

Pulling on my heartstrings: mechanotransduction in cardiac development and function

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Abstract**Purpose of Review**

Endothelial cells (ECs) line the surface of the cardiovascular system and display a large degree of heterogeneity due to developmental origin and location. Despite this heterogeneity, all ECs are exposed to wall shear stress imparted by the frictional force of flowing blood, which plays an important role in determining the EC phenotype. While the effects of WSS have been greatly studied in vascular ECs, less is known about the role of WSS in regulating cardiac function and cardiac ECs.

Recent Findings

Recent advances in genetic and imaging technologies have enabled a more thorough investigation of cardiac hemodynamics. Using developmental models, shear stress sensing by endocardial endothelial cells (EECs) has been shown to play an integral role in proper cardiac development including morphogenesis and formation of the conduction system. In the adult, less is known about hemodynamics and EECs but a clear role for WSS in the development of coronary and valvular disease is increasingly appreciated.

Summary

Future research will further elucidate a role for WSS in the developing and adult heart and understanding this dynamic relationship may represent a potential therapeutic target for the treatment of cardiomyopathies.

Keywords

endocardium, mechanotransduction, heart, development, shear stress

Introduction

Endothelial cells (ECs) line the inner surface of the heart and all blood vessels. They are present in all tissues and originate early in embryonic development. Despite a tremendous heterogeneity of ECs due to location and developmental origin, a common and critical feature of these cells is their exposure to fluid wall shear stress (WSS), which initiates as soon as the heart begins beating. Research over the last several decades has revealed an important role for the mechanical force of blood flow in regulating development of the cardiovascular system as well as in the adult. Here, we will review the role of hemodynamics and EC mechanotransduction in cardiovascular development with a focus on the embryonic and adult heart.

Endothelial cells and mechanotransduction

Endothelial cells were first recognized in the mid-1800s and following these early descriptions it took almost 150 years for their complex and diverse biology to be appreciated [1]. In the mid-1960s, Lord Florey was among the first to describe the heterogeneity of the vascular network and to introduce the first descriptions of specialization to individual organs [2]. A large degree of EC heterogeneity can be attributed to the microenvironment to which the cells are exposed during development. Gradients of signaling molecules, diverse cell-cell interactions, as well as exposure to mechanical forces drive specialization and further affect EC function.

Despite increased appreciation for their heterogeneous nature, one universal factor affecting ECs is their continuous exposure to WSS, the frictional force of blood flow. Shear stress varies by location and caliber of blood vessel and spatio-temporal heterogeneities have important consequences on EC biology [3]. In regions of undisturbed, laminar blood flow, such as the descending thoracic aorta, ECs express a repertoire of genes and regulate a number of pathways that maintain normal physiology [4]. In contrast, in regions of disturbed blood flow, which are characterized by flow reversals and typically occur at arterial segments with high curvatures, branch points and bifurcations, ECs are primed for the formation of disease, with reduced expression of protective genes and activation of pro-inflammatory pathways. These susceptible regions are typically the sites of atherosclerosis formation [5].

Given that WSS plays a key role in regulating EC form and function, a significant amount of work has focused on understanding the ways in which ECs sense and respond to flow. Using knockout cell lines and animals, a number of mechanosensors have been identified (reviewed in [6]). These include, but are not limited to, a mechanosensory complex of PECAM-1, VE-cadherin, and VEGFR2, ion channels, and integrins among others [7-9]. Furthermore, the downstream signaling from these mechanosensors has been studied and a canonical set of 'shear-responsive' genes and signaling events has been identified in vascular ECs [10].

Mechanical forces and mechanotransduction in cardiovascular development

The cardiovascular system is the first system to develop in vertebrate embryos and disruptions in this process have significant effects on viability. Genetic manipulation of *Drosophila*, zebrafish and mouse embryos have identified a complex milieu of signaling molecules (morphogens) and their cognate receptors that are required for proper cardiovascular development. These include Notch [11, 12], Sonic hedgehog (Shh) [13, 14] and bone morphogenic protein (BMP) signaling (reviewed in [15]) among many others. Increasingly studied, but less well understood, are the effects of mechanical forces on cells as they divide, migrate and differentiate [16].

