

The **developing** heart

The way in which blood flow dynamics can cause malformations in developing hearts is not well understood at present. **Dr Sandra Rugonyi** is using her expertise in engineering and computational modelling to investigate the haemodynamic origins of congenital heart disease

Firstly, what are the major goals of your project?

Blood flow during early embryonic development is essential for proper cardiac formation. Blood flow generates forces in the cardiac walls that determine the mechanical environment in which cardiac tissues develop. Cardiac genetic programmes are modulated by these mechanical forces so that cardiac cells are constantly adjusting to the growing

needs of the developing embryo, ensuring proper development under diverse conditions.

However, when haemodynamic forces are abnormal because of anomalous blood flow conditions through the embryonic heart, maladaptations can also occur and cardiac malformations develop. This is a fascinating area of research, not only because it implies that genetics do not completely determine who we are, but also because research in

this area will help us understand the very fundamentals of cardiac development and adaptation. Our goal is to understand how blood flow influences cardiac growth and development, understanding that might one day allow us to prevent congenital heart disease (CHD), and treat CHD babies more effectively.

How is your team hoping to gain a better understanding of the underlying mechanisms by which blood flow dynamics affect the behaviour of embryonic cardiac cells?

Using chicken embryos, we are studying changes in cardiac cells and tissues in response to altered blood flow conditions during early stages of embryonic development. The idea is to capture early modifications that will then lead to malformations, well before CHD is apparent. This approach allows us to investigate possible mechanisms that act in the process of cardiac malformation, and whether early cardiac tissue alterations are reversible. To study cardiac development, we are using a combination of biological, mechanical and computational methods.

In what ways do your own skills and experiences enable you to engage successfully with this project?

My formal scientific background is in engineering, and in particular mathematical modelling and computational mechanics. While not the experience you would expect for someone working on cardiac development and congenital heart disease, it is a vital area of expertise in the field. For a cardiac biologist the mechanical forces that influence the way in which the heart develops and grows might be difficult to quantify, but a computational mechanical engineer has the knowhow to develop tools to quantify these forces, which are essential for cardiac development.

Of course, it is also necessary to look at the biological changes in response to these forces during development, including changes in tissue composition and cell behaviour.



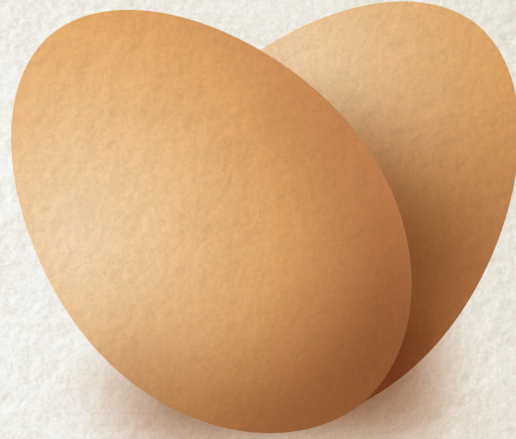
However, without proper quantification of the forces believed to be at the 'heart' of the matter, only half of the story can emerge. Thus, my expertise in computational mechanics and more recently image analysis, is enabling a deeper understanding of how mechanics affects the development of the heart.

Could you comment on your success to date?

For the first time, our group has reconstructed (from images) and modelled (computationally) the beating motion of the embryonic developing heart. These models enable detailed quantifications of blood flow velocities, tissue deformations and wall shear stresses, which stimulate cardiac growth and development. Importantly, wall stresses cannot be measured directly, and thus modelling is an essential component of studies to determine how mechanics and blood flow affect cardiac development. Presently, our group is the only one that has achieved computational, dynamic models of cardiac beating during development.

What are your research plans for the coming years? What might draw you closer to assembling the pieces of the heart defect 'puzzle'?

