Mechanical factors in the development of the vascular bed

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Abstract

During embryonic development, blood flow is needed not only to nourish the developing embryo but is also important for shaping the vascular network such that it becomes hemodynamically efficient. The first blood vessels form a network called the capillary plexus. After the onset of blood flow, the capillary plexus remodel into a more hierarchical tree-shaped network. Mechanical forces created by blood flow are required for remodelling to occur and these forces are believed to induce a maturation of the blood vessels that stabilizes the growing vascular network. The role of mechanical force has been extensively studied in the mature cardiovascular system. Though the events induced by blood flow during development are thought to be similar to what occurs in the adult, there are several important differences between the embryo and the adult. We therefore discuss what is known about the role of mechanical forces in vascular remodelling from the adult cardiovascular system and highlight how embryonic development differs from the adult. We consider the role of blood flow in altering branching morphology, arterial-venous identity and the formation of the blood vessel wall during early vascular development.

1. Introduction

The heart begins to beat almost as soon as a network of blood vessels forms in the embryo and normal blood flow is required for development of the embryonic vasculature. The developing cardiovascular system is exposed to a whole new set of cues when circulation is established, one of which is the presence of new mechanical forces created by the blood flow. Though hemodynamics, or blood fluid dynamics, are known to activate signalling pathways in the mature cardiovascular system, in events ranging from atherosclerosis to cancer angiogenesis, the role in development has not been as intensively studied.

The two most important mechanical forces created by blood flow are shear stress and circumferential stretch. Shear stress can be thought of as the friction created as the molecules of the fluid move against the vessel wall and is a force which is parallel to the direction of flow. The shear stress (τ) exerted by a fluid can be calculated by multiplying the viscosity of fluid (μ) by the slope of the velocity profile of the blood flow at the vessel wall (dV/dr at $r = R$). The slope of the velocity profile is also referred to as the shear rate (γ).

$$\tau = \mu \left. \frac{dV}{dr} \right|_{r=R} = \mu \gamma$$

Where τ is a force per unit area, μ is a mass per unit time per unit length and the shear rate (γ) has units of inverse time. Circumferential stretch, the second hemodynamic force, is present due to the effect of blood pressure which causes a distension of the vascular wall. For this reason, circumferential stretch acts perpendicular to the blood vessel wall. Changes in the level of either

of these forces are known to induce vascular remodelling in the adult. How mechanical forces affect early vascular development has been studied to a much lesser extent.

Flow is sensed in biological system by transduction of mechanically signals into altered gene expression, using a process called mechanotransduction. When endothelial cells are cultured in the presence of unidirectional steady flow, they alter their cytoskeleton and align in the direction of flow (Dewey et al., 1981). Mechanical forces can be transduced many different ways, from stretch-activated ion channels that provide fast responses to changes in blood flow, to more long-term changes associated with cytoskeleton rearrangement and integrin activation (Tzima et al., 2001). There are multiple lines of evidence indicating that biomechanical forces are required for proper vascular development as well, but the mechanism by which these forces translate into morphological events is poorly understood. Embryonic development is fundamentally different from vascular remodelling in the adult since many of the cell types which play a role in the adult have not yet differentiated when the embryo is undergoing vascular remodelling. Though we can gain some understanding of the role of biomechanical forces from the work in mature cardiovascular system, we need to understand how embryonic development differs since from this knowledge we can infer the minimum requirements for flow-induced remodelling of a vascular bed.

2. Vascular Development and Remodelling

The first cardiovascular cells form in the embryo shortly after gastrulation with the emergence of vascular endothelial growth factor receptor 2 (VEGF-R2) positive cells from the primitive streak.

These cells migrate towards the proximal yolk sac (Ferkowicz et al., 2003) and differentiate into a structure called the blood islands that is composed of both endothelial and hematopoietic cells. The endothelial cells of the blood islands expand to cover the entire yolk sac, forming the earliest vascular network, known as the capillary plexus. At the same time, a second site of vascular development arises in the embryo proper. Angioblasts, the precursors of endothelial cells, differentiate along the embryonic midline through signalling from the somites and the notochord (Fouquet et al., 1997; Lawson et al., 2002). These angioblasts migrate towards the midline and fuse into an angioblastic cord that becomes the dorsal aorta. A complete vascular loop is therefore present by 3-4 somites in mouse embryos (embryonic day 8.0 or E8.0, Table I), the same stage at which the embryonic heart begins to contract. Though a complete vascular network is present, erythroblasts remain confined to the blood islands. The entry of erythroblasts into circulation begins at 5 somites in mouse embryos with the first erythroblasts circulating in an intermittent fashion (Lucitti et al., 2007; McGrath et al., 2003). By 8 somites (E8.5), a continuous circulation of erythroblasts is present (Lucitti et al., 2007). Early vascular development occurs in a similar manner in other model organisms such as chick and zebrafish, but the model systems differ significantly with regards to the timing of events during early vascular development (Table I).

