Chapter 1

# Extracellular Matrix Organization, Structure, and Function

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## 1. Introduction

Heart valves are thin, complex, layered connective tissues that direct blood flow in one direction through the heart. There are four valves in the heart, located at the entrance to and exit from the ventricular chambers. The normal function of the heart valves is essential to cardiovascular and cardiopulmonary physiology. The opening and closing of valve leaflets at precise times during the cardiac cycles contributes to the generation of sufficiently high pressure to eject blood from the ventricles, and also prevents blood from flowing backwards into the heart instead of forward towards the systemic circulation and the lungs.

The ability of heart valves to open and close repeatedly, as well as the maintenance of the phenotypes of valvular cells, is made possible by their tissue microstructure, specifically the composition and orientation of extracellular matrix (ECM). The ECM within heart valves is primarily comprised of collagen, elastic fibers, and proteoglycans and glycosaminoglycans, although other ECM components are present as well. Taken together, the ECM performs several roles in heart valves. First, the ECM plays a biomechanical role: it is responsible for the unique mechanical behavior of the valve tissue and thus the overall valve function. Second, the valvular cells are bound to and surrounded by the ECM that is located within the immediate vicinity of the cell; this ECM is specifically known as the pericellular matrix (PCM). The PCM influences cell function by serving as a source of ligands for cell surface receptors, which transfers mechanical strains (experienced by the leaflet tissues) to the cells and initiates intracellular signaling pathways. Third, the various types of ECM have different innate mechanical behaviors, for example with collagen being stiffer than elastic fibers,

and a growing body of research has demonstrated that the phenotype and function of cells, including valve cells, are influenced by the stiffness of the substrate to which they are adhered [1]. These two latter functions of the ECM are considered to be mechanobiological as opposed to merely biomechanical since they affect cell behavior. Fourth, ECM has binding sites for growth factors and other soluble molecules found in the extracellular space, and thus the ECM serves as a reservoir for numerous bioactive factors than can affect cell behavior if they are released (such as when the ECM is degraded) or if a cell migrates close to this ECM reservoir.

Overall, the heart valve field is beginning to appreciate that there are numerous interactions between the ECM, valve cells, and valve mechanics. Given the complicated relationships that are being demonstrated, it is not surprising that alterations to the normal arrangement or composition of ECM, which frequently occur in valve disease, significantly and detrimentally impact valve function in a rather vicious cycle. For this reason, there has been an increasing effort to characterize the ECM within normal heart valves not only to elucidate valve biomechanics and mechanobiology, but also to obtain a solid basis for comparison with diseased valves.

This chapter will provide an overview of the ECM within heart valves, focusing on the aortic valve. After detailing the layered structure of the valve leaflets, each type of ECM component will be described and discussed in relation to its role in valve function and, in some instances, valve dysfunction.

## 2. The aortic valve leaflets are layered structures

Aortic valve leaflets consist of three main layers: the fibrosa, spongiosa, and ventricularis. Each layer has a distinct composition that aids in the normal mechanical and biochemical behavior of the valve. In diseased states, however, the composition of the layered structures can be altered compared to healthy tissues.

The fibrosa layer, close to the outflow surface, is mainly composed of collagen fibers with a small amount of elastic fibers, which are the major stress-bearing components and provide strength to maintain coaptation during diastole [2]. The circumferential alignment and orientation of collagen fibers contribute to the biological stress-strain relationship for aortic valve leaflets (Figure 1). This bilinear stress-strain curve represents the high extension with a low load and high elastic modulus with a high load applied [3]. Moreover, the particular architecture of collagen fibers contributes to the anisotropic mechanical behavior of the fibrosa layer. It has been found that the fibrosa is 4-6 times stiffer in the circumferential direction than in the radial direction [4].

The middle spongiosa layer of the leaflet predominantly consists of glycosaminoglycans and proteoglycans, particularly hyaluronan, which form a foam-like structure and bind a large amount of water. The spongiosa layer absorbs energy during compression, and facilitates the arrangement of collagen fibrils in the fibrosa and elastin in the ventricularis during the cardiac cycles [5].

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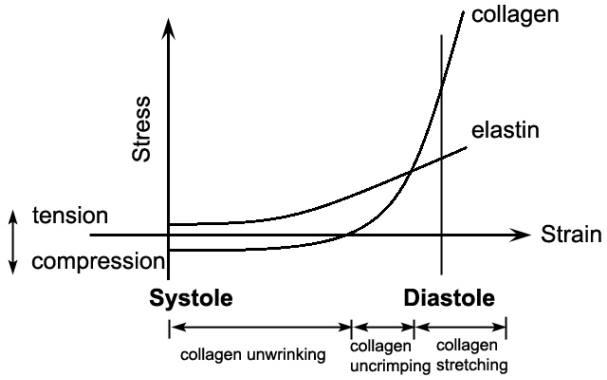


**Figure 1.** Schematic drawing of stress-strain relationships for collagen and elastin fibers during valve motion, reproduced with permission [3]

The ventricularis layer, close to the inflow surface, is rich in elastin with a moderate amount of collagen, which extends in diastole and recoils during systole [6]. The recoil of elastin restores the crimp of collagen fibrils and decreases the surface area of the stretched tissue from the closing phase [5]. The thickness of the three layers varies from the base to the free edge of the cusp [7].

It is worth noting that elastic fibers were found to span the whole leaflet, and connect or anchor three discrete layers together [6,8]. In addition, elastin provides intrafibrillar connections between collagen bundles in the fibrosa layer, whereas it forms a three-dimensional interconnected network in the spongiosa layer [8]. During unloading, the intrafiber elastin, which has high extensibility, helps the collagen fibers return to their wavy and crimped state [6]. These interconnected structures of elastic fibers anchor the discrete layers together, and prevent delamination, which therefore improves the continuity of material behavior of the whole leaflet. Table 1 summarizes the key ECM components in the layers and their major functions.

| Location | Main Component(s) | Major Function(s) |
|---|---|---|
| Fibrosa | Collagen | Stress bearing |
| Spongiosa | Glycosaminoglycans and proteoglycans | Conferring flexibility, dampening vibrations from closing, and resisting delamination |
| Ventricularis | Elastin | Restoration of the wavy and crimped state of collagen fibers |

**Table 1.** The key ECM components in each layer of the leaflet and their major functions

The structures of the leaflets described above provide the following critical functions [6,9–11]: 1) anisotropic mechanical behavior withstanding circumferential stress and extending radially; 2) bilinear biological stress-strain behavior allowing the leaflet to extend before bearing load in the closed phase; 3) elastic recoil to fully open the valve and restore the layer

structures for the next cycle. The particular shape of the leaflets and their unique macro- and micro-structures cause the anisotropic mechanical behavior along the circumferential and radial directions of the leaflets [9–11].

During the closed phase (diastole), the leaflets experience the maximum load. Collagen bundles in the fibrosa layer are the major stress-bearing component withstanding approximately 80 mm Hg pressure while the valve is closed and bulging back towards the ventricle [3]. Collagen fibrils are assembled into parallel collagen fiber bundles oriented along the circumferential direction in the leaflet, which are able to withstand such high tensile forces. However, collagen fibers cannot be compressed, making the alignment of collagen (waviness and crimping) important for decreasing the area of the stretched fibrosa layer. Although the collagen fibrils have limited extensibility (approximately 1-2% yield strain), the waviness and crimping allows the fibrosa to withstand roughly 40% strain under loading. Straightening of wavy fibers provides approximately 17% strain, whereas the crimping allows additional approximately 23% strain [6]. In addition, the strains of the cusps in the closed phase are anisotropic, i.e., the strains differ in the radial and circumferential directions [11].

During valve opening, cusps become relaxed through recoil of the elongated, taut elastin. This restores the wavy and crimped state of collagen fibers while decreasing the surface area of the cusps. The GAG-rich spongiosa layer facilitates the rearrangements of the collagen and elastic fibers during the cardiac cycle, dampens vibration from closing, and resists delamination between layers [6,8].

It is evident that normal aortic valve function is maintained, in part, by not only the composition but also the arrangement and orientation of ECM components, particularly collagen, elastin, and GAGs, in the leaflets. Furthermore, it is important to note that alteration of the composition [12] and mechanics [13] of ECM in the aortic valve leaflets was found in diseased conditions. In calcific aortic valve disease (CAVD), collagen bundles and elastin fibers in the fibrosa layer were disrupted and disorganized [14]; meanwhile, there was increased proteoglycan deposition [12]. Matrix metalloproteinases (MMPs) [14,15] and the potent elastase cathepsin S [16], which are produced by macrophages, contribute to this ECM remodeling. Moreover, ECM proteins related to bone, i.e., osteocalcin and osteonectin, were present in the calcified fibrosa layer [17]. These proteins promote mineralization, and their presence suggests the osteoblastic differentiation of valve interstitial cells (VICs).

In addition, excessive myofibroblast differentiation from VICs, leading to ECM accumulation and fibrosis, was influenced by remodeling of ECM in the fibrosa and facilitated by elastin degradation [18]. Furthermore, myofibroblast differentiation from VICs and calcification *in vitro* have been shown to be dependent on ECM composition [19].

Taken together, the macroscopic layered structure and the microscopic structure in each layer of the leaflets impart pronounced anisotropic mechanical behavior that allows the valve to open and close during a great number of cardiac cycles throughout life. These structures are tailored to fulfill the normal functions and maintain the homeostasis of the leaflets in a healthy condition. However, abnormal alteration of composition and mechanics of ECM in these structures may lead to calcific heart valve disease.