We plan to continue advancing our fascinating multidisciplinary project on cardiac development and investigating factors that lead to detrimental cardiac development during embryonic stages and beyond. We hope to expand the stages that we are studying and extend our investigations to the whole cardiovascular system, assessing possible reversibility of detrimental cardiac modifications as well as 'safe' cardiac adjustment limits. We also plan on expanding our suite of techniques to study the chick embryo cardiovascular system at disparate organisational levels (tissue, cellular, subcellular, molecular) including the use of multiscale imaging and modelling. Putting together the pieces of the heart defect puzzle will require extensive multidisciplinary collaborations, and we will continue to welcome such collaborations wholeheartedly.



Getting to the heart of cardiac defects

To understand more about how congenital heart defects form in unborn babies, a group at **Oregon Health & Science University** are using chick embryos to investigate dynamic changes in the developing heart

CONGENITAL HEART DISEASE (CHD) is characterised by one or more defects or malformations of the heart, such as cardiac valve or chamber anomalies, at the time a baby is born. About 1 per cent of babies in developing countries are born with the disease. While there have been tremendous advances in the treatment of patients – mainly due to the availability of new technologies resulting in more efficient interventions and procedures – CHD is still the leading non-infectious cause of infant and childhood death in developed countries. Cardiac defects originate during embryonic development, and are usually detected early during pregnancy. Babies and children with this condition typically undergo several surgical procedures to repair the defects, often soon after birth. While many children with heart defects survive into adulthood, their hearts can fail prematurely as their repaired systems wear out. As such, CHD represents an emotional and economic burden for both families and society.

BLOOD FLOW DYNAMICS

Although CHD is frequently associated with genetic anomalies that run in families, recent studies have shown that only a small percentage of heart defects can be unequivocally attributed to abnormalities in genes. The biochemical and mechanical environments in which the embryo and heart develop also play an important role in cardiac development. Among such environmental factors, blood circulation is emerging as a crucial determinant of heart formation: blood flow is essential for proper

cardiac development, and abnormalities in circulatory conditions during early embryonic stages have been shown to lead to cardiac defects. Altered blood flow can result from placental anomalies, poor nutrition on the mother's part or poorly constructed blood vessel networks. Furthermore, once a defect is present, the dynamics of blood flow in the baby's heart are distorted, and this leads to further complications.

However, the mechanisms by which blood flow dynamics cause cardiac malformations during development are not well understood and more work is needed here in order to eventually develop novel prevention strategies, innovative ways to correct abnormal conditions as they arise, and better surgical strategies for both infant and adult CHD patients.

CHICKEN EMBRYOS

One multidisciplinary research group currently focusing their investigations on the effects of blood flow on cardiac formation is based at Oregon Health & Science University (OHSU) in the US. Led by Dr Sandra Rugonyi, the team from the Biomedical Engineering Department and the Heart Research Center is using chicken embryos to study the influence of blood flow conditions on early stages of cardiac embryonic development: days three to six of incubation out of 21 days to hatching. Chicken embryos are an ideal animal model to study heart development. Genetic processes are highly conserved among vertebrate species, so that lessons learned from chicken embryos are likely to be directly applicable to

INTELLIGENCE

DYNAMIC CHANGES IN THE CHICK DEVELOPING HEART IN RESPONSE TO ALTERED HAEMODYNAMICS

OBJECTIVES

To unravel the mechanisms by which haemodynamic conditions modulate cardiac development at early embryonic stages. The current focus is to elucidate early cardiac modifications to altered haemodynamic conditions, and whether those early modifications can be reversed or if they set the heart in permanent detrimental paths that lead to congenital heart defects.

KEY COLLABORATORS

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DR SANDRA RUGONYI obtained her MS and PhD degrees in Mechanical Engineering from the Massachusetts Institute of Technology (MIT) in Cambridge, USA in 2001. Her background in computational mechanics has laid the foundation for her work on mechanobiology, which studies the response of cells to mechanical stimuli. In 2005, she joined the Biomedical Engineering Department at OHSU, where she started building her cardiac development programme looking at haemodynamic origins of congenital heart disease. Her main research interests include the analysis of how blood flow, biotransport and mechanics affect tissues. Rugonyi uses experimental data, together with mathematical and computational models that describe the tissue's biomechanics and biochemistry, and its responses to these conditions.

embryonic human hearts. Additionally, using non-mammal species such as chickens avoids the limitations presented by mammal species, in which many detailed physiological studies need to be conducted by first anaesthetising the mother and then removing the embryos. Chicken embryos are easily accessible for studies since all that is involved is the removal of a small piece of egg shell.