Once erythroblast circulation is established, the vascular network undergoes a remodelling process (Figure 1). The vessels of the capillary plexus are of fairly uniform diameter and interconnect randomly to form a honeycomb-shaped network. During remodelling, this plexus transforms into a hierarchical branching structure with visible arteries and veins. Though this second stage of vascular development is often referred to as angiogenesis, angiogenesis implies

only the sprouting of new vessels from existing vessels and vascular remodelling is a much more complex process than this. By time-lapse microscopy, it is possible to observe both the sprouting of new vessels but also the regression of existing vessels during the remodelling period (unpublished data in quail, chick and mouse). Vessel regression during embryonic development has been correlated to those vessels which carry little or no flow (Clark, 1918). The process of vascular remodelling also includes events that lead to the maturation of vessels. The dorsal aorta is present before blood flow begins but the large vessels of the capillary plexus, such as the vitelline artery, arise through the remodelling process. Smooth muscle cells and pericytes differentiate and migrate to encircle the blood vessels during vascular remodelling, thereby initiating the formation of the blood vessel wall components. In this way, vascular remodelling not only alters branching patterns and vascular density (angiogenesis) but also induces the structural changes that differentiate arteries, veins and capillaries.

Signals from blood flow are essential for vascular remodelling. If flow is stopped, either through physical manipulation or by ablating genes necessary for cardiac contraction, vascular development arrests at the capillary plexus stage (Chapman, 1918; Wakimoto et al., 2000). Altering or ablating blood flow affects several important physiological signals including mechanical cues, oxygen concentrations and nutrient delivery. We recently showed that mechanical cues are essential for vascular remodelling in the mouse (Lucitti et al., 2007). As mentioned, erythroblasts are confined to the blood islands for a period of time after the heart begins to contract. During this period of time, the blood plasma, devoid of any erythroblasts, flows through the plexus (Lucitti et al., 2007). The main component that makes blood viscous is the red blood cells and blood plasma alone is of low viscosity. We therefore created a situation

of low shear stress during vascular remodelling by preventing the entry of red blood cells into circulation. This was achieved by polymerizing a gel around the blood islands and physically cutting them off from circulation while still allowing blood plasma to flow normally through the capillary plexus and embryo proper. We found that when erythroblast circulation is prevented, vascular remodelling is inhibited (Figure 2). Though nutrients and signalling molecules delivery would be normal in this situation, both mechanical forces and oxygen delivery are impaired. To separate the role of the red blood cells in oxygen transport from its role in biomechanics, we next took embryos in which we had sequestered erythroblast in the blood islands and injected a starch into circulation that makes the blood plasma viscous in the absence of erythroblasts. Embryos treated in this way underwent vascular remodelling normally, indicating that it is the presence of a viscous fluid, and not the erythroblasts themselves, which is required for normal vascular remodelling.

When blood flow begins in the embryo, the embryo is still small enough for oxygen requirements to be met through diffusion alone (Burggren, 2004). Carbon monoxide binds irreversibly to haemoglobin and can ablate the oxygen-carrying ability of red blood cells. When fish (Pelster and Burggren, 1996), frog (Territo and Burggren, 1998), chick (Ciotto and Arangi, 1989) or mouse embryos (unpublished data) are cultured in the presence of 1-2% carbon monoxide during the first day that blood circulation is present, carbon monoxide has no effect on embryonic or cardiovascular development. Furthermore, when oxygen consumption is measured in carbon monoxide-treated zebrafish or frog embryos, no change in consumption is observed (Pelster and Burggren, 1996; Territo and Burggren, 1998). This led to the hypothesis, termed prosynchronotropy, that blood flow begins before it is required to nourish the tissues of the

developing embryo and remove waste (Burggren and Territo, 1995). This idea has important implications. According to this theory, the role of blood flow during the first day after the onset of circulation is to mould the developing cardiovascular system, such that transport of oxygen and nutrients is hemodynamically efficient by the time it is required for growth.