## 3. Collagen comprises a significant portion of the aortic valve leaflet fibrosa

Collagen is an essential component of the aortic valve's layered structure and is vital for maintaining the tissue's mechanical integrity. Mainly responsible for tensile strength, collagen is a strong load-bearing protein created and regulated by VICs. Although present throughout the entire valve, collagen is largely located in the fibrosa where it reduces high tensile stresses. In addition to its central role in valve mechanics, collagen acts as a regulator of VIC phenotype and calcification. Insight into the structure of collagen reveals its unique mechanical properties that support aortic valve function.

Fibrillar collagens are high strength fibers that comprise nearly all of the valve's collagen content. Fibrillar collagens are groups of 3 coiled polypeptide chains that assemble together in tightly packed parallel arrangements. These coils are approximately 300 nm long and join together in a staggered banding pattern with a periodicity of 67 nm [20]. The aortic valve is mainly composed of fibrillar collagen types I, III, and V. Each of these collagens is constructed from different types of alpha chains that govern the overall function of the collagen molecule. Together, these three collagen types work to provide the aortic valve with unique mechanical properties suited for maintaining unidirectional blood flow.

Synthesis of fibrillar collagen is an essential mechanism for maintaining the valve's mechanical integrity. This complex process originates within VICs and is completed in the valve ECM. Production of collagen begins with the intracellular creation of polypeptide alpha chains. There exist ten distinct polypeptide chains that consist of approximately 300 consecutive Gly-X-Y amino acid sequences flanked by small terminal domains. The secondary structure of collagen is created by folding alpha chains into a right-handed alpha helix with the peptide bonds localized at the backbone of the helix and the amino acid side chains facing outward. With slightly less than three residues per turn and a pitch of approximately 8.6 nm, glycine residues are positioned in such a way that the side chains of these residues allow for the formation of the helix. The single hydrogen side chains of these glycine residues allows for the formation of a triple helix structure [21].

The tertiary structure of collagen involves the formation of a left-handed triple helix constructed in the C to N direction. These triple helices exist as both homotrimers and heterotrimers of alpha chains. Collagen type III is a homotrimer of $\alpha$1(III) while collagen type I is a heterotrimer of $\alpha$1(I) and $\alpha$2(I). Additionally, collagen type V is a heterotrimer of $\alpha$1(V) and $\alpha$2(V). Known as procollagen, the tertiary structure molecule is approximately 1.5 nm wide and longer than 300 nm. For creation of the final supramolecular structure, the procollagen molecule is transported into the ECM for crosslinking and fibril formation. After modification in the extracellular space, procollagen is converted into tropocollagen, which undergoes fibrillogenesis where the triple helices are packed together into bundles. Crosslinking of the fibrils ensures the stability of the complex [21].

The arrangement of collagen fiber bundles is crucial to the proper functioning of the aortic valve. Collagen fibers are organized into multilayer structures linked by thin membranes

containing variably aligned collagen. Ranging from 10 to 50 μm in size, these membranes are believed to be much more extensible than the collagen fiber bundles they connect. These multilayer structures can easily slide past one another during valve movement, providing the combination of flexibility and tensile strength necessary for the required mechanics during valve opening and closing [22].

Collagen constitutes approximately 90% of the protein content of the valve insoluble matrix [23]. The vast majority of the valve's content is composed of collagens type I, III, and V. Together, these fibrillar collagens account for 60% of the valve's dry weight [24]. There is approximately 74% collagen type I, 24% collagen type III, and 2% collagen type V distributed throughout the valve [25–27]. Whereas collagen type I mainly exists in the fibrosa, collagen type III is expressed ubiquitously throughout all three layers [25].

Collagen fibers mainly function to reduce stress on the leaflets during systole and diastole. While elastin controls initial valve opening and closing, collagen fibers reduce peak stresses in the leaflet matrix by an estimated 60%. These fibers have an important role in stabilizing leaflet motion [28]. Throughout leaflet movement, collagen fibers adjust position to resist tensile forces. As transvalvular pressure increases, the ventricularis expands in the circumferential direction, causing collagen fibers to become highly aligned. This is believed to increase the cuspal stiffness of the valve during diastole and prevent overextension of the valve [29].

The heterogeneous distribution of collagen throughout the aortic valve provides high strength in areas of greater stress while also allowing the valve to achieve a large degree of flexibility. Within the fibrosa, the primary tensile load-bearing layer, collagen fibers are highly aligned in the circumferential direction, resulting in tissue anisotropy. The arrangement of these fibers corresponds to the direction of highest tensile stress. In contrast, the ventricularis endures smaller tensile forces involved with initial opening and closing of the valve [30]. In addition to circumferentially oriented collagen, the largest and strongest collagen fiber bundles are localized in the areas of greatest tensile stress along the lower part of the commissure and coapting regions [22]. This unique arrangement and positioning of collagen reduces high tensile loads on the valve while allowing flexibility to open and close.

Comparisons between the fibrosa and ventricularis indicate that the fibrosa has a greater elastic modulus in the circumferential direction but a similar elastic modulus in the radial direction. These mechanical differences are largely the result of the number of aligned collagen fibers in each direction. With fewer collagen fibers, the ventricularis is approximately half as stiff as the fibrosa in the circumferential direction. In the radial direction, however, each layer contains approximately the same amount of collagen fibers and has similar elastic moduli [31]. Taken together, the multilayer valve structure causes aortic valves to be less stiff and more extensible radially than circumferentially [32].

Collagen achieves high strength and extensibility with the aid of additional mechanisms that contribute to the valve's mechanical properties. These include collagen cross-links, collagen crimp, and layer corrugations. Collagen cross-links function to increase the strength of aligned collagen. In the circumferential direction, the number of collagen cross-links per col-

lagen molecule directly corresponds to the elastic modulus. However, this relationship does not apply to the radial direction, possibly due to the presence of elastin [31]. When there is no mechanical stress on the leaflet, the fibrosa exists as a number of folds in the radial direction known as corrugations. Large extensibility is achieved through these collagen corrugations in combination with collagen crimp. When stress is applied to the leaflet, initial extension is accomplished by straightening of the collagen crimp. Further stress causes the corrugations to unfold in the radial direction [30]. Together, collagen crimp and corrugations allow the fibrosa to extend further in the radial direction when compared to the circumferential direction.

Throughout the lifetime of the aortic valve, collagen synthesis and degradation are responsible for maintaining adequate valve strength and extensibility. Constant turnover of collagen allows the valve to adapt to regional changes in tensile strength. *In vitro* studies show that VICs respond to cyclic mechanical loading as a way to balance collagen synthesis and degradation. Cyclic stretch of valve leaflets stimulates VIC collagen type III production. In particular, the amount and duration of the stretching can have an effect on the amount of collagen produced [27]. Additionally, VICs in culture express collagen type I and collagen type III mRNA for new matrix synthesis [33]. New collagen production is localized to specific regions of the valve depending on the collagen type that is produced. Collagen type I synthesis occurs in the fibrosa around, but not within, areas of mature collagen. Collagen type III synthesis, however, mainly occurs outside of the fibrosa [34]. Collagen degradation is also an important function of VICs and acts as an essential control to collagen production. Studies have shown that VICs seeded into collagen scaffolds express MMPs that degrade the scaffold in a heterogeneous manner [33]. Thus, VICs continuously regulate the mechanical properties of the surrounding ECM through collagen synthesis and degradation.

Aside from its mechanical functions, collagen has been shown to regulate VIC phenotype and calcification potential. *In vitro* studies were unable to induce calcification in VICs cultured on collagen proteins in standard media. It is believed that collagen actively inhibits VIC calcification [19]. Other studies have shown that scaffold collagen content also affects VIC proliferation. Specifically, one study reported that VICs adhered and spread on collagen surfaces but were not able to proliferate [35]. Another study showed that VIC proliferation decreased on scaffolds containing higher collagen content [36]. An *in vitro* study indicated that matrix stiffness regulates VIC differentiation to myofibrogenic or osteogenic phenotypes in calcific conditions [37].

## 4. Elastic fibers comprise a significant portion of the ventricularis layer of the aortic valve leaflets

Elastic fibers are macromolecular assemblies of several different molecules. The majority of the elastic fiber consists of elastin, an insoluble protein generated by lysyl oxidase crosslinking of soluble tropoelastin monomers (approximately 70 kDa). The elastin tends to be located in the inner core of the elastic fiber and is surrounded by a fine mesh of microfibrils.

These microfibrils are predominantly fibrillin-1, but to a lesser extent Fibrillin-2. Microfibril associated glycoproteins (MAGPs), fibulins, and other proteins are also present in the microfibrillar sheath [38]. At the light microscope level, one can observe the fine elastic fibers by histological staining with Voerhoff's stain or related methods, but when tissue sections are viewed with transmission electron microscopy, there is a clear distinction between the electron-dense elastin core and the microfibrillar sheath [39].

The unique mechanical behavior of the elastic fiber is conferred primarily by the mechanical function of elastin and fibrillin. Crosslinked elastin is remarkable for its ability to undergo high amounts of deformation when subjected to small amounts of load, as well as to recoil back to its original dimensions, when the load is removed, with very little loss of energy. Fibrillin-1, the most widely studied of the microfibrillar components, is also highly extensible. Fibrillin and the other microfibrillar components also coordinate, in a complicated manner still under investigation [38], to aid in the cross-linking of tropoelastin and assemble the final elastic fiber. Interestingly, fibrillin is not always associated with elastic fibers. Fibrillin can often be found by itself, in which it may independently function as a mechanical, load-bearing but highly extensible scaffold [40]. Numerous domains in fibrillin exist for binding integrins, heparan sulafate proteoglycans, and growth factors, which point to substantial roles for alone fibrillin and mature elastic fibers in mediating cell signaling and adhesion [41].

