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Mechanical Factors Affecting Heart Morphogenesis

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Abstract

For years, the genetic element of heart morphogenesis has been studied. This review focuses on a relatively new area of study, namely, mechanical factors influencing heart morphogenesis. To understand the context of the role of mechanical factors in heart development, an extensive review of the stages of heart morphogenesis is provided.

It is found that shear stress, surface tension, fluid forces, and contractions of certain cells play a role in various stages of heart development. Numerous studies have shown that in cases where these mechanical forces were modified, abnormal heart defects were produced. These studies prove that the mechanical forces are essential for normal heart morphogenesis.

Although attempts have been made to define the mechanisms involved in these pathways, most of the research done so far has been inconclusive. While it has been proven that the mechanical forces play a role in heart development, it is still unclear exactly how the forces are involved in the developmental pathways, and what initiates them to proceed. As of current studies, it is also still unclear if there is any correlation between the genes and the mechanical factors involved in heart morphogenesis.

Introduction

One of the most widely studied biological topics is the field of morphology. Specifically focusing on embryonic morphology, much research has been done to link genetics with the various formations and stages of embryonic development. Throughout the years, genetics has been used to explain and account for countless aspects of development patterns and pathways found in embryonic development. Many genes and signaling molecules have been identified and used to formulate blueprints of morphogenic pathways in a developing embryo.

In recent years, there has been a shift in the study of embryonic development. New studies are beginning to focus on the possible role of mechanical and physical forces in morphogenesis. There are numerous physical forces found within the embryo during the stages of development. This review focuses on contractions of cells, fluid forces, shear stress, and surface tension, and their impact on cardiac morphogenesis. Breakthroughs and new technology in mechanical biology allowed for advanced studies of these forces.

Finally, the data will be analyzed to determine if these physical forces that are involved in cardiac development can potentially be linked to genetic factors. Recent studies have begun to attempt to relate the mechanical factors affecting heart morphology with the genetic factors which have been studied for many years. By bridging the gap between genetic and mechanical forces, we will have a better understanding of the control of the development of cardiac tissue and the heart within an embryo.

Methods

Critical analysis of peer reviewed journal articles and original clinical research papers was used to write this review. The articles and papers from which the research was gathered were obtained by using the PubMed search engine found on the government's National Center for Biotechnology Information website. Additional references were obtained from those sources. Keywords used were heart morphology, heart development, mechanical forces, and hemodynamics.

Heart Fields and the Heart Tube

Before analyzing mechanical factors involved in embryonic cardiac morphology, it is essential to have a solid understanding of the stages of heart development in an embryo. The current accepted model of cardiogenesis was first discovered during the last decade. Heart morphogenesis begins with cells with myocardial potential that are located in a specific region, known as a heart field (Buckingham et al., 2005). This was the first research done which classified two individual heart fields, both of which participate in cardiac development. Before this, there were others who identified a second heart field (Kelly et al., 2001). However, Buckingham was the first to explain the process by which the two different fields give rise to distinct portions of the human heart.

Much of this research was done with chick embryos and mouse embryos. Comparisons of gestation timelines of chicks or mice to humans can be made using the formulas outlined by Srivastava (Srivastava, 2006). The anterior lateral plate mesoderm gives rise to cells in the first heart field. A crescent shape is formed by the first heart field. This occurs at approximately the second week during human gestation. By the third week the ends of the crescent merge with each other, forming a basic heart tube. This heart tube is considered basic because it is simply made up of two layers: an interior endothelial cell layer, and an exterior myocardial cell layer (Srivastava, 2006).

Simultaneously, the second heart field is expanding in a formation which appears to encircle the first heart field. The first heart field in the basic heart tube acts as a platform upon which the second heart field is able to settle and begin the process of becoming the various chambers found in the heart (Buckingham et al., 2005). Figure 1 labels the heart fields and shows how each field ultimately gives rise to a different portion of the heart.

It also gives a clearer understanding to the anterior/posterior positioning of the first heart field (labeled "FHF") and the second heart field (labeled "SHF") at two and three weeks of human gestation. It depicts how the second heart field (shown in yellow) uses the first heart field (shown in red/pink) as a support in the initial stages of morphogenesis. Figure 1 also clearly illustrates how the second heart field completely surrounds the first heart field by three weeks of human gestation.