3. Biomechanical Effects on Luminal Diameter

In the adult vasculature, it has been shown repeatedly that changes in shear stress levels increase vascular diameter (Kamiya and Togawa, 1980; Zarins et al., 1987) whereas changes in blood pressure alter the thickness of the vessel wall (Levy et al., 1988; Wolinsky and Glagov, 1969). A similar theory was proposed during embryonic development by Thoma over a hundred years ago in his *Histomechanique Principien* (Thoma, 1893). He proposed that increases and decreases in flow regulated vessel diameter, that modifications in vessel length were dictated by longitudinal tension on vessels, and that the wall thickness was dependent upon blood pressure.

When changes in the levels of shear stress occur in the adult, blood vessels quickly alter their luminal diameter in order to normalize shear stress levels. The amount of shear stress through a given blood vessel can be calculated by Poiseuille's law, which states that:

$$\tau = \frac{4\mu Q}{\pi R^3}$$

Where τ is the shear stress, μ is the viscosity, Q is the volumetric flow rate and R is the vessel radius. Because radius is raised to the third power, small changes in vessel radius have tremendous effects on the level of shear stress present in a given vessel and changes in diameter

are the principle way in which blood vessels normalize shear stress. The endothelium is responsible for sensing the level of shear present. In the adult, if a vessel is denuded of endothelial cells, the luminal diameter of blood vessels does not change in response to chronic changes in shear stress (Langille and O'Donnell, 1986). Increased shear stress leads endothelial cells to produce vasodilators such as nitric oxide which reduce vascular tone and increase luminal diameter. Reduction in shear stress, on the other hand, results in the expression of vasoconstrictors such as endothelin-1. The change in vascular tone is almost instantaneous but it is also reversible. If changes in shear stress levels are chronic, however, a remodelling of the blood vessel wall will occur that permanently alters the luminal diameter (Langille et al., 1989).

Though smooth muscle cells play an active role in vascular adaptation to shear stress in the adult, they are not required for flow-induced remodelling to the luminal diameter. Embryos undergoing vascular remodelling are devoid of smooth muscle cells and yet embryonic blood vessels still adapt their luminal diameter to chronic changes in the flow rate. The formation of the vitelline arteries occurs within hours of the onset of blood flow during development, but smooth muscle cells only begin to differentiate and surround blood vessels approximately one day after the onset of blood flow both in mice and chick embryos. Furthermore, ligation of one vitelline artery results in increased flow through the contra-lateral vitelline artery such that the contralateral begins to enlarge within as little as 5 hours (Buschmann et al., 2010). Therefore, it is generally believed that smooth muscle cells play a supporting role rather than an instructive role during vascular remodelling in response to changes in shear stress.

The mechanism by which changes in lumenal diameter become entrenched appears to largely been driven by nitric oxide signalling in the adult. Mice which lack endothelial nitric oxide synthase (eNOS) fail to undergo chronic remodelling after changes in blood flow (Rudic et al., 1998). Though nitric oxide is paramount to vascular remodelling in the adult, its role during embryonic development is confusing at best. Decreasing shear stress levels just after the onset of blood flow in the embryo impedes endothelial nitric oxide synthase (eNOS) expression (Lucitti et al., 2007). There also appears to be gestational losses in eNOS^{-/-} litters during the period of vascular remodelling (Pallares et al., 2008), but these losses represent a fraction of the litter and many knock-out animals do survive until birth (Feng et al., 2002). Furthermore, the triple knockout of all three isoforms of NOS is viable though again, some gestational losses are present within a litter (Morishita et al., 2005). These results imply that nitric oxide is not necessary for vascular remodelling during embryonic development. Contradicting this, however, is work showing that addition of the general NOS inhibitor L-NMMA (NG-monomethyl-L-arginine) to mouse embryo in culture between E8.0 and E9.0 results in a failure of vascular remodelling and embryonic lethality (Nath et al., 2004). Control experiments with D-NMMA showed no inhibition of vascular remodelling and no embryonic lethality. It is possible that nitric oxide enhances but is not necessary for vascular remodelling during development, contrary to what is seen in mature cardiovascular networks. This raises the question, however, as to what the important modulator of shear-induced remodelling is during embryonic development if not nitric oxide.