In semilunar heart valves, elastin is found primarily within the ventricularis layer on the inflow side of the leaflet, but is also abundant in the middle spongiosa layer. A thin, frequently imperceptible layer of elastic fibers, the aterialis, is found atop the collagen-rich fibrosa layer. These elastic fibers merge with the intima of the adjacent arterial well, but the overall function of the arterialis has not been well characterized [42].

In the ventricularis, elastic fibers are present in dense and continuous sheets across the whole of the leaflet. These fiber sheets are the most significant contributor to the mechanical properties of the ventricularis [6,30], which can be demonstrated when all ECM components but elastin are removed when using NaOH digestion. After this treatment, the digested ventricularis matches the mechanical behavior of the undigested ventricularis radially, indicating a strong presence of elastin in the radial direction [30]. The elastic fibers within the ventricularis undergo considerable, continual stretch from the initial stage of closure, when blood flow vortices are starting to push the leaflets towards the valve orifice, to the final coapted position of the leaflets. The extension of these elastic fibers accommodates the unfolding of the fibrosa layer, which is normally corrugated in the unloaded position. During this unfolding process, the elastic fibers are bearing the loading of the entire leaflet [43]. Even at high strains, when the collagen in the fibrosa is considered to dominate mechanical properties, the elastin in the ventricularis still plays a significant role. This effect was shown when separated ventricularis was preloaded to mimic its intact configuration; the separated ventricularis was shown to bear load before the separated fibrosa [44]. It has been speculated that this response acts as a safety mechanism to prevent radial overextension of the aortic valve leaflet. Then, when the pressure across the valve is reduced, the elastic fiber sheet in the ventricularis recoils and retracts the leaflets back toward the annular attachment to the

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arterial wall, a process that involves the re-folding of the corrugations in of the fibrosa. This action restores the original shape and orientation of collagen quickly and consistently to prepare for the next cycle of valve closing. Although the elastic sheet in the ventricularis has fibers that are also oriented circumferentially as well as radially, elastin does not appear to play an important role in the mechanical behavior of the leaflet in the circumferential direction. Valve leaflets exposed to cyclic circumferential stretch and cultured under flow for 48 hours maintained a constant concentration of elastic, suggesting that elastogenesis was not activated during the duration of stretch [45]. However, it is speculated that connections between the elastic fiber and collagen networks facilitate the radial extensibility of the ventricularis layer and the overall leaflet [43]. There are also some elastic fibers in the fibrosa, which surround and connect the collagen fibers, thus preserving collagen crimp and the characteristic corrugated nature of the fibrosa [6,46,47].

The elastic fiber structure in the spongiosa has been characterized much less than in the ventricularis, partly due to the difficulty in isolating its structure from the rest of the leaflet [30]. This elastic structure, however, has been observed during microdissection separating the leaflet [30,48], with scanning electron microscopy (SEM) [6,46], micro-computed tomography (micro-CT) [6], immunohistochemistry (IHC) [25], and autofluorescence imaging [49,50], which all have shown a fine elastic fiber network emanating from the ventricularis and connecting to the fibrosa. We have recently reported that the thickness of this elastic fiber network in the spongiosa is significantly thicker in the hinge and coaptation region than in the belly region of the aortic valve leaflet [8]. We also found two distinct patterns of spongiosa elastic fibers within the leaflet: (i) a rectilinear pattern in the hinge and coaptation region; and (ii) a radially oriented stripe pattern in the belly. Overall, it is believed that the elastic fibers in the spongiosa contribute to valve function in three ways. First, they connect the elastic fibers in the ventricularis to collagen in the fibrosa, which allows coupling of the mechanics of the two layers and matrix components, while using elastic recoil to exert preload on the fibrosa. Second, they distribute stress between collagen and elastic fibers, particularly at low strains. Third, they passively allow relative movement and shear between the outer layers [5,6,48].

Given the presence of a thick, rectilinearly-arranged structure of elastic fibers in the spongiosa of the hinge and coaptation regions, it is speculated that this elastin structure plays a role in leaflet flexure [5,30]. Flexure of the leaflet towards the outflow direction compresses the fibrosa and applies tension to the ventricularis. Rather than undergoing compression, however, the fibrosa may attempt to buckle separately from the leaflet, thereby exaggerating its corrugated configuration. The leaflet would subsequently bend at the troughs of this corrugation, where the second moment of inertia would be locally reduced, albeit temporarily. Buckling would only occur with shearing between the fibrosa and ventricularis, which is allowed by both the compliant elastic fibers in the spongiosa connecting the two outer layers as well as by GAGs in the spongiosa lubricating the outer layer movement [5,30,51,52]. Recoil from the elastic fibers in the spongiosa would then return the fibrosa to its original configuration so it could undergo the next cycle of loading [5,30]. At the hinge, where bending occurs in the opposite direction, it is speculated that the elastic fiber-rich ventricularis com-

presses readily without buckling, most likely due to the tensile preload already exerted on the ventricularis, but that leaflet deflection may be limited by the stiff fibrosa, which would not allow the leaflet to bend [4,53]. Limited flexure at the hinge would allow the leaflet to absorb pressure from reverse blood flow in diastole, but prevents distention of the leaflet. Thus, our finding of a thicker spongiosa and elastic fiber structure in flexural regions provides evidence of a significant role for elastin in flexure [8]. In addition, the thick network of elastic fibers that we have observed in the spongiosa of the coaptation region may play a role in dampening vibrations that result from valve closing [5].

## 5. The middle layer, the spongiosa, is comprised mainly of glycosaminoglycans and proteoglycans

Glycosaminoglycans and proteoglycans (GAGs and PGs, respectively) comprise a significant part of the aortic valve leaflets. PGs and GAGs are mainly found in the spongiosa layer of the valve, located between the ventricularis and fibrosa, where they play a vital role in maintaining normal valve function. Previous work has shown that GAGs and PGs serve to not only provide mechanical support to the tissue but also aid in the normal biological functions of the valve [54]. Therefore, it is crucial to fully understand the function of GAGs and PGs in both the normal and possible diseased states of tissues.

GAGs are composed of long and unbranched chains of repeating disaccharides, which consist of a hexosamine and either, depending on the GAG type, uronic acid or galactose. There exist the following families of GAGs with each group being defined by its composition: hyaluronan (HA), heparin, heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), and keratan sulfate (KS) (Table 2) [55–58].

GAGs are primarily formed in the lumen of the Golgi apparatus. The formation process occurs, except in the case of HA, with glycosyltransferases alternatively adding a uronic acid or galactose with a hexosamine to a protein core. The attachment to the protein core varies based on the GAG type. Heparin, HS, CS, and DS are attached to a serine residue, connected to the protein core, via xylose. KS can attach to the protein core either by an asparagine residue at the N-terminus or linked to serine or threonine at the O-terminus. HA does not attach to a protein core. It is synthesized by the addition of sugars to the non-reducing termini of the forming polysaccharide by HA synthase, without a protein backbone. In all cases, modifications can be made to the resulting polysaccharides. Two noteworthy changes include sulfation of the chains and epimerization of the uronic acid. These changes do not occur, however, with HA. Sulfation and epimerization modifications can give a more distinct characteristic to the GAG chains. The epimerization of the uronic acid of CS leads to the production of DS. Epimerization also occurs on heparin and HS. Sulfation can occur in CS, DS, heparin, HS, and KS. N-sulfation takes place in heparin and HS; whereas, O-sulfation can take place in heparin, HS, CS, and DS. In addition to epimerization and sulfation, phosphorylation of the xylose linkage—occurring among CS, DS, heparin, and HS to their respective protein cores—can take place [54,58,59]. Through gel electrophoresis, it has been found that

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HA comprises approximately half of the total GAG content in aortic valves [60]. It is important to note that all GAGs, with the exception of HA, exist *in vivo* as components of PGs.

| Glycosaminoglycan | Uronic acid | Galactose | Hexosamine |
|---|---|---|---|
| Hyaluronan | Glucuronic | - | N-acetylglucosamine |
| Heparin | Glucuronic<br>Iduronic | - | N-acetylglucosamine |
| Heparan sulfate | Glucuronic<br>Iduronic | - | N-acetylglucosamine |
| Chondroitin sulfate | Glucuronic | - | N-acetylgalactosamine |
| Dermatan sulfate | Glucuronic<br>Iduronic | - | N-acetylgalactosamine |
| Keratan sulfate | - | + | N-acetylglucosamine |

**Table 2.** List of glycosaminoglycans and their composition [59]

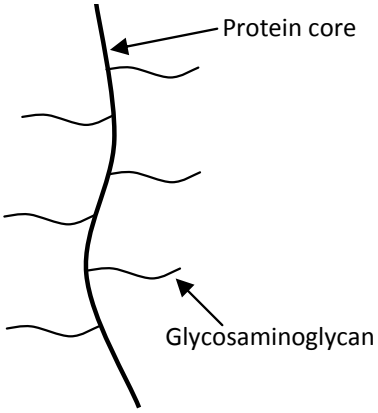


**Figure 2.** Proteoglycan structure

PGs are formed when GAGs are added to a protein core through a covalent linkage (Figure 2). During PG synthesis, a protein core moves from the endoplasmic reticulum of a cell to the Golgi apparatus, where GAGs are then added to the protein core [55]. PGs can be found in intracellular organelles, on the cell surface, and in the extracellular matrix (ECM) [59]. PGs found in the ECM can be divided into three categories: PGs found within the basement membrane, hyalectans or PGs that interact with HA and lectins, and small leucine-rich PGs (SLRPs) or PGs that contain a leucine motif and have considerably low molecular weights. These PGs can be further classified by the type of protein backbone they contain, as well as the amount, type, and sulfation pattern of the GAGs that are attached to the backbone. More than thirty PGs have been characterized [61]. For example, well-characterized PGs that exist in cardiovascular tissue include decorin, biglycan, and versican. Decorin and biglycan have a core protein

size of 40 kDa and are a part of the SLRP family of PGs. They contain CS and DS GAG chains [61]. Versican is a large, chondroitin sulfate proteoglycan. It interacts with HA, and therefore is a hyalectan PG [62]. Other significant PGs in mammalian tissues include perlecan—a basement membrane protein that contains HS and CS, aggrecan—a hyalectan containing CS, and syndecans—a family of cell surface heparan sulfate proteoglycans containing HS and CS [61].

