



King's Research Portal

DOI: 10.3390/app10114001

Document Version Peer reviewed version

Link to publication record in King's Research Portal

Citation for published version (APA): Chaher, N., Hajhosseiny, R., Phinikaridou, A., & Botnar, R. (2020). Imaging the Extracellular Matrix in Prevalent Cardiovascular Diseases. *Applied Sciences (Switzerland), 10*(11), Article 4001. https://doi.org/10.3390/app10114001

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

•Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research. •You may not further distribute the material or use it for any profit-making activity or commercial gain •You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.





Imaging the Extracellular Matrix in Prevalent Cardiovascular Diseases

4 Nadia Chaher ^{1*}, Reza Hajhosseiny ¹, Alkystis Phinikaridou ¹ and René M. Botnar ^{1,2,}

5 ¹ King's College London, School of Biomedical Engineering Imaging Sciences, 3rd Floor, Lambeth Wing, St
 6 Thomas' Hospital, London SE1 7EH, United Kingdom

7 ² Escuela de Ingeniería, Pontificia Universidad Católica de Chile, Santiago, Chile

- 8 * Correspondence: nadia.chaher@kcl.ac.uk
- 9 Received: 24/04/2020; Accepted: 04/06/2020; Published: date
- 10

1

Review

11 Abstract: The extracellular matrix (ECM) is a highly complex macromolecular network present in all 12 tissues and organs. The ECM is continuously remodelling under an orchestrated process facilitated by 13 many matrix-degrading and matrix-synthesising enzymes in both health and disease. Disturbance of 14 this balance can be the result of or can lead to various diseases. In cardiovascular diseases (CVDs) 15 changes to the ECM are evident in conditions including: atherosclerosis, myocardial infarction (MI), 16 venous thromboembolism (VTE) and abdominal aortic aneurysm (AAA). ECM proteins and ECM 17 regulating enzymes are differently expressed in various CVDs. Most importantly, the altered deposition, macromolecule arrangement and activity of the ECM makes it an attractive marker of 18 19 disease onset, pathogenesis and progression. Many medical imaging modalities allow disease 20 assessment by exploiting native image contrast, by using non-targeted or by using protein or cell 21 specific (targeted) imaging probes. However, the ability to directly visualise and quantify changes in 22 specific ECM proteins enhances our understanding of the biological role of these proteins, enables 23 monitoring of disease progression and response to treatment and may improve patient diagnosis and allocation of personalised therapies. This review focuses on the biochemistry of the major 24 25 extracellular matrix proteins and advancements in the development of ECM-targeted probes for 26 molecular imaging of CVD, particularly for applications of molecular MRI and PET imaging.

Keywords: Extracellular Matrix; Matrix Proteins; Cardiovascular Disease; Molecular Imaging; Imaging
 Probes; Collagen; Elastin; Fibrin; Matrix Metalloproteases

29

30 1. Introduction

The extracellular matrix (ECM) plays a key role in multicellular organism development [1]. Previously, it was believed that the ECM was an inert component that solely served to provide mechanical stability, but today it is viewed as a highly dynamic system that undergoes constant remodelling with post-translational modifications of its molecular components [2,3]. The ECM is found within all tissues 35 and organs and acts not only as a framework for cellular organelles but also has a crucial signal 36 transduction role in both healthy and diseased tissues [2,4]. The composition of the ECM is tissue 37 specific and heterogeneous and its unique structure is formed early during tissue development. The 38 ECM is comprised of many macromolecules that can be categorised into two key groups: fibre-forming 39 and non-fibre-forming molecules. As the ECM is a highly organised ensemble of different 40 macromolecules, even small changes to each subunit can have detrimental effects on the macromolecules' physiochemical properties and thus the properties of the tissue and the cellular 41 42 phenotypes, ultimately resulting in functional changes [3].

43 It is possible to identify changes in the ECM during disease. However, it is vital to distinguish between ECM changes causing disease and ECM changes that occur as a result of disease progression [3]. 44 45 During the early stages of development and in response to injury, the remodelling rate of the ECM is 46 increased and involves various molecules, including but not exclusively limited to, integrins and matrix 47 metalloproteins (MMPs) [5] and also changes of intra- or extracellular tension forces [5]. There is an 48 extensive list of diseases that involve disturbances of the ECM including: connective tissue disorders, 49 genetic disorders, liver cirrhosis, inflammatory bowel disease, chronic kidney disease and several 50 cardiovascular diseases [3,6]. In cardiovascular diseases, preserving ECM integrity by reducing excess 51 fibrosis or inhibiting degradation is a critical target of both treatment and prevention strategies [7]. 52 Given the critical role of the extracellular matrix in both physiological homeostasis and pathological 53 compensatory processes, the ECM has become an attractive new target for molecular imaging with 54 applications in numerous diseases.

55 The ECM can be imaged using both invasive (including tissue staining methods, nonlinear optic 56 microscopy, electron microscopy and fluorescence life time imaging [8-10]) and non-invasive 57 including [MRI (magnetic resonance imaging), PET (positron emission tomography) and CT (computed 58 tomography)] imaging methods. There are several review articles that provide extensive coverage of 59 the use of these imaging modalities in the context of imaging cardiac ECM [11,12]. The major limitation 60 with invasive imaging methods is that clinical translation can be limited due to the general use of ex 61 vivo tissue specimens requiring biopsies obtained by specialised surgical procedures and are therefore 62 not readily available. Non-invasive imaging methods overcome this limitation as they provide in vivo 63 imaging which can be applied safely to the patient. This review aims to address the latest 64 developments in the field of non-invasive imaging of ECM changes that are associated with CVD. 65 Whilst we briefly mention other imaging modalities, the scope and focus of this review is on MRI and 66 PET applications. The review also provides a summary of the basic components that comprise the ECM 67 and their biosynthetic pathways that enable a better understanding of the development of targeted 68 probes for imaging of the ECM.

69 2. Understanding the Key Extracellular Matrix Proteins

70 ECM Synthesis

The ECM is a complex, heterogenous collection of different molecules. Over the last two decades 71 72 intensive research into ECM proteins has generated new insights into ECM composition and function 73 in both healthy and diseased tissues [13]. The exact number of ECM proteins remains an open question 74 and is an active area of research; however, it is estimated that there are between 300-400 ECM genes, 75 with a third of them still to be identified [14]. ECM components are commonly categorised into two 76 groups: fibre-forming and non-fibre-forming molecules [3]. The typical fibre-forming molecules 77 include specific classes of collagen and elastin and the non-fibre-forming molecules include 78 proteoglycans and glycoproteins (Figure 1). It is paramount to have a tight regulation between the 79 molecules signalling ECM synthesis and degradation as this is essential for maintaining the structural 80 integrity and thereby the tissue and organ functions.



81

Figure 1- Components of the Extracellular Matrix.

83

82

84 ECM proteins are located within these two basic compartments (Figure 2): i) interstitial connective 85 tissue, that surrounds cells providing a structural matrix for the tissues and ii) specialised basement membrane that separates the epithelium and the stroma and is involved in the matrix-cell interactions 86 87 [15]. These two compartments of the ECM are characterised by different components with the interstitial matrix comprised of molecules including: type I collagen, fibronectin, proteoglycans (PGs), 88 89 glycosaminoglycans (GAGs), tenascin C and elastin, whereas the basement membrane is comprised of type IV collagen, laminins and nidogen. Dysregulated ECM composition either because of excessive 90 91 ECM production and/ or increased degradation is observed in various diseases including cardiac, lung,

- 92 liver, cancer, deep vein thrombosis and osteoarthritis [15]. A summary of these common ECM
- 93 components with respective functions are provided in **Table 1**.
- 94 **Table 1** Summary of common ECM components with their respective functions. The components have been categorised 95 into structural ECM proteins(green); adhesion proteins (orange); glycosaminoglycans and proteoglycans (blue).

<u>CM Component</u> <u>Function</u>			
	Type		<u>Reference</u>
Collagen	Fibrous protein	Main structural component of the ECM that provides strength, regulates adhesion and supports chemotaxis and migration.	Rozario et al, 2010 [16]
Elastin	Fibrous protein	Main structural component of the ECM that provides elasticity and reliance to tissues undergoing stretching.	Bailey, 1978 [17]
Laminin	Glycoprotein	Regulate vital ECM activities including cell adhesion, migration, differentiation and proliferation.	Hamill et al., 2009 [18]
Fibronectin	Glycoprotein	Interacts with cells to link the ECM to the intracellular cytoskeleton and signalling pathways	Magnusson et al., 1998 [19]
Tenascin	Glycoprotein	Carries out adhesive and counter adhesive activities upon binding of the ECM proteins to cell surface receptors	Jones et al., 2000 [20]
Glycosaminoglycans (GAG)	Heterogenous polysaccharide	Negatively charged molecules that interact (reversibly and irreversibly) with other ECM proteins and growth factors providing they exhibit a positive charge on their surface. These interactions play essential roles in normal physiology and pathogenic processes.	Rienks et al., 2014, Hileman et al., 1998 [21,22]
Proteoglycan (PG)	GAG covalently linked to a core protein	Retain water that hydrates that ECM generating a swelling pressure that aids the ECM to resist compressive forces.	Yanagishita, 1993 [23]





Figure 2- The two basic compartments of the Extracellular Matrix

Understanding the underlying biosynthesis and assembly of the ECM proteins is essential for various 99 100 disciplines including: development of molecular imaging probes and drugs, tissue engineering and 101 regenerative medicine. The turnover of ECM proteins is regulated by various cell types including: 102 fibroblasts, mast cells and macrophages; growth factors such as the transforming growth factor- βs 103 (TGF- β s) and enzymes including matrix metalloproteases (MMPs). In the late 1980s, it was first shown 104 that TGF- β has the ability to control synthesis of ECM proteins [24,25]. Subsequently, in 1986, Robert et al. demonstrated that injecting TGF- β in newborn mice activated the rough endoplasmic reticulum 105 106 (RER) of fibroblasts stimulating the production of extracellular collagen fibres [24][25]. The pro-fibrotic effect of TGF- β is complemented with its ability to inhibit the proteolytic degradation of ECM proteins 107 108 [25] by increasing the production of tissue inhibitors of matrix metalloproteases (TIMPs) [26]. MMPs 109 are a class of enzymes that have been studied since 1962 with 23 human MMPs identified to date [15]. MMPs are categorised into 6 groups including collagenases and gelatinases, based on their structure 110 111 and enzymatic substrates [27]. MMPs are secreted as inactive pro-enzymes, pro-MMPs, and are activated by proteolytic degradation of the amino terminals exposing the Zinc ion (Zn²⁺) binding pocket 112 113 of the catalytic domain [28]. Activated MMPs cleave at specific sites, and therefore, breakdown the ECM scaffold or they can modify bioactive molecules that exist within the ECM proteins [27]. The 114 activity of MMPs is counterbalanced by TIMPs that block the activation pathway and hence inhibit 115 116 MMPs binding to ECM substrates. There are four members in the TIMPs family, TIMP1-4. The ratio of 117 MMP to TIMP determines the overall ECM degradation. A disease example that illustrates the 118 importance of ECM homeostasis is cardiomyopathy, where increased levels of MMP-1 reduce collagen 119 content and thus reduced cardiac contractility [29]. The increased overexpression of MMP-1 is 120 associated with an increased ratio of MMP-1/TIPM-1 in dilated cardiomyopathy [30]. In vessel wall

121 development and remodelling, MMPs also have an inflammatory mediated function, similar to 122 cytokines, as they are actively expressed in diseased tissue but absent or expressed at very low levels 123 in healthy tissue [27]. In vascular tissues inflammatory cells, including macrophages and neutrophils, 124 are important sources of MMPs [27]. Tissue injury or pathologic conditions can also lead to excessive 125 production and accumulation of ECM proteins ultimately leading to fibrosis [15]. Fibrosis is the body's 126 natural response mechanism to injury whereby a scar is formed as part of the wound healing process. 127 However, if the tissue properties within the scar region are not the same as the native surrounding 128 tissue it can lead to organ failure, such as liver cirrhosis [1] and heart failure [31]. The fibrotic response 129 is driven by a variety of cell types, growth factors and cytokines and can be inhibited by signalling 130 mediators such as interferon- γ (IFN- γ), an antagonist to TGF β [15].

131

132 <u>Collagen</u>

133 Collagen comprises about one third of the total protein content in the human body [1]. Collagen is a 134 large family of molecules with more than 28 different types of collagen identified to date [32]. All 135 collagens have the same fundamental characteristic; a protein comprised of three polypeptide chains 136 that has at least one repeating amino acid sequence [32]. The most abundant fibrillar collagen, type I, 137 is composed of well organised fibrils that provide tensile strength and resistance to deformation and 138 stress to the ECM and is found in various tissues including the skin, the vasculature, the heart tissue 139 and bones [33]. Type I collagen is formed by two types of α helices; specifically, $[\alpha 1(I)]2\alpha 2(I)]$, thus 140 forming a triple helix consisting of two $\alpha 1(I)$ and one $\alpha 2(I)$ helix [34]. Other types of collagen include; 141 type II found mainly in cartilage; type III found in the skin, muscle and blood vessels and type IV found 142 in all basement membranes [35].

143 All collagens are characterised by the repeat sequence of: GLY-X-Y, where GLY is glycine, X is often 144 proline and Y is often hydroxyproline [36,37]. The precursor molecule of collagen is procollagen, a 145 glycoprotein, that consists of three extended polypeptide chains and is believed to have a stiff 146 structure with flexible short end regions [37]. The synthesis of collagen initiates in the nucleus where 147 the DNA is transcribed into mRNA that is then translated in the rough endoplasmic reticulum (RER), 148 synthesising collagen α -chains (Figure 3). These molecules are transported to the Golgi apparatus 149 where post-translational modifications generate the self-assembled triple helical procollagen 150 structure that is then secreted to the extracellular space [37]. During this exocytosis, procollagen is 151 converted into tropocollagen, by the proteolytic cleavage of the C- and N-terminus propeptides of the 152 procollagen molecule [38]. Collagen fibres are assembled by a process called fibrillogenesis [32] 153 through a variety of cross-linking mechanisms. Three key cross-liking mechanisms occur in vivo: lysyl

154 oxidase crosslinking, sugar mediated and transglutaminase crosslinking [38]. Lysyl Oxidase (LOX), a 155 copper-dependent enzyme, specifically crosslinks collagen and elastin [39] creating intramolecular 156 bonds by catalysing the deamination of lysine to allysine and hydroxylysine to hydroxyallysine (Figure 3). The allysine aldehyde product subsequently undergoes spontaneous aldol condensation resulting 157 in crosslinks between the fibrils. Alternatively, Schiff base crosslinks are formed as the aldehyde 158 159 groups react with the amino groups of lysine and hydroxylysine residues on adjacent molecules [39]. 160 The hydroxylysine pathway results in two cross linked molecules: ketoimine and aldimine, which 161 connect individual collagen triple helices [38]. Sugar-mediated crosslinking is characterised by a Maillard reaction, also known as a non-enzymatic browning reaction, which increase the turnover of 162 collagen linearly. In diabetic patients studies have shown that there is an acceleration of the browning 163 Finally, transglutaminases (TGase), that are thiol- and calcium dependent 164 reaction [40]. 165 multifunctional enzymes [31,41], also induce collagen crosslinking. However, not a lot is currently 166 known about their activity. To date, we know that TGase catalyses the formation of covalent bonds 167 between the γ -carboxamide group of the peptide-bound glutamine residue and the ϵ -amino group of lysine of the collagen molecules [31]. 168



169

Figure 3-Collagen Synthesis pathway and demonstration of LOX-medicated crosslinks: A. Triple stranded helix is formed in
 the endoplasmic reticulum (ER) followed by post-translational modification in the Golgi apparatus. B. Procollagen is processed
 and assembled intracellularly before being secreted to the extracellular space. C. The N and C terminus are removed to form

tropocollagen. D. Lysyl Oxidase (LOX) catalyses the deamination of the lysine. E. Conversion of a lysine residue into a reactive

aldehyde. F. Aldol condensation reaction between neighbouring aldehyde groups. G. Crosslinks both within and between
 triple-helical molecules [where X is C=C(CHO)].

176

177 <u>Elastin</u>

178 The mechanism of elastin synthesis is much better understood compared with collagen. The elastic 179 and resilient properties of the ECM are provided by elastin and microfibrils [42]. Elastin itself is the 180 second most abundant macromolecule in the ECM and serves an important role in the regulation of 181 the biomechanical properties of cells and tissues [36] such as blood vessels , lungs and skin [17]. Elastin 182 is an insoluble molecule with a finite turnover in healthy tissue. The appearance of elastin was first 183 reported in 1958, but the interest in understanding elastin biochemistry further sparked in 1963 when 184 the relationship between elastin structure and function was first reported [17]. The most abundant 185 amino acids present in elastin are glycine followed by alanine, valine and a low level of hydroxyproline 186 [43]. It has been shown that elastin and collagen are the only animal proteins that contain 187 hydroxyproline, with an abundance of 2-4% and 13%, respectively [44]. Elastin, just like collagen, is an 188 insoluble protein synthesised from intracellularly soluble monomers called tropoelastin (TE) that are 189 assembled extracellularly by crosslinking (Figure 4). After the tropoelastin mRNA is translated in the 190 RER, the protein migrates to the Golgi apparatus where it binds to the elastin-binding protein (EBP) to 191 inhibit self-aggregation and avoid early degradation [36]. The TE-EBP complex is then exocytosed and 192 the EBP releases the tropoelastin to the ECM environment. Extracellularly, the tropoelastin monomers 193 align and interact with the microfibrillar scaffold and acquire the correct orientation to form the 194 polymeric elastic fibres [36]. Tropoelastin has an intrinsic ability to undergo coacervation which is an endothermic process driven by entropy [45]. The process is believed to be largely due to the 195 196 hydrophobic domains of tropoelastin [45]. With increasing temperature to physiological conditions, 197 the tropoelastin molecules are correctly aligned for the subsequent enzymatic crosslinking of the molecules [45]. Lysyl oxidase (LOX) catalyses this reaction [36] in a similar way as for collagen. The 198 199 structure of elastin is reported to be highly elastic and amorphous when compared to inextensible 200 collagen [17].



