.

[95] I.P. Whitehead, K. Abe, J.L. Gorski, C.J. Der, CDC42 and FGD1 Cause Distinct Signaling and Transforming Activities, Mol. Cell. Biol. 18 (1998) 4689–4697. doi:10.1128/mcb.18.8.4689.

[96] X. Wu, M. Li, S.Q. Chen, S. Li, F. Guo, Pin1 facilitates isoproterenol-induced cardiac fibrosis and collagen deposition by promoting oxidative stress and activating the MEK1/2-ERK1/2 signal transduction pathway in rats, Int. J. Mol. Med. 41 (2018) 1573–1583. doi:10.3892/ijmm.2017.3354.

[97] P. Monje, J. Hernández-Losa, R.J. Lyons, M.D. Castellone, J.S. Gutkind, Regulation of the transcriptional

activity of c-Fos by ERK: A novel role for the prolyl isomerase Pin1, J. Biol. Chem. 280 (2005) 35081–35084. doi:10.1074/jbc.C500353200.

[98] P.Y. Mong, C. Petrulio, H.L. Kaufman, E. Cells, P.Y. Mong, C. Petrulio, H.L. Kaufman, Q. Wang, Activation in Human Pulmonary Microvascular, J. Immunol. 180 (2007) 550–558. doi:10.4049/jimmunol.180.1.550.

[99] Y. Zou, Y. Hu, B. Metzler, Q. Xu, Signal transduction in arteriosclerosis: mechanical stress-activated MAP kinases in vascular smooth muscle cells (review)., Int. J. Mol. Med. 1 (1998) 827–834. doi:10.3892/ijmm.1.5.827.

[100] E. Piddini, Dispatches Epithelial Homeostasis : A Piezo of the Puzzle, Curr. Biol. 27 (2017) R232–R234. doi:10.1016/j.cub.2017.02.002.

[101] B. Zhao, L. Li, Hippo signaling at a glance Hippo Signaling at a Glance, J. Cell Sci. 123 (2010) 4001–4006. doi:10.1242/jcs.069070.

[102] S. Dupont, L. Morsut, M. Aragona, E. Enzo, S. Giulitti, M. Cordenonsi, F. Zanconato, J. Le Digabel, M. Forcato, S. Bicciato, N. Elvassore, S. Piccolo, Role of YAP / TAZ in mechanotransduction, (2011). doi:10.1038/nature10137.

[103] K. Wang, Y. Yeh, P. Nguyen, E. Limqueco, J. Lopez, S. Thorossian, Flow-dependent YAP / TAZ activities regulate endothelial phenotypes and atherosclerosis, (2016) 1–6. doi:10.1073/pnas.1613121113.

[104] O. Dobrokhotov, M. Samsonov, M. Sokabe, H. Hirata, Mechanoregulation and pathology of YAP/TAZ via Hippo and non-Hippo mechanisms, Clin. Transl. Med. 7 (2018) 1–14. doi:10.1186/s40169-018-0202-9.

[105] Z. Meng, T. Moroishi, K. Guan, Mechanisms of Hippo pathway regulation, (2016) 1–17. doi:10.1101/gad.274027.115.1/2.

[106] Y. Zheng, W. Wang, B. Liu, H. Deng, E. Uster, D. Pan, Identification of Happyhour/MAP4K as Alternative Hpo/Mst-like Kinases in the Hippo Kinase Cascade, Dev. Cell. 34 (2015) 642–655. doi:10.1016/j.devcel.2015.08.014.

[107] C.S. Collins, J. Hong, L. Sapinoso, Y. Zhou, Z. Liu, K. Micklash, P.G. Schultz, G.M. Hampton, A small interfering RNA screen for modulators of tumor cell motility identifies MAP4K4 as a promigratory kinase, Proc. Natl. Acad. Sci. 103 (2006) 3775–3780. doi:10.1073/pnas.0600040103.

[108] L. V. Danai, A. Guilherme, K. V. Guntur, J. Straubhaar, S.M. Nicoloro, M.P. Czech, Map4k4 suppresses Srebp-1 and adipocyte lipogenesis independent of JNK signaling, J. Lipid Res. 54 (2013) 2697–2707. doi:10.1194/jlr.m038802.

[109] R.J. Roth Flach, A. Skoura, A. Matevossian, L. V. Danai, W. Zheng, C. Cortes, S.K. Bhattacharya, M. Aouadi, N. Hagan, J.C. Yawe, P. Vangala, L.G. Menendez, M.P. Cooper, T.P. Fitzgibbons, L. Buckbinder, M.P. Czech, Endothelial protein kinase MAP4K4 promotes vascular inflammation and atherosclerosis, Nat. Commun. 6 (2015) 1–11. doi:10.1038/ncomms9995.

[110] A. Totaro, M. Castellan, D. Di Biagio, S. Piccolo, Crosstalk between YAP/TAZ and Notch Signaling, Trends Cell Biol. 28 (2018) 560–573. doi:10.1016/j.tcb.2018.03.001.