GAGs, and in turn PGs, have a significant role in aortic valve tissue behavior. GAGs have been shown to enhance the viscoelastic properties of the valve leaflets through binding of water molecules [63]. The sulfation and carboxylation on the GAGs make them highly negatively-charged polysaccharides. This negative charge draws in water molecules. Once the tissue becomes hydrated, it acts like a sponge for the valve leaflets. As noted previously, GAGs and PGs are highly abundant in the middle layer of the aortic valve leaflet. One of the main functions of this cushioned layer, the spongiosa, is to provide a barrier between two other layers, the ventricularis and fibrosa, of the valve. This barrier allows for proper shearing between the layers as well as compressibility of the leaflet without compromising the leaflet's overall structural or biological integrity when mechanical stimuli are applied to aortic valve leaflets [63,64]. The mechanical competency that GAGs provide is crucial to the aortic valve leaflets. The aortic valve leaflets serve to ensure unidirectional blood flow from the left ventricle to the aorta. In order to guarantee normal blood flow, the leaflets must open and close properly. Therefore, the flexibility that GAGs provide to the leaflet is crucial to the normal valve's function. In addition, the space that GAGs occupy and form in the matrix serve to organize other molecules within the structure. The structure and hydration that GAGs provide also allow for biological cues to occur within the valve. Moreover, GAGs are known to aid in cell migration, proliferation, act as receptors for signaling molecules, bind growth factors, and serve in the recruitment of various cell types [54].

It is believed that GAGs/PGs likely play an active role in aortic valve tissue disease. Research has shown regional variation of decorin, biglycan, versican and HA in, near, and distal to regions of calcification in diseased aortic valves, suggesting the occurrence of remodeling in the tissue during an unhealthy state [65]. In addition, although the exact causation of calcific aortic valve disease is unknown, it is speculated that it may be due, at least in part, to an inflammatory process [17]. Interestingly, GAGs are thought to play an active role, quite often in the case of cellular injury, in many inflammatory processes for a variety of cell types and have shown to alter in structure and localization in these processes [66]. In addition, some researchers believe that lipid binding due to the unique structure of GAGs may be critical to the accumulation of lipids in calcified aortic valves, a characteristic that is hypothesized to aid in valvular calcification [67]. Although the specific mechanisms underlying calcific aortic valve disease are not quite understood, the complex nature and distinguishable differences of GAGs in both healthy and diseased tissue give rise to the possibility of GAGs being a key factor in valve calcification.

GAGs are very complex disaccharides that highly dictate the behavior of PGs. These polysaccharides are vital in maintaining mechanical, structural, and biological integrity of the aortic valve. Although there is growing interest in further elucidating the role of GAGs in

healthy tissues, the exact role of GAGs in diseased aortic valves needs further investigation, as well.

## 6. Minor ECM components in heart valves also play significant roles in normal valve function and in pathological states

The extracellular matrix of heart valves contain a number of minor components that perform a variety of functions. They are important in valve development, function, and pathology. The study and further characterization of these minor ECM components not only facilitates the development of targeted therapies but would also aid in the microenvironmental mimicry needed for potential tissue engineering applications.

Vitronectin is a glycoprotein that is approximately 75 kDa in size and is present in both serum and the ECM as an adhesive substrate [68]. It is involved in the inhibition of the complement system [68] and is associated with the regulation of hemostasis [69]. Vitronectin also promotes cellular attachment to ECM and is involved in cellular migration [68]. This glycoprotein, along with fibronectin, is found in moderate amounts in aortic, pulmonary, and mitral valves, localizing around valve endothelial cells (VEC) on the inflow layer [25]. In addition, both fibronectin and vitronectin have been shown to associate with collagen fibers in chordae tendinae [70].

Fibronectin is a dimer glycoprotein which consists of two ~250 kDa subunits and is a component of the extracellular matrix [71]. There are many various isoforms of fibronectin, which is the result of alternative mRNA splicing [71]. In addition to being an insoluble ECM component secreted primarily by fibroblasts, soluble fibronectin is also found in the plasma [71]. Fibronectin acts by binding to integrins, collagens, fibrin, and heparin sulfate proteoglycans [71], which allows it to participate in wound healing [72,73] and act as a critical player in embryogenesis [74]. Although not a major ECM component in heart valves, valve interstitial cells (VIC) secrete fibronectin in response to valve damage, providing a means for cell migration [75].

Additionally, fibronectin, along with osteonectin and periostin, confers stiffness to the fibrosa layer [76]. Periostin is a component of the ECM that acts as a ligand for $\alpha$-V/$\beta$-3 and $\alpha$-V/$\beta$-5 integrins and is known to support adhesion and epithelial migration [77]. It is present in the extracellular matrix of several types of tissues and is upregulated in several types of cancers [78]. Recombinant periostin has been shown to promote cardiomyocyte proliferation and angiogenesis after a myocardial infarction [79]. It has been shown previously that periostin plays a role in murine embryonic valve development and remains present in the valves throughout the lifespan even when there is no pathological calcification [80]. A recent study involving chick cardiac development suggests that the presence of periostin in the developing heart may provide a means of organizing other ECM molecules in order to facilitate early epithelial-mesenchymal transition (EMT) [81]. However, the overexpression of periostin and osteopontin can lead to valve calcification.

Osteopontin is a phosphoprotein, meaning that it contains chemically bound phosphoric acid. Originally found in bone, it also contains the arginine-glycine-aspartate (RGD) motif more commonly attributed to fibronectin and is also a constituent of ECM in other tissues [82]. It is secreted by various tissues such as fibroblasts [82] and immune cells, including dendritic cells, macrophages, and neutrophils [83]. Osteopontin is known to interact with various surface receptors that make it a crucial player in bone remodeling [84], wound healing, inflammation, and immune responses [83]. It is also known to be involved in vascular remodeling during endothelial injury [82]. Osteopontin is present in valves calcified as a result of disease as well as in calcified bioprosthetic heart valves [85]. The calcification process of aortic valves closely resembles osteoblast differentiation in regards to expression of genes characteristic of bone formation, such as osteopontin and osteocalcin [86].

Osteocalcin is a small, non-collagenous protein that is considered a late-stage marker for bone formation and is one of a small group of proteins that are osteoblast-specific [87,88]. It is present in general circulation [87] and its capacity for binding hydroxyapatite and calcium suggests that it is largely involved in mineral deposition [88], but it also has recently been shown to act in a hormone-like manner by enhancing insulin secretion [87]. Its traditional role as a product of bone indicates that valve calcification may actually be a result of active bone formation in the valve tissue [86]. This bone formation may be the result of VEGF secretion by endothelial cells during neoangiogenesis occurring in response to inflammation, as seen in rheumatic valve calcification [89]. Additionally, increased serum levels of osteocalcin were shown to be indicative of aortic valve disease in patients [90].

In addition to the matrix proteins, matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) are also found in heart valves. They assist in tissue development and remodeling and can be used as indicators of disease. It is also believed that the ECM degradation resulting from MMP activity serves to release growth factors bound to ECM components and thus alter the microenvironment chemically, as well as structurally [91]. Calcified leaflets from stenotic valves have been shown to express levels of MMP-2 that are similar to those of normal valves but express higher levels of MMP-3, MMP-9, and TIMP-1 [14]. MMP-1, produced by activated myofibroblasts and macrophages, is also prevalent in calcific aortic valve stenosis and may be related to high TNF-$\alpha$ levels resulting from inflammation [92].

## 7. The basement membrane supports valve endothelial cells and acts as a barrier between circulating blood and subendothelial components

The basement membrane is a myriad of proteins, proteoglycans, and glycoproteins that not only supply a substrate to anchor the valve endothelial cells, but also has a large array of biological activities that regulate spatial organization, sequester growth factors, modulate angiogenesis and migration, and regulate the diffusion of nutrients through it towards the underlying valve interstitial cells [93]. The major constituents of the basement membrane are laminin, perlecan, collagen type IV and VIII, nidogen, and the glycoprotein SPARC (secreted protein, acidic and rich in cysteine). Each of these constituents play a role in the overall func-

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tion of the basement membrane. In addition, MMPs contribute to the biological activity that occurs within the basement membrane. Understanding basement membrane composition and behavior, during both healthy and diseased states of the aortic valve, may lead to a better understanding of calcific aortic valve disease.