Figure 4-Elastin biosynthesis pathway: A. Tropoelastin (TE), precursor of elastin, is translated from mRNA in the rough
 endoplasmic reticulum. B. TE and elastin binding protein (EBP) form a complex in the Golgi apparatus. C. The TE-EBP complex
 is secreted to the extracellular space. D. The EBP detaches from TE, allowing TE to interact and align along the microfibrillar
 scaffold. E. Coacervation of TE together with the enzymatic reaction, catalysed by lysyl oxidase (LOX), result in the crosslinking
 of the TE molecules into the polymeric elastin fibre.

207

208 *Fibrin*

209 The precursor of fibrin is the glycoprotein fibrinogen. Each unit of fibrinogen comprises of two copies 210 of three polypeptide chains; A α , B β and γ . These polypeptide chains are connected by 29 disulphide 211 bonds and each fibrinogen molecule has outer D domains that are connected via a coiled central E 212 domain [46,47]. The N-termini of all six chains are contained within the E domains, while the carboxyl termini of both Bß and y are located within the D domain [48]. The overview of the synthesis of fibrin 213 214 is illustrated in (Figure 5). If the vessel wall sustains an injury, a series of local events ultimately lead to production of thrombin, a protease, that convers fibrinogen to fibrin [48]. It does so by cleaving 215 216 peptides at the N-terminus of fibrinogen, causing the release of fibrinopeptides generating fibrin monomers [48]. Fibrin monomers then undergo polymerisation to effectively produce fibres to 217 stabilise the clot. 218



221 Figure 5-Synthesis of fibrinogen and the conversion pathway into fibrin. A. The three polypeptide chains that make up 222 fibrinogen are A-Alpha (A α), B-Beta (B β) and Gamma (γ) **B.** Each of the polypeptide chains are translated and independently 223 translocated to the endoplasmic reticulum with the signal protein cleaved from each chain. It is then processed and assembled 224 in the Golgi apparatus. Disulphide bridges are formed between two of the A α B $\beta\gamma$ units and post translation occurs before the 225 fibrinogen molecule is excreted. C. The fibrinogen unit is comprised of two sets of the polypeptides, $(A\alpha B\beta \gamma)^2$, and is 226 characterised by a D Domain, on the outer regions, and an E domain in the central region. D. When thrombin cleaves the 227 fibrinogen molecule a fibrin monomer and two fibrinopeptides are removed leaving the knobs exposed. These knobs are 228 complementary to the holes that are on the surface of the interacting fibrin molecule. E. The knob-hole interaction occurs 229 which generates the protofibril. F. Protofibrils aggregate laterally resulting in fibrin formation.

230 3. Cardiovascular Disease

231 Clinical Relevance

232 Cardiovascular Disease (CVD) is a collective term to characterise various conditions of the heart and 233 blood vessels. CVD can occur from genetic and acquired diseases. CVD itself includes, but is not limited to, diseases such as coronary artery disease, cerebrovascular disease, aortic disease and Marfan 234 235 syndrome, deep vein thrombosis, heart attacks, heart failure, cardiomyopathy, inflammatory diseases 236 and atrial fibrillation. CVD is the primary cause of death worldwide and it is reported by the World 237 Health Organisation (WHO) that around 17.9 million people died from CVD in 2016, representing 31% 238 of deaths globally [49]. Even with improved patient management it is projected that CVD will account 239 for 10 million deaths globally by 2030 [50]. Sections 3.1-3.4 will focus on the latest developments in 240 non-invasive MRI and PET imaging of four prevalent CVDs: atherosclerosis, myocardial infarction, deep 241 vein thrombosis and abdominal aortic aneurysms. A summary of the various targeting probes and 242 their respective applications in imaging the ECM are presented in Table 2.

244

Table 2- Summary of recent probe development in the field of CVD with indication to the ECM structure that they target.

<u>Protein</u>	<u>Probe</u>	Model and Species	<u>Reference</u>	General Application
Collagen				
	EP -3533	 Fibrosis in various disease 	Caravan et al 2007	Imaging of Type I collagen in
		including myocardial infarction (MI)	Caravan et al., 2007	fibrosis
		model- mice (in vivo)		
		Atherosclerotic model of plaque	Chan at al. 2012	
		progression and regression- mice (in	Chen et al., 2013	
		vivo)		
		Healed myocardial infarction (MI)-	Holes at al. 2000	
		mice (in vivo)	Heim et al., 2008	
	CNA-35	♦Atherosclerotic arteries- mice (ex	Manage at al. 2007	Binds to all fibrillar collagens
		vivo)	Megens et al., 2007	and collagen type IV
		Aortic aneurysms and rupture	Klink et al., 2011	
	Platelet Collagen Receptor	◆Carotid atherosclerotic plaques-	Schulz et al 2008	Targeted to selectively visualise
Glycoprotein (GP) VI		human (ex vivo) and atherosclerotic		type I and III
		model -mice (in vivo)		
Elastin				
	ESMA	Arterial remodelling post stent-	Von Bary et a., 2011	Quantification of elastin in
		swine (in vivo)		plaque

Fibrin

TESMA	 Plaque rupture- rabbit (in vivo) Matrix remodelling in a MI model- mice (in vivo) Aortic aneurysm remodelling- mice (in vivo) Elastin remodelling in aortic wall in Marfan mouse model (in vivo) Atherosclerosis model- mice and 	Phinikaridou et al., 2014 Wildgruber et al., 2014 Botnar et al., 2014 Okamura et al., 2014 Phinikaridou et al., 2018	Quantification of elastin in myocardium Tropoelastin selectively	
	rabbits (in vivo) ❖Model of abdominal aortic aneurysm- mice (in vivo)	Lavin et al., 2019	visualises dysfunctional elastogenesis or elastolysis	
Lipid encapsulated perfluorocarbon nanoparticle	↔Human thrombus (ex vivo)	Yu et al., 2000	Imaging of fibrin clots	
Para-magnetic nanoparticle	◆Jugular vein -canine (in vivo)	Flacke et al., 2001	Targets and enhances signal in thrombi	
EP-1242	 Acute plaque thrombus-guinea pig (in vivo) 	Sirol et al., 2005		
EP-1873	 Plaque rupture model -rabbit (in vivo) 	Botnar et al., 2004	Imaging subacute plaque thrombosis	

MMP

EP-2014R	Acute coronary thrombosis -swine (in vivo) and human translation	Botnar et al., 2004 Spuentrup et al., 2008	Detection of acute coronary thrombosis
	✤ Mouse model of DVT – mice (in vivo)	Vymazal et al., 2009 Andia et al., 2014	
FTP11-cy+NIRF	✤Deep venous thrombosis (DVT) model – mice (in vivo)	Hara et al., 2012	
⁶⁴ Cu-FBP8	Carotid artery and femoral vein thrombosis- rodent (in vivo)	Blasi et al., 2015	
P947	♦Atherosclerotic plaque -mice (in vivo) and rabbit (ex vivo)	Lancelot et al., 2008	Detects arterial wall remodelling
	aneurysm – rat (in vivo)	Bazeli et al., 2010	
ACPPs	◆Plaque rupture model -rabbit (ex vivo)	Hua et al., 2015	Selectively differentiates stable and unstable plaques
Monoclonal Antibody	☆Atherosclerosis model-rabbit (in vivo)	Kunge et al., 2010	Targets MMP in atherosclerosis
MPI	✦Atherosclerosis model -rabbit (ex vivo)	Fujimoto et al., 2008	

13 of 55

Appl. Sci. 2020, 10, x FOR PEER REVIEW

¹¹¹ In- labelled assessing MMP	Zhana at al 2008	Assess MMP activation in	
activation in an induced vascular	Zhung et ul., 2008	vascular remodelling	
remodelling model- mice (in vivo)			
◆Post-MI model- mice (in vivo)	Su et al., 2005		
Aneurysm biology and outcome prediction- mice (in vivo)	Golestani et al. 2015		
	 ¹¹¹In- labelled assessing MMP activation in an induced vascular remodelling model- mice (in vivo) Post-MI model- mice (in vivo) Aneurysm biology and outcome prediction- mice (in vivo) 	 *¹¹¹In- labelled assessing MMP activation in an induced vascular remodelling model- mice (in vivo) *Post-MI model- mice (in vivo) Su et al., 2005 *Aneurysm biology and outcome prediction- mice (in vivo) 	

246 3.1 Atherosclerotic Arterial Disease

247 Clinical Need

Atherosclerosis is a disease of the vessels and is characterised by build-up of atheromatous plaques [51]. The plaques are formed by the deposition of fatty substrates, cells and other molecules within the vessel wall [52]. This accumulation of plaque eventually causes partial or total obstruction of the vessel [52]. Atherosclerosis is a major contributor to overall cardiovascular morbidity and mortality [53]. Clinical manifestations include ischaemic heart disease, stroke and peripheral arterial disease [53].

254 ECM in Atherosclerosis

255 ECM changes are observed in various CVD conditions with MMPs playing a key role in remodelling of 256 the ECM. In atherosclerosis, excessive activation of MMPs contributes to disease progression and 257 plaque destabilisation. In atherosclerotic vessels MMPs act as inflammatory mediators linking 258 inflammation with vascular remodelling. In disease vessels MMPs function as inflammatory cytokines 259 and their expression and activity increases. However, MMPs are only present in low concentration 260 and activity in healthy vessels. An important cell type in atherosclerosis is vascular smooth muscle 261 cells (VSMCs). VSMCs are present in a quiescent/contractile state in healthy arteries and switch to a 262 proliferative/synthetic state in atherosclerosis [54] rendering atherosclerotic plaques rich in collagen 263 [54]. VSMC proliferation is dependent on type I collagen and results in plaque progression [54]. Adhesion of the ECM to cells is largely regulated by integrins, a large family of heterodimeric cell 264 adhesion molecules that anchor cells to ECM and neighbouring cells. In this regard, integrin β 1 is the 265 266 most predominant integrin expressed on the surface of VSMCs and all collagen-binding integrins share 267 the common β 1 subunit [55,56]. Important extracellular matrix components and imaging targets in 268 atherosclerosis include elastin, collagen, fibrin and metalloproteinases.

269 <u>Elastin</u>

270 Taking advantage of the increased abundance of elastin within the extracellular matrix of 271 atherosclerotic plaque compared to the normal arterial wall, a novel elastin-specific magnetic 272 resonance contrast agent (ESMA, BMS753951) was used for the non-invasive quantification of plaque 273 burden and arterial remodelling in a mouse and swine model of atherosclerosis [57,58]. ESMA is a 274 paramagnetic Gd-labeled $C_{32}H_{40}N_7O_{11}Gd$ low-molecular-weight contrast agent (855.95 Da), which has 275 high vessel wall and plaque uptake (peaking at 30 minutes post injection in an apolipoprotein Edeficient ApoE^{-/-} mouse) and relatively low uptake within other tissues (e.g. heart, liver, lung and 276 muscle). ApoE^{-/-} mice fed a high fat diet have become a popular animal model of atherosclerosis 277

278 because they reproducibly develop plagues in the aorta, aortic root and brachiocephalic arteries with 279 little variability between animals. It is also the quickest animal model that can achieve advanced atherosclerosis within 3 months of high-fat feeding in comparison, for example, to LDLR^{-/-} mice that 280 require around an additional month to display the same plaque burden and features as Apoe^{-/-} mice 281 [59]. In an ApoE^{-/-} mouse model fed a high fat diet (HFD), the plaque contrast to noise ratio (CNR) post 282 283 ESMA injection was significantly higher at 8 weeks and 12 weeks compared with control mice. Furthermore, there was a significant reduction in the post ESMA CNR at 12 weeks in mice who were 284 285 treated with pravastatin compared with mice on a high fat diet alone; demonstrating the potential for 286 non-invasive quantitative detection of plaque elastin content and size after therapeutic treatment 287 (Figure 6) [57].



289 Figure 6- In vivo assessment of plaque burden by morphometric measurements. (a) Cross-sectional views of brachiocephalic 290 arteries by MRI of control and ApoE^{-/-} mice 4, 8 and 12 weeks after the onset of HFD (n = 8 per group). High-resolution 291 delayed-enhancement images overlaid on TOF images with corresponding sections from histology (H&E and EvG stain). (b) 292 Comparison of average PAMV, calculated from morphometric measurement on high-resolution DE images after the injection 293 of ESMA (n = 8 per group). (c,d) Scatter plots showing significant (P < 0.05) correlation between morphometric PAMV 294 measurements (c) and lumen CSA measurements (d) on high-resolution DE-MRI images and on corresponding EvG-stained 295 histological sections (n = 15). Scale bars: white, 250 μ m; black, 100 μ m. Values are expressed as means \pm s.d. MRI – Magnetic 296 Resonance Imaging, HFD – High Fat Diet, TOF – Time of Flight, PAMV - percentage atheroma / media volume, CSA – cross-297 sectional area. Reproduced with permission from Makowski et al, 2011 [57]

In a swine model of arterial remodelling post stenting, significantly increased CNR was observed within
 the coronary arteries post ESMA injection compared with a non-targeted gadolinium agent [60].
 Furthermore in a rabbit model of plaque rupture, ESMA enabled a more accurate assessment of

301 vascular remodelling and identification of unstable plaque that rupture and form thrombus compared

302 with gold-standard native T1 weighted black blood imaging (Figure 7) [61].



303

Figure 7-A, E, Cross-sectional pre-contrast MR images. B, F, Corresponding DE MR images obtained after administration of
 elastin-specific contrast agent. C, G, DE MR images fused with angiographic images. D, H, Corresponding R1 maps obtained
 after administration of elastin-specific contrast agent. Uptake of elastin-specific contrast agent and R1 values are higher in
 diseased compared with control aortas. Reproduced with permission from Phinikaridou et al, 2014 [61]

309 More recently, Phinikaridou et al introduced tropoelastin as a new and attractive biomarker for plaque 310 progression and instability [62]. As elastogenesis and elastolysis favour the accumulation of 311 tropoelastin, rather than mature cross-linked elastin, tropoelastin may serve as a more sensitive 312 imaging biomarker of active but yet incomplete elastogenesis to detect plaque progression and 313 instability. MRI imaging of tropoelastin using a gadolinium labelled tropoelastin-binding contrast agent (TESMA) detected increased pathologic elastogenesis during atherosclerosis progression in 314 ApoE^{-/-} mice that was reduced with statin treatment. Moreover tropoelastin MRI was more sensitive 315 316 at detecting unstable plaque in a rabbit model of plaque rupture compared with ESMA [62]. This is 317 because TESMA binds only to tropoelastin that accumulates in higher proportion in unstable plagues as a result of a dysfunctional cross-linking or elastolysis in the presence of disease whereas ESMA binds 318 319 equally to both tropoelastin and endogenously present polymeric elastin. Thus, ESMA could be used as an imaging probe to assess the net elastin increase and plaque burden, whilst TESMA could be used 320 321 to selectively visualise dysfunctional elastogenesis or elastolysis (Figure 8) [62].



324 Figure 8- In vivo magnetic resonance imaging (MRI) comparison of vessel wall enhancement using the elastin (elastin-specific 325 magnetic resonance contrast agent [ESMA]) and tropoelastin (TESMA) binding contrast agents in mice. A, Fused maximum 326 intensity projection (MIP) reconstructed magnetic resonance angiography and delayed-enhanced-MRI after administration 327 of Gd-TESMA show focal uptake of Gd-TESMA in the brachiocephalic artery (BCA) of an atherosclerotic apolipoprotein E-328 deficient mouse. B-E, MRI of the BCA acquired from a control animal, scanned 24 h apart, showed vessel wall uptake of Gd-329 ESMA (B and C), but no uptake of Gd-TESMA (D and E) because of the lack of tropoelastin in the absence of disease. F-M, 330 MRI of the BCA acquired from 2 different diseased animals showed enhancement of the vessel wall after administration of 331 both agents because of the presence of both cross-linked elastin and tropoelastin in the atherosclerotic lesion. Ao indicates 332 aortic; and L., left. Reproduced with permission from Phinikaridou et al, 2018 [62].

333

334 <u>Collagen</u>

335 The differential expression of various collagen sub-types within atherosclerotic plaque and at different 336 stages of plaque progression makes it an attractive target for atherosclerosis imaging. Type I collagen 337 is the most abundant sub-type and accounts for up to two thirds of all collagen [63]. In an ex vivo 338 immunoblotting study, Chung et al investigated the abundance of different subtypes of collagen in atherosclerotic carotid arteries in patients presenting with diabetes. They demonstrated significant 339 340 accumulation of type III collagen in diabetic vessels and found that type III collagen was more predominant than type I [64]. Caravan et al developed a Gd-DTPA-based MRI probe that binds to type 341 342 I collagen (EP-3533) for molecular imaging of fibrosis [65]. More recently, Chen et al extended this approach by conjugating high density lipoprotein (HDL) based nanoparticles with EP-3533 and used it 343 344 to image collagen in atherosclerotic plaque progression and regression in mice in vivo (Figure 9) [66]. 345 Molecular imaging of collagen using EP-3533 has been extensively discussed in a review article by Haas et al [12]. Using a different approach, a collagen binding adhesion protein found in Staphylococcus 346 347 aureus, called CNA35, was shown to selectively bind to type I and type II collagen [67,68]. Healthy and

atherosclerotic arteries have been imaged with both gadolinium and fluorescently labelled liposomes
conjugated with CNA35 [69,70]. Using positron emission tomography (PET), Schulz et al demonstrated
the feasibility of a novel platelet-collagen-receptor-glycoprotein (GP) VI radiotracer to target and
selectively visualise collagen type I and III ex vivo in human carotid atherosclerotic plaques and in vivo
in an ApoE^{-/-} mouse model [71].