[111] J.W. Haskins, D.X. Nguyen, D.F. Stern, Neuregulin 1-activated ERBB4 interacts with YAP to induce Hippo pathway target genes and promote cell migration, Sci. Signal. 7 (2014) 1–11. doi:10.1126/scisignal.2005770.

[112] N.G. Kim, B.M. Gumbiner, Adhesion to fibronectin regulates Hippo signaling via the FAK – Src – PI3K pathway, 210 (2015) 503–515. doi:10.1083/jcb.201501025.

[113] G. Halder, S. Dupont, S. Piccolo, Transduction of mechanical and cytoskeletal cues by YAP and TAZ, Nat. Rev. Mol. Cell Biol. 13 (2012) 591–600. doi:10.1038/nrm3416.

[114] K. Hayakawa, H. Tatsumi, M. Sokabe, Actin filaments function as a tension sensor by tension-dependent binding of cofilin to the filament, J. Cell Biol. 195 (2011) 721–727. doi:10.1083/jcb.201102039.

[115] O. Wiggan, B. Schroder, D. Krapf, J.R. Bamburg, J.G. Deluca, Cofilin Regulates Nuclear Architecture through a Myosin-II Dependent Mechanotransduction Module, Sci. Rep. 7 (2017) 1–15. doi:10.1038/srep40953.

[116] M. Aragona, T. Panciera, A. Manfrin, S. Giulitti, F. Michielin, N. Elvassore, S. Dupont, S. Piccolo, A

mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors, Cell. 154 (2013) 1047–1059. doi:10.1016/j.cell.2013.07.042.

[117] B.D. Cosgrove, K.L. Mui, T.P. Driscoll, S.R. Caliari, K.D. Mehta, R.K. Assoian, J.A. Burdick, R.L. Mauck, N-cadherin adhesive interactions modulate matrix mechanosensing and fate commitment of mesenchymal stem cells, Nat. Mater. 15 (2016) 1297–1306. doi:10.1038/nmat4725.

[118] F. Zanconato, M. Forcato, G. Battilana, L. Azzolin, E. Quaranta, B. Bodega, A. Rosato, S. Bicciato, M. Cordenonsi, S. Piccolo, Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth, Nat. Cell Biol. 17 (2015) 1218–1227. doi:10.1038/ncb3216.

[119] A. Mitani, T. Nagase, K. Fukuchi, H. Aburatani, R. Makita, H. Kurihara, Transcriptional coactivator with PDZ-binding motif is essential for normal alveolarization in mice, Am. J. Respir. Crit. Care Med. 180 (2009) 326–338. doi:10.1164/rccm.200812-1827OC.

[120] Azad, Ghahremani, Yang, The Role of YAP and TAZ in Angiogenesis and Vascular Mimicry, Cells. 8 (2019) 407. doi:10.3390/cells8050407.

[121] F. Neto, A. Klaus-Bergmann, Y.T. Ong, S. Alt, A.-C. Vion, A. Szymborska, J.R. Carvalho, I. Hollfinger, E. Bartels-Klein, C.A. Franco, M. Potente, H. Gerhardt, YAP and TAZ regulate adherens junction dynamics and endothelial cell distribution during vascular development, Elife. 7 (2018) 1–30. doi:10.7554/elife.31037.

[122] A. Totaro, M. Castellan, G. Battilana, F. Zanconato, L. Azzolin, S. Giulitti, M. Cordenonsi, S. Piccolo, YAP/TAZ link cell mechanics to Notch signalling to control epidermal stem cell fate, Nat. Commun. 8 (2017) 1–13. doi:10.1038/ncomms15206.

[123] G. Rusanescu, R. Weissleder, E. Aikawa, Notch Signaling in Cardiovascular Disease and Calcification, Curr. Cardiol. Rev. 4 (2008) 148–156. doi:10.2174/157340308785160552.

[124] T. Nakano, D. Fukuda, J.I. Koga, M. Aikawa, Delta-like ligand 4-notch signaling in macrophage activation, Arterioscler. Thromb. Vasc. Biol. 36 (2016) 2038–2047. doi:10.1161/ATVBAHA.116.306926.

[125] D.Y. Tian, X.R. Jin, X. Zeng, Y. Wang, Notch signaling in endothelial cells: Is it the therapeutic target for vascular neointimal hyperplasia?, Int. J. Mol. Sci. 18 (2017). doi:10.3390/ijms18081615.

[126] J.J. Mack, T.S. Mosqueiro, B.J. Archer, W.M. Jones, H. Sunshine, G.C. Faas, A. Briot, R.L. Aragón, T. Su, M.C. Romay, A.I. McDonald, C.-H. Kuo, C.O. Lizama, T.F. Lane, A.C. Zovein, Y. Fang, E.J. Tarling, T.Q. de Aguiar Vallim, M. Navab, A.M. Fogelman, L.S. Bouchard, M.L. Iruela-Arispe, NOTCH1 is a mechanosensor in adult arteries., Nat. Commun. 8 (2017) 1620. doi:10.1038/s41467-017-01741-8.