Laminins belong to a family of heterotrimeric glycoproteins composed of combinations of $\alpha$, $\beta$, and $\gamma$ chains that form a cross-like structure averaging between 400-900 kDa in size [94,95]. Laminins play an integral role in the formation of the supportive ECM network. The unique cross-like shape allows laminin molecules to bind with neighboring laminins and ECM via the three short chains, and use the long alpha chain as a cell anchoring site [96]. In addition to their structural contributions to the basement membrane, laminins are essential for proper biological activity. These glycoproteins have been shown to promote cell adhesion, migration, differentiation, and maintenance of cellular phenotype [94,97,98]. Dysfunction in laminin expression has been linked to diseases with improper tissue formation such as muscular dystrophy, epidermolysis bullosa, and various nephritic syndromes [94,99].

Although laminin is not as ubiquitous as collagen, this basement membrane component has been highly investigated as an ECM substrate for *in vitro* cultures. However, this glycoprotein may influence valve cell types differently. *In vivo* and *in vitro* studies have shown that laminin interacts with endothelial and epithelial cells, and can help maintain physiological functionality of the cells [97,100,101]. However, VICs cultured on laminin have been found to support high quantities of calcific nodule formation in the presence of TGF-$\beta$, when compared to subendothelial ECM components collagen type I and fibronectin [19,102]. The various regions of laminin protein have been reported to mediate specific cell responses. The G-domains of laminin $\alpha$ chains are associated with heparin binding and cell adhesion, whereas regions along the laminin $\beta$ chains promote cell differentiation [98,100,101]. The peptide sequence YIGSR from the laminin $\beta$-1 chain has been shown to promote endothelial cell adhesion and proliferation, however, it also influences other cell types including smooth muscle and tumor cells [98,101]. VICs cultured on YIGSR were also shown to promote calcific nodule formation, although less than those seeded on fibronectin derived RGDS peptides. However, when the 67-kDa laminin cell receptor was blocked, the YIGSR seeded VIC cultures significantly increased in nodule formation and gene expression for various myogenic and osteogenic markers, suggesting that disruption in laminin binding may be linked to valve calcification [103]. IKVAV, another peptide sequence derived from the laminin $\alpha$1-chain, has been linked to promoting angiogenesis, cell migration and spreading [97,98,104]. Though most work with this peptide has been done with endothelial and tumor cells, its ability to promote angiogenesis may also be a future area of interest in studying how angiogenesis mediates valve tissue calcification. Furthermore, laminin influence on cell activity varies between cell types, and may promote VIC activation and tissue calcification in diseased states.

Perlecan (Pln) is one of the more abundant heparan sulfate proteoglycans and is found in several tissues including in the endochondral barrier in bones [105]; however, it is primarily localized in vascular basement membranes. It has a major role in regulating the development of blood vessels, the heart, cartilage, and the nervous system. Physiologically, perlecan plays a prominent role in regulating cellular proliferation, differentiation, organization, and

mediating inflammation [106]. Perlecan derives its functionality from five protein subdomains which share their sequence homology with several other proteins [107]. Domain 1 contains an SEA (Sperm protein, Enteokinase, Agrin) module and three SGD (Ser-Gly-Asp) tripeptide sequences to which three heparan sulfate (HS) glycosaminoglycans attach. These HS can bind and sequester several important growth factors for determining endothelial quiescence in a process known as matricrine signaling. The SEA section is unique to perlecan, and it has no known function other than to influence the O-linked glycosylation of the SGD domain. Interestingly, it has been shown that several factors that determine the activity of these sugar chains vary greatly by the cell source that is producing them [108]. These factors can include the ratio of heparan sulfate to chondroitin sulfate, the length of the chains, and the sulfation level of the chains which all affect how the chains modulate the bioactivity of nearby growth factors. Domain II contains 4 low-density lipoprotein receptor sequences and one immunoglobulin-like repeat. Domain III contains three laminin-like domain modules and eight epidermal growth factor-like repeats. Domain IV, the largest domain, contains many N-CAM-like Ig repeats. Domain V has been demonstrated to be the major cell-binding domain of perlecan due to the laminin and agrin homologies that it contains. Domain V can also be glyocosylated, which can contribute along with domain I to the matricrine signaling capabilities of perlecan, which could potentially contribute to the development of CAVD [109].

Matricrine signaling occurs when the ECM modulates cell behavior by controlling the local levels of growth factor concentrations by sequestering or releasing them when the underlying matrix is intact or degraded, respectively [109]. Proteoglycans, like perlecan, and their GAG chains are the major sites for matricrine signaling due to their heparan sulfate and chondroitin sulfate chains electrostatically binding free growth factors. Their role in the pathology of CAVD is widely unexplored despite their presence in normal valves and their increased production in diseased valves [67]. It is known that PGs and GAGs play an integral role in the progression of atherosclerosis via sequestering of inflammatory molecules and lipids [110–113] and mediating angiogenesis into the vessel supplying an entry way for additional inflammatory entities. Both of these factors are seen histologically in CAVD, but their role is merely speculative at this moment.

Collagens in the basement membrane can form lateral, axial, and linear connections with surrounding ECM. Of the basement membrane collagens, collagen IV (COL IV) is the most abundant and essential for network formation. Only found in basement membrane tissues, COL IV molecules are approximately 400 kDa, and composed of two $\alpha_1$, and one $\alpha_2$ [115–117]. COL IV proteins have many biologically active domains that can influence specific cellular responses, as well as have specific affinities to other molecules such as BMP-4, fibronectin, Von Hippel Lindau protein, and factor IX [115,117]. Mapping of COL IV protein reveals 3 major integrin motifs that are located in strategic regions to promote cell activity or protein degradation when activated [115]. During angiogenesis and tumor invasion, COL IV is degraded by MMP-2 and MMP-9 enzymes to allow for cell migration and infiltration into the matrix. Studies have found that the cleavage sites also overlap with many integrin binding domains such as $\alpha_1\beta_1$, resulting in the availability of $\alpha_v\beta_3$ integrin binding sites known to

promote neutrophil binding [115,118]. Collagen IV networks are highly adhesive to all cells types except erythrocytes [115,119]. Furthermore, cell binding has been found to be enhanced in the presence of various ECM molecules such as perlecan, SPARC, and von Willebrand factor (vWF) [115,118,119]. Interestingly, COL IV also has numerous anti-angiogenic domains that are activated after MMP degradation at the non-collagenous (NC) 1 domain, thereby limiting angiogenesis or migration of endothelial and tumor cells [115]. The changes in COL IV bioactivity depending on the domain region and cleavage state can greatly affect the functionality of surrounding cells. Dysfunctional COL IV expression or mutations in the heterotrimer formation have been found to be extremely detrimental and cause matrix disorders such as Goodpasture's syndrome or Alport syndrome [94,115]. Therefore, additional studies should be done to investigate how the highly bioactive COL IV meshworks may promote the onset of calcification in valve tissues.

COL VIII has also been found to play a network forming role, maintaining the sheet-like structure ECM, while sequestering various integrin binding sites and growth factors. COL VIII is smaller than COL IV, and can form tetrahedral and hexagonal assemblies [117,118]. Though work on COL VIII in regards to valve tissues has been limited, vascular basement membrane studies have found that COL VIII plays a large role in interacting with subendothelial cells such as smooth muscle cells and fibroblasts. In vitro, COL VIII promotes fibroblast proliferation and migration [114]. Furthermore, COL VIII may be linked to atherogenesis, a pathology similar to CAVD, as its expression in cells is upregulated during vessel injury [114,120]. This collagen has even been found to interact with elastic fibers in liver tissues, suggesting it may have a bridging function between the basement membrane components and subendothelial ECM [118]. Therefore, COL VIII could play an integral role in mediating valve interstitial and endothelial cell communication. Recent studies have found after enzyme cleavage at the NC1 domain, the resulting C-terminal fragment known as vastatin will prevent endothelial cell proliferation and induce cell apoptosis [120]. While some work has investigated using vastatin as an anti-angiogenic agent, further studies are needed to elucidate how it may affect the functionality of surrounding cells and ECM, especially in older valve tissue.

Similar to perlecan, nidogen is a 150 kDa glycoprotein that has sequence homologies with other basement membrane proteins. It consists of two amino (G1, G2) and one carboxyl (G3) terminal globular domains that are connected by a rod domain composed primarily of endothelial growth factor repeats [121]. Nidogen binds collagen type IV, perlecan, and laminin. This binding contributes to the hypothesis that nidogen is important in basement membrane assembly; although some recent animal studies have demonstrated that nidogen may not be necessary for basement membrane formation [121]. The role of nidogen in CAVD is unexplored, but it may play a role in maintaining valvular basement membrane functionality by regulating infiltration of inflammatory agents [93].

SPARC positive neovascularisation is a documented histological change in CAVD [122]. Secreted protein acidic and rich in cysteine (SPARC), also known as osteonectin, is a small basement membrane protein. It interacts with cells, binds to other members of the basement membrane, growth factors, various proteases, and is found in newly developing neovessels.

Intact SPARC protein inhibits cellular proliferation and has anti-angiogenic activity in vitro [123]. However, enzymatic degradation of SPARC can release matricryptic fragments with the KGHK motif that may induce angiogenic activity both *in vitro* and *in vivo* [124]. SPARC has been observed lining blood vessels in early to mid stage calcified valves suggesting the presence of a fully formed basement membrane lining these vessels [125]. However, the presence of the other constituents of the basement membrane is merely speculative at this point as the studies investigating their presence during CAVD have not been completed.