Figure 9- (A) Typical MR images, (B) Normalised Enhancement Ratio of the aortic wall to muscle (NER_w) and (C) The difference between the contrast-to-noise ratio from the aortic wall to muscle pre- and post-contrast injection of abdominal atherosclerotic plaques for pre- and 24 h post-injection (Δ CNR_w) of HDL, EP3533-HDL, and EP3612-HDL at day 0 (yellow bars) and day 28 (green bars) of Reversa mice in the regression group. The red arrows point to the aortas. Error bars are representing mean±SD. Statistical significance at p<0.05 (n=25) is indicated by the asterixs (*). (HDL – high density lipoprotein) Reproduced with permission from Chen et al, 2013 [66]

360

353

361 <u>Fibrin</u>

Fibrin plays a crucial role during plaque progression, development of intraplaque haemorrhage and formation of thrombus following plaque rupture [72,73]. It is therefore an important target for molecular imaging of atherosclerotic plaque. Yu et al demonstrated the feasibility of molecular MRI of fibrin clots ex vivo using a novel fibrin-targeted contrast agent with a lipid-encapsulated perfluorocarbon nanoparticle incorporating numerous Gd-DTPA complexes into its outer surface [74]. The ability to visualise human clots of variable sizes (0.5-7.0mm) ex vivo was significantly improved 368 after administration of this contrast agent compared to non-contrast T1 weighted imaging. In another study, the use of fibrin-specific paramagnetic nanoparticles enabled efficient targeting and signal 369 370 enhancement of thrombi in the external jugular vein of a canine model in vivo [75]. Instead of 371 nanoparticles, Sirol et al used a gadolinium-based, small molecular weight, fibrin-binding MRI agent 372 (EP-1242; EPIX Pharmaceutical) to image acute carotid plaque thrombus in guinea pigs [76]. Later on, 373 Botnar et al demonstrated the feasibility of in vivo molecular MRI of acute and subacute plaque 374 thrombosis following plaque rupture using the gadolinium-based fibrin-binding MRI contrast agent 375 (EP-1873) in a rabbit model of plaque rupture and thrombosis [77] (Figure 10). The same group also 376 demonstrated the feasibility of acute coronary thrombus detection using a similar gadolinium based 377 fibrin-binding contrast agent, EP-2104R, in a swine model [78] (Figure 11). EP-2104R was subsequently successfully translated into humans, demonstrating the in-vivo potential of this fibrin-specific MRI 378 379 contrast agent for the detection of acute venous and arterial thrombus in patients for the first time 380 [79,80] (Figure 12).



381

Figure 10-A, Reformatted view of a coronal 3D data set shows sub-renal aorta ≈20 hours after EP-1873 administration. Three well-delineated mural thrombi (arrows) can be observed, with good contrast between thrombus (numbered), arterial blood (dotted arrow), and vessel wall (dashed arrow). The in-plane view of the aorta allows simultaneous display of all thrombi, showing head, tail, length, and relative location. B to D, Corresponding cross-sectional views show good agreement with

386 histopathology (*E to G*). Reproduced with permission from Botnar et al, 2004 [77]



Figure 11-In vivo MRI of Gd-labelled fibrinogen clots. A and D, Coronary MRA before (A) and after (D) thrombus delivery. On
 both scans, no apparent thrombus is visible (circle). B and E, Black-blood inversion recovery TFE scans before (B) and after (E)
 clot delivery (same view as A and D). After thrombus delivery (E), 3 bright areas are readily visible (arrows and circle),
 consistent with location of thrombus. No apparent thrombus was visible on prethrombus (B) images (arrow and circle). C, X ray angiogram confirming MR finding of thrombus in mid-LAD (circle). F, Magnified view of C. LM indicates left main; LAD
 indicates left anterior descending artery; LCX indicates left circumflex artery. Reproduced with permission from Botnar et al,
 2004 [78]

396

397



398

Figure 12-a Molecular MR imaging of thrombus in the descending thoracic aorta in an 82-year-old female patient using
 inversion recovery black-blood gradient-echo imaging (IR). In this patient parasagittal IR post-contrast imaging was also
 performed to demonstrate the extent of the clot. The high local signal amplification allows for white spot imaging of the
 clot (arrow). b Corresponding multiplanar reconstruction from contrast-enhanced multislice CT demonstrating
 corresponding plaque in the aortic wall. At the cranial end of the plaque a small calcification is also visible (arrowhead).

- 404 Reproduced with permission from Spuentrup et al, 2008 [79]
- 405
- 406

408 *Matrix metalloproteinases*

409 Matrix metalloproteinases (MMPs) have an important role in extracellular matrix and plaque 410 remodelling and are associated with the thinning of the fibrous cap and the destabilization of 411 atherosclerotic plaques [81,82]. Lancelot et al introduced a novel gadolinium-based MRI contrast agent (P947) to target MMPs in atherosclerotic plaques [83]. P947 was evaluated in vivo using ApoE^{-/-} 412 413 mice and ex vivo in hyperlipidaemic rabbits and human carotid artery endarterectomy specimens. This 414 study demonstrated the preferential accumulation of P947 in atherosclerotic lesions compared with 415 non-targeted Gd-DOTA (Figure 13). This contrast agent has also been shown to distinguish dietary-416 induced variations in MMP-related enzymatic activity within plagues in an atherosclerotic model, 417 supporting its utility as a clinical imaging tool for in vivo detection of arterial wall remodelling [84]. 418 Haas et al have systematically reviewed alternative applications of the P947 probe for imaging the 419 ECM [12]



420

Figure 13-In vivo MRI of an ApoE^{-/-} mouse before (arrow) and after P947 injection (arrowhead). After injection, significant
 contrast-enhancement appears in the atherosclerotic aortic wall as shown on the inset images of the aorta. Delineation of
 plaque morphology after contrast-enhancement is clearly improved (arrowhead). The bottom-right panel is the matched
 pathologic section. Reproduced with permission from Lancelot et al, 2008 [83]

425

426

427 In a more recent study, fluorescent-labelled activatable cell penetrating peptides (ACPPs) designed to

428 target MMPs were tested in a rabbit model of plaque rupture [85]. Fluorescence enhancement was

429 significantly higher in ruptured plaques ex vivo, suggesting that ACPP probes can selectively 430 differentiate unstable from stable plaques (Figure 14). Taking advantage of the higher sensitivity of 431 single photon emission computed tomography (SPECT), Kuge et al proposed a technetium-99m-432 labelled monoclonal antibody targeted to MMP-1, for imaging atherosclerosis in a rabbit model [86]. 433 Probe uptake was significantly higher in atherosclerotic arteries compared with control arteries, with 434 further increased uptake in grade IV atheroma in comparison with neointimal or more stable lesions. In another study, technetium-99m-labelling of the broad MMP inhibitor (MPI) enabled ex vivo 435 436 quantification of the reduction of MMP after dietary modification or statin therapy in atherosclerotic rabbit aortas [87]. In a further study, Zhang et al used a novel indium ¹¹¹In-labelled tracer (RP782) with 437 438 specificity for activated MMPs for molecular imaging of MMP activation in injury-induced vascular remodeling in ApoE^{-/-} mice [88] using micro SPECT/CT. 439

440

441



443 Figure 14-Comparison of fluorescence signals from thrombi and the surrounding atherosclerotic plaque in the experimental 444 group. HCD + MMP-ACPP (a,b) and HCD + Thrombin-ACPP(c). The images from left to right in a are: closed view fluorescence 445 image, opened and zoomed-in fluorescence/reflected images with the thrombus (green arrows) attached at its original site, 446 opened and zoomed-in fluorescence/reflected images with the thrombus removed. The dark signal of the aorta on the 447 reflected image comes from clotted blood and relatively healthy vessel wall (without obvious fatty streaks). Plaques appeared 448 as brighter (gray-ish) signal. The bar graphs (b,c) give the statistical analysis of fluorescence signal for the underlying 449 disrupted plaques (DP) and the overlaying thrombi (p<0.001). HCD - high cholesterol diet; ACPP - Activatable Cell Penetrating 450 Peptides. Reproduced with permission from Hua et al, 2015 [85]

451 3.2 Myocardial Infarction

452 *Clinical need*

453 Myocardial Infarction (MI) followed by permanent structural damages and adverse cardiac 454 remodelling can gradually lead to Heart Failure (HF). HF is an increasingly prevalent clinical condition, 455 accounting for 1–2% of morbidity in the adult population in developed countries, rising to ≥10% among 456 people >70 years of age. The lifetime risk of developing heart failure at age 55 years is 33% for men 457 and 28% for women [89–91]. Ischaemic heart disease and myocardial infarction account for the vast 458 majority of all cases of heart failure [92] and therefore pose a significant health burden on patients 459 and an economic burden on healthcare systems.

460

461 Biology of Myocardial of infarction

462 Myocardial infarction involves a sudden and transient occlusion of the coronary arteries (either 463 following acute atherosclerotic plaque rupture or a thrombo-embolic event), depriving the 464 cardiomyocytes of vital nutrients and oxygen. The myocardial matrix network is primarily fibrillar 465 collagen and is organised into three distinct interconnected structures: endomysium (surrounding 466 cardiomyocytes), perimysium (surrounding major bundles) and the epimysium (encasing the whole 467 cardiac muscle). Within these interconnected myocardial structures there are different types of 468 collagen, with the epimysium and perimysium containing approximately 85-90% type I and 6-11% type 469 III collagen in the endomysium of the total collagen content [93]. The cardiac ECM has the structural 470 role to preserve the shape of the ventricles, facilitate the translation of force and transduce signals 471 [94]. Cardiomyocytes have no ability to regenerate and therefore, depending on the extent of the 472 injury, myocardial structural integrity can be severely disrupted with necrosed cardiomyocytes 473 replaced with fibrotic tissue leading to life-threatening arrhythmias and development of heart failure. After myocardial infarction (MI), MMPs are important in myocardial repair and scar formation, 474 475 however if MMPs become excessively activated leading to an imbalance in MMP:TIMP ratio it can 476 cause LV dysfunction and eventually lead to HF. The resulting insult subsequently triggers a cascade 477 of molecular and cellular compensatory or damaging inflammatory, proliferative and maturation 478 responses. The inflammatory phase is triggered by the death of cardiomyocyte cells that 479 consequentially activates pro-inflammatory cytokines, enhancing the activity of MMPs promoting 480 ECM degradation and also activates chemokines and other cell adhesion molecules. Neutrophils, leukocytes and macrophages are recruited into the infarcted area and as the debris is cleared from 481 482 the area, the initial pro-inflammatory stimulus is suppressed by signals from mediators like Interlukin483 10 (IL-10) and transforming growth factor- β (TGF- β). These molecules, activate TIMPs that help 484 preserve the ECM by inhibiting MMPs activity, and initiate the proliferative phase [95]. During the 485 proliferative phase fibroblasts proliferate and transform/ differentiate into myofibroblasts and secrete 486 ECM proteins. The ECM subsequently undergoes extensive remodelling and cross-linking during the 487 maturation phase leading to the formation of a fibrous scar. Fibroblasts are quiescent and along with 488 endothelial cells they are also subjected to apoptosis at this stage [95]. Throughout the myocardial 489 remodelling process, the ECM plays a crucial role and molecular imaging of specific ECM components 490 could offer a wealth of diagnostic and therapeutic insights into heart failure post myocardial infarction 491 that is an incredibly challenging clinical syndrome.

492 *Imaging Methods*

493 Non-contrast imaging

494 Cardiovascular magnetic resonance (CMR) is clinically established as the gold standard non-invasive 495 imaging modality for the assessment of myocardial injury in the context of ischaemic heart disease 496 and heart failure. In addition to highly reproducible 3D volumetric assessment of biventricular 497 dimensions and function, CMR offers a spectrum of non-contrast and contrast enhanced myocardial 498 tissue characterisation functions. Simonetti et al proposed a short-inversion-time inversion-recovery 499 (STIR) magnetic resonance imaging sequence with preparatory radio-frequency pulses to eliminate 500 signal from flowing blood to distinguish between normal and abnormal myocardial tissues and image 501 focal high signal intensity consistent with myocardial oedema following an acute myocardial infarction 502 [96]. Here, a segmented rapid acquisition with relaxation enhancement (turbo spin echo) readout is 503 used, with the inversion-recovery delay adjusted to null fat. To enable a more quantifiable and 504 reproducible assessment of myocardial oedema and inflammation post-MI, 2D and more recently free 505 breathing 3D whole heart myocardial T2 mapping sequences have been proposed [97,98]. It is also 506 possible to take advantage of native myocardial T1 values to differentiate between normal and 507 pathological myocardium including myocardial fibrosis, oedema, inflammation and infiltrative 508 processes such as Fabry disease, amyloidosis, and hemosiderosis [99-102]. Furthermore, recent pre 509 and post contrast myocardial T1 mapping techniques have enabled a non-invasive and quantifiable 510 assessment of the myocardial extracellular volume (ECV), a technique that was previously only feasible 511 through invasive histopathological analysis [103]. This is particularly important in assessing 512 pathological remodelling within the myocardial extracellular matrix, such as extracellular volume 513 expansion caused by oedema, inflammation, infiltrative processes, diffuse and focal fibrosis [103].

515 Contrast-enhanced imaging

Leveraging the differential washout time of gadolinium-based contrast agents within normal and pathological myocardial tissue (e.g. fibrotic scar tissue), T1-weighted inversion recovery prepared late gadolinium enhancement (LGE) CMR is able to directly visualise the transmural extent and pattern of myocardial scar (e.g. following acute myocardial infarction) with a high degree of accuracy, offering both prognostic and therapeutic guidance to clinicians [104].

521 *Collagen:*

522 Recent studies have demonstrated that early gadolinium enhancement (EGE) myocardial imaging 523 identifies both reversibly and irreversibly injured myocardium following an acute MI, thus detecting 524 and quantifying potential areas at risk [105–107] However, none of these techniques are able to 525 directly visualise and assess individual components of the extracellular matrix and its underlying 526 biological processes. To overcome this limitation, targeted molecular imaging of individual 527 extracellular matrix components is required. One example is EP-3533, the same collagen-binding 528 agent used for atherosclerotic imaging [108]. In a mouse model of myocardial infarction, Helm et al 529 demonstrated significantly longer washout time for EP-3533 compared to gadopentetate 530 dimeglumine in regions of post-MI scarring and in normal myocardium, with good correlation on 531 histologic sections stained for collagen and EP-3533 enhanced areas seen on inversion-recovery CMR 532 images (Figure 15) [108]. Furthermore, there was a two-fold higher concentration of EP-3533 within 533 post infarction tissues compared to normal myocardium.

534 *Elastin:*

In a separate approach, Wildgruber et al used ESMA to image elastin fibres for in vivo assessment of extracellular matrix remodelling in a mouse model of myocardial infarction [109]. Despite the final measured area of infarction being similar to that measured with a conventional Gd-based contrast agent, the authors observed progressively increased ESMA uptake within the infarcted area, peaking at day 21 post-MI, which correlated with increased synthesis of tropoelastin and improvement in ejection fraction; suggesting some compensatory properties of elastin post myocardial injury in a mouse of permanent occlusion (**Figure 16**) [109,110].



Figure 15- (a, b, d, e) Gradient-echo IR MR images and (c, f) corresponding picrosirius red-stained histologic sections of the
 LV. Arrows point to area of scarring. (a, d) Standard anatomic MR images acquired by using a double IR gradient-echo
 sequence (8.0/3.7/R-R interval). (b, e) Regions of contrast enhancement on midventricular short-axis MR images
 (7.1/3.0/430) of the LV at two section locations obtained 40 minutes after EP-3533 injection correlate closely with (c,
 f)photomicrographs of picrosirius red-stained tissue sections shown at nine times their original size. LV – Left ventricle.
 Reproduced with permission from Helm et at, 2008 [108].

549

550



Figure 16-Gadolinium-based elastin-specific MR contrast agent (Gd-ESMA) for imaging of myocardial scar. **A**, Chemical structure of Gd-ESMA. **B**, Cross-sectional cardiac MR images of C57BL/6J mouse on postoperative day 7 after myocardial infarction. Two-chamber view and short-axis view obtained at the level of the blue lines in first 4-chamber (image) show accumulation of Gd-ESMA (second and third image) in the myocardial scar, corresponding to the infarct area on triphenyltetrazolium chloride (TTC) staining (last image). Reproduced with permission from Wildgruber et al, 2014 [109]

In an alternative approach, perfluorocarbons were combined with elastin MRI to enable simultaneous assessment of inflammation and elastin remodelling in a murine model of myocardial infarction using a single loop multinuclear ${}^{1}H/{}^{19}F$ send receive coil [111]. This confirmed an early inflammatory response (peaking at day 7 post infarction) followed by elevated elastin remodelling (peaking at day 21 post-MI) (**Figure 17**) [111].