[127] A. Zhong, Z. Mirzaei, C.A. Simmons, The Roles of Matrix Stiffness and ß-Catenin Signaling in Endothelial-to-Mesenchymal Transition of Aortic Valve Endothelial Cells, Cardiovasc. Eng. Technol. 9 (2018) 158–167. doi:10.1007/s13239-018-0363-0.

[128] J.H. Chen, W.L.K. Chen, K.L. Sider, C.Y.Y. Yip, C.A. Simmons, B-Catenin Mediates Mechanically Regulated, Transforming Growth Factor-B1-Induced Myofibroblast Differentiation of Aortic Valve Interstitial Cells, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 590–597. doi:10.1161/ATVBAHA.110.220061.

[129] H. Li, Q. Zhao, L. Chang, C. Wei, H. Bei, Y. Yin, M. Chen, H. Wang, J. Liang, Y. Wu, LncRNA MALAT1 modulates ox-LDL induced EndMT through the Wnt/β-catenin signaling pathway, Lipids Health Dis. 18 (2019) 1–10. doi:10.1186/s12944-019-1006-7.

[130] M.-L. Bochaton-Piallat, M. Bäck, Novel concepts for the role of smooth muscle cells in vascular disease: towards a new smooth muscle cell classification, Cardiovasc. Res. 114 (2018) 477–480. doi:10.1093/cvr/cvy031.

[131] M. Corada, D. Nyqvist, F. Orsenigo, A. Caprini, C. Giampietro, M.M. Taketo, M.L. Iruela-Arispe, R.H. Adams, E. Dejana, The Wnt/β-catenin pathway modulates vascular remodeling and specification by upregulating Dll4/notch signaling, Dev. Cell. 18 (2010) 938–949. doi:10.1016/j.devcel.2010.05.006.

[132] J.T.Y. Kung, D. Colognori, J.T. Lee, Long Noncoding RNAs: Past, Present, and Future, 193 (2013) 651–669. doi:10.1534/genetics.112.146704.

[133] C.K. Yu, T. Xu, R.K. Assoian, D.J. Rader, Mining the Stiffness-Sensitive Transcriptome in Human Vascular Smooth Muscle Cells Identifies Long Noncoding RNA Stiffness Regulators, Arterioscler.

Thromb. Vasc. Biol. 38 (2018) 164–173. doi:10.1161/ATVBAHA.117.310237.

[134] G. Wang, Y. Li, Y. Peng, J. Tang, H. Li, Association of polymorphisms in MALAT1 with risk of coronary atherosclerotic heart disease in a Chinese population, Lipids Health Dis. 17 (2018) 1–7. doi:10.1186/s12944-018-0728-2.

[135] S. Cremer, K.M. Michalik, A. Fischer, L. Pfisterer, N. Jaé, C. Winter, S. Dimmeler, Hematopoietic Deficiency of the Long Non-Coding RNA MALAT1 Promotes Atherosclerosis and Plaque Inflammation, Circulation. (2019). doi:10.1161/circulationaha.117.029015.

[136] J. Guo, A. Fujiyoshi, B. Willcox, J. Choo, A. Vishnu, T. Hisamatsu, V. Ahuja, N. Takashima, E. Barinas-Mitchell, A. Kadota, R.W. Evans, K. Miura, D. Edmundowicz, K. Masaki, C. Shin, L.H. Kuller, H. Ueshima, A. Sekikawa, Increased Aortic Calcification Is Associated with Arterial Stiffness Progression in Multiethnic Middle-Aged Men, Hypertension. 69 (2017) 102–108. doi:10.1161/HYPERTENSIONAHA.116.08459.

[137] K. Carrion, J. Dyo, V. Patel, R. Sasik, S.A. Mohamed, G. Hardiman, V. Nigam, The long non-coding HOTAIR is modulated by cyclic stretch and WNT/β-CATENIN in human aortic valve cells and is a novel repressor of calcification genes, PLoS One. 9 (2014) 1–7. doi:10.1371/journal.pone.0096577.

[138] R.A. Gupta, N. Shah, K.C. Wang, J. Kim, H.M. Horlings, D.J. Wong, M.C. Tsai, T. Hung, P. Argani, J.L. Rinn, Y. Wang, P. Brzoska, B. Kong, R. Li, R.B. West, M.J. Van De Vijver, S. Sukumar, H.Y. Chang, Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis, Nature. 464 (2010) 1071–1076. doi:10.1038/nature08975.

[139] F. Guo, C. Tang, Y. Li, Y. Liu, P. Lv, W. Wang, Y. Mu, The interplay of LncRNA ANRIL and mir-181b on the inflammation-relevant coronary artery disease through mediating NF-κb signalling pathway, J. Cell. Mol. Med. 22 (2018) 5062–5075. doi:10.1111/jcmm.13790.

[140] M.H. Bao, V. Szeto, B.B. Yang, S.Z. Zhu, H.S. Sun, Z.P. Feng, Long non-coding RNAs in ischemic stroke review-article, Cell Death Dis. 9 (2018) 1–12. doi:10.1038/s41419-018-0282-x.