## 8. Summary

In conclusion, the last several years have witnessed significant acceleration in the number of studies characterizing specific types of extracellular matrix in heart valves, although there is still much to be learned. The basement membrane of heart valves, and its role in regulating valvular endothelial cell function, are particularly understudied. The broad scope of cell-matrix and matrix-matrix interactions within heart valves, and how these are regulated by the local, dynamic signaling environment, is another subject that merits further investigation. We expect that insights gained from these research endeavors will lead to novel treatments for valve diseases in the future.

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## References

[1] Nemir S, West JL. Synthetic materials in the study of cell response to substrate rigidity. *Annals of Biomedical Engineering*. 2010 Jan;38(1):2–20.

[2] Sacks MS, Schoen FJ, Mayer, Jr. JE. Bioengineering challenges for heart valve tissue engineering. *Annual Review of Biomedical Engineering*. 2009 Jan;11:289–313.

[3] Schoen FJ, Levy RJ. Founder's Award, 25th Annual Meeting of the Society for Biomaterials, perspectives. Providence, RI, April 28-May 2, 1999. Tissue heart valves: current challenges and future research perspectives. *Journal of Biomedical Materials Research*. 1999 Dec 15;47(4):439–65.

[4] Vesely I, Lozon A. Natural preload of aortic valve leaflet components during glutaraldehyde fixation: effects on tissue mechanics. *Journal of Biomechanics*. 1993 Feb;26(2): 121–31.

[5] Schoen FJ. Aortic valve structure-function correlations: role of elastic fibers no longer a stretch of the imagination. *Journal of Heart Valve Disease*. 1997 Jan;6(1):1–6.

[6] Scott MJ, Vesely I. Aortic valve cusp microstructure: the role of elastin. *Annals of Thoracic Surgery*. 1995;60(Fig 2):S391–S394.

[7] Filion RJ, Ellis CG. A finite difference model of O2 transport in aortic valve cusps: importance of intrinsic microcirculation. *American Journal of Physiology. Heart and Circulatory Physiology*. 2003 Nov;285(5):H2099–104.

[8] Tseng H, Grande-Allen KJ. Elastic fibers in the aortic valve spongiosa: A fresh perspective on its structure and role in overall tissue function. *Acta Biomaterialia*. 2011 Jan 19;7(5):2101–8.

[9] Missirlis YF, Chong M. Aortic valve mechanics–Part I: material properties of natural porcine aortic valves. *Journal of Bioengineering*. 1978;2(3-4):287.

[10] Sauren AAHJ, van Hout MC, van Steenhoven AA, Veldpaus FE, Janssen JD. The mechanical properties of porcine aortic valve tissues. *Journal of Biomechanics*. 1983 Jan; 16(5):327–37.

[11] Lee JM, Courtman DW, Boughner DR. The glutaraldehyde-stabilized porcine aortic valve xenograft. I. Tensile viscoelastic properties of the fresh leaflet material. *Journal of Biomedical Materials Research*. 1984 Jan;18(1):61–77.

[12] Hinton RB, Lincoln J, Deutsch GH, Osinska H, Manning PB, Benson DW, et al. Extracellular matrix remodeling and organization in developing and diseased aortic valves. *Circulation Research*. 2006 Jun 9;98(11):1431–8.

[13] Chen WLK, Simmons CA. Lessons from (patho)physiological tissue stiffness and their implications for drug screening, drug delivery and regenerative medicine. *Advanced Drug Delivery Reviews*. 2011 Apr 30;63(4-5):269–76.

[14] Fondard O, Detaint D, Iung B, Choqueux C, Adle-Biassette H, Jarraya M, et al. Extracellular matrix remodelling in human aortic valve disease: the role of matrix metalloproteinases and their tissue inhibitors. *European Heart Journal*. 2005 Jul;26(13): 1333–41.

[15] Edep ME, Shirani J, Wolf P, Brown DL. Matrix metalloproteinase expression in nonrheumatic aortic stenosis. *Cardiovascular Pathology*. 9(5):281–6.

[16] Aikawa E, Aikawa M, Libby P, Figueiredo J-L, Rusanescu G, Iwamoto Y, et al. Arterial and aortic valve calcification abolished by elastolytic cathepsin S deficiency in chronic renal disease. *Circulation*. 2009 Apr 7;119(13):1785–94.

[17] Mohler, III ER, Gannon FH, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. *Circulation*. 2001 Mar 20;103(11):1522–8.

[18] Simionescu A, Simionescu DT, Vyavahare NR. Osteogenic responses in fibroblasts activated by elastin degradation products and transforming growth factor-beta1: role of myofibroblasts in vascular calcification. *American Journal of Pathology*. 2007 Jul; 171(1):116–23.

[19] Rodriguez KJ, Masters KS. Regulation of valvular interstitial cell calcification by components of the extracellular matrix. *Journal of Biomedical Materials Research. Part A*. 2009 Sep 15;90(4):1043–53.

[20] Bailey AJ, Paul RG, Knott L. Mechanisms of maturation and ageing of collagen. *Mechanisms of Ageing and Development*. 1998 Dec 1;106(1-2):1–56.

[21] Ottani V, Martini D, Franchi M, Ruggeri A, Raspanti M. Hierarchical structures in fibrillar collagens. *Micron*. 2002 Jan;33(7-8):587–96.

[22] Doehring TC, Kahelin M, Vesely I. Mesostructures of the aortic valve. *Journal of Heart Valve Disease*. 2005 Sep;14(5):679–86.

[23] Eriksen HA, Satta J, Risteli J, Veijola M, Väre P, Soini Y. Type I and type III collagen synthesis and composition in the valve matrix in aortic valve stenosis. *Atherosclerosis*. 2006 Nov;189(1):91–8.

[24] Kunzelman KS, Cochran RP, Murphree SS, Ring WS, Verrier ED, Eberhart RC. Differential collagen distribution in the mitral valve and its influence on biomechanical behaviour. *Journal of Heart Valve Disease*. 1993 Mar;2(2):236–44.

[25] Latif N, Sarathchandra P, Taylor PM, Antoniw J, Yacoub MH. Localization and pattern of expression of extracellular matrix components in human heart valves. *Journal of Heart Valve Disease*. 2005 Mar;14(2):218–27.

[26] Cole WG, Chan D, Hickey AJ, Wilcken DEL. Collagen composition of normal and myxomatous human mitral heart valves. *Biochemical Journal*. 1984 Apr 15;219(2):451–60.

[27] Ku C-H, Johnson PH, Batten P, Sarathchandra P, Chambers RC, Taylor PM, et al. Collagen synthesis by mesenchymal stem cells and aortic valve interstitial cells in response to mechanical stretch. *Cardiovascular Research*. 2006 Aug 1;71(3):548–56.

[28] de Hart J, Peters GWM, Schreurs PJG, Baaijens FPT. Collagen fibers reduce stresses and stabilize motion of aortic valve leaflets during systole. *Journal of Biomechanics*. 2004 Mar;37(3):303–11.

[29] Sacks MS, Smith DB, Hiester ED. The aortic valve microstructure: effects of transvalvular pressure. *Journal of Biomedical Materials Research*. 1998 Jul;41(1):131–41.

[30] Vesely I, Noseworthy R. Micromechanics of the fibrosa and the ventricularis in aortic valve leaflets. *Journal of Biomechanics*. 1992 Jan;25(1):101–13.

[31] Balguid A, Rubbens MP, Mol A, Bank RA, Bogers AJJC, van Kats JP, et al. The role of collagen cross-links in biomechanical behavior of human aortic heart valve leaflets--relevance for tissue engineering. *Tissue Engineering*. 2007 Jul;13(7):1501–11.

[32] Stephens EH, de Jonge N, McNeill MP, Durst CA, Grande-Allen KJ. Age-related changes in material behavior of porcine mitral and aortic valves and correlation to matrix composition. *Tissue Engineering. Part A*. 2010 Mar;16(3):867–78.

[33] Dreger SA, Thomas PS, Sachlos E, Chester AH, Czernuszka JT, Taylor PM, et al. Potential for synthesis and degradation of extracellular matrix proteins by valve interstitial cells seeded onto collagen scaffolds. *Tissue Engineering*. 2006 Sep;12(9):2533–40.

[34] Stephens EH, Grande-Allen KJ. Age-related changes in collagen synthesis and turnover in porcine heart valves. *Journal of Heart Valve Disease*. 2007 Nov;16(6):672–82.

[35] Masters KS, Shah DN, Walker GA, Leinwand LA, Anseth KS. Designing scaffolds for valvular interstitial cells: cell adhesion and function on naturally derived materials. *Journal of Biomedical Materials Research. Part A*. 2004 Oct 1;71(1):172–80.

[36] Taylor PM, Sachlos E, Dreger SA, Chester AH, Czernuszka JT, Yacoub MH. Interaction of human valve interstitial cells with collagen matrices manufactured using rapid prototyping. *Biomaterials*. 2006 May;27(13):2733–7.

[37] Yip CYY, Chen J-H, Zhao R, Simmons CA. Calcification by valve interstitial cells is regulated by the stiffness of the extracellular matrix. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2009 Jun;29(6):936–42.

[38] Wagenseil JE, Mecham RP. New insights into elastic fiber assembly. *Birth Defects Research Part C: Embryo Today: Reviews*. 2007 Dec;81(4):229–40.

[39] Mecham RP, Davis EC. Elastic fiber structure and assembly. In: Yurchenco PD, Birk DE, Mecham RP, editors. *Extracellular Matrix Assembly and Structure*. 1994. p. 281–314.