As mentioned above, WSS is a key epigenetic factor in EC biology. Shear stress occurs simultaneously with the onset of a heartbeat, which occurs early in embryogenesis (Table 1). The initiation of WSS plays an important role in the development of all components of the cardiovascular system, including remodeling of blood vessels [21, 22], hematopoiesis [18, 23], angiogenesis [24, 25] as well as lymphangiogenesis [26, 27]. In the following section we focus on the specific requirements of shear stress in aspects of heart development.

Mechanical forces in heart development

Heart development is a complex and tightly regulated process and disruption is linked to a number of congenital birth defects, including hypoplastic left heart syndrome (HLHS), tetralogy of Fallot and atrioventricular septal defects. The process of cardiogenesis has been well-studied and revealed important spatiotemporal information

regarding activation and expression of key signaling molecules (e.g. Notch, Tbx2, Nkx2-5) [28, 29]. In addition to signaling molecules and transcription factors, the role for hemodynamic force in heart development is also becoming increasingly appreciated. The availability of models that allow surgical or genetic manipulation of contraction and visualization of the cardiac flow environment has greatly advanced the field [30-35].

Like blood vessels, the embryonic heart chambers are lined by a monolayer of ECs. These *endocardial* endothelial cells (EECs) are formed prior to the initiation of contraction and contribute significantly to heart development [36]. *In vivo* developmental studies suggest that EECs are shear stress responsive but the mechanisms by which they sense shear stress are still under investigation. A role for the primary cilium has been described [37-39] but additional mechanosensors have yet to be identified. Many of the studies that implicate a role for mechanotransduction in heart development rely on detection of endothelial shear-responsive transcription factors and signaling molecules (e.g. BMP, neuregulin-1 (NRG1), Krüppel-like factor 2 (KLF2), endothelin-1 (ET-1), endothelial nitric oxide synthase (eNOS)) [40]. Recent evidence suggests that mechanosensitive ion channels are important as well [41]. It is highly likely that EC mechanosensors previously identified in blood vessel mechanotransduction also contribute to heart development, but a role for many of these proteins has not yet been explored in this context. Additionally, it is entirely possible that EECs utilize other mechanisms of mechanotransduction.

One key aspect in studying hemodynamics in heart development is the ability to visualize *and* characterize the forces. With the advent of new imaging modalities, as well as the use of models that allow *in vivo* assessment of mechanical forces, is it possible to link the two. These techniques range from *in vivo* confocal microscopy [32, 42], micro-computed tomography (CT) [43], high frequency ultrasound, and optical coherence tomography (OCT) [44, 45]. Detailed analysis of cardiac flow has revealed that despite the existence of unidirectional flow through the developing blood vessels, flow in the embryonic heart is bi-directional which exposes the heart to unique spatial shear stress [46].

Hemodynamics plays an important role in all aspects of heart development, including looping, trabeculation, cardiac cushion/valve formation, and maturation of the

conduction system (Figure 1). The role WSS plays in each will be described in detail below.

Looping

One of the earliest and most fundamental processes in heart development is cardiac looping. The primitive heart begins as a straight tube with an atrial and ventricular pole and is composed of three layers: myocardium and endocardium separated by cardiac jelly. At this stage, flow through the heart is based on its function as a suction pump [47]. Early in development, the heart tube begins to elongate and then transform into a c-shaped heart loop. In mice, this occurs around E8.5, which is approximately the time at which the heart starts beating. Following looping, a primitive atria, ventricle and outflow tract form. Using an elegant model of flow disruption in zebrafish in which a bead was placed in the heart to obstruct flow, Hove et al demonstrated that a 10-fold decrease in WSS impaired cardiac looping [32]. Additionally, after cardiac looping, activation of shear-responsive transcription factors is required for ballooning of the cardiac chambers [48], which is important for defining the ventricular chamber.

Trabeculation

Following looping, the heart becomes highly trabeculated. Trabeculae are specialized sheets of developing myocardium that form endocardial cell-lined protrusions into the ventricular lumen. Functionally, they are proposed to increase surface area for oxygen-nutrient exchange in the developing myocardium [49]. A zebrafish model of altered flow through the left ventricle (LV) demonstrated that blood flow is required for this process to occur [34]. In these studies, a mutation in the *atrial myosin heavy chain (amhc)* gene leads to a non-contractile atrium causing a reduction in LV flow without altering LV function. Under these conditions the authors observe reduced invaginations in the lumen. Interestingly, the authors also demonstrate a requirement for endothelial NRG-1 in trabeculation and speculate that EEC-cardiomyocyte (CM) signaling may be required for this process to proceed.