[141] L. Elia, M. Quintavalle, Epigenetics and Vascular Diseases: Influence of Non-coding RNAs and Their Clinical Implications, Front. Cardiovasc. Med. 4 (2017) 1–7. doi:10.3389/fcvm.2017.00026.

[142] A. Kumar, S.K. Thomas, K.C. Wong, V. Lo Sardo, D.S. Cheah, Y.H. Hou, J.K. Placone, K.P. Tenerelli, W.C. Ferguson, A. Torkamani, E.J. Topol, K.K. Baldwin, A.J. Engler, Mechanical activation of noncoding-RNA-mediated regulation of disease-associated phenotypes in human cardiomyocytes, Nat. Biomed. Eng. 3 (2019) 137–146. doi:10.1038/s41551-018-0344-5.

[143] S. Nanoudis, M. Pikilidou, M. Yavropoulou, P. Zebekakis, The role of MicroRNAs in arterial stiffness and arterial calcification. An update and review of the literature, Front. Genet. 8 (2017) 1–11. doi:10.3389/fgene.2017.00209.

[144] T. Bertero, A.L. Handen, S.Y. Chan, Factors associated with heritable pulmonary arterial hypertension exert convergent actions on the miR-130/301-vascular matrix feedback loop, Int. J. Mol. Sci. 19 (2018) 1–17. doi:10.3390/ijms19082289.

[145] S. Kumar, R.A. Boon, L. Maegdefessel, S. Dimmeler, H. Jo, Role of Noncoding RNAs in the Pathogenesis of Abdominal Aortic Aneurysm, Circ. Res. 124 (2019) 619–630. doi:10.1161/CIRCRESAHA.118.312438.

[146] D. Hori, B. Dunkerly-Eyring, Y. Nomura, D. Biswas, J. Steppan, J. Henao-Mejia, H. Adachi, L. Santhanam, D.E. Berkowitz, C. Steenbergen, R.A. Flavell, S. Das, MiR-181b regulates vascular stiffness age dependently in part by regulating TGF-β signaling, PLoS One. 12 (2017) 1–20. doi:10.1371/journal.pone.0174108.

[147] Y. Wei, A. Schober, C. Weber, Pathogenic arterial remodeling: the good and bad of microRNAs, Am. J. Physiol. Circ. Physiol. 304 (2013) H1050–H1059. doi:10.1152/ajpheart.00267.2012.

[148] X. Lin, J.-K. Zhan, Y.-J. Wang, P. Tan, Y.-Y. Chen, H.-Q. Deng, Y.-S. Liu, Function, Role, and Clinical Application of MicroRNAs in Vascular Aging, Biomed Res. Int. 2016 (2016) 1–15. doi:10.1155/2016/6021394.

[149] V. Ulrich, N. Rotllan, E. Araldi, A. Luciano, P. Skroblin, M. Abonnenc, P. Perrotta, X. Yin, A. Bauer, K.L. Leslie, P. Zhang, B. Aryal, R.L. Montgomery, T. Thum, K. Martin, Y. Suarez, M. Mayr, C. Fernandez-Hernando, W.C. Sessa, Chronic miR-29 antagonism promotes favorable plaque remodeling in atherosclerotic mice, EMBO Mol. Med. 8 (2016) 643–653. doi:10.15252/emmm.201506031.

[150] S. Lee, E. Choi, M.J. Cha, A.J. Park, C. Yoon, K.C. Hwang, Impact of miRNAs on cardiovascular aging, J. Geriatr. Cardiol. 12 (2015) 569–574. doi:10.11909/j.issn.1671-5411.2015.05.011.

[151] Y.C. Liao, Y.S. Wang, E. Hsi, M.H. Chang, Y.Z. You, S.H.H. Juo, MicroRNA-765 influences arterial stiffness through modulating apelin expression, Mol. Cell. Endocrinol. 411 (2015) 11–19. doi:10.1016/j.mce.2015.04.006.

[152] A. Sayed, K. Xia, F. Li, X. Deng, U. Salma, T. Li, H. Deng, D. Yang, Z. Haoyang, T. Yang, J. Peng, The diagnostic value of circulating microRNAs for middle-aged (40–60-year-old) coronary artery disease patients, Clinics. 70 (2015) 257–263. doi:10.6061/clinics/2015(04)07.

[153] J. Huynh, N. Nishimura, K. Rana, J.M. Peloquin, J.P. Califano, C.R. Montague, M.R. King, C.B. Schaffer, C.A. Reinhart-king, Age-Related Intimal Stiffening Enhances Endothelial Permeability and Leukocyte Transmigration, Sci. Transl. Med. 3 (2011) 112–122.

[154] M.W. Tibbitt, K.S. Anseth, Hydrogels as extracellular matrix mimics for 3D cell culture, Biotechnol. Bioeng. 103 (2009) 655–663. doi:10.1002/bit.22361.