[40] Haston JL, Engelsen SB, Roessle M, Clarkson J, Blanch EW, Baldock C, et al. Raman microscopy and X-ray diffraction, a combined study of fibrillin-rich microfibrillar elasticity. *Journal of Biological Chemistry*. 2003 Oct 17;278(42):41189–97.

[41] Bax DV, Mahalingam Y, Cain S, Mellody K, Freeman L, Younger K, et al. Cell adhesion to fibrillin-1: identification of an Arg-Gly-Asp-dependent synergy region and a heparin-binding site that regulates focal adhesion formation. *Journal of Cell Science*. 2007 Apr 15;120(Pt 8):1383–92.

[42] Stephens EH, Kearney DL, Grande-Allen KJ. Insight into pathologic abnormalities in congenital semilunar valve disease based on advances in understanding normal valve microstructure and extracellular matrix. *Cardiovascular Pathology*. 2011 Feb 22.

[43] Schoen FJ. Cardiac valve prostheses: pathological and bioengineering considerations. *Journal of Cardiac Surgery*. 1987 Mar;2(1):65–108.

[44] Klövekorn WP, Meisner H, Paek SU, Sebening F. Long-term results after right ventricular outflow tract reconstruction with porcine and allograft conduits. *Thoracic and Cardiovascular Surgeon*. 1991 Dec;39 Suppl 3:225–7.

[45] Misfeld M, Sievers H-H. Heart valve macro- and microstructure. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 2007 Aug 29;362(1484):1421–36.

[46] Scott MJ, Vesely I. Morphology of porcine aortic valve cusp elastin. *Journal of Heart Valve Disease*. 1996 Sep;5(5):464–71.

[47] Vesely I. The role of elastin in aortic valve mechanics. *Journal of Biomechanics*. 1998 Feb;31(2):115–23.

[48] Stella JA, Sacks MS. On the biaxial mechanical properties of the layers of the aortic valve leaflet. *Journal of Biomechanical Engineering*. 2007 Oct;129(5):757–66.

[49] Konig K, Schenke-Layland K, Riemann I, Stock UA. Multiphoton autofluorescence imaging of intratissue elastic fibers. *Biomaterials*. 2005;26(5):495–500.

[50] Christov AM, Liu L, Lowe S, Icton C, Dunmore-Buyze J, Boughner DR, et al. Laser-induced fluorescence (LIF) recognition of the structural composition of porcine heart valves. *Photochemistry and Photobiology*. 1999 Mar;69(3):382–9.

[51] Talman EA, Boughner DR. Glutaraldehyde fixation alters the internal shear properties of porcine aortic heart valve tissue. *Annals of Thoracic Surgery*. 1995 Aug;60(2 Suppl):S369–73.

[52] Talman EA, Boughner DR. Effect of altered hydration on the internal shear properties of porcine aortic valve cusps. *Annals of Thoracic Surgery*. 2001 May;71(5 Suppl):S375–8.

[53] Vesely I, Boughner DR, Song T. Tissue buckling as a mechanism of bioprosthetic valve failure. *Annals of Thoracic Surgery*. 1988;46(3):302–8.

[54] Esko JD, Kimata K, Lindahl U. Proteoglycans and Sulfated Glycosaminoglycans. In: Varki A, Cummings RD, Esko JD, editors. *Essentials of Glycobiology*. 2nd editio. 2009.

[55] Silbert JE, Sugumaran G. Biosynthesis of chondroitin/dermatan sulfate. *IUBMB Life*. 2002 Oct;54(4):177–86.

[56] Bodevin-Authelet S, Kusche-Gullberg M, Pummill PE, DeAngelis PL, Lindahl U. Biosynthesis of hyaluronan: direction of chain elongation. *Journal of Biological Chemistry*. 2005 Mar 11;280(10):8813–8.

[57] Sasisekharan R, Venkataraman G. Heparin and heparan sulfate: biosynthesis, structure and function. *Current Opinion in Chemical Biology*. 2000 Dec;4(6):626–31.

[58] Funderburgh JL. Keratan sulfate: structure, biosynthesis, and function. *Glycobiology*. 2000 Oct;10(10):951–8.

[59] Prydz K, Dalen KT. Synthesis and sorting of proteoglycans. *Journal of Cell Science*. 2000 Jan;113 Pt 2:193–205.

[60] Murata K. Acidic glycosaminoglycans in human heart valves. *Journal of Molecular and Cellular Cardiology*. 1981 Mar;13(3):281–92.

[61] Iozzo RV. Matrix proteoglycans: from molecular design to cellular function. *Annual Review of Biochemistry*. 1998 Jan;67:609–52.

[62] Wight TN. Versican: a versatile extracellular matrix proteoglycan in cell biology. *Current Opinion in Cell Biology*. 2002 Oct;14(5):617–23.

[63] Bhatia A, Vesely I. The effect of glycosaminoglycans and hydration on the viscoelastic properties of aortic valve cusps. *Conference Proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society*. 2005 Jan;3:2979–80.

[64] Lincoln J, Lange AW, Yutzey KE. Hearts and bones: shared regulatory mechanisms in heart valve, cartilage, tendon, and bone development. *Developmental Biology*. 2006 Jun 15;294(2):292–302.

[65] Stephens EH, Saltarrelli JG, Baggett LS, Nandi I, Kuo JJ, Davis AR, et al. Differential proteoglycan and hyaluronan distribution in calcified aortic valves. *Cardiovascular Pathology*. 2010 Dec 23;

[66] Taylor KR, Gallo RL. Glycosaminoglycans and their proteoglycans: host-associated molecular patterns for initiation and modulation of inflammation. *FASEB Journal*. 2006 Jan;20(1):9–22.

[67] Grande-Allen KJ, Osman N, Ballinger ML, Dadlani HM, Marasco S, Little PJ. Glycosaminoglycan synthesis and structure as targets for the prevention of calcific aortic valve disease. *Cardiovascular Research*. 2007 Oct 1;76(1):19–28.

[68] Felding-Habermann B, Cheresh DA. Vitronectin and its receptors. *Current Opinion in Cell Biology*. 1993 Oct;5(5):864–8.

[69] Preissner KT, Seiffert D. Role of vitronectin and its receptors in haemostasis and vascular remodeling. *Thrombosis Research*. 1998 Jan 1;89(1):1–21.

[70] Akhtar S, Meek KM, James V. Immunolocalization of elastin, collagen type I and type III, fibronectin, and vitronectin in extracellular matrix components of normal and myxomatous mitral heart valve chordae tendineae. *Cardiovascular Pathology*. 1999;8(4):203–11.

[71] Pankov R, Yamada KM. Fibronectin at a glance. *Journal of Cell Science*. 2002 Oct 15;115(Pt 20):3861–3.

[72] Valenick LV, Hsia HC, Schwarzbauer JE. Fibronectin fragmentation promotes alpha4beta1 integrin-mediated contraction of a fibrin-fibronectin provisional matrix. *Experimental Cell Research*. 2005 Sep;309(1):48–55.

[73] Ffrench-Constant C, Van de Water L, Dvorak HF, Hynes RO. Reappearance of an embryonic pattern of fibronectin splicing during wound healing in the adult rat. *The Journal of Cell Biology*. 1989 Aug;109(2):903–14.

[74] George EL, Georges-Labouesse EN, Patel-King RS, Rayburn H, Hynes RO. Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. *Development*. 1993 Dec;119(4):1079–91.

[75] Fayet C, Bendeck MP, Gotlieb AI. Cardiac valve interstitial cells secrete fibronectin and form fibrillar adhesions in response to injury. *Cardiovascular Pathology*. 2007;16(4):203–11.

[76] Combs MD, Yutzey KE. Heart valve development: regulatory networks in development and disease. *Circulation Research*. 2009 Aug 28;105(5):408–21.

[77] Gillan L, Matei D, Fishman DA, Gerbin CS, Karlan BY, Chang DD. Periostin secreted by epithelial ovarian carcinoma is a ligand for alpha(V)beta(3) and alpha(V)beta(5) integrins and promotes cell motility. *Cancer Research*. 2002 Sep 15;62(18):5358–64.

[78] Tilman G, Mattiussi M, Brasseur F, van Baren N, Decottignies A. Human periostin gene expression in normal tissues, tumors and melanoma: evidences for periostin production by both stromal and melanoma cells. *Molecular Cancer*. 2007 Jan;6:80.

[79] Polizzotti BD, Arab S, Kühn B. Intrapericardial delivery of gelfoam enables the targeted delivery of Periostin peptide after myocardial infarction by inducing fibrin clot formation. *PloS One*. 2012 Jan;7(5):e36788.

[80] Kruzynska-Frejtag A, Machnicki M, Rogers R, Markwald RR, Conway SJ. Periostin (an osteoblast-specific factor) is expressed within the embryonic mouse heart during valve formation. *Mechanisms of Development*. 2001 May;103(1-2):183–8.

[81] Kern CB, Hoffman S, Moreno R, Damon BJ, Norris RA, Krug EL, et al. Immunolocalization of chick periostin protein in the developing heart. *The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology*. 2005 May;284(1):415–23.

[82] Ashizawa N, Graf K, Do YS, Nunohiro T, Giachelli CM, Meehan WP, et al. Osteopontin is produced by rat cardiac fibroblasts and mediates A(II)-induced DNA synthesis and collagen gel contraction. *Journal of Clinical Investigation*. 1996 Nov;98(10):2218–27.

[83] Wang KX, Denhardt DT. Osteopontin: role in immune regulation and stress responses. *Cytokine & Growth Factor Reviews*. 2008;19(5-6):333–45.