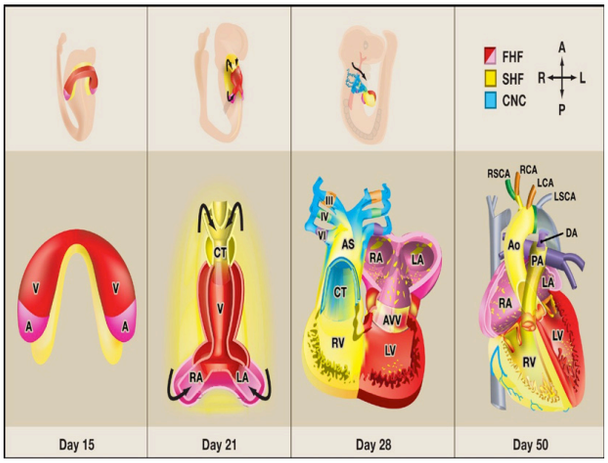

Figure 1. Mammalian Heart Development (Srivastava, 2006).

It is unclear what exactly causes the crescent shaped first heart field to bend into a heart tube. It is theorized that contraction of the crescent, caused by microfilaments in the apical regions of the epithelial cells results in the production of the tube. The contractions force the cells to assume a wedge-shape, causing the plane of the cell sheet to begin to bend into a tube-like formation (Taber, 1998). Unrelated research supported this theory by reporting that administering pharmacological agents that inhibited actin filament function prevented the heart tube formation and resulted in the persistence of the crescent shape (Ettensohn, 1985). Because no experimental work has definitively linked contractions of the actin microfilament with the crescent bending and heart tube formation, it would be inappropriate to say that this is definitely the mechanism which forms the heart tube. Nonetheless, the research provided is enough to strongly suggest that this is the correct method of heart tube formation.

Dextral Looping

Before the heart can fully develop into its various components and chambers, it must go through an event known as looping. Looping produces the c-shaped structure present in the third segment of Figure 1. There are various stages of this looping process, also known as "dextral looping." To analyze this process research was performed using chick embryos. Before looping begins, in a stage referred to as the prelooping stage, the heart tube is essentially bilaterally symmetrical. As the heart tube undergoes rapid elongation, interventricular grooves are formed. These grooves ultimately become the bending points upon which the bottom curve of the "c" looping is established. Upon the completion of c-looping, three bended bands are easily noticeable: the truncus arteriosus (which will later develop into the aorta, its branching arteries, and the pulmonary trunk), the primitive atria region, and the primitive ventricular region (Manner, 2000). Figure 2 gives detailed pictures of the process of dextral looping.

It can be seen clearly in Figure 2 that throughout the process of dextral looping, the heart loses the symmetry and linearity it possessed during the previous stages of the heart tube (Manner,