[155] M. Kapałczyńska, T. Kolenda, W. Przybyła, M. Zajączkowska, A. Teresiak, V. Filas, M. Ibbs, R. Bliźniak, Ł. Łuczewski, K. Lamperska, 2D and 3D cell cultures – a comparison of different types of cancer cell cultures, Arch. Med. Sci. 14 (2018) 910–919. doi:10.5114/aoms.2016.63743.

[156] R. Edmondson, J.J. Broglie, A.F. Adcock, L. Yang, Three-Dimensional Cell Culture Systems and Their Applications in Drug Discovery and Cell-Based Biosensors, Assay Drug Dev. Technol. 12 (2014) 207–218. doi:10.1089/adt.2014.573.

[157] F. Brandl, F. Sommer, A. Goepferich, Rational design of hydrogels for tissue engineering: Impact of physical factors on cell behavior, Biomaterials. 28 (2007) 134–146. doi:10.1016/j.biomaterials.2006.09.017.

[158] A.K. Denisin, B.L. Pruitt, Tuning the Range of Polyacrylamide Gel Stiffness for Mechanobiology Applications, ACS Appl. Mater. Interfaces. 8 (2016) 21893–21902. doi:10.1021/acsami.5b09344.

[159] A. Buxboim, K. Rajagopal, A.E.X. Brown, D.E. Discher, How deeply cells feel: Methods for thin gels, J. Phys. Condens. Matter. 22 (2010) 1–10. doi:10.1088/0953-8984/22/19/194116.

[160] R.J. Pelham, Y.-L. Wang, Cell locomotion and focal adhesions are regulated by substrate flexibility ROBERT, Proc. Am. Thorac. Soc. 94 (1997) 13661–13665. doi:10.1073/pnas.94.25.13661.

[161] T. Grevesse, M. Versaevel, S. Gabriele, Preparation of Hydroxy-PAAm Hydrogels for Decoupling the Effects of Mechanotransduction Cues, J. Vis. Exp. (2014) 1–8. doi:10.3791/51010.

[162] D. Calvet, J.Y. Wong, S. Giasson, Rheological monitoring of polyacrylamide gelation: Importance of cross-link density and temperature, Macromolecules. 37 (2004) 7762–7771. doi:10.1021/ma049072r.

[163] W. Zhang, D.S. Choi, Y.H. Nguyen, J. Chang, L. Qin, Studying cancer stem cell dynamics on PDMS surfaces for microfluidics device design, Sci. Rep. 3 (2013) 1–8. doi:10.1038/srep02332.

[164] B. Trappmann, J.E. Gautrot, J.T. Connelly, D.G.T. Strange, Y. Li, M.L. Oyen, M.A. Cohen Stuart, H. Boehm, B. Li, V. Vogel, J.P. Spatz, F.M. Watt, W.T.S. Huck, Extracellular-matrix tethering regulates stem-cell fate, Nat. Mater. 11 (2012) 642–649. doi:10.1038/nmat3339.

[165] I.D. Johnston, D.K. McCluskey, C.K.L. Tan, M.C. Tracey, Mechanical characterization of bulk Sylgard 184 for microfluidics and microengineering, J. Micromechanics Microengineering. 24 (2014). doi:10.1088/0960-1317/24/3/035017.

[166] M. Liu, J. Sun, Q. Chen, Influences of heating temperature on mechanical properties of polydimethylsiloxane, Sensors Actuators, A Phys. 151 (2009) 42–45. doi:10.1016/j.sna.2009.02.016.

[167] S.W. Lee, S.S. Lee, Shrinkage ratio of PDMS and its alignment method for the wafer level process, Microsyst. Technol. 14 (2008) 205–208. doi:10.1007/s00542-007-0417-y.

[168] J.H. Wen, L.G. Vincent, A. Fuhrmann, Y.S. Choi, K.C. Hribar, H. Taylor-Weiner, S. Chen, A.J. Engler, Interplay of matrix stiffness and protein tethering in stem cell differentiation, Nat. Mater. 13 (2014) 979–987. doi:10.1038/nmat4051.

[169] Y. Berdichevsky, J. Khandurina, A. Guttman, Y.H. Lo, UV/ozone modification of poly(dimethylsiloxane) microfluidic channels, Sensors Actuators, B Chem. 97 (2004) 402–408. doi:10.1016/j.snb.2003.09.022.

[170] A. Oláh, H. Hillborg, G.J. Vancso, Hydrophobic recovery of UV/ozone treated poly(dimethylsiloxane):

Adhesion studies by contact mechanics and mechanism of surface modification, Appl. Surf. Sci. 239 (2005) 410–423. doi:10.1016/j.apsusc.2004.06.005.

[171] M.K. Saums, W. Wang, B. Han, L. Madhavan, L. Han, D. Lee, R.G. Wells, Mechanically and chemically tunable cell culture system for studying the myofibroblast phenotype, Langmuir. 30 (2014) 5481–5487. doi:10.1021/la4047758.

[172] C.C. Lin, K.S. Anseth, PEG hydrogels for the controlled release of biomolecules in regenerative medicine, Pharm. Res. 26 (2009) 631–643. doi:10.1007/s11095-008-9801-2.

