Antenatal architecture and activity of the human heart

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We construct the components for a family of computational models of the electrophysiology of the human foetal heart from 60 days gestational age (DGA) to full term. This requires both cell excitation models that reconstruct the myocyte action potentials, and datasets of cardiac geometry and architecture. Fast low-angle shot and diffusion tensor magnetic resonance imaging (DT-MRI) of foetal hearts provides cardiac geometry with voxel resolution of approximately 100 μm. DT-MRI measures the relative diffusion of protons and provides a measure of the average intravoxel myocyte orientation, and the orientation of any higher order orthotropic organization of the tissue. Such orthotropic organization in the adult mammalian heart has been identified with myocardial sheets and cleavage planes between them. During gestation, the architecture of the human ventricular wall changes from being irregular and isotropic at 100 DGA to an anisotropic and orthotropic architecture by 140 DGA, when it has the smooth, approximately 120° transmural change in myocyte orientation that is characteristic of the adult mammalian ventricle. The DT obtained from DT-MRI provides the conductivity tensor that determines the spread of potential within computational models of cardiac tissue electrophysiology. The foetal electrocardiogram (fECG) can be recorded from approximately 60 DGA, and RR, PR and QT intervals between the P, R, Q and T waves of the fECG can be extracted by averaging from approximately 90 DGA. The RR intervals provide a measure of the pacemaker rate, the QT intervals an index of ventricular action potential duration, and its rate-dependence, and so these intervals constrain and inform models of cell electrophysiology. The parameters of models of adult human sinusotrial node and ventricular cells that are based on adult cell electrophysiology and tissue molecular mapping have been modified to construct preliminary models of foetal cell electrophysiology, which reproduce these intervals from fECG recordings. The PR and QR intervals provide an index of conduction times, and hence propagation velocities (approx. 1–10 cm s⁻¹, increasing during gestation) and so inform models of tissue electrophysiology. Although the developing foetal heart is small and the cells are weakly coupled, it can support potentially lethal re-entrant arrhythmia.

1. Introduction

The rhythmic pumping of the mammalian heart sustains the circulation throughout life, and loss of this rhythm (as in an arrhythmia) can lead to sudden death. The rhythm is generated by pacemaking activity in specialized cardiomyocytes that drive propagating waves of excitation in the myocardium and conducting system, and that trigger the alternating contractions of the atria and ventricles. The computational biology of the electrodynamics of the heart [1] is largely based on models and datasets that have been constructed from in vitro and in vivo experiments on isolated cells, tissue and organs extracted from laboratory animals. These computational models require datasets of cardiac geometry, its...
spatially heterogeneous architecture and electrophysiology, from which the spatio-temporal patterns of excitation and the resulting deformations can be computed [2,3]. Such virtual cardiac tissues have proved effective tools in the simulation of cardiac activity, and for developing quantitative, predictive hypotheses that can be tested experimentally.

The application of this approach to the adult human is not straightforward, as physiologically viable, healthy human cardiac tissue is rarely available [4], and experiments have to be largely replaced by non-invasive clinical observations. Detailed models of human cardiomyocytes have been constructed for pacemaking [5], atrial [6] and ventricular cells [7,9], and been embedded into tissue architecture and organ models based on post-mortem anatomy and architecture [8,9], or atlases of cardiac anatomy [10]. Patient-specific modelling is becoming technically possible.

Adult human cardiac models can be validated by experiments on isolated cells and by non-invasive electrocardiograms (ECGs), endocardial electrophysiological data obtained via catheter electrodes during interventional procedures or epicardial surface recordings during open chest surgery [11]. These models allow the interpretation of the surface ECG in terms of epicardial activation patterns [12].

We outline the extension of this approach to the developing foetal human heart. During gestation, the human heart develops from a simple tube into the four-chambered heart supporting the foetal and placental circulation, and at birth supporting the pulmonary and systemic circulations. After premature or full term delivery, the neonatal heart continues to develop with changes in both electrophysiology and structure.