563

564 *Matrix metalloproteinases:*

Targeting the extensive role of MMPs in post-MI remodelling, Su et al proposed a novel and highly 565 sensitive MMP-targeted imaging approach in a murine model [112]. This study used a MMP-targeted 566 ^{99m}Tc-labeled compound (^{99m}Tc-RP805), which acted on the activated catalytic domain and therefore 567 exhibit enzyme inhibitory effects. Using SPECT imaging, ^{99m}Tc-RP805 uptake was increased three-fold 568 569 and two-fold within the infarcted and remote myocardium regions, respectively, compared with 570 control mice; confirming the significant role of MMPs within both the infarcted and remote 571 myocardium following an ischaemic insult. In a review article by Haas et al, further applications of the RP805 probe for imaging the ECM in CVD are discussed [12]. 572

573



575Figure 17- A: Assessment of inflammatory response after myocardial infarction in mice at 3T magnetic resonance imaging576(MRI) using 19F perfluorocarbons.Representative short-axis views of co-registered 1H+19F images (left column; N=8 MI577animals/time-point; N=6 SHAM-operated animals/time-point) and macrophage immunohistochemistry (IHC; macrophages578identified as MAC-3 positive, brown; N=4 MI animals/time-point; N=3 SHAM-operated animals/time-point) from the heart at

3, 7, 14, and 21 days after MI. B: In vivo imaging of extracellular matrix remodeling after myocardial infarction with a
 gadolinium-based elastin/tropoelastin-specific contrast agent Representative short-axis images of relaxation rate (R1, left
 columns) maps and tropoelastin immunohistochemistry (IHC; right columns) of the hearts sections at 7, 14, and 21 days post myocardial infarction (MI) at 3T magnetic resonance imaging (MRI). Tropoelastin fibers were identified as black fine fiber
 network. Reproduced with permission from Ramos et at, 2018 [111]

584

585 3.3 Venous thromboembolism

586 Clinical Need

587 Venous thromboembolism (VTE) predominantly manifests as deep vein thrombosis (DVT) in the veins 588 of the pelvis or lower limb [113], with or without pulmonary embolism (PE), and annual incidence and 589 mortality of up to 600,000 and 100,000, respectively in the United States [114,115]. Approximately 590 30% of patients with DVT develop pulmonary embolism (PE), of which 10% die; while 50% of patients with DVT develop post-thrombotic syndrome (PTS) characterised by pain, swelling and chronic 591 ulceration [114,116]. PTS is associated with a reduced quality of life [117]. VTE causes a substantial 592 financial burden to health systems in Europe with the range of costs for in Europe ranging from 1.5 to 593 594 13.2 billion euro[118]. Given the burden of the disease early diagnosis and treatment are essential to 595 improve patient prognosis, quality of life and management is needed [113]. Venous thrombi mainly 596 contain erythrocytes, fibrin and collagen [119].

597

598 Biology of venous thromboembolism

599 The factors contributing to the formation of DVT were first described in 1856 as stasis in the venous 600 system, injury to vasculature and hypercoagulability that collectively increase the risk of clot formation 601 [120]. The initiation of this coagulation cascade along with fibrin deposition in the vessel leads to the 602 formation of the venous thrombus. Thrombus resolution occurs slowly through a natural process of 603 organisation involving the replacement of fibrin with collagen and recanalisation of the vein 604 [116,121,122]. Inflammation has been strongly associated with DVT thrombus resolution as the 605 inflammatory response causes the activation of pro-inflammatory cytokines (including IL-6 and IL-8) and growth factors (including transforming growth factor beta-1 (TGF-B1) and -2 tumour growth factor 606 607 $(TGF-\beta 2)$ [123–125]. Consequently, macrophages, neutrophils and monocytes are recruited within 608 the thrombus further enhancing the inflammatory response by producing more inflammatory 609 cytokines, amalgamating the inflammation and growth factors [126]. With time, this existing fibrin 610 fibre network thickens or is replaced by other structural proteins, such as collagen [127]. An important 611 factor of MMP activation in DVT is that MMP are activated proximally by serine proteases; in particular 612 plasmin that is the main mediator of fibrinolysis [126]. Fibrinolysis, breakdown of fibrin, along with collagen remodelling are essential for the resolution of the thrombus [128]. Fibrinolysis occurs at an
increased rate at the early phases and as the thrombus matures, fibrin is replaced by collagen [128].
Only acute thrombi are considered for thrombolytic treatment, as they are more fibrin-rich; while
older thrombi are more collagenous and therefore resistant to plasmin-mediated degradation [129–
133].

618 Imaging venous thromboembolisms

619 Direct MRI thrombus imaging

Moody et al [134] demonstrated that it is possible exploit the intrinsic T1 properties of tissues to image 620 thrombus with MRI. Proton binding to Fe³⁺ within accumulating methemoglobin in thrombi produces 621 T1 shortening leading to high signal intensity on T1-weighted images of thrombi such as in DVT. In a 622 623 murine model of DVT, Saha et al assessed the longitudinal relaxation time (T1) of thrombus over a 28-624 day evolutionary phase of the thrombus [135]. They demonstrated that the T1 relaxation time of 625 thrombus was shortest at 7 days following thrombus induction and returned to that of blood as the 626 thrombus resolved (Figure 18). T1 relaxation time was related to thrombus methemoglobin formation 627 and was a good predictor of successful thrombolysis with a cut-off point of <747ms; with a sensitivity 628 and specificity to predict successful lysis of 83% and 94%, respectively.

- 629
- 630
- 631

632

633

634

635

636

637

638



640





642



A	d1 suture thrombu	d4 suture thrombu	d7 s thrombu	s suture	d14 bus thromm	d21 suture thrombus	d28 suture thrombus
в							
C							
	DAY 1	DAY 4	DAY 7	DAY 10	DAY 14	DAY 21	DAY 28



Day

645 Figure 18- Magnetic resonance T1 mapping of experimental venous thrombi. A, MRI venography demonstrates the presence 646 of thrombus in the murine inferior vena cava, which recanalizes over 28 days. **B**, MSB sections of venous thrombus (T) during 647 its resolution (yellow=red cells, red=fibrin, blue=collagen; ×200; bar, 200 μm). C, Corresponding T1 maps were generated by 648 the use of a customized program (MATLAB software, MathWorks) before importation into OSIRIX as shown. Short 649 T1relaxation times (ms) appear red and revert back to blood (black) as thrombus ages. D, Mean T1 relaxation times (ms) of 650 the thrombus change during its resolution (scatter plot and mean of thrombus T1 relaxation time [ms] is shown, n=88 651 mice, P<0.0001, 1-way ANOVA). Mouse procedures were performed under the Animals (Scientific Procedures) Act, 1986, UK. 652 ANOVA indicates analysis of variance; d, day; and MSB, Martius Scarlet Blue. Reproduced with permission from Saha et al, 653 2013 [135].

654

- In a similar experimental murine model of DVT, Phinikaridou et al assessed the feasibility of in-vivo
- magnetization transfer contrast (MTC) and diffusion-weighted (DW) MRI for thrombus imaging [136].
- They demonstrated that quantitative MTC imaging is able to differentiate between young (days 1 and
- 658 7) and old (>day 14) thrombus, while the combination of quantitative MTC and DW imaging can
- successfully identity thrombus between days 7 and 14 (Figure 19).



Figure 19- Percentage of magnetization transfer rate (MTR) and apparent diffusion coefficient (ADC) values measured at different time points of venous thrombosis organization. The combination of the percentage of MTR (<7620%/cm3) and ADC (>0.81×10-3 mm2/s) had a sensitivity of 87.5% (95% confidence interval [CI], 47–99) and specificity of 83.3% (95% CI, 51–97) to identify thrombus between 7 and 14 days old, when the fibrin content would be larger. Reproduced with permission from Phinikaridou et al, 2013 [136].

⁶⁷⁷ Recently, Wu et al demonstrated the feasibility of non-contrast diffusion weighted MRI for in vivo 678 discrimination of acute (<14 days) from non-acute (>14 days) DVT [137]. In this study, 85 patients with 679 lower limb DVT who underwent diffusion weighted MRI could be successfully differentiated based on 680 their apparent diffusion coefficient (ADC) images. The mean ADC was higher in acute DVT than non-681 acute DVT ($0.56 \pm 0.17 \times 10^{-3}$ vs. $0.22 \pm 0.12 \times 10^{-3}$ mm²/s, *P*<0.001). Using 0.32×10^{-3} mm²/s as the

- 682 cut-off, the sensitivity and specificity of ADC to discriminate acute from non-acute DVT were 93 and
- 683 90%, respectively (**Figure 20**).



Figure 20- A comparison between acute and non-acute DVT. Acute thrombus (first line) and non-acute thrombus (second line) were hypointense on b = 0 (a, d), b = 800 s/mm2 (b, e) image. The signal intensity was about the same for acute and non-acute thrombus, while ADC values differed greatly (c, f). Mean ADC of this acute thrombus was above the cutoff of $0.32 \times 10-3$ mm2/s, while that of non-acute thrombus was below the cutoff. Reproduced with permission from We et al, 2019 [137]

In a different study, Chen et al demonstrated the potential of black blood non-contrast direct thrombus imaging in a cohort of 15 healthy volunteers and 30 patients with acute DVT. Thrombus appeared either iso-or hyperintense in the acute phase with comparable diagnostic confidence to contrast enhanced CMR venography: sensitivity (95%), specificity (99%), positive predictive value (96%), negative predictive value (98%), and accuracy (98%)(**Figure 21**) [138]



Figure 21- Representative images obtained by CE-CMRV and BBTI from a patient with DVT symptom onset at 10 days. The
 small thrombus can also be detected by BBTI and matched well with that seen with CE-CMRV (yellow arrows). BBTI - black blood thrombus imaging; CE-CMRV - Contrast-enhanced CMR venography. Reproduced with permission from Chen et al, 2018
 [138]

701

702 Molecular thrombus imaging

703 *<u>Fibrin:</u>*

Fibrin is heavily involved in thrombus formation [72,73] and is therefore an important molecular target for imaging of DVT. In a pre-clinical murine model of DVT, Andia et al investigated the fibrin-specific MRI contrast agent (EP-2104R) for the in vivo quantification of thrombus fibrin content the identification of thrombus suitable for thrombolysis [139] (**Figure 22**). Contrast uptake positively

correlated with the fibrin content of the thrombus measured by Western blotting (R^2 =0.889; P<0.001).

Furthermore, thrombus relaxation rate post contrast and the change in visualised thrombus size pre

and post EP-2104R injection were the best predictors for successful thrombolysis.

- 711
- 712



Figure 22- A, Changes in thrombus relaxation rate ΔR1 (s-1) pre–EP-2104R and post–EP-2104R administration at different time point during thrombus organization. Greatest differences in ΔR1 pre and post contrast are observed at days 7 and 10. B, Thrombus gadolinium concentration (µmol/L) mirrored the changes in R1 over time. C, R1mapping images pre–EP-2104R and post–EP-2104R injection and Martius Scarlet Blue (MSB) histology sections at the corresponding levels and fibrin selective segmentation. Gd-DTPA indicates gadolinium with diethylenetriaminepentacetate (Magnevist, Schering AG, Berlin, Germany). Reproduced with permission from Andia et al, 2014 [139].

730 In another study, Spuentrup et al demonstrated the successful imaging of thrombus (both freshly 731 engineered thrombi and human thrombus removed from patients) by using EP-2104R in a swine 732 model of pulmonary embolism [140]. In a clinical translational study, Vymazal et al assessed the use of EP-2104R in patients with confirmed PE or DVT [80]. Use of the agent enhanced the signal of the 733 734 thrombus and enabled visualisation of additional thrombus features, not visible on the pre-contrast 735 images (Figure 23). In a different approach, Hara et al combined a novel fibrin-targeted probe (FTP 11-736 Cy7) with near-infrared fluorescence (NIRF) in a mouse model of DVT [141]. In vitro human clot-737 binding analyses showed a 6-fold higher NIRF signal for the clot target-to-background ratio (TBR) using 738 the FTP11-Cy7 probes compared to the free Cy7. Moreover, the thrombus TBR of acute and sub-acute 739 femoral DVT with FTP11-Cy7 obtained by intravital fluorescence microscopy was 4-fold higher than 740 control free Cy7. Indeed, in vivo imaging of fibrin in jugular DVT via fluorescence molecular computed

- tomography demonstrated strong NIRF signal in thrombi compared to sham-operated jugular veins
- using FTP11-Cy7. Recently, Blasi et al combined whole-body PET/MRI with the fibrin-binding probe
- ⁶⁴Cu-FBP8 in a rodent model of carotid artery and femoral vein thrombosis [142]. A single whole-body
- 744 PET/MRI scan revealed the location of both arterial and venous thrombi after the administration of
- ⁶⁴Cu-FBP8. PET imaging showed that probe uptake was greater in younger, fibrin-rich clots compared
- 746 with older collagen-rich clots in both arterial and venous thrombosis (P<0.0001), which was also
- 747 confirmed on quantitative histopathology analysis.

749



750

Figure 23- Left panel, met-Hb enhanced thrombus in the lower femoral/popliteal (knee) veins. Centre panel, Additional
 thrombus apparent in the mid and upper femoral vein when image acquired 2 hours post EP-2104R injection. Right panel, 20
 hours postcontrast agent image demonstrates persistent enhancement of the thrombus. Reproduced with permission from
 Vymazal et al, 2009 [80]

755

756 3.4 Abdominal Aortic Aneurysms

757 Clinical Relevance of AAA

758 Abdominal aortic aneurysms (AAA or triple A) are a localized enlargement of the abdominal aorta such that the diameter is greater than 3cm or more than 50% larger than normal. AAA's are becoming 759 760 increasingly more prevalent with rupture of an AAA having devastating clinical consequences, with up 761 to 90% mortality [143,144]. This rise is driven by a maelstrom of risk factors including connective tissue 762 disorders (e.g., Marfan syndrome, Ehler Danlos syndrome), autoimmune and inflammatory disorders (e.g., Takayasu disease, Kawasaki disease), infective disorders (e.g. brucellosis, salmonellosis, 763 764 and tuberculosis), modifiable factors (e.g. smoking, hyperlipidaemia, obesity, hypertension) and nonmodifiable factors (increasing age and male sex) [145,146]. The size and rate of progression of AAA 765

has important prognostic implications and it currently serves as the screening marker for monitoring
disease progression and for following up treatments prior to pre-emptive surgical intervention [147].
However, anatomical assessment alone is highly unreliable as the rate of disease progression and
aortic dilatation varies significantly between affected individuals [148]. ECM components could
potentially offer a complementary tool to that of anatomical imaging, for improved and early diagnosis
and therapeutic targeting of AAA at a biological and molecular level [149].

772 Biology of AAA

773 Components of the extracellular matrix play significant roles in the remodelling and dilatation of the 774 abdominal aorta. Based on ex-vivo tissue studies there is strong evidence that the breakdown of two major ECM fibre components, elastin and collagen, is what primarily weakens the aortic wall resulting 775 776 in gradual enlargement and formation of a balloon-like swelling, called an aneurysm [150]. With the 777 continuous enlargement of the aneurysm, the media and adventitial layers of the vessel wall might 778 separate, and an intramural hematoma might be formed which is frequently rich in fibrin. Through 779 time the aneurysm tissue matures and the hematoma composition changes from fibrin-rich to 780 collagen and elastin rich, ultimately improving the hematomas' stability [150]. Acute aortic dissection 781 (AAD) is characterised by the formation of an intimal flap separating the true (normal pathway for 782 blood) and false (newly created pathway for blood) lumens [151]. The false lumen is located in the 783 outer portion of the aorta and there are generally indications of slow flow along some, if not all, of 784 the lumen [151].

785 In AAAs, the most common type of proteinases are the MMPs [152]. MMP expression is increased in 786 comparison to normal tissue and TIMPs expression is also changed, However the MMP/TIMP equilibrium is shifted towards the degradation of proteins [152]. In healthy conditions many vascular 787 788 cells secrete MMP, including both endothelial and VSMC, however in AAA additional secretion is 789 adopted by macrophages and lymphocytes [153,154]. Aortic wall elastin can be degraded and 790 fragmented by MMPs, leading to subsequent instability of the aortic wall [155]. Consequently, the 791 pulsatile strength of the vessel wall is reduced and as a result there is initially compensatory 792 production of collagen, which however, is also degraded over time [152]. When collagen degradation 793 surpasses the production of collagen the tensile strength of the vessel wall attenuates even more 794 resulting in vessel wall thinning and ultimately rupture [152]. Therefore, prognosis of AAA is highly 795 determined by the synthesis and degradation of elastin and collagen proteins.

796 *Elastin:*

- 797 Elastin underpins the structural integrity of the aortic wall and constitutes up to a third of its weight
- [156]. In a mouse model of aortic aneurysm, Botnar et al demonstrated the feasibility of an elastin-
- specific MR agent (ESMA) to monitor the in vivo stages of aortic wall remodelling prior to and during
- aortic dilatation, with subsequent histopathological validation (Figure 24) [157].



802 Figure 24- In vivo assessment of the aortic rupture site prior to the dilation of the aorta. In an ApoE^{-/-} mouse 1 week after 803 continuous infusion of angiotensin II, the angiogram demonstrates an aortic lumen without dilatation or luminal irregularities 804 (A1). The red line indicates the location of the corresponding transverse images. On elastin-specific magnetic resonance 805 molecular imaging agent (ESMA)-MRI, the rupture of elastic laminae could be clearly visualized in vivo (A4, A5). 806 Corresponding ex vivo histological sections (A6, A7) confirmed the rupture of elastic laminae in this area (magnification of 807 A6, orange arrows). On pre-contrast (A2) and gadolinium diethylenetriamine penta-acetic acid enhanced images (A3), only 808 minor enhancement of the arterial wall was observed. aA indicates abdominal aorta; EvG, Elastica van Gieson; HE, 809 hematoxylin and eosin; rRA, right renal artery; and TOF, time of flight. Reproduced with permission from Botnar et al, 2014 810 [157]

811

812

813 Similarly, in a Marfan mouse model Okamura et al demonstrated the potential of ESMA to detect 814 decreased aortic wall levels of elastin compared with wild-type controls [158]. Recently, Lavin et al advanced our understanding of dysfunctional elastogenesis in AAA disease by employing a newly 815 816 developed gadolinium labelled tropoelastin-binding MRI agent (TESMA). This study assessed whether 817 quantifying regional tropoelastin turnover correlates with aortic expansion in an Angio-II-infused 818 murine model (Figure 25) [159]. The authors demonstrated that tropoelastin overexpression and the 819 uptake of the contrast agent were confined within aneurysmal walls. Significantly, a parallel 820 longitudinal imaging study demonstrated a greater proportion of tropoelastin:elastin in dilating

- 821 compared to non-dilating aortas, which correlated with the rate of aortic expansion. Interestingly,
- treatment with aspirin and statins did not affect tropoelastin turnover or aortic dilatation in a small
- cohort of mice. Finally, TESMA was able to identify accumulation of tropoelastin in a small number of
- 824 excised human aortic aneurysmal tissue as confirmed by histology.