The mechanism by which vessels change diameter during embryonic development is poorly understood. As the vasculature remodels just after the onset of blood flow, surprisingly low

levels of endothelial replication are present in mouse embryos, with only 2-4% of endothelial cells staining positive for phospho-histone3 (unpublished data). Vascular development was recently observed by time-lapse microscopy using transgenic quail which express a nuclear-specific yellow fluorescent protein (YFP) driven by an endothelial-specific promoter, allowing individual endothelial cells to be tracked (Sato et al., 2010). These studies showed that extensive cell migration are present after the onset of blood flow (personal communication Charles Little & Rusty Lansford). Large vessels appear to grow by incorporating endothelial cells from regressing vessels or by the fusion of many smaller vessels into one vessel (often termed reverse intussusception). The regression of smaller vessels also appears to shape the arterial-venous network; when small capillary branches disconnect from the arterial network, they reconnect to venous cells and begin to express venous markers (le Noble et al., 2004). Therefore, vascular remodelling in the embryo appears to largely represent a re-shuffling of the endothelial cells and not the proliferation/apoptosis as new vessels sprout and regress.

4. The Effect of Flow on Arterial-Venous Differentiation

Vascular remodelling not only changes the luminal diameter of vessels but also induces the differentiation of the morphological characteristics of arteries and veins. This differentiation was initially believed to be controlled solely by mechanical factors but more recent discoveries have shown that certain genes that are specific either to arteries or to veins are expressed before the onset of blood flow (Wang et al., 1998). Neuropilin-1, EphrinB2 and Uncoordinated-5b (Unc5b) are specifically expressed in arteries, whereas genes such as Coup-TFII (Chicken ovalbumin upstream promoter transcription factor II), Neuropilin-2 and EphB4 are expressed in veins. Many

of these proteins which are differentially expressed by arteries and veins were initially identified from work on growth cone guidance in the nervous system but later found to have a role in cardiovascular development. They fall into four main families of neurovascular guidance proteins; Slit/Robos, Uncoordinated(Unc)/Netrins, Semaphorins/Plexins/Neuropilins and Eph/Ephrins. During sprouting angiogenesis, these proteins have a clear role in guiding sprouting vessels since genetic ablation of many of these genes results in abnormal vascular density (Hellstrom et al., 2007; Kawasaki et al., 1999; Lobov et al., 2007; Lu et al., 2004; Suchting et al., 2007) or ectopic vessel formation (Gitler et al., 2004; Gu et al., 2005; Helbling et al., 2000; Torres-Vazquez et al., 2004). Their expression is not limited to sprouting vessels, however, and their role in morphologically distinct arteries and veins is not known.

The molecular differentiation of arteries and veins appears to be largely controlled by the Notch signalling pathway. During the formation of the primary embryonic axial artery and vein in zebrafish, the notochord produces Sonic Hedgehog (Shh) which induces the somites to produce Vascular Endothelial Growth Factor (VEGF) leading to a dorsal/ventral gradient of VEGF (Lawson et al., 2002). VEGF activates the Notch pathway in more dorsal endothelial cells and activation of the Notch pathway leads to expression of a series of arterial specific genes, the first of which to be expressed is EphrinB2 (Lawson et al., 2001). Overexpression of Shh leads to ectopic expression of EphrinB2 in vessels which should be veins (Lawson et al., 2002). Ablation of Shh signalling during vascular development abolishes EphrinB2 expression in arteries. Though Shh from the notochord explains arterial-venous patterning in the embryo proper, most of the blood vessels in the early embryo are located in extra-embryonic tissue and not exposed to these signals. The expression pattern of EphB4/EphrinB2 demonstrates a very clear demarcation

of the arterial-venous boundary in the yolk sac before the onset of blood flow (Wang et al., 1998). Neither differential expression of VEGF nor an arterial-specific source of Shh has been shown to exist in the early yolk sac. This highlights the possibility that other signals can induce arterial-venous gene expression.