Figure 1 illustrates the time line of human gestation (in weeks). During normal gestation there may be a routine ultrasound scan within the first trimester to check gestational age, monitor heart rate and look for specific problems, e.g. during weeks 9–13 to look for signs of Down’s syndrome. During the second or third trimester, ultrasound is used to identify any additional developmental problems. Non-invasive recordings of the foetal electrocardiogram (fECG) are not routine, unless previous heart rate recordings have suggested arrhythmia, but are possible from week 8. Magnetic resonance imaging (MRI) of the foetus can provide approximately 2 mm pixel images of structure and detect abnormalities in cardiac development, but is only used when an ultrasound scan has suggested possible abnormalities in development.

Computational models of cardiac structure and electrophysiology have already provided useful insights into normal and pathological cardiac activity in the adult [8,9,11]. The construction of similar models for the developing foetal human heart will aid a detailed interpretation of the fECG and offer insight into the initiation and persistence of arrhythmia in the normally developing foetal heart. Foetal re-entrant ventricular tachycardia may account for in utero foetal deaths that precede some stillbirths. These computational models will add necessary details and knowledge to clinical datasets currently available, facilitating and extending their interpretation. This extends the application of computational methods in the field of cardiology from the adult into the developing human foetus. It increases the spatial resolution from the ‘millimetre’ range of clinical visualization to the approximately 100 μm range of computer simulations. It will provide increased understanding of and a firm basis for foetal cardiology, with possible applications in predicting the effects of maternally administered chemicals that impact on the human foetal cardiac electrophysiology, and may provide insights into sudden cardiac death of the foetus.

Histology and MRI [13–15] can map the orientation of myocytes and their organization (representing cardiac architecture) and models of cellular excitation may be embedded into this architecture. At the millimetre, tissue level scale, propagation appears continuous and can be modelled by a reaction–diffusion equation:

$$\frac{\partial V}{\partial t} = \nabla(D \nabla V) - I_{\text{ion}}.$$  \hspace{1cm} (1.1)

where $V$ is the membrane potential (mV), $t$ is time (ms), $\nabla$ is a spatial gradient operator, $D$ is the electrical diffusion coefficient tensor (mm$^2$ ms$^{-1}$) that characterizes the electrotonic spread of voltage, and $I_{\text{ion}}$ is the total membrane ionic current density ($\mu$A/$\mu$F). The electrical or conductivity diffusion tensor (DT) $D$ is defined within the geometry of the heart and changes with location within the heart, and is determined by the tissue architecture, principally by the average myocyte orientation at any given location.

The heterogeneity of the heart—spatial differences in cell electrophysiology and protein expression—can be mapped and incorporated in equation (1.1) as spatially varying parameters.

2. Constructing anisotropic and orthotropic geometric models of foetal heart

Here, we outline the imaging and computational methods used to construct models for the DT $D$ for foetal hearts.

2.1. Material and methods

Human foetal hearts with an age range of 99–143 days gestational age (DGA) were obtained from abortions performed under the UK 1990 Human Fertilization and Embryology Act, which restricts legal abortions to under-24-week gestational age, except in the cases of grave risk. Temporary storage of tissue for imaging was in premises licensed by the 2004 Human Tissues Act, and all procedures were approved by both hospital and university ethics committees, and informed maternal consent had been obtained for use of foetal material in research.

The hearts were stored in Tyrode solution containing 0 mM CaCl$_2$, 4 per cent formaldehyde and immersed in Fomblin (a perfluoropolyether used as a wetting and embedding agent as it has few $^4$H nuclei and so a low MRI signal) for MRI and DT-MRI imaging. For the fast low-angle shot (FLASH)
MRI protocols, the hearts were kept in 4 per cent formaldehyde solution containing 0.1 per cent Gd-DTPA (gadopentetate dimeglumine, Magnevist, Bayer Schering Pharma).

Magnetic resonance image acquisition was performed in a Bruker BioSpin (Ettlingen, Germany) 9.4 T vertical MRI/S system using standard Bruker protocols.