826 Figure 25- MRI of tropoelastin shows enhancement at sites of aortic aneurysm or dissection. (A) A reformatted MRA of an 827 Ang II-infused ApoE^{-/-} mouse shows two regions of aortic dilation. (B-G) MRA, LGE-MRI, and histology of a non-dilated 828 segment show a normal aortic size, low enhancement after administration of Gd-TESMA, and lack of tropoelastin, 829 respectively. (H–M) At the level of the first aortic dilation, there is vascular enhancement of the aneurysm after administration 830 of Gd-TESMA that co-localized with the accumulation of tropoelastin as verified histologically. (N-P and T-V) At the level of 831 the second aortic dilation, MRI images show the formation of a false lumen indicative of an aortic dissection, with aortic 832 enhancement after administration of Gd-TESMA and retrograde blood flow. (Q-S) Histology verified the formation of two 833 lumens and the accumulation of tropoelastin in areas where vascular enhancement was observed in vivo using the 834 tropoelastin agent. Ao, aorta; FL, false lumen. Reproduced with permission from Lavin et al, 2019 [159]

835

836 *Collagen:*

Despite an initial compensatory increase in collagen synthesis in the early stages of AAA onset, enzymatic (e.g. MMPs and cathepsins) collagen degradation dominates the subsequent progressive stages of AAA development, paving the way for aortic dilatation [160,161]. In a mouse model of aortic aneurysm and rupture, Klink et al assessed the feasibility of in vivo molecular MRI of collagen using paramagnetic/fluorescent micellar nanoparticles functionalized with the collagen-binding protein CNA-35 [162]. Injection of CNA-35 micelles resulted in a significantly higher MRI signal enhancement in aneurysmal walls compared with nonspecific micelles. Histological analysis demonstrated that CNA- 844 35 colocalized with type I collagen, although it is known to bind to all fibrillar collagens and collagen 845 type IV [162]. Furthermore, the investigators also showed that stable aneurysms were associated with 846 increased expression of collagen in the aortic wall and high CNA-35 uptake, whilst ruptured aneurysms 847 displayed significant collagen degradation and low CNA-35 uptake demonstrating the potential of 848 collagen imaging in the diagnosis and risk stratification of AAA. Given the most abundant collagens in 849 the aortic wall are type I and III collagen, Satta el al investigated whether changes of the propeptide of type III collagen (PIIINP) in serum could be associated with characteristics of AAA. PIINP measures 850 851 the turnover of type III collagen [163]. Satta et al. quantified the levels of animoterminal PIIINP and 852 carboxyterminal propeptide of type I collagen by radioammunoassays in patient samples of AAA and 853 reported an increase in type III collagen in the serum of patients with AAA compared with controls. 854 These result could be attributed to increased synthesis of type III collagen, increased degradation or 855 both in these patients [163].

856 <u>*Fibrin:*</u>

857 Fibrin is a key protein in the formation of focal hematoma associated with aortic dissection and the 858 development of larger thrombi during the progression of AAAs [155]. Botnar et al used the fibrin-859 specific molecular MRI probe (EP2104R) in an angiotensin-II-infused ApoE^{-/-} mouse model of AAA 860 [150]. EP2104R enabled visualization of the small fibrin-rich hematoma located at the site of aortic 861 dissection prior to aortic dilation and during the progression of AAA (Figure 26). In early thrombi, a 862 strong in vivo signal and increased fibrin deposition was measured, which was confirmed by ex vivo 863 analysis. In advanced thrombi, an increased remodelling of the matrix of the thrombus was observed and a relative decrease in fibrin expression was measured in vivo. This could potentially enable 864 865 differentiation between early fibrin-rich and advanced remodelled aortic aneurysm thrombi.



867 Figure 26-Assessment of a focal fibrin-rich intramural hematoma before the dilation of the aorta by fibrin MRI. A, On 868 the TOF angiogram, a nondilated aortic lumen without luminal irregularities can be appreciated in an ApoE^{-/-}mouse 1 week 869 after continuous infusion of angiotensin II. The red line indicates the alignment of in vivo MRI sequences and ex vivo histology. 870 No significant enhancement of the aorta was measured on pre-contrast scans (A2) and following the administration of the 871 nonspecific control agent (Gd-DTPA, A3). Following the administration of the fibrin-specific molecular probe, a strong focal 872 enhancement was measured at the dissection side (A4-A6). On the EvG stain, the dissection of elastic laminae can be clearly 873 visualized on magnified images (B2). In between the dissection, a fibrin-rich mural hematoma can be visualized (B2-B4). It's 874 a strong signal from the fibrin that antibody was measured at the location of the intramural hematoma (B4). No relevant 875 formation of elastin or collagen fibers was measured in the hematoma on histologic sections (B1-B3), indicating that no 876 remodeling of the hematoma has occurred. aA indicates abdominal aorta; Gd-DTPA, gadopentetate dimeglumine; MRI, 877 magnetic resonance imaging; rRA, right renal artery; TOF, time of flight. Reproduced with permission from Botnar et al, 2018 878 [150]

879 *Matrix metalloproteinases:*

880 Similar to atherosclerotic arterial disease, MMPs play a significant role in aortic remodelling during 881 the evolution of AAA (e.g. the degradation of elastin and collagen) [154,155]. Bazeli et al used the 882 MMP targeting MRI probe (P947) in an elastase-infused rat model of AAA [164]. After 5 days of 883 elastase infusion, a significantly higher molecular signal was detected in rats injected with P947 884 compared with rats injected with an untargeted-scrambled agent or Gd-DOTA. Histologic analysis confirmed colocalization of areas of contrast enhancement and the periarterial inflammatory area of 885 the aneurysms, representing a potential non-invasive method to detect AAAs at high risk of rupture. 886 887 Using SPECT imaging, Golestani et al used a technetium-99m-labeled MMP-specific tracer (RP805) for 888 the detection of aneurysm biology and prediction of outcome in angiotensin II-infused mice [165]. 889 RP805 uptake was significantly higher in animals with AAA when compared with angiotensin II-infused 890 animals without AAA or control animals. A cohort of angiotensin II-infused animals were imaged at 1 891 week and followed up for an additional 3 weeks. RP805 uptake at 1 week was significantly higher in 892 mice that later developed rupture or AAA. Furthermore, tracer uptake at 1 week correlated with aortic

diameter at 4 weeks, demonstrating the potential of RP805 as a risk stratification tool for future aortic
 expansion/rupture.

4. Clinical Relevance of Non-invasive Imaging Probes

896 The ability to image the individual components of the ECM to gain an insight into the live biological 897 processes that ultimately manifest as various CVDs is of great clinical value. The clinical translational 898 scope is multifaceted. Patients can be identified at an earlier stage in the disease process, potentially 899 prior to phenotypical or symptomatic presentation. This will subsequently enable the initiation of 900 either prophylactic or therapeutic treatment at a much earlier stage, which may translate into 901 improved clinical outcomes. The dynamic nature of ECM biology can be leveraged to monitor patients 902 over time, including their response to treatment. Furthermore, the quantitative and targeted imaging 903 of ECM components could be utilised as an additional tool to titrate and if needed alter therapeutic 904 strategies. Similarly, molecular imaging of ECM components could play a significant role in therapeutic 905 drug development both in pre-clinical and clinical trials to assess the efficacy of pharmacological 906 interventions at both the cellular and molecular levels.

907 However, these probes are largely still at the developmental stage and no single probe has yet been 908 established for routine clinical decision-making. They remain expensive to implement and are mainly 909 used by a select group of academic and commercial institutions. In addition to large clinical efficacy 910 trials, long-term data on the potential effect these imaging probes may have on the physiological 911 function of the ECM is required before large-scale clinical trials take place. Nuclear imaging probes 912 have a distinct advantage over MRI probes in the sense that a significantly lower concentration of 913 probe is required to achieve the required threshold for detection. This lowers the developmental cost 914 of probes and accelerates human trials as expensive and time-consuming toxicity studies in humans 915 can often be extenuated due to micromolar dosing. However, MRI retains the advantage of superior 916 spatial resolution, intrinsic tissue characterisation and lack of ionising radiation. Furthermore, once 917 the initial hurdle of probe safety and efficacy has been overcome, long-term production, storage and 918 maintenance of MRI probes is cheaper and more scalable compared to nuclear imaging probes, for 919 instance no requirement for cyclotrons in close proximity to scanners, longer and safer storage once 920 manufactured. Furthermore, access to MRI scanners is relatively cheaper compared to PET. Hybrid 921 scanners (e.g. PET/MR) have been developed to take advantage of the higher sensitivity of PET whilst 922 at the same time enjoying the benefits of MRI (e.g. higher spatial resolution). However, they are very 923 expensive, limited to a select group of centres and ionising radiation remains a persistent drawback.

925 One area that is attracting interest is the idea of using ECM probes with PET in clinical proof of concept 926 studies by taking advantage of the significantly lower dose that is required as a pragmatic bridge to 927 full MRI only probe development. Once a clear proof of concept in man is established, resources and 928 focus can shift towards toxicity and safety studies for MRI only probes. This strategy can streamline 929 the pathway and significantly increase the likelihood of successful probe development.

930 5. Future Directions

931 Understanding the biosynthesis and biochemical composition of ECM components enables the design 932 and development of targeted imaging probes for molecular imaging using modalities, such as MRI and 933 PET. This information is of importance not only to the field of molecular imaging but also in for tissue 934 engineering and regenerative medicine to produce biomaterials to mimic the properties of ECM 935 proteins. Existing probes in research still require further development and clinical validation in clinical 936 trials. Despite the considerable advantages of MRI; including high spatial resolution images and 937 radiation free imaging; clinical translation of MRI probes is impeded because of concerns with toxicity 938 and financial viability. There is therefore need for future studies into methods that can improve the 939 safety of these MRI probes to bridge the gap that currently exists between probe development and 940 clinical translation. The limitations of the use of gadolinium-based contrast agents are well 941 documented for instance in patients with renal impairment. There is therefore a renewed interest in 942 manganese, a biogenic compound, as a non-gadolinium MR imaging contrast which displays similar 943 physical properties of gadolinium, however, differs by means of clearance as it is excreted via the 944 hepatobiliary system. Targeted iron oxide nanoparticles are currently been explored as contrast 945 agents and have shown great potential. Prospective development of MRI agents is likely to involve 946 multi-modal approaches, integrating systems and new theranostic applications combining diagnosis 947 and therapy in a single exam.

948 6. Conclusion

949 The ECM plays vital roles in multiple cardiovascular diseases and maintaining ECM homeostasis is a 950 key target of effective treatment and prevention. Different CVDs share changes in common ECM 951 proteins e.g., fibrin, collagen and elastin and ECM-related enzymes e.g., MMPs, LOX that have become 952 interesting imaging and therapeutic targets. Understanding the biochemistry of the major ECM 953 proteins is essential for the development of drugs that specifically modulate the ECM and novel 954 imaging approaches for assessing changes in the composition of ECM proteins in disease conditions. 955 In the past decade there have been major advancements in both non-contrast imaging of the ECM 956 and also protein and cell specific imaging probe development for imaging the biology of ECM proteins. 957 These imaging technologies could be used to improve diagnosis and guide personalised treatments 958 for improving patient outcomes. Future studies should focus on further refinement of the imaging
959 probes to enable safe and efficient imaging and to ultimately translate and evaluate their use in the
960 clinical setting.

961

962 Acknowledgements:

This work was supported by the following grants: (1) EPSRC EP/P032311/1, EP/P001009/1 and EP/P007619/1, (2) BHF programme grant RG/20/1/34802, (3) King's BHF Centre for Research Excellence RE/18/2/34213 (4) Wellcome EPSRC Centre for Medical Engineering (NS/A000049/1), and (5) the Department of Health via the National Institute for Health Research (NIHR) Cardiovascular Health Technology Cooperative (HTC) and comprehensive Biomedical Research Centre awarded to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust.

970

971

 Schuppan, D. Structure of the extracellular matrix in normal and fibrotic liver: Collagens and glycoproteins. *Semin. Liver Dis.* **1990**, *10*, 1–10, doi:10.1055/s-2008-1040452.

- 974 2. Gullberg, D.; Ekblom, P. Extracellular matrix and its receptors during development. *Int. J. Dev.* 975 *Biol.* 1995, doi:10.1387/ijdb.8645569.
- Järveläinen, H.; Sainio, A.; Koulu, M.; Wight, T.N.; Penttinen, R. Extracellular matrix
 molecules: Potential targets in pharmacotherapy. *Pharmacol. Rev.* 2009, *61*, 198–223,
 doi:10.1124/pr.109.001289.
- Frantz, C.; Stewart, K.M.; Weaver, V.M. The extracellular matrix at a glance. *J. Cell Sci.* 2010, 123, 4195–4200, doi:10.1242/jcs.023820.
- 9815.Daley, W.P.; Peters, S.B.; Larsen, M. Extracellular matrix dynamics in development and982regenerative medicine. J. Cell Sci. 2008, 121, 255–264, doi:10.1242/jcs.006064.
- Wallace, K.; Burt, A.D.; Wright, M.C. Liver fibrosis. *Biochem. J.* 2008, 411, 1–18, doi:10.1042/BJ20071570.
- 985 7. Wight, T.N.; Merrilees, M.J. Proteoglycans in atherosclerosis and restenosis: Key roles for
 986 versican. *Circ. Res.* 2004, doi:10.1161/01.RES.0000126921.29919.51.
- Caorsi, V.; Toepfer, C.; Sikkel, M.B.; Lyon, A.R.; MacLeod, K.; Ferenczi, M.A. Non-Linear Optical
 Microscopy Sheds Light on Cardiovascular Disease. *PLoS One* 2013,
 doi:10.1371/journal.pone.0056136.
- Jo, J.A.; Park, J.; Pande, P.; Shrestha, S.; Serafino, M.J.; De Jesus Rico Jimenez, J.; Clubb, F.;
 Walton, B.; Buja, L.M.; Phipps, J.E.; et al. Simultaneous morphological and biochemical
 endogenous optical imaging of atherosclerosis. *Eur. Heart J. Cardiovasc. Imaging* 2015,
 doi:10.1093/ehjci/jev018.

- Schipke, J.; Brandenberger, C.; Rajces, A.; Manninger, M.; Alogna, A.; Post, H.; Mühlfeld, C.
 Assessment of cardiac fibrosis: A morphometric method comparison for collagen
 quantification. J. Appl. Physiol. 2017, doi:10.1152/japplphysiol.00987.2016.
- Pinkert, M.A.; Hortensius, R.A.; Ogle, B.M.; Eliceiri, K.W. Imaging the Cardiac Extracellular
 Matrix. In *Advances in Experimental Medicine and Biology*; 2018; Vol. 1098, pp. 21–44 ISBN
 978-3-319-97421-7.
- De Haas, H.J.; Arbustini, E.; Fuster, V.; Kramer, C.M.; Narula, J. Molecular imaging of the
 cardiac extracellular matrix. *Circ. Res.* 2014, *114*, 903–915,
 doi:10.1161/CIRCRESAHA.113.302680.
- Skandalis, S.S.; Dobra, K.; Götte, M.; Karousou, E.; Misra, S. Impact of Extracellular Matrix on
 Cellular Behavior: A Source of Molecular Targets in Disease. *Biomed Res. Int.* 2015, 2015,
 doi:10.1155/2015/482879.
- Manabe, R.I.; Tsutsui, K.; Yamada, T.; Kimura, M.; Nakano, I.; Shimono, C.; Sanzen, N.;
 Furutani, Y.; Fukuda, T.; Oguri, Y.; et al. Transcriptome-based systematic identification of
 extracellular matrix proteins. *Proc. Natl. Acad. Sci. U. S. A.* 2008, *105*, 12849–12854,
 doi:10.1073/pnas.0803640105.
- 1010 15. Bonnans, Caroline Chou, Jonathan Werb, Z. Remodelling the extracellular matrix in
 1011 development and disease. *Nat. Rev. Mol. Cell Biol.* 2014, *15*, 786–801,
 1012 doi:10.1038/nrm3904.Remodelling.
- 1013 16. Rozario, T.; DeSimone, D.W. The extracellular matrix in development and morphogenesis: A dynamic view. *Dev. Biol.* **2010**, doi:10.1016/j.ydbio.2009.10.026.
- 1015 17. Bailey, A.J. Collagen and elastin fibres. *J. Clin. Pathol.* 1978, *31*, 49–58,
 1016 doi:10.1136/jcp.31.Suppl_12.49.
- 1017 18. Hamill, K.J.; Kligys, K.; Hopkinson, S.B.; Jones, J.C.R. Laminin deposition in the extracellular
 1018 matrix: A complex picture emerges. *J. Cell Sci.* 2009, doi:10.1242/jcs.041095.
- 101919.Magnusson, M.K.; Mosher, D.F. Fibronectin: Structure, assembly, and cardiovascular1020implications. Arterioscler. Thromb. Vasc. Biol. 1998, doi:10.1161/01.ATV.18.9.1363.
- 102120.Jones, F.S.; Jones, P.L. The tenascin family of ECM glycoproteins: Structure, function, and1022regulation during embryonic development and tissue remodeling. *Dev. Dyn.* 2000,1023doi:10.1002/(SICI)1097-0177(200006)218:2<235::AID-DVDY2>3.0.CO;2-G.
- Rienks, M.; Papageorgiou, A.P.; Frangogiannis, N.G.; Heymans, S. Myocardial extracellular
 matrix: An ever-changing and diverse entity. *Circ. Res.* 2014,
 doi:10.1161/CIRCRESAHA.114.302533.
- 1027 22. Hileman, R.E.; Fromm, J.R.; Weiler, J.M.; Linhardt, R.J. Glycosaminoglycan-protein
 1028 interactions: Definition of consensus sites in glycosaminoglycan binding proteins. *BioEssays*1029 1998, 20, 156–167, doi:10.1002/(SICI)1521-1878(199802)20:2<156::AID-BIES8>3.0.CO;2-R.
- Yanagishita, M. Function of proteoglycans in the extracellular matrix. *Pathol. Int.* 1993,
 doi:10.1111/j.1440-1827.1993.tb02569.x.
- 1032 24. Roberts, A.B.; Sporn, M.B.; Assoian, R.K.; Smith, J.M.; Roche, N.S.; Wakefield, L.M.; Heine,
 1033 U.I.; Liotta, L.A.; Falanga, V.; Kehrl, J.H. Transforming growth factor type β: Rapid induction of
 1034 fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc. Natl.*1035 *Acad. Sci. U. S. A.* **1986**, *83*, 4167–4171, doi:10.1073/pnas.83.12.4167.
- 1036 25. Roberts, A.B.; McCune, B.K.; Sporn, M.B. TGF-β: Regulation of extracellular matrix. *Kidney Int.*