Cardiac cushion and valve formation

Concomitant with trabeculation, constrictions develop at the outflow tract (OFT) and atrioventricular canal (AVC), which specify where the heart valves will develop. EECs in these regions undergo a process of endothelial to mesenchymal transition (EndoMT) and proliferate to initiate endocardial cushion formation, which remodel into the four major valves: mitral, tricuspid, pulmonary and aortic. There is a great deal known about the signaling pathways involved, which include Notch, BMP/TGF β , Wnt/B-catenin, VEGF among others (reviewed in [50]).

In addition to activation of signaling pathways, WSS has been demonstrated to greatly influence cardiac cushion formation and valvulogenesis [30, 51]. During early looping, flow-mediated expression of the microRNA, *miR-21*, is required for constriction of the region that will eventually become the AV valve [52]. In E9.5-E12.5 hearts, mice lacking endocardial cilia (*Kif3a*^{-/-} embryos) exhibit abnormal cardiac cushion development [39]. This implicates flow sensing as an important factor in cushion development. Furthermore, it has been shown that in the AVC, EECs experience higher WSS than other regions of the developing heart [46, 53]. An additional component of WSS, flow reversal, is also important for proper valvulogenesis, as it induces elevated expression of shear-related genes KLF2a, Notch1b and BMP4, which are required for valve formation [46]. In the absence of these forces, there is reduced gene expression and inhibition of valvulogenesis [32, 46, 54]. These observations were further validated by Heckel et al who demonstrated that oscillatory flow amplitude mediates KLF2 activation, as well as an endocardial calcium response, via activation of the mechanosensitive ion channels Trpv2 and Trpp2 [41]. Interestingly, these ion channels have also been identified as mechanosensors in adult blood vessels [55].

Conduction system

Also critical for proper cardiogenesis is development of the cardiac conduction system (CCS), which is required for coordinated contraction of the heart. Improper formation can lead to the presence of ectopic pacemakers and arrhythmias. The CCS is derived from cardiomyocytes that express markers of the CCS early after looping [56, 57]. In addition to the importance of CMs, ECs are, primarily through paracrine

signaling, critical for development of this system [58]. Importantly, shear stress in the developing embryo has been shown to influence expression of endothelin converting enzyme (ECE), which converts “big endothelin” to active endothelin (ET-1) [59, 60]. More recently identified is the role of WSS in the expression and patterning of genes necessary for the proper development of the atrial conduction system [61].

Hemodynamics and mechanotransduction in the adult heart

While the role of hemodynamics in heart development has been established, significantly less is known about WSS in the adult heart. Most current knowledge relates to the coronary arteries and valves since most EC-associated cardiac pathologies occur at these sites. The relevance of hemodynamics to the different EC populations of the heart will be discussed in the following sections (Figure 2).

Coronary

Due to the difficulty in imaging the coronary arteries, unlike other regions of the vasculature, the hemodynamic environment and measurements of WSS in these vessels is still under investigation [62]. Despite detailed WSS measurements it is clear that coronary ECs are mechanoresponsive and that this characteristic plays an important role in the focal development and progression of coronary artery disease (CAD) [63-66]. ECs in regions of the coronary arteries exposed to low, disturbed flow upregulates common atherosusceptible pathways including endoplasmic reticulum stress and reactive oxygen species [66, 67]. Interestingly, coronary ECs express these pathways at higher levels than their non-coronary counterparts, which may indicate why these vessels are more susceptible to atherosclerotic plaque formation and disease progression [67]. Furthermore, low WSS sites correlate with increased lipid insudation and modification [68]. It is generally accepted that coronary ECs utilize the same mechanosensors identified in vascular ECs but this has not been thoroughly investigated [6].