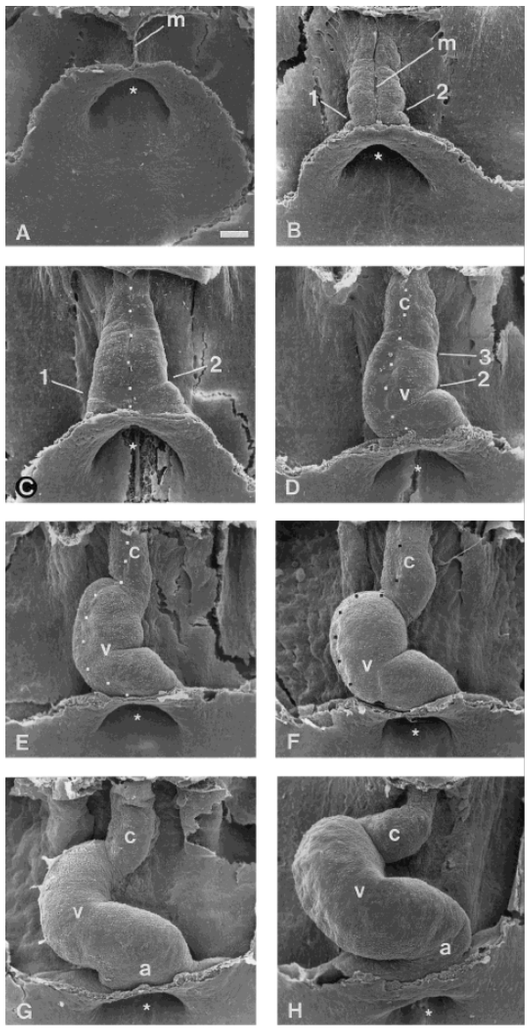

Figure 2. Stages of Dextral-Looping. Slides A and B represent the prelooping stage. In Slide B, "1" and "2" represent the interventricular grooves. Slides C – G show the incremental changes in the heart tube as it proceeds through the process of dextral looping. In Slide H, upon completion of dextral-looping, "c" represents the conus, "v" represents the primitive ventricular region, and "a" represents the primitive atria region (Manner, 2000).

2000). The conus will ultimately give rise to the aorta, the left and right subclavian arteries, the left and right carotid arteries, and the pulmonary trunk. The primitive ventricular region will ultimately give rise to the left and right ventricles. The primitive atria region will ultimately give rise to the left and right atria (Srivastava, 2006).

While the slides in Figure 2 show actual images of the stages of dextral looping, the third segment in Figure 1 diagrams how the developing heart appears after dextral looping of the heart tube is completed. Upon completion of looping, the aorta and its various branching arteries are not fully developed. The aorta appears as a simple structure known as the aortic sac (labeled AS in Figure 1). At this stage of development, the branching arteries of the aorta (left and right subclavian arteries and left and right carotid arteries) are bilaterally symmetric branches (labeled "III" and "IV" in Figure 1) stemming off of the aortic sac. As Figure 1 illustrates, following dextral looping, the aortic sac and its branches are not yet positioned between the atria (Srivastava, 2006).

Transformation of C-Shape to S-Shape

Following dextral looping, the heart goes through another morphogenic process, converting the c-shaped heart loop (created by dextral looping) into an s-shaped heart loop, resulting in the placement of the ultimate locations of the chambers and modifying the position of the craniocaudal axis. This process pushes the right atrium to be positioned superior to the right ventricle, and drags the left atrium towards the right atrium, aligning the left atrium almost directly above the left ventricle. This process causes the aortic sac and its branches to become positioned between the atria. Because the ultimate location of the aorta will be parallel to what will eventually be the interatrial septum,

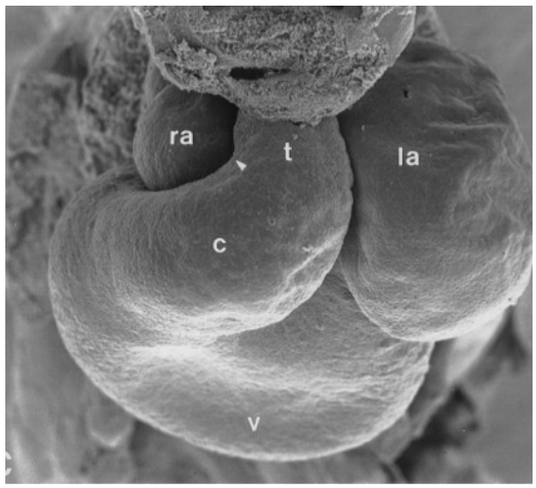

Figure 3. S-Shaped Heart Loop. The left and right atria are represented by "la" and "ra", respectively, the ventricles are represented by "v", and the aortic sac is represented by "c" and "t" (Manner, 2000).

this positioning of the aortic sac and its branches between the atria is essential for the process of heart morphogenesis to proceed (Manner, 2000). Figure 3 shows the resulting s-shaped heart loop with the aortic sac positioned between the atria, which have become aligned superiorly to the segment destined to be split into the left and right ventricles.