- 1037 **1992**, *41*, 557–559, doi:10.1038/ki.1992.81.
- Bujak, M.; Frangogiannis, N.G. The role of TGF-β signaling in myocardial infarction and cardiac
 remodeling. *Cardiovasc. Res.* **2007**, doi:10.1016/j.cardiores.2006.10.002.
- 1040 27. Chen, Q.; Jin, M.; Yang, F.; Zhu, J.; Xiao, Q.; Zhang, L. Matrix metalloproteinases: Inflammatory
 1041 regulators of cell behaviors in vascular formation and remodeling. *Mediators Inflamm.* 2013,
 1042 2013, doi:10.1155/2013/928315.
- 104328.Perrine Susan Extracellular Matrix Remodeling During the Progression of Volume Overload-1044Induced Heart Failure. Bone 2005, 23, 1–7, doi:10.1038/jid.2014.371.
- 104529.Kim, H.E.; Dalal, S.S.; Young, E.; Legato, M.J.; Weisfeldt, M.L.; D'Armiento, J. Disruption of the1046myocardial extracellular matrix leads to cardiac dysfunction. J. Clin. Invest. 2000, 106, 857–1047866, doi:10.1172/JCI8040.
- 1048 30. Picard, F.; Brehm, M.; Fassbach, M.; Pelzer, B.; Scheuring, S.; Küry, P.; Strauer, B.E.;
 1049 Schwartzkopff, B. Increased cardiac mRNA expression of matrix metalloproteinase-1 (MMP-1)
 1050 and its inhibitor (TIMP-1) in DCM patients. *Clin. Res. Cardiol.* 2006, doi:10.1007/s00392-0061051 0373-z.
- 1052 31. Rodríguez-Pascual, F.; Díez, J. Myocardial fibrosis in response to pressure overload:
 1053 Elucidating the contribution of tissue transglutaminase. *Cardiovasc. Res.* 2017, *113*, 841–843,
 1054 doi:10.1093/cvr/cvx105.
- 1055 32. Pawelec, K.M.; Best, S.M.; Cameron, R.E. Collagen: A network for regenerative medicine. *J.*1056 *Mater. Chem. B* 2016, *4*, 6484–6496, doi:10.1039/c6tb00807k.
- 105733.Boraschi-Diaz, I.; Wang, J.; Mort, J.S.; Komarova, S. V. Collagen type i as a ligand for receptor-1058mediated signaling. Front. Phys. 2017, doi:10.3389/fphy.2017.00012.
- 105934.Baynes, J. and Dominiczak, M. *Medical Biochemistry*; 2nd ed.; Elsevier Mosby: Edinburgh,10602004;
- 1061 35. Copes, F.; Pien, N.; Van Vlierberghe, S.; Boccafoschi, F.; Mantovani, D. Collagen-based tissue
 1062 engineering strategies for vascular medicine. *Front. Bioeng. Biotechnol.* 2019, 7, 1–15,
 1063 doi:10.3389/fbioe.2019.00166.
- 1064 36. Miranda-Nieves, D.; Chaikof, E.L. Collagen and Elastin Biomaterials for the Fabrication of
 1065 Engineered Living Tissues. ACS Biomater. Sci. Eng. 2017, 3, 694–711,
 1066 doi:10.1021/acsbiomaterials.6b00250.
- 1067 37. Veis, A. The biochemistry of collagen. *Ann. Clin. Lab. Sci.* **1975**, *5*, 123–131.
- Sorushanova, A.; Delgado, L.M.; Wu, Z.; Shologu, N.; Kshirsagar, A.; Raghunath, R.; Mullen,
 A.M.; Bayon, Y.; Pandit, A.; Raghunath, M.; et al. The Collagen Suprafamily: From Biosynthesis
 to Advanced Biomaterial Development. *Adv. Mater.* 2019, *31*, 1–39,
 doi:10.1002/adma.201801651.
- Siegel, R.C. Collagen cross linking. Synthesis of collagen cross links in vitro with highly purified
 lysyl oxidase. J. Biol. Chem. 1976, 251, 5786–5792.
- Monnier, V.M.; Kohn, R.R.; Cerami, A. Accelerated age-related browning of human collagen in
 diabetes mellitus. *Proc. Natl. Acad. Sci. U. S. A.* **1984**, *81*, 583–587,
 doi:10.1073/pnas.81.2.583.
- 1077 41. Griffin, M, Collighan, RJ, Chau, D.& V.E. Transglutaminase Crosslinked Collagen Biomaterial
 1078 for Medical Implant Materials 2006.

- 1079 42. Wise, S.G.; Weiss, A.S. Tropoelastin. *Int. J. Biochem. Cell Biol.* 2009, *41*, 494–497,
 1080 doi:10.1016/j.biocel.2008.03.017.
- 1081 43. Debelle, L.; Tamburro, A.M. Elastin: Molecular description and function. *Int. J. Biochem. Cell*1082 *Biol.* 1999, doi:10.1016/S1357-2725(98)00098-3.
- 1083 44. Bentley, J.P.; Hanson, A.N. The hydroxyproline of elastin. *BBA Protein Struct.* 1969, doi:10.1016/0005-2795(69)90011-7.
- 1085 45. Vrhovski, B.; Jensen, S.; Weiss, A.S. Coacervation characteristics of recombinant human
 1086 tropoelastin. *Eur. J. Biochem.* **1997**, *250*, 92–98, doi:10.1111/j.1432-1033.1997.00092.x.
- Henschen, A.; Lottspeich, F.; Kehl, M.; Southan, C. Covalent Structure of Fibrinogen. *Ann. N. Y. Acad. Sci.* 1983, 408, 28–43, doi:10.1111/j.1749-6632.1983.tb23232.x.
- Mosesson, M.W. Fibrinogen and fibrin structure and functions. *J. Thromb. Haemost.* 2005, 3–
 26, doi:10.1007/978-4-431-78847-8_1.
- 109148.Diane E. Handy Rita Castro Joseph Loscalzo Molecular mechanisms affecting fibrin structure1092and stability Susan. Bone 2011, 23, 1–7, doi:10.1161/CIRCULATIONAHA.110.956839.
- 109349.WHO Cardiovascular Disease- Key Facts Available online: https://www.who.int/news-1094room/fact-sheets/detail/cardiovascular-diseases-(cvds) (accessed on Feb 21, 2020).
- 1095 50. Cannon, B. Cardiovascular disease: Biochemistry to behaviour. *Nature* 2013, doi:10.1038/493S2a.
- 1097 51. Phinikaridou, A.; Andia, M.E.; Lacerda, S.; Lorrio, S.; Makowski, M.R.; Botnar, R.M. Molecular
 1098 MRI of atherosclerosis. *Molecules* 2013, doi:10.3390/molecules181114042.
- 1099 52. Herrington, W.; Lacey, B.; Sherliker, P.; Armitage, J.; Lewington, S. Epidemiology of
 1100 Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease.
 1101 *Circ. Res.* 2016, *118*, 535–546, doi:10.1161/CIRCRESAHA.115.307611.
- 1102 53. Benjamin, E.J.; Virani, S.S.; Callaway, C.W.; Chamberlain, A.M.; Chang, A.R.; Cheng, S.; Chiuve,
 1103 S.E.; Cushman, M.; Delling, F.N.; Deo, R.; et al. Heart disease and stroke statistics-2018
 1104 update: A report from the American Heart Association. *Circulation* 2018, 137, e67--e492,
 1105 doi:10.1161/CIR.0000000000558.
- 1106 54. Chistiakov, D.A.; Sobenin, I.A.; Orekhov, A.N. Vascular extracellular matrix in atherosclerosis.
 1107 *Cardiol. Rev.* 2013, *21*, 270–288, doi:10.1097/CRD.0b013e31828c5ced.
- Hillis, G.S.; Mlynski, R.A.; Simpson, J.G.; MacLeod, A.M. The expression of β1 integrins in human coronary artery. *Basic Res. Cardiol.* **1998**, doi:10.1007/s003950050098.
- 1110 56. Hong, Z.; Reeves, K.J.; Sun, Z.; Li, Z.; Brown, N.J.; Meininger, G.A. Vascular smooth muscle cell
 1111 stiffness and adhesion to collagen I modified by vasoactive agonists. *PLoS One* 2015,
 1112 doi:10.1371/journal.pone.0119533.
- 1113 57. Makowski, M.R.; Wiethoff, A.J.; Blume, U.; Cuello, F.; Warley, A.; Jansen, C.H.P.; Nagel, E.;
 1114 Razavi, R.; Onthank, D.C.; Cesati, R.R.; et al. Assessment of atherosclerotic plaque burden
 1115 with an elastin-specific magnetic resonance contrast agent. *Nat. Med.* 2011, *17*, 383–388,
 1116 doi:10.1038/nm.2310.
- 1117 58. Bary, C. Von; Makowski, M.; Preissel, A.; Keithahn, V.; Warley, A.; Spuentrup, E.; Buecker, A.;
 1118 Lazewatsky, J.; Cesati, R.; Onthank, D.; et al. MRI of coronary wall remodeling in a swine
 119 model of coronary injury using an elastin-binding contrast agent. *Circ. Cardiovasc. Imaging*1120 **2011**, *4*, 147–155, doi:10.1161/CIRCIMAGING.109.895607.

- 1121 59. Rosenfeld, M.E.; Polinsky, P.; Virmani, R.; Kauser, K.; Rubanyi, G.; Schwartz, S.M. Advanced
 1122 atherosclerotic lesions in the innominate artery of the apoE knockout mouse. *Arterioscler*.
 1123 *Thromb. Vasc. Biol.* 2000, doi:10.1161/01.ATV.20.12.2587.
- Bary, C. Von; Makowski, M.; Preissel, A.; Keithahn, V.; Warley, A.; Spuentrup, E.; Buecker, A.;
 Lazewatsky, J.; Cesati, R.; Onthank, D.; et al. MRI of coronary wall remodeling in a swine
 model of coronary injury using an elastin-binding contrast agent. *Circ. Cardiovasc. Imaging*2011, doi:10.1161/CIRCIMAGING.109.895607.
- Phinikaridou, A.; Andia, M.E.; Indermuehle, A.; Onthank, D.C.; Cesati, R.R.; Smith, A.;
 Robinson, S.P.; Saha, P.; Botnar, R.M. Vascular remodeling and plaque vulnerability in a rabbit
 model of atherosclerosis: Comparison of delayed-enhancement MR imaging with an elastinspecific contrast agent and unenhanced black-blood MR imaging. *Radiology* 2014, 271, 390–
 399, doi:10.1148/radiol.13130502.
- Phinikaridou, A.; Lacerda, S.; Lavin, B.; Andia, M.E.; Smith, A.; Saha, P.; Botnar, R.M.
 Tropoelastin: A novel marker for plaque progression and instability. *Circ. Cardiovasc. Imaging* **2018**, *11*, doi:10.1161/CIRCIMAGING.117.007303.
- 113663.Murata, K.; Motayama, T.; Kotake, C. Collagen types in various layers of the human aorta and1137their changes with the atherosclerotic process. Atherosclerosis 1986, 60, 262,1138doi:10.1016/0021-9150(86)90172-3.
- 1139 64. Chung, A.W.Y.; Luo, H.; Tejerina, T.; van Breemen, C.; Okon, E.B. Enhanced cell cycle entry
 and mitogen-activated protein kinase-signaling and downregulation of matrix
 1141 metalloproteinase-1 and -3 in human diabetic arterial vasculature. *Atherosclerosis* 2007,
 1142 doi:10.1016/j.atherosclerosis.2007.01.011.
- 114365.Caravan, P.; Das, B.; Dumas, S.; Epstein, F.H.; Helm, P.A.; Jacques, V.; Koerner, S.; Kolodziej,1144A.; Shen, L.; Sun, W.C.; et al. Collagen-targeted MRI contrast agent for molecular imaging of1145fibrosis. Angew. Chemie Int. Ed. 2007, 46, 8171–8173, doi:10.1002/anie.200700700.
- 1146 66. Chen, W.; Cormode, D.P.; Vengrenyuk, Y.; Herranz, B.; Feig, J.E.; Klink, A.; Mulder, W.J.M.;
 1147 Fisher, E.A.; Fayad, Z.A. Collagen-Specific Peptide Conjugated HDL Nanoparticles as MRI
 1148 Contrast Agent to Evaluate Compositional Changes in Atherosclerotic Plaque Regression.
 1149 JACC Cardiovasc. Imaging **2013**, *6*, 373–384, doi:10.1016/j.jcmg.2012.06.016.
- Patti, J.M.; Boles, J.O.; Höök, M. Identification and Biochemical Characterization of the Ligand
 Binding Domain of the Collagen Adhesin from Staphylococcus aureus. *Biochemistry* 1993, *32*,
 11428–11435, doi:10.1021/bi00093a021.
- 1153 68. Xu, Y.; Rivas, J.M.; Brown, E.L.; Liang, X.; Höök, M. Virulence Potential of the Staphylococcal
 1154 Adhesin CNA in Experimental Arthritis Is Determined by Its Affinity for Collagen. *J. Infect. Dis.*1155 2004, 189, 2323, doi:10.1086/420851.
- Sanders, H.M.H.F.; Strijkers, G.J.; Mulder, W.J.M.; Huinink, H.P.; Erich, S.J.F.; Adan, O.C.G.;
 Sommerdijk, N.A.J.M.; Merkx, M.; Nicolay, K. Morphology, binding behavior and MRproperties of paramagnetic collagen-binding liposomes. *Contrast Media Mol. Imaging* 2009,
 4, 81–88, doi:10.1002/cmmi.266.
- 116070.Megens, R.T.A.; Oude Egbrink, M.G.A.; Cleutjens, J.P.M.; Kuijpers, M.J.E.; Schiffers, P.H.M.;1161Merkx, M.; Slaaf, D.W.; Van Zandvoort, M.A.M.J. Imaging collagen in intact viable healthy and1162atherosclerotic arteries using fluorescently labeled CNA35 and two-photon laser scanning1163microscopy. Mol. Imaging 2007, 6, 246–260, doi:10.2310/7290.2007.00021.
- 1164 71. Schulz, C.; Penz, S.; Hoffmann, C.; Langer, H.; Gillitzer, A.; Schneider, S.; Brandl, R.; Seidl, S.;