[84] Choi ST, Kim JH, Kang E-J, Lee S-W, Park M-C, Park Y-B, et al. Osteopontin might be involved in bone remodelling rather than in inflammation in ankylosing spondylitis. *Rheumatology*. 2008 Dec;47(12):1775–9.

[85] Srivatsa SS, Harrity PJ, Maercklein PB, Kleppe L, Veinot J, Edwards W, et al. Increased cellular expression of matrix proteins that regulate mineralization is associated with calcification of native human and porcine xenograft bioprosthetic heart valves. *Journal of Clinical Investigation*. 1997 Mar 1;99(5):996–1009.

[86] Rajamannan NM, Subramaniam M, Rickard D, Stock SR, Donovan J, Springett M, et al. Human aortic valve calcification is associated with an osteoblast phenotype. *Circulation*. 2003 May 6;107(17):2181–4.

[87] Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell*. 2007 Aug 10;130(3):456–69.

[88] Gundberg CM, Hauschka PV, Lian JB, Gallop PM. Osteocalcin: isolation, characterization, and detection. *Methods in Enzymology*. 1984 Jan;107(1975):516–44.

[89] Rajamannan NM, Nealis TB, Subramaniam M, Pandya S, Stock SR, Ignatiev CI, et al. Calcified rheumatic valve neoangiogenesis is associated with vascular endothelial growth factor expression and osteoblast-like bone formation. *Circulation*. 2005 Jun 21;111(24):3296–301.

[90] Levy RJ, Zenker JA, Lian JB. Vitamin K-dependent calcium binding proteins in aortic valve calcification. *Journal of Clinical Investigation*. 1980 Feb;65(2):563–6.

[91] Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular Research*. 2006 Feb;69(3):562–73.

[92] Kaden JJ, Dempfle C-E, Grobholz R, Fischer CS, Vocke DC, Kiliç R, et al. Inflammatory regulation of extracellular matrix remodeling in calcific aortic valve stenosis. *Cardiovascular Pathology*. 2005;14(2):80–7.

[93] Yip CYY, Simmons CA. The aortic valve microenvironment and its role in calcific aortic valve disease. *Cardiovascular Pathology*. 2011;20(3):177–82.

[94] Kreis T, Vale R. Guidebook to the Extracellular Matrix, Anchor, and Adhesion Proteins. 2nd ed. Kreis T, Vale R, editors. 1999.

[95] Timpl R, Rohde H, Robey PG, Rennard SI, Foidart J-M, Martin GR. Laminin--a glycoprotein from basement membranes. *Journal of Biological Chemistry*. 1979 Oct 10;254(19):9933–7.

[96] Durbeej M. Laminins. *Cell and Tissue Research*. 2010 Jan;339(1):259–68.

[97] Okazaki I, Suzuki N, Nishi N, Utani A, Matsuura H, Shinkai H, et al. Identification of biologically active sequences in the laminin alpha 4 chain G domain. *Journal of Biological Chemistry*. 2002 Oct;277(40):37070–8.

[98] Pradhan S, Farach-Carson MC. Mining the extracellular matrix for tissue engineering applications. *Regenerative Medicine*. 2010 Nov;5(6):961–70.

[99] McGowan KA, Marinkovich MP. Laminins and human disease. *Microscopy Research and Technique*. 2000 Nov 1;51(3):262–79.

[100] Hozumi K, Suzuki N, Nielsen PK, Nomizu M, Yamada Y. Laminin alpha1 chain LG4 module promotes cell attachment through syndecans and cell spreading through integrin alpha2beta1. *Journal of Biological Chemistry*. 2006 Oct;281(43):32929–40.

[101] Ponce ML, Nomizu M, Delgado MC, Kuratomi Y, Hoffman MP, Powell S, et al. Identification of endothelial cell binding sites on the laminin gamma 1 chain. *Circulation Research*. 1999 Apr;84(6):688–94.

[102] Gwanmesia P, Ziegler H, Eurich R, Barth M, Kamiya H, Karck M, et al. Opposite effects of transforming growth factor-β1 and vascular endothelial growth factor on the degeneration of aortic valvular interstitial cell are modified by the extracellular matrix protein fibronectin: implications for heart valve engineering. *Tissue Engineering. Part A*. 2010 Dec;16(12):3737–46.

[103] Gu X, Masters KS. Regulation of valvular interstitial cell calcification by adhesive peptide sequences. *Journal of Biomedical Materials Research. Part A*. 2010 Jun 15;93(4): 1620–30.

[104] Genové E, Shen C, Zhang S, Semino CE. The effect of functionalized self-assembling peptide scaffolds on human aortic endothelial cell function. *Biomaterials*. 2005 Jun; 26(16):3341–51.

[105] Ishijima M, Suzuki N, Hozumi K, Matsunobu T, Kosaki K, Kaneko H, et al. Perlecan modulates VEGF signaling and is essential for vascularization in endochondral bone formation. *Matrix Biology*. 2012 May;31(4):234–45.

[106] Knox SM, Whitelock JM. Perlecan: how does one molecule do so many things? *Cellular and Molecular Life Sciences*. 2006 Nov;63(21):2435–45.

[107] Farach-Carson MC, Carson DD. Perlecan--a multifunctional extracellular proteoglycan scaffold. *Glycobiology*. 2007 Sep;17(9):897–905.

[108] Ellis AL, Pan W, Yang G, Jones K, Chuang C, Whitelock JM, et al. Similarity of recombinant human perlecan domain 1 by alternative expression systems bioactive heterogenous recombinant human perlecan D1. *BMC Biotechnology*. 2010 Jan;10:66.

[109] Chen J-H, Simmons CA. Cell-matrix interactions in the pathobiology of calcific aortic valve disease: critical roles for matricellular, matricrine, and matrix mechanics cues. *Circulation Research*. 2011 Jun;108(12):1510–24.

[110] Wilkinson TS, Bressler SL, Evanko SP, Braun KR, Wight TN. Overexpression of hyaluronan synthases alters vascular smooth muscle cell phenotype and promotes monocyte adhesion. *Journal of Cellular Physiology*. 2006 Feb;206(2):378–85.

[111] Nakashima Y, Fujii H, Sumiyoshi S, Wight TN, Sueishi K. Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by mac-

rophage infiltration. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2007 May;27(5): 1159–65.

[112] Wight TN. Cell biology of arterial proteoglycans. *Arteriosclerosis*. 1989;9(1):1–20.

[113] Yan J, Stringer SE, Hamilton A, Charlton-Menys V, Götting C, Müller B, et al. Decorin GAG synthesis and TGF-β signaling mediate Ox-LDL-induced mineralization of human vascular smooth muscle cells. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2011 Mar;31(3):608–15.

[114] Plenz GAM, Deng MC, Robenek H, Völker W. Vascular collagens: spotlight on the role of type VIII collagen in atherogenesis. *Atherosclerosis*. 2003 Jan;166(1):1–11.

[115] Parkin JD, San Antonio JD, Pedchenko V, Hudson B, Jensen ST, Savige J. Mapping structural landmarks, ligand binding sites, and missense mutations to the collagen IV heterotrimers predicts major functional domains, novel interactions, and variation in phenotypes in inherited diseases affecting basement membranes. *Human Mutation*. 2011 Feb;32(2):127–43.

[116] Soininen R, Haka-Risku T, Prockop DJ, Tryggvason K. Complete primary structure of the alpha 1-chain of human basement membrane (type IV) collagen. *FEBS Letters*. 1987 Dec;225(1-2):188–94.

[117] Shuttleworth CA. Type VIII collagen. *International Journal of Biochemistry & Cell Biology*. 1997 Oct;29(10):1145–8.

[118] Wells RG. Function and metabolism of collagen and other extracellular matrix proteins. In: Rodés J, Benhamou J-P, Blei A, Reichen J, Rizzetto M, editors. *Textbook of Hepatology*. 3rd ed. 2002. p. 264–73.

[119] Tsilibary EC, Reger LA, Vogel AM, Koliakos GG, Anderson SS, Charonis AS, et al. Identification of a multifunctional, cell-binding peptide sequence from the a1(NC1) of type IV collagen. *Journal of Cell Biology*. 1990 Oct;111(4):1583–91.

[120] Xu R, Yao Z-Y, Xin L, Zhang Q, Li T-P, Gan R-B. NC1 domain of human type VIII collagen (alpha 1) inhibits bovine aortic endothelial cell proliferation and causes cell apoptosis. *Biochemical and Biophysical Research Communications*. 2001 Nov 23;289(1): 264–8.

[121] Kang SH, Kramer JM. Nidogen is nonessential and not required for normal type IV collagen localization in Caenorhabditis elegans. *Molecular Biology of the Cell*. 2000 Nov;11(11):3911–23.

[122] Charest A, Pépin A, Shetty R, Côté C, Voisine P, Dagenais F, et al. Distribution of SPARC during neovascularisation of degenerative aortic stenosis. *Heart*. 2006 Dec; 92(12):1844–9.

[123] Chlenski A, Liu S, Crawford SE, Volpert OV, DeVries GH, Evangelista A, et al. SPARC is a key Schwannian-derived inhibitor controlling neuroblastoma tumor angiogenesis. *Cancer Research*. 2002 Dec 15;62(24):7357–63.

[124] Sage H. Pieces of eight: bioactive fragments of extracellular proteins as regulators of angiogenesis. *Trends in Cell Biology*. 1997 May;7(5):182–6.

[125] Kalluri R, Zeisberg E. Controlling angiogenesis in heart valves. *Nature Medicine*. 2006 Oct;12(10):1118–9.