Though early endothelial cells are pre-specified as arteries or veins, their fate remains plastic. If arterial endothelial cells are transplanted into a vein, they will change their gene expression based on the new environment and they begin to express venous marker (Moyon et al., 2001). Similarly, if venous endothelial cells are placed in an artery, they begin to express arterial markers. Though signalling from neighboring cells could induce these changes, it is more likely that the biomechanical signals from flow cause the change from arterial to venous gene expression. Ligation of the vitelline artery to redirect blood flow in the yolk sac causes arterial blood vessels adjacent to the ligation to be exposed to venous blood flow patterns (le Noble et al., 2004). In this situation, arteries ceased to express arterial genes such as EphrinB2 and begin expressing venous genes such as EphB4.

Shear stress can activate the Notch signalling pathway in endothelial cells and therefore many research groups are investigating whether the plasticity of arterial-venous fate arises from shear-induced Notch expression. Endothelial cells of primitive capillary plexus of mouse embryos are exposed to shear stress levels between 1-5 dyn/cm² (Jones et al., 2004). Exposing bone-marrow derived endothelial progenitor cells (EPCs) to laminar shear stress between 0.1 and 2.5 dyn/cm² induces expression of the Notch pathway including *hey1*, *hey2*, *notch1* and *notch3* (Obi et al., 2009). Laminar shear stress has also been shown to increase both the expression of EphrinB2

and of Notch1 in VEGFR2+ cells derived from embryonic stem cells (Masumura et al., 2009). Inhibition of the Notch pathway prevented shear-induced EphrinB2 expression indicating that Notch is upstream of EphrinB2 in this system. Furthermore, shear stress increased expression of EphrinB2 in VEGFR2+ cells derived from embryonic stem cells to the same extent as exposure to VEGF.

Endothelial cells in the adult are known not only to be sensitive to presence of flow but also the type of flow to which they are exposed. Though laminar shear stress can activate Notch and EphrinB2 in endothelial cells derived from EPCs and from embryonic stem cells, it fails to activate EphrinB2 when more mature endothelial cell types are used (Buschmann et al., 2010; Goettsch et al., 2004; Korff et al., 2008). Arteries are exposed to pulsatile blood flow and not constant velocity laminar flow. The pulsatile nature of blood flow diminishes throughout the vasculature and disappears by the time it reaches the veins, where velocities change very little between systole and diastole. Buschmann et al. recently showed that exposing HUAECs (human umbilical arterial endothelial cells, which are representative of endothelial cells from an arterial source) to pulsatile but not to flow of constant velocity induced the expression of arterial genes such as *hey1*, *hey2*, *ephrinB2* and *neuropilin1* (Buschmann et al., 2010). In contrast, exposing HUVECs (human umbilical vein endothelial cells, representative of endothelial cells from a venous source) to pulsatile flow had no effect on the expression level of venous genes, but exposing them to constant velocity flow increased the expression of the venous marker Coup-TFII (Chicken ovalbumin upstream promoter transcription factor II). Coup-TFII is believed to maintain venous identity by repressing the Notch signalling pathway (You et al., 2005).

The level of shear stress and the pulsatile nature of blood flow is not the only hemodynamic difference between arteries and veins. Blood pressure is high in arteries and almost non-existent in veins. In a femoral artery ligation model, Korff et al. found that EphrinB2 expression was increased in the femoral artery proximal to the site ligation (Korff et al., 2008). They found no change in VEGF expression in the blood vessels being studied. Ligation decreases flow and shear stress through the femoral artery but increases blood pressure in that vessel. They therefore investigated whether increased stretch on endothelial cells affect EphrinB2 expression levels and found that exposure of HUVECs to cyclic stretch (15%, 0.5 Hz) for 3 to 18 hours increased both the levels of protein and mRNA for EphrinB2.

There is an increased level of interest in understanding how an endothelial can be defined as an arterial or venous endothelial cell at a molecular level. The involvement of many of these arterio-venous genes in sprouting and angiogenesis make them attractive therapeutic targets. Though the Notch pathway is clearly of interest in determining how flow can affect arterial-venous identity at a molecular level, studies into the effect of flow on this pathway are recent and further work is required to decipher the mechanism by which biomechanics can alter arterial-venous identity in endothelial cells.