- Massberg, S.; Pichler, B.; et al. Platelet GPVI binds to collagenous structures in the core region
 of human atheromatous plaque and is critical for atheroprogression in vivo. *Basic Res. Cardiol.* 2008, *103*, 356–367, doi:10.1007/s00395-008-0722-3.
- 1168 72. Nörenberg, D.; Ebersberger, H.U.; Diederichs, G.; Hamm, B.; Botnar, R.M.; Makowski, M.R.
 1169 Molecular magnetic resonance imaging of atherosclerotic vessel wall disease. *Eur. Radiol.*1170 **2016**, *26*, 910–920, doi:10.1007/s00330-015-3881-2.
- 1171 73. Tavora, F.; Cresswell, N.; Li, L.; Ripple, M.; Burke, A. Immunolocalisation of fibrin in coronary
 1172 atherosclerosis: implications for necrotic core development. *Pathology* 2010, *42*, 15–22,
 1173 doi:10.3109/00313020903434348.
- 117474.Yu, X.; Song, S.K.; Chen, J.; Scott, M.J.; Fuhrhop, R.J.; Hall, C.S.; Gaffney, P.J.; Wickline, S.A.;1175Lanza, G.M. High-resolution MRI characterization of human thrombus using a novel fibrin-1176targeted paramagnetic nanoparticle contrast agent. *Magn. Reson. Med.* 2000, 44, 867–872,1177doi:10.1002/1522-2594(200012)44:6<867::aid-mrm7>3.0.co;2-p.
- Flacke, S.; Fischer, S.; Scott, M.J.; Fuhrhop, R.J.; Allen, J.S.; McLean, M.; Winter, P.; Sicard,
 G.A.; Gaffney, P.J.; Wickline, S.A.; et al. Novel MRI contrast agent for molecular imaging of
 fibrin implications for detecting vulnerable plaques. *Circulation* 2001, *104*, 1280–1285,
 doi:10.1161/hc3601.094303.
- 118276.Sirol, M.; Aguinaldo, J.G.S.; Graham, P.B.; Weisskoff, R.; Lauffer, R.; Mizsei, G.; Chereshnev, I.;1183Fallon, J.T.; Reis, E.; Fuster, V.; et al. Fibrin-targeted contrast agent for improvement of in vivo1184acute thrombus detection with magnetic resonance imaging. *Atherosclerosis* 2005, 182, 79–118585, doi:10.1016/j.atherosclerosis.2005.02.013.
- 1186 77. Botnar, R.M.; Perez, A.S.; Witte, S.; Wiethoff, A.J.; Laredo, J.; Hamilton, J.; Quist, W.; Parsons,
 1187 E.C.; Vaidya, A.; Kolodziej, A.; et al. In Vivo Molecular Imaging of Acute and Subacute
 1188 Thrombosis Using a Fibrin-Binding Magnetic Resonance Imaging Contrast Agent. *Circulation*1189 2004, 109, 2023–2029, doi:10.1161/01.CIR.0000127034.50006.C0.
- 1190 78. Botnar, R.M.; Buecker, A.; Wiethoff, A.J.; Parsons, E.C.; Katoh, M.; Katsimaglis, G.; Weisskoff,
 1191 R.M.; Lauffer, R.B.; Graham, P.B.; Gunther, R.W.; et al. In Vivo Magnetic Resonance Imaging
 1192 of Coronary Thrombosis Using a Fibrin-Binding Molecular Magnetic Resonance Contrast
 1193 Agent. *Circulation* 2004, *110*, 1463–1466, doi:10.1161/01.CIR.0000134960.31304.87.
- 1194 79. Spuentrup, E.; Botnar, R.M.; Wiethoff, A.J.; Ibrahim, T.; Kelle, S.; Katoh, M.; Özgun, M.; Nagel,
 1195 E.; Vymazal, J.; Graham, P.B.; et al. MR imaging of thrombi using EP-2104R, a fibrin-specific
 1196 contrast agent: initial results in patients. *Eur. Radiol.* 2008, *18*, 1995–2005,
 1197 doi:10.1007/s00330-008-0965-2.
- 119880.Vymazal, J.; Spuentrup, E.; Cardenas-Molina, G.; Wiethoff, A.J.; Hartmann, M.G.; Caravan, P.;1199Parsons, E.C. Thrombus imaging with fibrin-specific gadolinium-based MR Contrast Agent EP-12002104R Results of a Phase II Clinical Study of Feasibility. Invest. Radiol. 2009,1201doi:10.1097/RLI.0b013e3181b092a7.
- 1202 81. Matrisian, L.M. The matrix-degrading metalloproteinases. *BioEssays* 1992, *14*, 455–463, doi:10.1002/bies.950140705.
- 120482.Galis, Z.S.; Khatri, J.J. Matrix metalloproteinases in vascular remodeling and atherogenesis:1205The good, the bad, and the ugly. *Circ. Res.* **2002**, *90*, 251–262, doi:10.1161/res.90.3.251.
- 1206 83. Lancelot, E.; Amirbekian, V.; Brigger, I.; Raynaud, J.-S.; Ballet, S.; David, C.; Rousseaux, O.; Le
 1207 Greneur, S.; Port, M.; Lijnen, H.R.; et al. Evaluation of Matrix Metalloproteinases in
 1208 Atherosclerosis Using a Novel Noninvasive Imaging Approach. *Arterioscler. Thromb. Vasc.*

- 1209 *Biol.* **2008**, *28*, 425–432, doi:10.1161/ATVBAHA.107.149666.
- 1210 84. Hyafil, F.; Vucic, E.; Cornily, J.-C.; Sharma, R.; Amirbekian, V.; Blackwell, F.; Lancelot, E.; Corot,
 1211 C.; Fuster, V.; Galis, Z.S.; et al. Monitoring of arterial wall remodelling in atherosclerotic
 1212 rabbits with a magnetic resonance imaging contrast agent binding to matrix
 1213 metalloproteinases. *Eur. Heart J.* 2011, *32*, 1561–1571, doi:10.1093/eurheartj/ehq413.
- Hua, N.; Baik, F.; Pham, T.; Phinikaridou, A.; Giordano, N.; Friedman, B.; Whitney, M.; Nguyen,
 Q.T.; Tsien, R.Y.; Hamilton, J.A. Identification of high-risk plaques by MRI and fluorescence
 imaging in a rabbit model of atherothrombosis. *PLoS One* 2015, *10*, e0139833,
 doi:10.1371/journal.pone.0139833.
- 1218 86. Kuge, Y.; Takai, N.; Ogawa, Y.; Temma, T.; Zhao, Y.; Nishigori, K.; Ishino, S.; Kamihashi, J.;
 1219 Kiyono, Y.; Shiomi, M.; et al. Imaging with radiolabelled anti-membrane type 1 matrix
 1220 metalloproteinase (MT1-MMP) antibody: Potentials for characterizing atherosclerotic
 1221 plaques. *Eur. J. Nucl. Med. Mol. Imaging* 2010, *37*, 2093–2104, doi:10.1007/s00259-0101222 1521-2.
- Fujimoto, S.; Hartung, D.; Ohshima, S.; Edwards, D.S.; Zhou, J.; Yalamanchili, P.; Azure, M.;
 Fujimoto, A.; Isobe, S.; Matsumoto, Y.; et al. Molecular Imaging of Matrix Metalloproteinase
 in Atherosclerotic Lesions. Resolution With Dietary Modification and Statin Therapy. J. Am. *Coll. Cardiol.* 2008, *52*, 1847–1857, doi:10.1016/j.jacc.2008.08.048.
- 1227 88. Zhang, J.; Nie, L.; Razavian, M.; Ahmed, M.; Dobrucki, L.W.; Asadi, A.; Edwards, D.S.; Azure,
 1228 M.; Sinusas, A.J.; Sadeghi, M. Molecular imaging of activated matrix metalloproteinases in
 1229 vascular remodeling. *Circulation* **2008**, *118*, doi:10.1161/CIRCULATIONAHA.108.789743.
- 1230 89. Mosterd, A.; Hoes, A.W. Clinical epidemiology of heart failure. *Heart* 2007, *93*, 1137–1146,
 1231 doi:10.1136/hrt.2003.025270.
- 1232 90. Redfield, M.M.; Jacobsen, S.J.; Burnett, J.C.; Mahoney, D.W.; Bailey, K.R.; Rodeheffer, R.J.
 1233 Burden of systolic and diastolic ventricular dysfunction in the community: Appreciating the
 1234 scope of the heart failure epidemic. *J. Am. Med. Assoc.* 2003, *289*, 194–202,
 1235 doi:10.1001/jama.289.2.194.
- 1236 91. Bleumink, G.S.; Knetsch, A.M.; Sturkenboom, M.C.J.M.; Straus, S.M.J.M.; Hofman, A.; Deckers,
 1237 J.W.; Witteman, J.C.M.; Stricker, B.H.C. Quantifying the heart failure epidemic: Prevalence,
 1238 incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. *Eur. Heart J.*1239 2004, 25, 1614–1619, doi:10.1016/j.ehj.2004.06.038.
- Ponikowski, P.; Voors, A.A.; Anker, S.D.; Bueno, H.; Cleland, J.G.F.; Coats, A.J.S.; Falk, V.;
 González-Juanatey, J.R.; Harjola, V.P.; Jankowska, E.A.; et al. 2016 ESC Guidelines for the
 diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis
 and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC).
 Developed with the special contribution. *Eur. J. Heart Fail.* 2016, *37*, 2129–2200,
 doi:10.1002/ejhf.592.
- Bashey, R.I.; Martinez-Hernandez, A.; Jimenez, S.A. Isolation, characterization, and
 localization of cardiac collagen type VI: Associations with other extracellular matrix
 components. *Circ. Res.* 1992, *70*, 1006–1017, doi:10.1161/01.res.70.5.1006.
- 1249 94. Frangogiannis, N.G. The extracellular matrix in myocardial injury, repair, and remodeling. *J.*1250 *Clin. Invest.* 2017, 127, 1600–1612, doi:10.1172/JCI87491.
- 125195.Dobaczewski, M.; Gonzalez-Quesada, C.; Frangogiannis, N.G. The extracellular matrix as a1252modulator of the inflammatory and reparative response following myocardial infarction. J.

- 1253 *Mol. Cell. Cardiol.* **2010**, *48*, 504–511, doi:10.1016/j.yjmcc.2009.07.015.
- Simonetti, O.P.; Finn, J.P.; White, R.D.; Laub, G.; Henry, D.A. "Black blood" T2-weighted
 inversion-recovery MR imaging of the heart. *Radiology* 1996, 199, 49–57,
 doi:10.1148/radiology.199.1.8633172.
- 1257 97. Verhaert, D.; Thavendiranathan, P.; Giri, S.; Mihai, G.; Rajagopalan, S.; Simonetti, O.P.;
 1258 Raman, S. V Direct T2 quantification of myocardial edema in acute ischemic injury. *JACC*1259 *Cardiovasc. Imaging* 2011, *4*, 269–278, doi:10.1016/j.jcmg.2010.09.023.
- Bustin, A.; Milotta, G.; Ismail, T.F.; Neji, R.; Botnar, R.M.; Prieto, C. Accelerated free-breathing
 whole-heart 3D T2 mapping with high isotropic resolution. *Magn. Reson. Med.* 2020, *83*, 988–
 1002, doi:10.1002/mrm.27989.
- 126399.Bull, S.; White, S.K.; Piechnik, S.K.; Flett, A.S.; Ferreira, V.M.; Loudon, M.; Francis, J.M.;1264Karamitsos, T.D.; Prendergast, B.D.; Robson, M.D.; et al. Human non-contrast T1 values and1265correlation with histology in diffuse fibrosis. *Heart* **2013**, *99*, 932–937, doi:10.1136/heartjnl-12662012-303052.
- 1267 100. Ugander, M.; Bagi, P.S.; Oki, A.J.; Chen, B.; Hsu, L.Y.; Aletras, A.H.; Shah, S.; Greiser, A.;
 1268 Kellman, P.; Arai, A.E. Myocardial edema as detected by pre-contrast T1 and T2 CMR
 1269 delineates area at risk associated with acute myocardial infarction. *JACC Cardiovasc. Imaging*1270 **2012**, *5*, 596–603, doi:10.1016/j.jcmg.2012.01.016.
- 1271 101. Sado, D.M.; White, S.K.; Piechnik, S.K.; Banypersad, S.M.; Treibel, T.; Captur, G.; Fontana, M.;
 1272 Maestrini, V.; Flett, A.S.; Robson, M.D.; et al. Identification and assessment of anderson-fabry
 1273 disease by cardiovascular magnetic resonance noncontrast myocardial T1 mapping. *Circ.*1274 *Cardiovasc. Imaging* 2013, *6*, 392–398, doi:10.1161/CIRCIMAGING.112.000070.
- 1275 102. Sado, D.M.; Maestrini, V.; Piechnik, S.K.; Banypersad, S.M.; White, S.K.; Flett, A.S.; Robson,
 1276 M.D.; Neubauer, S.; Ariti, C.; Arai, A.; et al. Noncontrast myocardial T1 mapping using
 1277 cardiovascular magnetic resonance for iron overload. *J. Magn. Reson. Imaging* 2015, *41*,
 1278 1505–1511, doi:10.1002/jmri.24727.
- 1279 103. Miller, C.A.; Naish, J.H.; Bishop, P.; Coutts, G.; Clark, D.; Zhao, S.; Ray, S.G.; Yonan, N.;
 1280 Williams, S.G.; Flett, A.S.; et al. Comprehensive validation of cardiovascular magnetic
 1281 resonance techniques for the assessment of myocardial extracellular volume. *Circ.*1282 *Cardiovasc. Imaging* 2013, *6*, 373–383, doi:10.1161/CIRCIMAGING.112.000192.
- 104. Kim, R.J.; Wu, E.; Rafael, A.; Chen, E.-L.; Parker, M.A.; Simonetti, O.; Klocke, F.J.; Bonow, R.O.;
 1284 Judd, R.M. The use of contrast-enhanced magnetic resonance imaging to identify reversible
 1285 myocardial dysfunction. *N. Engl. J. Med.* **2000**, *343*, 1445–1453,
 1286 doi:10.1056/NEJM200011163432003.
- Hammer-Hansen, S.; Leung, S.W.; Hsu, L.Y.; Wilson, J.R.; Taylor, J.; Greve, A.M.; Thune, J.J.;
 Køber, L.; Kellman, P.; Arai, A.E. Early Gadolinium Enhancement for Determination of Area at
 Risk: A Preclinical Validation Study. *JACC Cardiovasc. Imaging* 2017, *10*, 130–139,
 doi:10.1016/j.jcmg.2016.04.009.
- 1291 106. Matsumoto, H.; Matsuda, T.; Miyamoto, K.; Shimada, T.; Mikuri, M.; Hiraoka, Y. Peri-infarct
 1292 zone on early contrast-enhanced CMR imaging in patients with acute myocardial infarction.
 1293 JACC Cardiovasc. Imaging 2011, 4, 610–618, doi:10.1016/j.jcmg.2011.03.015.
- 107. Matsumoto, H.; Matsuda, T.; Miyamoto, K.; Shimada, T.; Ushimaru, S.; Mikuri, M.; Yamazaki,
 T. Temporal change of enhancement after gadolinium injection on contrast-enhanced CMR in
 reperfused acute myocardial infarction. *J. Cardiol.* 2015, 65, 76–81,

- doi:10.1016/j.jjcc.2014.04.005.
- 1298 108. Helm, P.A.; Caravan, P.; French, B.A.; Jacques, V.; Shen, L.; Xu, Y.; Beyers, R.J.; Roy, R.J.;
 1299 Kramer, C.M.; Epstein, F.H. Postinfarction myocardial scarring in mice: Molecular MR imaging
 1300 with use of a collagen-targeting contrast agent. *Radiology* 2008, 247, 788–796,
 1301 doi:10.1148/radiol.2473070975.
- 109. Wildgruber, M.; Bielicki, I.; Aichler, M.; Kosanke, K.; Feuchtinger, A.; Settles, M.; Onthank,
 D.C.; Cesati, R.R.; Robinson, S.P.; Huber, A.M.; et al. Assessment of myocardial infarction and
 postinfarction scar remodeling with an elastin-specific magnetic resonance agent. *Circ. Cardiovasc. Imaging* 2014, 7, 321–329, doi:10.1161/CIRCIMAGING.113.001270.
- 1306 110. Protti, A.; Lavin, B.; Dong, X.; Lorrio, S.; Robinson, S.; Onthank, D.; Shah, A.M.; Botnar, R.M.
 1307 Assessment of Myocardial Remodeling Using an Elastin/Tropoelastin Specific Agent with High
 1308 Field Magnetic Resonance Imaging (MRI). J. Am. Heart Assoc. 2015, 4, e001851,
 1309 doi:10.1161/JAHA.115.001851.
- 1310 111. Ramos, I.T.; Henningsson, M.; Nezafat, M.; Lavin, B.; Lorrio, S.; Gebhardt, P.; Protti, A.; Eykyn,
 1311 T.R.; Andia, M.E.; Flögel, U.; et al. Simultaneous Assessment of Cardiac Inflammation and
 1312 Extracellular Matrix Remodeling after Myocardial Infarction. *Circ. Cardiovasc. Imaging* 2018,
 1313 11, e007453, doi:10.1161/CIRCIMAGING.117.007453.
- 1314 112. Su, H.; Spinale, F.G.; Dobrucki, L.W.; Song, J.; Hua, J.; Sweterlitsch, S.; Dione, D.P.; Cavaliere,
 1315 P.; Chow, C.; Bourke, B.N.; et al. Noninvasive targeted imaging of matrix metalloproteinase
 1316 activation in a murine model of postinfarction remodeling. *Circulation* 2005, *112*, 3157–3167,
 1317 doi:10.1161/CIRCULATIONAHA.105.583021.
- 1318 113. Abas Osman, A.; Ju, W.; Sun, D.; Qi, B. Deep venous thrombosis: a literature review. Int J Clin
 1319 Exp Med 2018, 11, 1551–1561.
- 1320 114. Silverstein, M.D.; Heit, J.A.; Mohr, D.N.; Petterson, T.M.; O'Fallon, W.M.; Melton, L.J. Trends
 1321 in the Incidence of Deep Vein Thrombosis and Pulmonary Embolism. *Arch. Intern. Med.* 1998,
 1322 158, 585, doi:10.1001/archinte.158.6.585.
- 1323 115. Beckman, M.G.; Hooper, W.C.; Critchley, S.E.; Ortel, T.L. Venous Thromboembolism. *Am. J.* 1324 *Prev. Med.* 2010, *38*, S495--S501, doi:10.1016/j.amepre.2009.12.017.
- 1325 116. Bates, S.M. and J.S.G. Clinical practice. Treatment of deep-vein thrombosis. *N. Engl. J. Med.*1326 2004, 268–77.
- 1327 117. Kahn, S.R., A. Hirsch, and I.S. Effect of postthrombotic syndrome on health-related quality of 1328 life after deep venous thrombosis. *Arch Intern Med* **2002**, 1144–8.
- 1329 118. Barco, S.; Woersching, A.L.; Spyropoulos, A.C.; Piovella, F.; Mahan, C.E. European Union-28:
 1330 An annualised cost-of-illness model for venous thromboembolism. *Thromb. Haemost.* 2016,
 1331 doi:10.1160/TH15-08-0670.
- 1332 119. Stone, J.; Hangge, P.; Albadawi, H.; Wallace, A.; Shamoun, F.; Knuttien, M.G.; Naidu, S.; Oklu,
 1333 R. Deep vein thrombosis: Pathogenesis, diagnosis, and medical management. *Cardiovasc.*1334 *Diagn. Ther.* 2017, 7, S276–S284, doi:10.21037/cdt.2017.09.01.
- 1335 120. Kesieme; Kesieme Deep vein thrombosis: a clinical review. J. Blood Med. 2011, 59, doi:10.2147/jbm.s19009.
- 1337 121. Kahn, S.R.; Ginsberg, J.S. Relationship between Deep Venous Thrombosis and the
 1338 Postthrombotic Syndrome. *Arch. Intern. Med.* 2004, doi:10.1001/archinte.164.1.17.
- 1339 122. Raju, S.; Neglén, P. Chronic venous insufficiency and varicose veins. N. Engl. J. Med. 2009,