[173] D. Aydin, I. Louban, N. Perschmann, J. Blümmel, T. Lohmüller, E.A. Cavalcanti-Adam, T.L. Haas, H. Walczak, H. Kessler, R. Fiammengo, J.P. Spatz, Polymeric substrates with tunable elasticity and nanoscopically controlled biomolecule presentation, Langmuir. 26 (2010) 15472–15480. doi:10.1021/la103065x.

[174] W.G. Herrick, T. V. Nguyen, M. Sleiman, S. McRae, T.S. Emrick, S.R. Peyton, PEG-phosphorylcholine hydrogels as tunable and versatile platforms for mechanobiology, Biomacromolecules. 14 (2013) 2294–2304. doi:10.1021/bm400418g.

[175] S.R. Peyton, C.B. Raub, V.P. Keschrumrus, A.J. Putnam, The use of poly(ethylene glycol) hydrogels to investigate the impact of ECM chemistry and mechanics on smooth muscle cells, Biomaterials. 27 (2006) 4881–4893. doi:10.1016/j.biomaterials.2006.05.012.

[176] K.H. Son, J.W. Lee, Synthesis and characterization of poly(ethylene glycol) based thermo-responsive hydrogels for cell sheet engineering, Materials (Basel). 9 (2016) 1–13. doi:10.3390/ma9100854.

[177] K.B. Keys, F.M. Andreopoulos, N.A. Peppas, Poly(ethylene glycol) star polymer hydrogels, Macromolecules. 31 (1998) 8149–8156. doi:10.1021/ma980999z.

[178] A. Khavari, M. Nydén, D.A. Weitz, A.J. Ehrlicher, Composite alginate gels for tunable cellular microenvironment mechanics, Sci. Rep. 6 (2016) 1–11. doi:10.1038/srep30854.

[179] J.A. Rowley, G. Madlambayan, D.J. Mooney, Alginate hydrogels as synthetic extracellular matrix materials, Biomaterials. 20 (1999) 45–53. doi:10.1016/S0142-9612(98)00107-0.

[180] C. Lee, J. Shin, J.S. Lee, E. Byun, J.H. Ryu, S.H. Um, D.I. Kim, H. Lee, S.W. Cho, Bioinspired, calcium-free alginate hydrogels with tunable physical and mechanical properties and improved biocompatibility, Biomacromolecules. 14 (2013) 2004–2013. doi:10.1021/bm400352d.

[181] K.I. Draget, G. Skjåk-Bræek, O. Smidsrød, Alginate based new materials, Int. J. Biol. Macromol. 21 (1997) 47–55. doi:10.1016/S0141-8130(97)00040-8.

[182] H.J. Kong, E. Wong, D.J. Mooney, Independent control of rigidity and toughness of polymeric hydrogels, Macromolecules. 36 (2003) 4582–4588. doi:10.1021/ma034137w.

[183] M.S. Shoichet, R.H. Li, M.L. White, S.R. Winn, Stability of hydrogels used in cell encapsulation: An in vitro comparison of alginate and agarose, Biotechnol. Bioeng. 50 (1996) 374–381. doi:10.1002/(SICI)1097-0290(19960520)50:4<374::AID-BIT4>3.0.CO;2-I.

[184] O. Jeon, K.H. Bouhadir, J.M. Mansour, E. Alsberg, Photocrosslinked alginate hydrogels with tunable biodegradation rates and mechanical properties, Biomaterials. 30 (2009) 2724–2734. doi:10.1016/j.biomaterials.2009.01.034.

[185] A. Forget, J. Christensen, S. Ludeke, E. Kohler, S. Tobias, M. Matloubi, R. Thomann, V.P. Shastri, Polysaccharide hydrogels with tunable stiffness and provasculogenic properties via -helix to -sheet switch in secondary structure, Proc. Natl. Acad. Sci. 110 (2013) 12887–12892. doi:10.1073/pnas.1222880110.

[186] X. Bao, K. Hayashi, Y. Li, A. Teramoto, K. Abe, Novel agarose and agar fibers: Fabrication and characterization, Mater. Lett. 64 (2010) 2435–2437. doi:10.1016/j.matlet.2010.08.008.

[187] M. Lahaye, C. Rochas, Chemical structure and physico-chemical properties of agar, Hydrobiologia. 221 (1991) 137–148. doi:10.1007/bf00028370.

[188] J.J. Roberts, A. Earnshaw, V.L. Ferguson, S.J. Bryant, Comparative study of the viscoelastic mechanical behavior of agarose and poly(ethylene glycol) hydrogels, J. Biomed. Mater. Res. - Part B Appl. Biomater. 99 B (2011) 158–169. doi:10.1002/jbm.b.31883.

[189] P. Aymard, D.R. Martin, K. Plucknett, T.J. Foster, A.H. Clark, I.T. Norton, Influence of thermal history

on the structural and mechanical properties of agarose gels, Biopolymers. 59 (2001) 131–144. doi:10.1002/1097-0282(200109)59:3<131::AID-BIP1013>3.0.CO;2-8.