Cardiac Valves

Valvular endothelial cells are exposed to hemodynamics throughout the cardiac cycle. Most often studied are the effects of WSS on the mitral and aortic valves as these are closely associated with the development of human disease such as mitral stenosis and calcific aortic valve disease (CAVD). These hemodynamic studies have demonstrated that the aortic side of the aortic valve is most susceptible to disease. Laser Doppler Velocimetry (LDV) has demonstrated that the two sides of the aortic valve are exposed to different flow patterns [69, 70]. Using healthy pig tissue, Simmons et al investigated gene expression on the aortic and ventricular side of the aortic valve and showed that there was differential gene expression, with upregulation of BMP-4 among others [71].

Intracardiac

Similarly to vascular ECs, EECs lining the adult heart chambers are constantly exposed to WSS. Unlike vascular ECs, the effect of WSS on EECs remains understudied and poorly defined. *In vitro* studies using primary EECs suggest that these cells are shear stress responsive as they upregulate prostacyclin production in response to flow [72]. While informative, these studies lack detailed information regarding the hemodynamic environment and rely on WSS values derived from the vasculature. Studies *in vivo* support this hypothesis, demonstrating that manipulating the hemodynamic environment influences EEC phenotype [73-75]. Again, these studies lack information about the hemodynamic environment, which presumably is altered through manipulation of heart rate and pressure. The use of 4D flow MRI has allowed the more thorough description of cardiovascular hemodynamics, in particular intracardiac blood flow [76, 77]. Our recent data suggests that like both vascular ECs and embryonic EEC, adult EECs are sensitive to their hemodynamic environment (manuscript in review). Using 4D flow MRI, we have recently measured WSS in adult humans and pigs and isolated EECs from these regions. Using next-generation RNA-sequencing, we show that EECs from the different regions have dramatically different phenotypes, suggesting that in a normal heart, WSS has an important effect.

Contribution of EC mechanotransduction in cell-cell communication

Studies using isolated preparations of myocardium have demonstrated the requirement for EECs in regulating cardiac function [78-81]. Specifically, early studies of endocardial surface damage resulted in altered myocardial contraction, suggesting that the endothelial layer functions to regulate peak contraction and relaxation of the myocardial layer [78]. Other studies identified a role of ET-1, NO, prostacyclin and NRG-1 (all factors released from ECs) in regulation of cardiomyocyte contractility. Brutsaert provides an excellent review of this in [82]. Importantly, not only are these paracrine factors similar to those identified in EC-vascular smooth muscle cell (VSMC) crosstalk, but several are known to be regulated by shear stress in vascular ECs. Recent work from our laboratory has identified a role for the mechanosensor PECAM-1 in the regulation of cardiac contractility and function. We showed that aberrant release of NRG-1 in PECAM-1 knockout hearts and ECs results in increased ErbB2 phosphorylation and signaling [83]. Although this might seem at odds with the described cardioprotective effects of NRG-1 [84], it does underscore the importance of precise spatio-temporal regulation of NRG-1/ErbB2 signaling and the significance of EC mechanotransduction in EEC-CM crosstalk [83]. Current work is focused on identifying the molecular mechanisms by which PECAM-1 regulates NRG-1 release and the role of hemodynamics in this pathway.

Conclusion

Here we discuss the importance of shear stress and mechanotransduction in numerous aspects of cardiac biology from development, to the adult and in disease. A deeper understanding of hemodynamic forces in the heart may represent a new pharmacologically tractable area for treating cardiac disease.

Key Points

- Endothelial cells (ECs) are continuously exposed to wall shear stress, the frictional force imparted by blood flow, and are able to adapt to changing shear stress conditions.
- The response of vascular ECs in both development and in the adult has been studied in detail but much less is known about endocardial ECs (EECs), which line the chambers of the heart.
- Recent studies have shown that shear stress and EEC mechanotransduction play a critical role in all aspects of heart development, including chamber morphogenesis and valve development,
- Significantly less is known about adult EECs and whether and how they respond to shear stress.

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Conflicts of Interest

None.

Figure Legends

Figure 1. The major stages of cardiac development in which WSS is involved. A, Cardiac looping coincides with the onset of the heartbeat and therefore flow. After looping, the heart chambers begin to take shape. B, Trabeculation occurs following looping and increased the surface area of the ventricular chambers. C, Concomitant with trabeculation is cardiac valve formation, which involves a process of EndoMT and remodeling of the tissue. D, Conduction system formation results from hemodynamically-driven differentiation of CMs. RV, right ventricle; LV, left ventricle; OFT, outflow tract; AVC, atrioventricular canal; CM, cardiomyocyte; CJ, cardiac jelly; EC, endothelial cell; EndoMT, endothelial to mesenchymal transition; CCS, cardiac conduction system.