Blood Flow during Heart Formation

By the time that looping is completed, there is already blood flow present in the developing heart. At this point, septation of the atria into right and left components has not yet occurred. The blood flows from the portion of the primitive atria region destined to be the left atrium down into the primitive ventricular region. It flows through an area known as the AV canal, the walls of which will ultimately give rise to the region of the atrioventricular valve. Because septation of the ventricles has also not yet occurred, the blood must flow through the primitive ventricular region with enough force to navigate the entire ventricular loop. The blood enters the primitive ventricular region via the inlet tract, ultimately destined to become the left ventricle, and exits the ventricular loop via the outlet tract, ultimately destined to become the right ventricle. The inlet and outlet tracts are delineated by the interventricular foramen, which is an opening separating what will ultimately be the left and right ventricles. As will be described later, septation of the ventricles occurs by the closing of the interventricular foramen (Moorman et al., 2003).

Development of the Cardiac Chambers (Septation)

Changes in the atria and ventricles occur over the same period of time. As the developing lung forms, a network of vessels surrounds the lung buds. This network of vessels connects with the primary atrium (the unseptated atria) and attaches at a point on the left portion, in its inferior region. (This network of vessels, ultimately destined to become the pulmonary veins, does not expand and become fixated on the roof of the left atrium until after septation is completed.) In the right portion of the primary atrium, the sinus venous, the small cavity where the superior and inferior vena cava drain, attaches to the right atrium.

As this group of vessels attach to the primary atrium, the left and right atrial appendages begin to grow out of the walls of the primary atrium. The appendages are sacs which form in the muscular walls of the left and right atria. While the right atrial appendage is quite large and expands distally in a continuous path parallel with the right atrium, the left atrial appendage is narrower, and positioned in the superior portion of the left atrial wall. This is because the formation of the left atrium consists of a much larger portion of the primary atrium. The appendages are notably the first appearance of morphological differentiation

between the left and right atria. Known as morphological sidedness, this development is controlled by a pathway governed by the Pitx2 gene (Moorman et al., 2003).

As all of this is occurring, septation of the atria is also progressing. Cardiac jelly found in the edges of the region of the septum begin to fuse into ridges, eventually giving rise to an almost complete atrial septum dividing the primary atrium into the left and right atria. Blood resists entering into pulmonary circulation because the lungs do not become inflated until the baby is born. The foramen ovale is an opening found in the atrial septum. The foramen ovale allows for the blood in the right atrium to shunt into the left atrium, avoiding pulmonary circulation. It is formed by the overlapping of the two bands of the septa, resulting in a unidirectional valve (Bressler, 1990). At birth, the foramen ovale closes, and is represented in the adult heart as the fossa ovalis. Figure 4 shows a drawing of the embryonic heart with the foramen ovale. It is clear from the diagram how the foramen ovale is formed by the overlapping of the two bands of the septa.

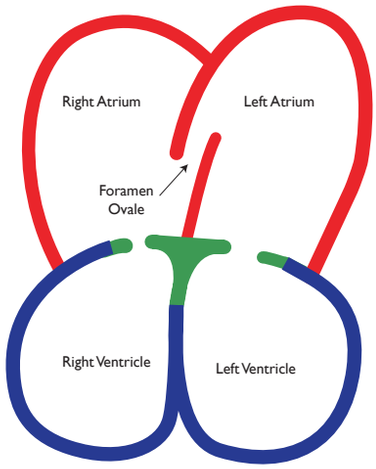

Figure 4. Diagram of Foramen Ovale Shunting Blood from the Right Atrium to the Left Atrium

Simultaneously, development of the interventricular septum is occurring. By the time that heart tube looping finished, the primary interventricular foramen (which connects the left and right ventricles before the interventricular septum develops) becomes noticeable (Moorman et al., 2003). The dextral loop is made up of an inner curve and an outer curve. The outer curve balloons out, extending the ventricular wall and increasing the size of the lumen (Davis, 1927).