[190] A. Forget, R.A. Pique, V. Ahmadi, S. Lüdeke, V.P. Shastri, Mechanically tailored agarose hydrogels through molecular alloying with β-sheet polysaccharides, Macromol. Rapid Commun. 36 (2015) 196–203. doi:10.1002/marc.201400353.

[191] X. Li, S. Chen, J. Li, X. Wang, J. Zhang, N. Kawazoe, G. Chen, 3D culture of chondrocytes in gelatin hydrogels with different stiffness, Polymers (Basel). 8 (2016). doi:10.3390/polym8080269.

[192] L. Möller, A. Krause, J. Dahlmann, I. Gruh, A. Kirschning, G. Dräger, Preparation and evaluation of hydrogel-composites from methacrylated hyaluronic acid, alginate, and gelatin for tissue engineering, Int. J. Artif. Organs. 34 (2011) 93–102. doi:10.5301/IJAO.2011.6397.

[193] J.-D. Christine, H. Domini, D. Madeleine, All gelatin networks: 1. Biodiversity and physical chemistry, Langmuir. 18 (2002) 7208–7217. doi:10.1021/la020189n.

[194] J.A. Benton, C.A. DeForest, V. Vivekanandan, K.S. Anseth, Photocrosslinking of Gelatin Macromers to Synthesize Porous Hydrogels That Promote Valvular Interstitial Cell Function, Tissue Eng. Part A. 15 (2009) 3221–3230. doi:10.1089/ten.tea.2008.0545.

[195] E. Hoch, C. Schuh, T. Hirth, G.E.M. Tovar, K. Borchers, Stiff gelatin hydrogels can be photo-chemically synthesized from low viscous gelatin solutions using molecularly functionalized gelatin with a high degree of methacrylation, J. Mater. Sci. Mater. Med. 23 (2012) 2607–2617. doi:10.1007/s10856-012-4731-2.

[196] M.C. Cushing, K.S. Anseth, Hydrogel cell cultures, Science 316 (2007) 1133–1134. doi:10.1126/science.1140171.

[197] H. Jarvelainen, A. Sainio, M. Koulu, T.N. Wight, R. Penttinen, H. Ja, Extracellular matrix molecules: potential targets in pharmacotherapy, Pharmacol. Rev. 61 (2009) 198–223. doi:10.1124/pr.109.001289.provided.

[198] D. Missirlis, J.P. Spatz, Combined effects of PEG hydrogel elasticity and cell-adhesive coating on fibroblast adhesion and persistent migration, Biomacromolecules. 15 (2014) 195–205. doi:10.1021/bm4014827.

[199] K.M. Hakkinen, J.S. Harunaga, A.D. Doyle, K.M. Yamada, Direct Comparisons of the Morphology, Migration, Cell Adhesions, and Actin Cytoskeleton of Fibroblasts in Four Different Three-Dimensional Extracellular Matrices, Tissue Eng. Part A. 17 (2011) 713–724. doi:10.1089/ten.tea.2010.0273.

[200] C.H. Streuli, Integrins as architects of cell behavior, Mol. Biol. Cell. 27 (2016) 2885–2888. doi:10.1091/mbc.e15-06-0369.

[201] H. Wolfenson, I. Lavelin, B. Geiger, Dynamic Regulation of the Structure and Functions of Integrin Adhesions, Dev. Cell. 24 (2013) 447–458. doi:10.1016/j.devcel.2013.02.012.

[202] J.C. Kohn, D.W. Zhou, A.L. Zhou, B.N. Mason, M.J. Mitchell, M.R. King, C.A. Reinhart-king, Article Cooperative Effects of Matrix Stiffness and Fluid Shear Stress on Endothelial Cell Behavior, 108 (2015). doi:10.1016/j.bpj.2014.12.023.

[203] N. Zaari, P. Rajagopalan, S.K. Kim, A.J. Engler, J.Y. Wong, Photopolymerization in microfluidic gradient generators: Microscale control of substrate compliance to manipulate cell response, Adv. Mater. 16 (2004) 2133–2137. doi:10.1002/adma.200400883.

[204] X.Q. Brown, K. Ookawa, J.Y. Wong, Evaluation of polydimethylsiloxane scaffolds with physiologically-relevant elastic moduli: Interplay of substrate mechanics and surface chemistry effects on vascular smooth muscle cell response, Biomaterials. 26 (2005) 3123–3129. doi:10.1016/j.biomaterials.2004.08.009.

[205] R.N. Palchesko, L. Zhang, Y. Sun, A.W. Feinberg, Development of Polydimethylsiloxane Substrates with Tunable Elastic Modulus to Study Cell Mechanobiology in Muscle and Nerve, PLoS One. 7 (2012). doi:10.1371/journal.pone.0051499.