TRULY MULTIDISCIPLINARY RESEARCH

Rugonyi's background is in computational mechanics, and this has underpinned her work on mechanobiology, an emerging field that focuses on the way physical forces and changes in cell or tissue mechanics contribute to development, physiology and disease. She joined the Biomedical Engineering Department at OHSU in 2005, where she began to build her cardiac development programme, using her engineering and modelling expertise to look at the haemodynamic origins of CHD.

The ultimate goal of the project is to understand how mechanics modulate cardiac development and to be able to predict the effects of this modulation. One aspect that the group is hoping to clarify is whether early cardiac changes in response to haemodynamics are reversible or permanent and, if reversible, at what point they become permanent. This knowledge will enable early detection of CHD, as well as the development of novel strategies to treat the disease in babies, possibly reversing detrimental cardiac modifications. If we can predict how blood flow will affect cardiac growth, we can develop optimal strategies to intervene in babies suffering from CHD.

MEASUREMENTS, IMAGING AND MODELLING TECHNIQUES

Rugonyi is utilising a combination of physiological measurements, imaging techniques (*in vivo* imaging and immunohistochemistry) and computational modelling. The physiological measurements consist of blood pressure data and blood flow velocities. Blood pressure is measured in the embryos invasively using a servo-null system, in which a micropipette is inserted in the blood vessels of interest (including the heart) and pressure signals obtained.

The team further uses state-of-the art imaging of the developing heart for their studies. *In vivo* imaging techniques include ultrasound and optical coherence tomography (OCT), both of which enable scientists to view the beating heart and reconstruct its beating motion. Furthermore, these techniques allow measurement of flows using Doppler velocimetry, and thus have been used to determine blood flow velocities in the developing heart. These *in vivo* imaging modalities are complemented by immunohistochemistry on fixed cardiac tissues. Immunohistochemistry techniques allow labelling and visualisation of specific molecules and proteins in the cardiac tissues in 3D, enabling localisation of cell and molecular distributions and their progression

over developing stages, both under normal and altered haemodynamic conditions.

In addition, the team has developed advanced computational modelling of the developing heart that allows the investigators to determine tissue strains (deformations) and stresses (forces) over the cardiac cycle. This gives them the opportunity to determine how mechanics affect cardiac development. Combining mechanical data and localised cell/molecular data means the group can study how mechanical stimuli result in cell functional changes *in vivo*. They are also planning on using other techniques, such as proteomics profiling, transcriptomics and electron microscopy to further understand how cells adjust to changes in mechanics.

NEW PERSPECTIVE ON CHD

The group's reconstruction and modelling of the beating motion of the embryonic developing heart is pioneering – no other group has achieved computational, dynamic models of cardiac beating during development. Furthermore, they have found remarkable relationships between the degree of alterations in haemodynamic conditions and the degree of cardiac tissue changes.

In a conotruncal banded model of altered haemodynamics – in which a surgical suture is placed and tightened around the embryonic heart outflow tract – they found that blood pressure increases in relationship to the band tightness. Thus, the more the band is tightened, the more the pressure increases. "After 24 hours of banding, we found that collagen deposition increases in a localised manner and in relation to the degree of banding. This is really exciting as it shows that mechanical stimuli directly influence cardiac tissue formation during embryonic development," Rugonyi enthuses.

Certainly, the OHSU team's efforts to understand the roots of CHD from this new perspective are unprecedented. By combining the effects of cardiac biomechanics, physiology and cell biology, and understanding how they interact, they hope to be able to transmit the resulting benefits to CHD patients and their families.

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