Numerous studies have attributed ventricular development to consolidation of myocardial trabeculae found in the primitive ventricular region. Myocardial trabeculae are spongy jagged folds of endocardium running along the circumference of the primary heart tube (Icardo, Fernandez-Teran, 1987). As these jagged edges become more compact and consolidated, the ventricular walls are formed (Moorman et al., 2003). Unfortunately, due to the geometric randomness and inconsistencies of the folding and compacting of ventricular trabeculae, so far no one has been able to successfully produce an accurate and definitive model of the methods involved in ventricular trabeculation (Taber, Perucchio, 2000).

Valvulogenesis

As the heart chambers undergo morphological pathways to attain their final shape, sizes, and orientations, the atrioventricular valves are forming. In the right side of the heart, the atrioventricular canal expands in order for the right atrium to become continuous with the right ventricle. During this process, the muscle tissue found in the right half of the atrioventricular canal becomes integrated with the right atrium, forming the region where the tricuspid valve will develop. In the left side of the heart, the left atrium is already continuous with the left ventricle due to a mechanism which occurs during looping. Similar to the right side, the muscle tissue found in a portion of the atrioventricular canal becomes integrated with the left atrium, forming the region where the mitral valve will develop (Moorman et al., 2003).

The function of the heart valves is to prevent backflow of blood from the ventricles into the atria. The valves are often described as multileaflet structures (Bartman, Hove, 2005). There is an abundance of a gelatinous substance known as cardiac jelly found in between the endocardium and the myocardium. It is believed that as the cardiac jelly in specific regions of the heart begins to swell, endocardial bulges are formed. These cushions are further enhanced by migrating cardiac endothelial cells, changing the cardiac jelly into cardiac mesenchymal cells, causing the endocardial bulges to begin to grow. Once their growth is completed, they are referred to as endocardial cushions (Markwald et al. 1977). These endocardial cushions then proceed through another stage of growth, ultimately forming heart valves made of fibrous tissue. The fibrous tissue found in the heart valves allows for them to open and close as the heart pumps blood throughout its chambers (Bartman, Hove, 2005).

The pulmonary valve and aortic valve have similar functions to the atrioventricular valves. They prevent backflow into the right ventricle and left ventricle, respectively. The mechanism for their morphogenesis is very similar to the growths of endocardial cushions described above in the formation of the atrioventricular valves (Bartman, Hove, 2005).

Development of the Arterial Trunks

Throughout heart development, the aortic sac undergoes changes to become the outflow tracts of the heart. As the distal outflow portion of the original ventricular loop begins to

extend out of the pericardial cavity, it is divided into two, forming the ascending aorta and the pulmonary trunk. The ascending aorta and the pulmonary trunk are two separate tubes, divided by the aorticopulmonary septum. Figure 5 shows a scanning electron micrograph image of the bend formed in the tube. The edge of the distal outflow portion of the original ventricular loop completely extends to the edge of the pericardial cavity. The distal outlet portion of the aortic sac develops into the distal portions of the aorta and its branching arteries, completing the systemic circulation path. The pulmonary trunk extends and attaches to the lungs to complete the pulmonary circulation path (Moorman et al., 2003).

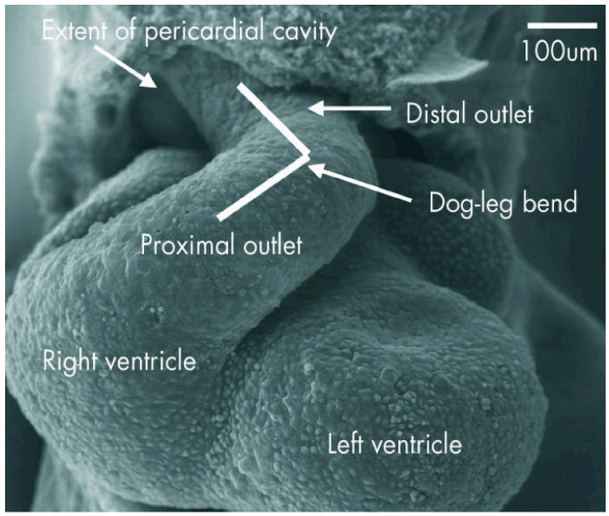

Figure 5. Bending of the distal outflow portion of the ventricular loop (Moorman et al., 2003).