Figure 2. Different populations of endothelial cells in the heart. A, Coronary vessels supply blood to the myocardium and are found on the surface of the heart. B, Valvular ECs line the surface of the major heart valves: mitral, tricuspid, aortic and pulmonary. Shown are the mitral and aortic. C, Endocardial ECs line the entire surface area of the four heart chambers. Shown are cross sections of the left atria and ventricle. Ao, Aorta; LV, left ventricle; LA, left atrium; CT, chordae tendinae; EEC, endocardial endothelium.

Tables

Table 1. Onset of heartbeat in organisms

Species	Heart Tube Formation	Onset Heartbeat	Reference
Human	Day 19	Day 27	[17]
Mouse	E8-8.5	E8.5	[18]
Chick	HH-stage 9-10	HH-stage 10-11	[19]
Zebrafish	24 hpf	24 hpf	[20]

E, embryonic day; HH, Hamburger-Hamilton Stage; hpf, hours post fertilization

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A. Cardiac Looping + Chamber Ballooning

B. Trabeculation

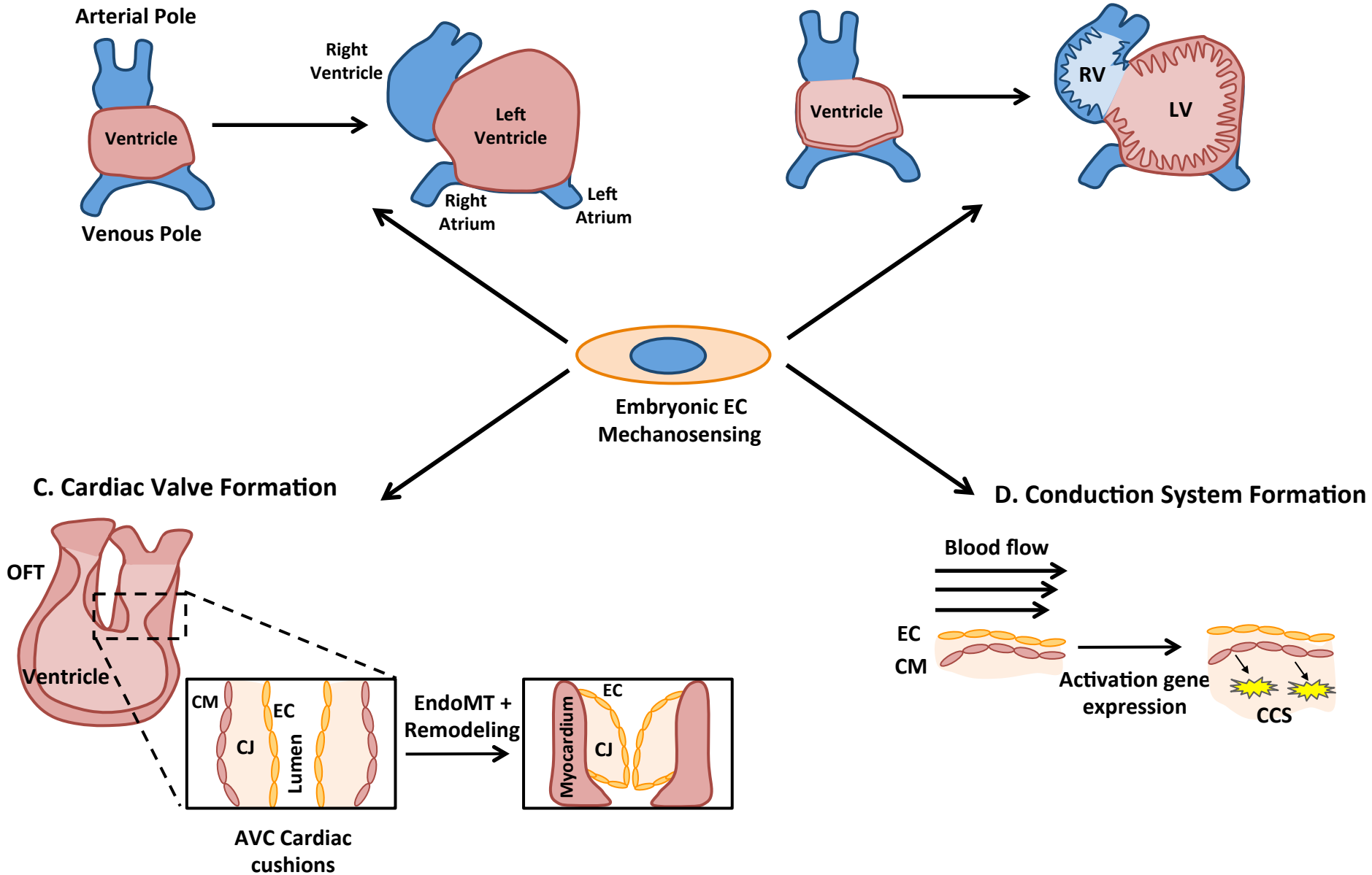
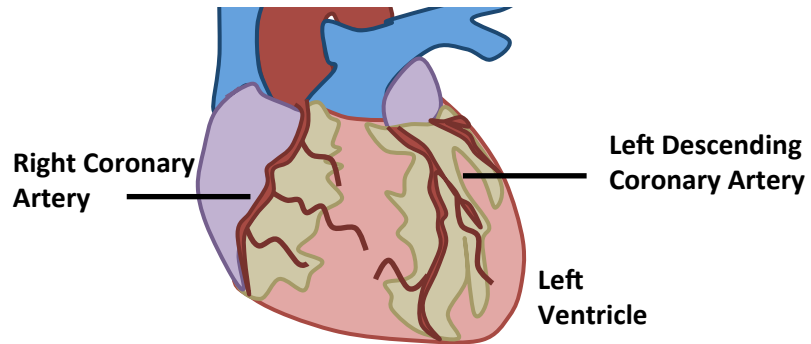
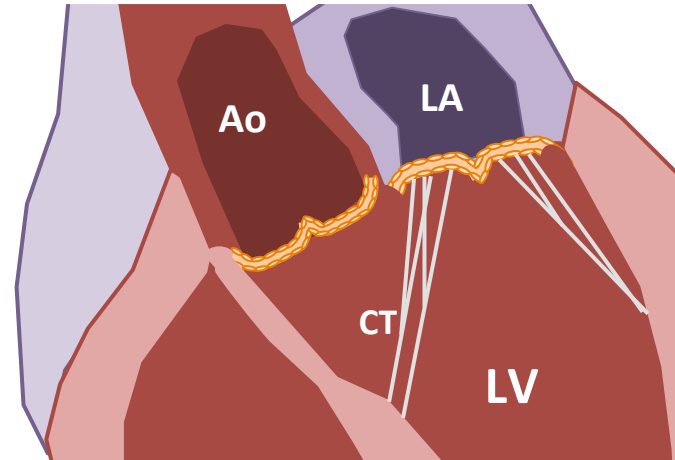


Figure 1.

A. Coronary



B. Cardiac Valves



C. Intracardiac

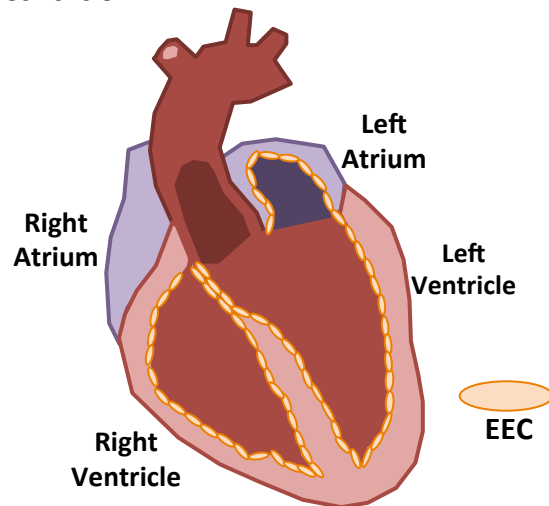


Figure 2.