5. Vessel Wall Development and the Effect of Blood Pressure

As large diameter vessels form and arteries and veins differentiate, the recruitment of mural cells to the vessel wall is essential to stabilize the vascular network. Both extra-cellular matrix proteins and mural cells such as pericytes and vascular smooth muscle cells (VSMCs) define

vessel characteristics including elasticity, vascular tone and control of peripheral resistance (Hungerford and Little, 1999). If blood flow is stopped, smooth muscle cell recruitment, measured by the presence of α -smooth muscle actin (α -SMA) positive cells around blood vessels, is inhibited (unpublished data). These results alone do not necessarily prove that flow is necessary for smooth muscle cell recruitment since there is a general inhibition of vascular development in the absence of flow. In fact, when embryoid bodies are allowed to differentiate, they will produce cells that are α -SMA and calponin positive even though flow is never produced by embryoid bodies. These presumptive smooth muscle cells do not, however, mature to produce smooth muscle- myosin heavy chain (SM-MHC) positive cells (Gerecht-Nir et al., 2003). Though the initial differentiation of smooth muscle cells is possible in the absence of flow, formation of a proper vessel wall consists not only of differentiation but also migration of the presumptive smooth muscle cells and production of basement membrane. Several lines of evidence support the idea that flow-induced signals are required for proper assembly of the vascular wall.

During development, smooth muscle cells originate from two distinct locations. The smooth muscle cells of the ascending aorta and the branchial arch arteries are of cranial neural crest origin (Le Lievre and Le Douarin, 1975) while the remainder of smooth muscle cells are of mesodermal origin. Vascular smooth muscle cells first appear as aggregates of mesodermal cells near the vessel and are described as “fibroblast-like” (reviewed in (Drake et al., 1998). By transmission electron microscopy, a mesodermal cell layer can be observed surrounding endothelial cells which are believed to represent smooth muscle cell precursors (Murphy and Carlson, 1978). The study of smooth muscle cells differentiation has been limited by the lack of

definitive markers of presumptive smooth muscle cells. Most smooth muscle genes are expressed at least transiently during skeletal and/or cardiac muscle development (Li et al., 1996; Miano and Olson, 1996; Ruzicka and Schwartz, 1988; Samaha et al., 1996). Though the initial differentiation is poorly understood, by E9.0 in mouse and Hamburger Hamilton Stage 15 in chick, α -SMA positive cells can be observed surrounding larger blood vessels (unpublished results). As the presumptive smooth muscle cells associates with endothelial cells, they produce the extracellular matrix that defines a vessel's elasticity and material properties (Mecham et al., 1991; Tan et al., 1991). This appears to be their main function until quite late in development (embryonic day 18 out of a gestational period of 21 days in chick embryos), at which point these cells convert from a synthetic to a contractile phenotype (Hungerford and Little, 1999).

Rather than shear stress, work from adult cardiovascular system links blood pressure and cyclic stretch to the development of vessel wall characteristics. If VEGFR2⁺ cells derived from ES cells are exposed to shear stress, they differentiate into endothelial cells (Yamamoto et al., 2005). If they are exposed to cyclic stretch, on the other hand, these cells differentiate into smooth muscle cells, expressing α -SMA and SM-MHC (Shimizu et al., 2008). Activation of the receptor for platelet derived growth factor-B (PDGF- β R), is required for smooth muscle cell proliferation during development (Hellstrom et al., 1999). Cyclic stretch induces the phosphorylation of PDGF- β R independently of its ligand, platelet derived growth factor-B, since conditioned media from cells exposed to stretch does not prevent phosphorylation of the receptor nor does exposing these cells to cyclic stretch in the presence of blocking antibodies to platelet derived growth factor-B (Shimizu et al., 2008).