As can be seen in this image, the dog-leg bend produced by the outflow tract helps align the aorta with aortic arches III and IV, and the pulmonary trunk with arch VI (Moorman et al., 2003).

Mechanical Forces Involved in Heart Morphogenesis

When analyzing mechanical factors affecting heart morphogenesis, the initial, and probably most striking piece of data, is that the heart begins to function before it is completely developed. Blood begins to flow through the primitive heart chambers by the time that initial stages of heart looping are completed. This suggests that the actual pumping of the heart, along with other forces produced by blood flowing through the heart chambers, might actually be the cause of later steps of heart morphogenesis (Bartman, Hove, 2005).

One of the accepted mechanisms of heart tube formation involves forces caused by contracting actin microfilaments. The forces caused by the contractions mold and bend the crescent into a tube shape, forming the heart tube (Taber, 1998).

Many theories have been produced to try to link biomechanics with heart looping. As early as 1970, a theory was suggested that looping is caused by differential growth of portions of the heart tube. Differential growth is when cells of the same structure grow at different rates, causing the structure to undergo a physical change of shape and other physical properties (Stalsberg, 1970). Others suggested that heart looping is caused by pressures placed on the heart tube due to the expanding cardiac jelly (Manasek et al., 1984). Later scientists proposed that looping is caused by residual stress which causes the dorsal mesocardium to shorten and bend (Taber, 1995). Although these theories give possible mechanisms for the cause of heart looping, they do not provide a clear explanation for what causes the heart tube to bend in a specific orientation.

Additionally, all of these theories are based on the assumption that the forces controlling heart looping are forces from within the heart tube (Bartman, Hove, 2005). In 2002, an experiment was run to attempt to prove that heart looping is caused by forces placed on the heart tube from external adjacent tissues pressing on the embryo. The splanchnopleure is an extraembryonic membrane which presses against the heart tube on its ventral side. Because heart looping involves ventral bending and rightward rotation, the forces applied by the splanchnopleure assist the heart in rotating in the rightward direction. In an experiment, the splanchnopleure was removed from 24 chick embryos. In all 24 cases, abnormal heart looping was reported, proving that forces applied by the splanchnopleure have a direct effect on heart looping. In most of the cases, slightly skewed ventral bending was reported, along with reports of minimal rightward rotation of the heart tube.

In most ex vivo experiments that analyze heart development, such as this one, conditions required to analyze the growth are dependent on factors which cause surface tension. Under normal in vivo conditions, surface tension forces have not been found to affect heart looping. It is interesting to note that in a parallel experiment, it was found that by placing the chick embryos in a liquid medium and thereby eliminating standard ex vivo surface tension forces, some abnormalities were found with respect to heart looping. This suggests that there are in fact surface tension forces present in vivo which play a role in heart looping (Voronov, Taber, 2002).

Another pathway regulating heart looping begins with secreted proteins flowing through the heart tube. Although very little is known about this pathway and the forces involved, it is known that it in some manner provides the forces required to initiate rightward rotation of the heart tube. This pathway is controlled by the Pitx2 gene, the nodal gene, and the lefty gene (Moorman et al., 2003).

Myocardial trabeculae are known to have many effects on the developing heart. While they are mainly known for their assistance in helping blood flow in the appropriate direction prior to formation of the atrial and ventricular septa, myocardial trabeculae also produce peristaltic contractions within the myocardium. These contractions help the trabeculae to become more compact and to fold along each other, thereby producing the ventricular walls (Thompson et al., 2000). A result of producing and solidifying the ventricular walls is that the intramyocardial blood flow increases. This increase in blood flow will cause an increase in the fluid forces affecting heart morphogenesis, and will also cause these stresses to be more evenly distributed within the portions of the heart upon which they are working (Taber, 1998).