Cardiac geometry was imaged in Fomblin using a T1 weighted FLASH MRI sequence with 300 averages, echo time (TE) = 5.3 ms, repetition time (TR) = 15 ms, taking a total of 115 h 12 min to acquire at a resolution of 38 × 38 × 38 μm, a matrix size of 256 × 256 × 360 for a field of view of 14.8 × 14.8 × 20.8 mm and a flip angle of 40°. This method is similar to that previously described [16]. Cardiac geometry, anisotropy and orthotropy were imaged in Fomblin with DT-MRI to acquire a resolution of 100–170 μm, a matrix size of 125 × 125 × 128, TE = 15 ms, TR = 500 ms and a b value of 1000 s mm⁻². In each scan, diffusion-weighted images were obtained in 12 directions. The fractional anisotropy (FA) and angles at each voxel were calculated from the primary, secondary and tertiary eigenvalues (λ₁, λ₂ and λ₃) and the orthogonal components of their eigenvectors.

The FA is a measure of the average local anisotropy of diffusion within the tissue and calculated at each voxel throughout a tissue using

\[
FA = \sqrt{\frac{3}{2} \left( \frac{(\lambda_1 - \langle \lambda \rangle)^2 + (\lambda_2 - \langle \lambda \rangle)^2 + (\lambda_3 - \langle \lambda \rangle)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2} \right)}
\]

and

\[
\langle \lambda \rangle = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}
\]

Propagation velocity is fastest in the direction of the primary eigenvector and slowest in the direction of the normal to the tertiary eigenvector.

The helix ‘H’ and transverse ‘T’ angles, representing the average myocyte orientation within each voxel, were calculated from the primary eigenvectors and the sheet normal angles ‘S’ were calculated from the secondary and tertiary eigenvectors (figure 2b). For every voxel within the cardiac tissue these angles are all defined within a coordinate system formed by three orthogonal axes: (i) the base–apex axis of the left ventricle (figure 2b), (ii) the radial axis joining the centroid of the left ventricle with the voxel within the transverse plane normal to the base–apex axis and (iii) the tangential axis normal to the transverse plane [17]. These angles define the architecture of the ventricular myocardium and are of modulus 180°. –90° has the same orientation as +90°, as the muscle fibres have an orientation and not a direction. This coordinate system is not appropriate for visualizing atrial architecture, but is suitable for computing spatio-temporal patterns of propagation within the atria [8]. All three-dimensional visualizations were performed in the publicly available ParaView and VolView (www.kitware.com).

2.2. Foetal cardiac anatomy

The left ventricle develops from the early heart tube with the recruitment of myocardial tissue of the atria and right ventricle from mesodermal cells from multiple cardiac fields. The outer curvatures of the looping heart balloon out from the outer to form the four-chambered heart [18]. The cells of the early myocardium structurally resemble those of nodal tissue of the adult cardiac pacemaking and conducting system. The molecular and cellular mechanisms of the formation of the heart have been explored in model systems [19] but the human time line and quantitative three-dimensional geometry need human data. An atlas of two-dimensional data stacks of MRI and EFIC (episodic fluorescence image capture) images of human foetal hearts from the first trimester that allow three-dimensional reconstruction is available online [20]. Distinct left and right chambers are visible by week 8, with ventricular septation completed by nine weeks, but the developing ventricular myocardium is not yet compact and appears as an irregular, spongy network.

2.3. Magnetic resonance imaging

Clinical in vivo MRI can provide images of the foetal heart which are good enough for the identification of developmental
abnormalities, and with a pixel resolution of about 2 mm. However, three-dimensional reconstruction of the geometry of the in vivo foetal heart is impractical, because of both foetal movements and the motion of the foetal heartbeat, and fECG-gated MRI is still at an early stage of development.