Though cyclic stretch appears to be the main force affecting smooth muscle cells in the adult, it is not clear that this force is even present when smooth muscle cells begin to differentiate during development. Blood pressure is quite low during vascular remodelling. Systolic arterial pressure is around 1-2 mmHg in the embryo as compared to 120 mmHg in the adult (Keller et al., 1991). Elastogenesis has not begun during vascular remodelling and the extracellular matrix surrounding blood vessels is primarily composed of fibronectin (Risau and Lemmon, 1988). Cyclic stretch, as measured by distensibility of a vessel, is only present in the dorsal aorta of chicken embryos at the 30-33 somite stage (Buschmann et al., 2010), a stage at which many vessels of the embryonic and extraembryonic vascular network are already surrounded by α -SMA positive VSMCs. Therefore, it is possible that shear stress and not cyclic stretch is important for smooth muscle cell recruitment during development. Several known mitogens for smooth muscle cells can be upregulated by shear stress, including transforming growth factor- β (TGF- β), basic fibroblast growth factor (bFGF) and platelet derived growth factor B (PDGF-B) (Mack et al., 2009; Malek et al., 1993). If VSMCs are cultured in media which has been conditioned by endothelial cells exposed to flow patterns that are similar to those present in vessels undergoing arteriogenesis, they increase their expression of calponin, myocardin and α -SMA (Mack et al., 2009). Genetic ablation of Kruppel-like factor 2 (KLF2), a transcription factor for many mechanotransduced genes, results in impaired smooth muscle recruitment. KLF2 expression is restricted to the endothelium and upregulated by shear stress but not by cyclic stretch (Dekker et al., 2005), indicating a role for endothelial cell derived signals in VSMC recruitment (Kuo et al., 1997). Therefore, formation of the vessel wall, as opposed to thickening of the vessel wall, may be initiated by shear stress rather than cyclic strain during

development. Current evidence, however, is extremely limited and further studies are required to understand the role of mechanical forces in the formation of the blood vessel wall.

6. Conclusions

The developing cardiovascular system is a unique system in which to study the effect of biomechanical forces on the cardiovascular system. It is a more minimal system with fewer cell types than mature vascular networks. Cardiovascular remodelling in the adult is known to involve smooth muscle cells but embryonic blood vessels are devoid of smooth muscle cells early in development and even once these cells are present, they exhibit a synthetic and not a contractile phenotype until later in development. Furthermore, multiple organ systems can affect cardiovascular morphology in the adult. The kidney produces renin which controls blood pressure. The autonomic nervous system can alter flow patterns and vascular tone. The endocrine system can also release a multitude of hormones and signalling factors that affect cardiovascular physiology. Therefore, the embryo presents itself as a simplified *in vivo* system compared to the adult and for this reason, understanding the process by which flow induces maturation of the embryonic vessels is essential.

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Figure Legend

Figure 1 – The Capillary Plexus Remodels into a Hierarchical Vascular Network. Antibody staining against CD31 (PECAM-1), an endothelial cell specific antibody, is used to highlight changes in the vascular network during embryonic development. The vasculature first forms as a capillary plexus (A). Through vascular remodelling, the morphological characteristics of arteries and veins appear and the endothelial cells of larger arteries align in the direction of flow, resulting in a hierarchical network of blood vessels (B). Scale bar: 100 μm .

Figure 2 – Vascular Remodelling Depends on Blood Viscosity. The vasculature was highlighted by injection of Texas-Red-dextran (A, D, G) in mice that expressed green fluorescent protein (B, E, H) in their erythroblasts. Circulation of erythroblasts was prevented by caging the erythroblasts in the blood islands before the onset of circulation (D-F). Vascular remodelling was inhibited and endothelial nitric oxide synthase (eNOS) was downregulated (C,F,I). Viscosity could be returned to more normal levels by the injection of a viscous starch solution in the absence of red blood cells (G-I). Remodelling and eNOS expression could be restored even without circulating erythroblasts. Scale bar: 500 μm & 20 μm . Reprinted with permission from Lucitti & Jones, et al., 2007.

Table I

Table I – Comparison of Vascular Development in Model Systems

	Human	Mouse	Avian	Zebrafish
Differentiation of angioblastic cells	19/20 days Carnegie Stage 8	E7.0 ¹	19-22 hours of incubation ⁴ HH5	6 hpf ⁵
Formation of dorsal aorta		E8.0 (3 somites) ²	29-33 hours (7 somites) ⁴ HH9	16-18 hpf ⁵
Onset of heart beat	22/23 days Carnegie Stage 10	E8.0 (3 somites)	33-38 hours (11 somites) HH10	24 hpf ⁵
Initiation of circulation		E8.5 (5-8 somites) ³	40-45 hours (14 somites) HH11	30 hpf ⁵

* E0.0 embryonic day; hpf hours post-fertilization; HH Hamburger Hamilton Stage. ¹ (Ferkowicz et al., 2003); ² (Iida et al., 2010); ³ (Lucitti et al., 2007; McGrath et al., 2003); ⁴ (Pardanaud et al., 1987); ⁵ (Baldessari and Mione, 2008)

Figure 1
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Figure 2
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