The role of hemodynamics in septation has been debated for many years. The "flow molding" hypothesis suggests that cardiac septa form due to opposite pressure gradients formed by two antiparallel streams of blood flowing through the heart simultaneously (Jaffee, 1963). Scientists who support the flow molding hypothesis believe that the cells lining the heart tube are able to detect changes in pressure caused by different flow velocities and directions. When such changes are detected, morphological changes are induced, causing the septa to form. Using this biomechanical mechanism as a springboard, they also suggested that these fluid forces assist in shaping and molding the cardiac jelly into firm structures in order to form the walls of the heart chambers (Dewey et al., 1981). Furthermore, experiments proved that modifying the fluid velocities in developing chick hearts can result in many heart malformations, including deformed ventricular septum formation (Clark et al., 1989). Challengers of the flow molding theory argue that the whole foundation of the theory is incorrect. They have used advancements in imaging technologies to show that the initial blood flow through the heart chambers does not appear as two antiparallel streams. This data suggests that perhaps septa formation is not at all affected by fluid forces. Possibly, it is the formation of the septa which may actually create and direct the streams of fluid; the heart septa might be the initial cause of formation of certain fluid forces found in the heart (Yoshida et al., 1983).

The frictional force caused by fluid flowing along the surface of a cell is known as shear stress. In the developing heart, shear stress is caused by the blood which is being pumped throughout the developing chambers. Shear stress is known to play a very major role in valve formation. In a study done with zebrafish, reduced fluid forces resulted in complete prevention of valve formation (Hove et. al., 2003).

In a recent study, a positive displacement pump was used to simulate the fluid forces and shear stress produced in the developing heart. Because the experiment focused on valvulogenesis, embryonic chick hearts with endocardial cushions were isolated. They were placed inside a tubular collagen scaffold, and remained there untouched for 72 hours. The purpose of this 72 hour period was to guarantee that the cells adhered to the inside of the tube (Tan et al., 2012). It is known that hemodynamic forces increase as heart development progresses (Biechler et al., 2010). Therefore, the forces applied by the pump were divided into two periods, one of a low force, followed by one of a higher force. Obviously, the actual level of fluid forces and shear stress found in vivo could not be completely replicated, for had these forces been applied, the cushions would have been pushed out of the tubular scaffold. The forces used in the experiment were based on formulas used to determine the highest magnitude of force that the cushions would be able to withstand without being dislodged from the tube. Initially, through the use of a positive displacement pump, a force with a frequency of 5.38 x 10-2 Hz with an average velocity of 0.07mm/s was applied in the tube for a period of three days. The shear stress calculated based on these numbers is 7.6 x 10-4 Pa and a positive pressure of 4.3 x 10-3 Pa. After this three day period, the flow rate was raised to a force with a frequency of 0.29 Hz with an average velocity of 0.86mm/s, and was applied for an additional four days. The shear stress calculated based on these numbers is 9.4 x 10-3 Pa and a positive pressure of 0.053 Pa. The shear stress in the second flow phase is equivalent to a 92% increase. Control groups were set up in tubes where no pump was attached. The control tubes were attached to bioreactors to ensure a closed loop would exist, thereby maintaining comparable oxygen levels and other conditions with the flow tubes.

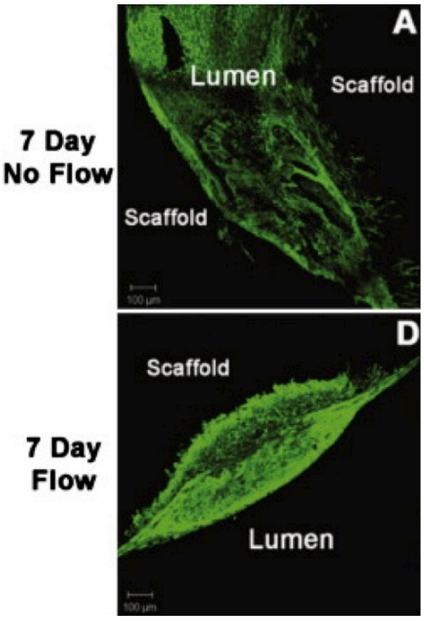

Figure 6. Comparison of flow and no-flow cushions (Tan et al., 2012).