[206] A. Forget, R. Gianni-Barrera, A. Uccelli, M. Sarem, E. Kohler, B. Fogli, M.G. Muraro, S. Bichet, K. Aumann, A. Banfi, V.P. Shastri, Mechanically Defined Microenvironment Promotes Stabilization of Microvasculature, Which Correlates with the Enrichment of a Novel Piezo-1 + Population of Circulating CD11b + /CD115 + Monocytes, Adv. Mater. 1808050 (2019) 1–10. doi:10.1002/adma.201808050.

[207] P. Kim, A. Yuan, K.H. Nam, A. Jiao, D.H. Kim, Fabrication of poly(ethylene glycol): Gelatin

methacrylate composite nanostructures with tunable stiffness and degradation for vascular tissue engineering, Biofabrication. 6 (2014). doi:10.1088/1758-5082/6/2/024112.

[208] H. Ko, K. Suthiwanich, H. Mary, S. Zanganeh, S.K. Hu, S. Ahadian, Y. Yang, G. Choi, K. Fetah, Y. Niu, J.J. Mao, A. Khademhosseini, A simple layer-stacking technique to generate biomolecular and mechanical gradients in photocrosslinkable hydrogels, Biofabrication. 11 (2019) 1–9. doi:10.1088/1758-5090/ab08b5.

[209] S. Sant, M.J. Hancock, J.P. Donnelly, D. Iyer, A. Khademhosseini, Biomimetic gradient hydrogels for tissue engineering, Can. J. Chem. Eng. 88 (2010) 899–911. doi:10.1002/cjce.20411.

[210] R. Sunyer, V. Conte, J. Escribano, A. Elosegui-Artola, A. Labernadie, … Valon, L., X. Trepat, Collective cell durotaxis emerges from long-range intercellular force transmission., Science 353 (2016) 1157–1161. doi:10.1126/science.aaf7119.

[211] W.J. Hadden, J.L. Young, A.W. Holle, M.L. McFetridge, D.Y. Kim, P. Wijesinghe, H. Taylor-Weiner, J.H. Wen, A.R. Lee, K. Bieback, B.-N. Vo, D.D. Sampson, B.F. Kennedy, J.P. Spatz, A.J. Engler, Y.S. Choi, Stem cell migration and mechanotransduction on linear stiffness gradient hydrogels, Proc. Natl. Acad. Sci. 114 (2017) 5647–5652. doi:10.1073/pnas.1618239114.

[212] P. Tseng, D. Di Carlo, Substrates with patterned extracellular matrix and subcellular stiffness gradients reveal local biomechanical responses, Adv. Mater. 26 (2014) 1242–1247. doi:10.1002/adma.201304607.

[213] L. Wang, Y. Li, G. Huang, X. Zhang, B. Pingguan-Murphy, B. Gao, T.J. Lu, F. Xu, Hydrogel-based methods for engineering cellular microenvironment with spatiotemporal gradients, Crit. Rev. Biotechnol. 36 (2016) 553–565. doi:10.3109/07388551.2014.993588.

[214] J.Y. Wong, A. Velasco, P. Rajagopalan. Directed Movement of Vascular Smooth Muscle Cells on Gradient-Compliant Hydrogels, Langmuir. 19 (2003) 1908–1913. doi:10.1021/la026403p.

[215] C. Chen, D. Hirdes, A. Folch, Gray-scale photolithography using microfluidic photomasks, Proc. Natl. Acad. Sci. 100 (2003) 1499–1504. doi:10.1073/pnas.0435755100.

[216] S. Ahadian, R. Civitarese, D. Bannerman, M.H. Mohammadi, R. Lu, E. Wang, L. Davenport-Huyer, B. Lai, B. Zhang, Y. Zhao, S. Mandla, A. Korolj, M. Radisic, Organ-On-A-Chip Platforms: A Convergence of Advanced Materials, Cells, and Microscale Technologies, Adv. Healthc. Mater. 7 (2018) 1–53. doi:10.1002/adhm.201700506.

[217] S. Lee, J. Ko, D. Park, S.R. Lee, M. Chung, Y. Lee, N.L. Jeon, Microfluidic-based vascularized microphysiological systems, Lab Chip. 18 (2018) 2686–2709. doi:10.1039/c8lc00285a.

[218] T. Pasman, D. Grijpma, D. Stamatialis, A. Poot, Flat and microstructured polymeric membranes in organs-on-chips, J. R. Soc. Interface. 15 (2018). doi:10.1098/rsif.2018.0351.

[219] W. Zhang, Y.S. Zhang, S.M. Bakht, J. Aleman, S.R. Shin, K. Yue, M. Sica, J. Ribas, M. Duchamp, J. Ju, R.B. Sadeghian, D. Kim, M.R. Dokmeci, A. Atala, A. Khademhosseini, Elastomeric free-form blood vessels for interconnecting organs on chip systems, Lab Chip. 16 (2016) 1579–1586. doi:10.1039/c6lc00001k.

[220] Q. Ramadan, M.A.M. Gijs, In vitro micro-physiological models for translational immunology, Lab Chip. 15 (2015) 614–636. doi:10.1039/c4lc01271b.