- 1340 doi:10.1056/NEJMcp0802444.
- Hoch, R.C.; Schraufstätter, I.U.; Cochrane, C.G. In vivo, in vitro, and molecular aspects of
 interleukin-8 and the interleukin-8 receptors. *J. Lab. Clin. Med.* **1996**, *128*, 134–145,
 doi:10.1016/S0022-2143(96)90005-0.
- 124. Zgheib, C.; Xu, J.; Liechty, K.W. Targeting Inflammatory Cytokines and Extracellular Matrix
 1345 Composition to Promote Wound Regeneration. *Adv. Wound Care* 2014, *3*, 344–355,
 1346 doi:10.1089/wound.2013.0456.
- 1347 125. Olczyk, P.; Mencner, Ł.; Komosinska-Vassev, K. The role of the extracellular matrix
 1348 components in cutaneous wound healing. *Biomed Res. Int.* 2014, 2014, 12–14,
 1349 doi:10.1155/2014/747584.
- 126. Deatrick, K.B.; Eliason, J.L.; Lynch, E.M.; Moore, A.J.; Dewyer, N.A.; Varma, M.R.; Pearce, C.G.;
 1351 Upchurch, G.R.; Wakefield, T.W.; Henke, P.K. Vein wall remodeling after deep vein
 1352 thrombosis involves matrix metalloproteinases and late fibrosis in a mouse model. *J. Vasc.*1353 Surg. 2005, 42, 140–148, doi:10.1016/j.jvs.2005.04.014.
- 127. Lee, Y.U.; Lee, A.Y.; Humphrey, J.D.; Rausch, M.K. Histological and biomechanical changes in a
 mouse model of venous thrombus remodeling. *Biorheology* 2015, *52*, 235–245,
 doi:10.3233/BIR-15058.
- 128. Mukhopadhyay, S.; Johnson, T.A.; Duru, N.; Buzza, M.S.; Pawar, N.R.; Sarkar, R.; Antalis, T.M.
 Fibrinolysis and inflammation in venous thrombus resolution. *Front. Immunol.* 2019, *10*, 1–
 14, doi:10.3389/fimmu.2019.01348.
- 129. Kearon, C.; Kahn, S.R.; Agnelli, G.; Goldhaber, S.; Raskob, G.E.; Comerota, A.J. Antithrombotic
 therapy for venous thromboembolic disease: American College of Chest Physicians evidencebased clinical practice guidelines (8th edition). *Chest* 2008, doi:10.1378/chest.08-0658.
- 1363 130. Comerota, A.J. The ATTRACT trial: Rationale for early intervention for iliofemoral DVT.
 1364 *Perspect. Vasc. Surg. Endovasc. Ther.* 2009, doi:10.1177/1531003509359311.
- 1365
 131. Enden, T.; Sandvik, L.; Kløw, N.E.; Hafsahl, G.; Holme, P.A.; Holmen, L.O.; Ghanima, W.;
 1366
 1367 Njaastad, A.M.; Sandbæk, G.; Slagsvold, C.E.; et al. Catheter-directed Venous Thrombolysis in
 1367 acute iliofemoral vein thrombosis-the CaVenT Study: Rationale and design of a multicenter,
 1368 randomized, controlled, clinical trial (NCT00251771). *Am. Heart J.* 2007,
 1369 doi:10.1016/j.ahj.2007.07.010.
- 1370 132. Cesarman-Maus, G.; Hajjar, K.A. Molecular mechanisms of fibrinolysis. *Br. J. Haematol.* 2005, doi:10.1111/j.1365-2141.2005.05444.x.
- 1372 133. Mirshahi, M.; Soria, J.; Lu, H.; Soria, C.; Samama, M.; Caen, J.P. Defective thrombolysis due to
 1373 collagen incorporation in fibrin clots. *Thromb. Res.* **1988**, doi:10.1016/S0049-3848(88)800091374 4.
- 1375 134. Moody, A.R. Direct imaging of deep-vein thrombosis with magnetic resonance imaging.
 1376 Lancet (London, England) 1997, 350, 1073, doi:10.1016/s0140-6736(97)24041-9.
- 1377 135. Saha, P.; Andia, M.E.; Modarai, B.; Blume, U.; Humphries, J.; Patel, A.S.; Phinikaridou, A.;
 1378 Evans, C.E.; Mattock, K.; Grover, S.P.; et al. Magnetic Resonance T ₁Relaxation Time of Venous
 1379 Thrombus Is Determined by Iron Processing and Predicts Susceptibility to Lysis. *Circulation* 1380 **2013**, *128*, 729–736, doi:10.1161/CIRCULATIONAHA.113.001371.
- 1381 136. Phinikaridou, A.; Andia, M.E.; Saha, P.; Modarai, B.; Smith, A.; Botnar, R.M. In vivo
 1382 magnetization transfer and diffusion-weighted magnetic resonance imaging detects

- 1383thrombus composition in a mouse model of deep vein thrombosis. Circ. Cardiovasc. Imaging1384**2013**, 6, 433–440, doi:10.1161/CIRCIMAGING.112.000077.
- 1385 137. Wu, G.; Morelli, J.; Xiong, Y.; Liu, X.; Li, X. Diffusion weighted cardiovascular magnetic
 1386 resonance imaging for discriminating acute from non-acute deep venous Thrombus. *J.*1387 *Cardiovasc. Magn. Reson.* 2019, *21*, 667, doi:10.1186/s12968-019-0552-5.
- 1388 138. Chen, H.; He, X.; Xie, G.; Liang, J.; Ye, Y.; Deng, W.; He, Z.; Liu, D.; Liu, X.; et al.
 1389 Cardiovascular magnetic resonance black-blood thrombus imaging for the diagnosis of acute
 1390 deep vein thrombosis at 1.5 Tesla. *J. Cardiovasc. Magn. Reson.* 2018, 20, 1556,
 1391 doi:10.1186/s12968-018-0459-6.
- 1392 139. Andia, M.E.; Saha, P.; Jenkins, J.; Modarai, B.; Wiethoff, A.J.; Phinikaridou, A.; Grover, S.P.;
 1393 Patel, A.S.; Schaeffter, T.; Smith, A.; et al. Fibrin-Targeted Magnetic Resonance Imaging
 1394 Allows In Vivo Quantification of Thrombus Fibrin Content and Identifies Thrombi Amenable
 1395 for Thrombolysis. *Arterioscler. Thromb. Vasc. Biol.* 2014, *34*, 1193–1198,
 1396 doi:10.1161/ATVBAHA.113.302931.
- 140. Spuentrup, E.; Katoh, M.; Buecker, A.; Fausten, B.; Wiethoff, A.J.; Wildberger, J.E.; Haage, P.;
 Parsons, E.C.; Botnar, R.M.; Graham, P.B.; et al. Molecular MR Imaging of Human Thrombi in
 a Swine Model of Pulmonary Embolism Using a Fibrin-Specific Contrast Agent. *Invest. Radiol.*2007, 42, 586–595, doi:10.1097/RLI.0b013e31804fa154.
- 1401 141. Hara, T.; Bhayana, B.; Thompson, B.; Kessinger, C.W.; Khatri, A.; McCarthy, J.R.; Weissleder,
 1402 R.; Lin, C.P.; Tearney, G.J.; Jaffer, F.A. Molecular imaging of fibrin deposition in deep vein
 1403 thrombosis using fibrin-targeted near-infrared fluorescence. *JACC Cardiovasc. Imaging* 2012,
 1404 5, 607–615, doi:10.1016/j.jcmg.2012.01.017.
- 142. Blasi, F.; Oliveira, B.L.; Rietz, T.A.; Rotile, N.J.; Naha, P.C.; Cormode, D.P.; Izquierdo-Garcia, D.;
 1406 Catana, C.; Caravan, P. Multisite Thrombus Imaging and Fibrin Content Estimation With a
 1407 Single Whole-Body PET Scan in Rats. *Arterioscler. Thromb. Vasc. Biol.* 2015, *35*, 2114–2121,
 1408 doi:10.1161/ATVBAHA.115.306055.
- 143. Lloyd-Jones, D.; Adams, R.J.; Brown, T.M.; Carnethon, M.; Dai, S.; De Simone, G.; Ferguson,
 1410 T.B.; Ford, E.; Furie, K.; Gillespie, C.; et al. Heart Disease and Stroke Statistics—2010 Update.
 1411 *Circulation* 2010, *121*, doi:10.1161/CIRCULATIONAHA.109.192667.
- 1412144.NICE NATIONAL INSTITUTE FOR HEALTH AND CLINICAL EXCELLENCE Overview Endovascular1413stents for abdominal aortic aneurysms; 2008;
- 1414 145. Sakalihasan, N.; Limet, R.; Defawe, O.D. Abdominal aortic aneurysm. *Lancet* 2005, 1577–
 1415 1589, doi:10.1016/S0140-6736(05)66459-8.
- 1416 146. Alcorn, H.G.; Wolfson, S.K.; Sutton-Tyrrell, K.; Kuller, L.H.; O'Leary, D. Risk factors for
 1417 abdominal aortic aneurysms in older adults enrolled in the Cardiovascular Health Study.
 1418 Arterioscler. Thromb. Vasc. Biol. 1996, 16, 963–970, doi:10.1161/01.ATV.16.8.963.
- 1419 147. Nevitt, M.P.; Ballard, D.J.; Hallett, J.W. Prognosis of Abdominal Aortic Aneurysms. *N. Engl. J.* 1420 *Med.* 1989, *321*, 1009–1014, doi:10.1056/NEJM198910123211504.
- 1421148.Hong, H.; Yang, Y.; Liu, B.; Cai, W. Imaging of Abdominal Aortic Aneurysm: the present and1422the future. *Curr. Vasc. Pharmacol.* **2010**, *8*, 808–819, doi:10.2174/157016110793563898.
- 1423149.Brangsch, J.; Reimann, C.; Collettini, F.; Buchert, R.; Botnar, R.M.; Makowski, M.R. Molecular1424Imaging of Abdominal Aortic Aneurysms. *Trends Mol. Med.* 2017, 150–164.
- 1425 150. Botnar, R.M.; Brangsch, J.; Reimann, C.; Janssen, C.H.P.; Razavi, R.; Hamm, B.; Makowski, M.R.

- 1426 In Vivo Molecular Characterization of Abdominal Aortic Aneurysms Using Fibrin-Specific
 1427 Magnetic Resonance Imaging. J. Am. Heart Assoc. 2018, 7, e007909,
 1428 doi:10.1161/JAHA.117.007909.
- 1429 151. Erbel, R.; Alfonso, F.; Boileau, C.; Dirsch, O.; Eber, B.; Haverich, A.; Rakowski, H.; Struyven, J.;
 1430 Radegran, K.; Sechtem, U.; et al. Diagnosis and management of aortic dissection:
 1431 Recommendations of the Task Force on Aortic Dissection, European Society of Cardiology.
 1432 *Eur. Heart J.* 2001, doi:10.1053/euhj.2001.2782.
- 1433 152. Kadoglou, N.P.; Liapis, C.D. Matrix metalloproteinases: Contribution to pathogenesis,
 1434 diagnosis, surveillance and treatment of abdominal aortic aneurysms. *Curr. Med. Res. Opin.*1435 2004, doi:10.1185/030079904125003143.
- 1436 153. Sosa, S.E.Y.; Flores-Pliego, A.; Espejel-Nuñez, A.; Medina-Bastidas, D.; Vadillo-Ortega, F.; Zaga1437 Clavellina, V.; Estrada-Gutierrez, G. New insights into the role of matrix metalloproteinases in
 1438 preeclampsia. *Int. J. Mol. Sci.* 2017, doi:10.3390/ijms18071448.
- 1439
 154.
 Thompson, R.W.; Parks, W.C. Role of matrix metalloproteinases in abdominal aortic

 1440
 aneurysms. Ann. N. Y. Acad. Sci. **1996**, 800, 157–174, doi:10.1111/j.1749

 1441
 6632.1996.tb33307.x.
- 1442 155. Hellenthal, F.A.M.V.I.; Buurman, W.A.; Wodzig, W.K.W.H.; Schurink, G.W.H. Biomarkers of
 1443 AAA progression. Part 1: extracellular matrix degeneration. *Nat. Rev. Cardiol.* 2009, *6*, 464–
 1444 474, doi:10.1038/nrcardio.2009.80.
- 1445 156. Krettek, A.; Sukhova, G.K.; Libby, P. Elastogenesis in human arterial disease: a role for
 1446 macrophages in disordered elastin synthesis. *Arterioscler. Thromb. Vasc. Biol.* 2003, *23*, 582–
 1447 587, doi:10.1161/01.ATV.0000064372.78561.A5.
- 1448 157. Botnar, R.M.; Wiethoff, A.J.; Ebersberger, U.; Lacerda, S.; Blume, U.; Warley, A.; Jansen,
 1449 C.H.P.; Onthank, D.C.; Cesati, R.R.; Razavi, R.; et al. In vivo assessment of aortic aneurysm wall
 1450 integrity using elastin-specific molecular magnetic resonance imaging. *Circ. Cardiovasc.*1451 *Imaging* 2014, 7, 679–689, doi:10.1161/CIRCIMAGING.113.001131.
- 1452 158. Okamura, H.; Pisani, L.J.; Dalal, A.R.; Emrich, F.; Dake, B.A.; Arakawa, M.; Onthank, D.C.;
 1453 Cesati, R.R.; Robinson, S.P.; Milanesi, M.; et al. Assessment of elastin deficit in a Marfan
 1454 mouse aneurysm model using an elastin-specific magnetic resonance imaging contrast agent.
 1455 *Circ. Cardiovasc. Imaging* 2014, 7, 690–696, doi:10.1161/CIRCIMAGING.114.001658.
- 1456 159. Lavin, B.; Lacerda, S.; Andia, M.E.; Lorrio, S.; Bakewell, R.; Smith, A.; Rashid, I.; Botnar, R.M.;
 1457 Phinikaridou, A. Tropoelastin: an in vivo imaging marker of dysfunctional matrix turnover
 1458 during abdominal aortic dilation. *Cardiovasc. Res.* 2019, doi:10.1093/cvr/cvz178.
- 1459
 160. Shimizu, K.; Mitchell, R.N.; Libby, P. Inflammation and cellular immune responses in abdominal aortic aneurysms. *Arterioscler. Thromb. Vasc. Biol.* 2006, *26*, 987–994, doi:10.1161/01.ATV.0000214999.12921.4f.
- 1462 161. Abdul-Hussien, H.; Soekhoe, R.G. V; Weber, E.; von der Thüsen, J.H.; Kleemann, R.; Mulder,
 1463 A.; van Bockel, J.H.; Hanemaaijer, R.; Lindeman, J.H.N. Collagen degradation in the abdominal
 1464 aneurysm: a conspiracy of matrix metalloproteinase and cysteine collagenases. *Am. J. Pathol.*1465 2007, *170*, 809–817, doi:10.2353/ajpath.2007.060522.
- 1466 162. Klink, A.; Heynens, J.; Herranz, B.; Lobatto, M.E.; Arias, T.; Sanders, H.M.H.F.; Strijkers, G.J.;
 1467 Merkx, M.; Nicolay, K.; Fuster, V.; et al. In Vivo Characterization of a New Abdominal Aortic
 1468 Aneurysm Mouse Model With Conventional and Molecular Magnetic Resonance Imaging. J.
 1469 Am. Coll. Cardiol. 2011, 58, 2522–2530, doi:10.1016/j.jacc.2011.09.017.

- 1470 163. Satta, J.; Juvonen, T.; Haukipuro, K.; Juvonen, M.; Kairaluoma, M.I. Increased turnover of
 1471 collagen in abdominal aortic aneurysms, demonstrated by measuring the concentration of
 1472 the aminoterminal propeptide of type III procollagen in peripheral and aortal blood samples.
 1473 J. Vasc. Surg. 1995, doi:10.1016/S0741-5214(95)70110-9.
- 1474 164. Bazeli, R.; Coutard, M.; Duport, B.D.; Lancelot, E.; Corot, C.; Laissy, J.-P.; Letourneur, D.;
 1475 Michel, J.-B.; Serfaty, J.-M. In Vivo Evaluation of a New Magnetic Resonance Imaging Contrast
 1476 Agent (P947) to Target Matrix Metalloproteinases in Expanding Experimental Abdominal
 1477 Aortic Aneurysms. *Invest. Radiol.* 2010, *45*, 662–668, doi:10.1097/RLI.0b013e3181ee5bbf.
- 1478 165. Golestani, R.; Razavian, M.; Nie, L.; Zhang, J.; Jung, J.-J.; Ye, Y.; de Roo, M.; Hilgerink, K.; Liu,
 1479 C.; Robinson, S.P.; et al. Imaging Vessel Wall Biology to Predict Outcome in Abdominal Aortic
 1480 Aneurysm. *Circ. Cardiovasc. Imaging* 2015, *8*, e002471,
 1481 doi:10.1161/CIRCIMAGING.114.002471.
- 1482
- 1483
- 1484
- 1485
- 1486