Following the first (days 1-3) and second (days 4-7) phases of pumping forces, the "flow" cushions and the "no-flow" cushions were analyzed. After the first phase, no significant differences were found between the flow and no-flow cushions. However, analysis after the second phase revealed amazing results. The no-flow cushions formed a scattered network of cells in the lumen of the tube. This network was very loose, and did not exhibit signs of the rigidness or compactness normally found in heart valves. In contrast, the flow cushions formed a very compact mass of cells. The mass was wedge-shaped, and had leaflet-like structures extending off of it (Tan et al., 2012). Figure 6 shows confocal laser scanning microscope images comparing the cushions.

A signaling molecule, rhoA was also studied in this experiment. rhoA regulates the stiffening of the AV cushions, a prominent characteristic. The flow cushions were found to be much stiffer than the no-flow cushions. It was found that rhoA message levels were increased by flow forces. In addition to that, it was also reported that inhibiting a downstream effector of rhoA, rhoA coiled-coil containing kinase (ROCK), caused a decrease in stiffness of the fibers in the AV cushions. Figure 7 shows a graph comparing the expression of rhoA in flow and no-flow cushions (Tan et al., 2012).

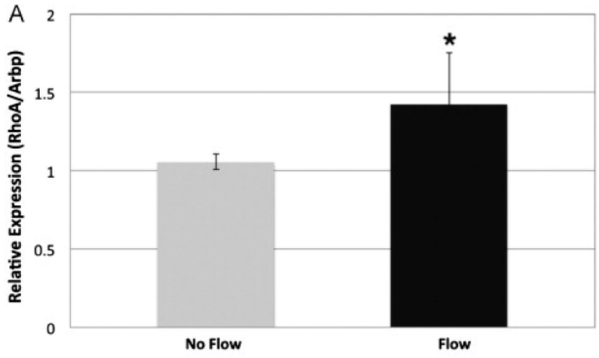

Figure 7. rhoA expression in flow and no-flow cushions (Tan et al., 2012).

This study was a major breakthrough in relating biomechanical forces to heart development. Even though the shear stress and fluid forces applied in this study were significantly below values found in vivo, the cushions and proteins still responded appropriately. The flow cushions still developed into wedge-shaped stiff masses, and rhoA was clearly upregulated.

Discussions

Formation and development of the human heart involve many precise and intricate steps. New studies have analyzed how many of these steps are initiated by mechanical factors. A major element of this hypothesis is that the heart begins to pump blood before it is completely developed. The fluid rushing through the developing heart causes shear stress, flow pressure, and surface tension. With experiments, these forces have been proven to play a role in propagating various stages of heart development, with specific focuses on heart looping and valvulogenesis.

Other mechanical factors, such as contractions and expansion of cardiac jelly, have also been linked to heart development. With regard to all of these forces, studies have been done to show abnormal heart development in experiments where the forces have been eliminated. This provides a strong basis for theories suggesting that these mechanical forces play a major role in heart development.

Because the study of mechanical factors affecting heart development is relatively new, much of the research is still inconclusive. While in many cases it has been proven that mechanical factors play a role in specific stages of heart development, much of the research has not been able to specify exactly which mechanical factors affect which steps of heart development. Additionally, the research done to date has not been able to prove, in many cases, if the mechanical factors involved are causes or effects of certain stages. Specifically with regard to septation, it has been debated for years whether the blood flow paths cause the septation to occur, or is a result of septation. Nonetheless, it has been documented that mechanical factors are definitely present in heart development, and regardless of whether they are the cause or effect of one step, they definitely affect propagation of further steps of heart development.

An interesting facet of studying the role mechanical forces play in heart development is to attempt to link these forces to the genes and genetic pathways which have already been discovered. Due to the relative novelty of studying mechanical forces in heart development, minimal breakthroughs have been made with respect to linking these forces to genetics. Although a few genes have been recognized as working simultaneously with mechanical factors, it is unclear whether these genes are influencing the mechanical factors, or are merely present at the same time and only affecting other, non-mechanical, elements of heart morphogenesis. Future studies need to be performed in order to determine whether or not these mechanical force are linked to genetic factors. If they are proven to be linked, future studies will be necessary to determine to what extent they are linked and to determine the exact mechanisms relating the genes to the mechanical forces. Relating mechanical factors to genetics could lead to major breakthroughs in diagnosing and preventing heart developmental abnormalities.

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