Fourteen foetal hearts, with wet weights from 0.2 g (at 99 DGA) to 1.4 g (at 143 DGA), were imaged, reconstructed in three dimensions and visualized. This increase in size with gestational age is illustrated by their surface visualizations for the diffusion weighted image datasets of five hearts in figure 2a. Coronary arteries are visible on the surface, and the development of the atrial appendage is apparent. The construction of the axes of the coordinate system (base-apical, radial and tangential) for quantifying the geometry and architecture (orientation of the eigenvectors, and hence fibre angles) is illustrated in figure 2b, where a colour scale (same as in figure 4) is used to display a long-axis view of the transmural changes in the helix angle $\alpha_{H}$ of the 143 DGA heart.

The geometry of the heart can be visualized from the diffusion-weighted images (as in figure 2a) and by FLASH imaging (as in figure 3), with contrast enhanced by Gd-DTPA. Figure 3 presents long- and short-axes views of a human foetal heart obtained by FLASH MRI, after immersion of the heart in 0.1 per cent Gd-DTPA. Unlike the adult heart, in which the left ventricular wall is thicker than the right ventricular wall, in the foetal heart the left and right ventricular walls have a similar thickness. The long- and short-axes sections of the ventricular myocardium in figure 3 clearly show transmural changes in the structure of the ventricular wall, with the subepicardial tissue being more compact than the more interior sub-endocardial tissue. An orientated texture is also discernable, that appears to correspond to the cleavage planes between myolaminae. The adult mammalian myocardium is structured as myolaminae or sheets of myocytes [21], with regions of abrupt transmural change in laminar organization. The motion of cleavage myolaminae has been visualized in a contracting rat heart, and their three-dimensional orientation by contrast-enhanced MRI [16].

2.4. Diffusion tensor magnetic resonance imaging

Histology and DT-MRI have demonstrated that myocardium is composed of myocytes whose orientation has a helical architecture [21], with a smooth transmural change in the helix angle $\alpha_{H}$, and with myocytes being organized in ‘sheets’. The average orientation of the myocytes ($\alpha_{H}$) within these sheets varies smoothly in a radial section through the ventricular wall, through approximately $120^\circ$ from endocardium to epicardium. As a result of this myofibre and myolaminar structure, each point in the myocardium has an orthotropic architecture, with three orthogonal structural directions: (i) along the myocyte axis; (ii) perpendicular to the myocyte axis in the sheet plane; and (iii) normal to the sheet plane. These orthogonal structural axes influence the electrical coupling and the spread of activation of the myocardium [22].

The organization of the myocardium is measured by the FA, and defined by the three angles: the fibre helix $\alpha_{H}$ and transverse $\alpha_{T}$ angles, representing the average myocyte orientation within a voxel, and the sheet normal angle $\alpha_{S}$. Figure 4 displays the FA, fibre helix, transverse angles and sheet normal angles of a mid-equatorial ventricular slice of three foetal hearts, arranged in increasing gestational age (from 100 to 143 DGA). The clear transmural change in helix angle from negative to positive, the clear organization of the fibre transverse angle and the changes in the sheet normal angle that are characteristic of adult hearts are only seen in the oldest heart (143 DGA, bottom panel of figure 4). The $116$ DGA heart (illustrated in the middle panel of figure 4) presents only a visual hint of organization in the distribution of myofibre angles.
All the foetal hearts with age less than 116 DGA show a low (less than 0.2) FA. This follows from the primary eigenvalues being greater, but not very much greater than the secondary and tertiary eigenvalues. Qualitatively, this could be explained by the foetal cardiac cells not having a profoundly elongated shape or not being more tightly coupled at their poles. It could also be explained by the cells being organized more in an irregular branching network, or due to the less compact structure of the myocardial tissue. Quantitatively, low FA would account for the irregularity of the angles (described in figure 4), and would imply that propagation in the foetal ventricle of hearts with ages less than 116 DGA is approximately isotropic.

The transmural fibre helix angle distribution across the left ventricular free wall is illustrated for these three foetal hearts in figure 5. A smooth change in transmural distribution from positive on the epicardial surface to negative is apparent early in development, against the organized pattern observed in the 143 DGA foetal heart.

3. Constructing electrophysiological models of foetal cardiac cells and tissue

The periodicity of the foetal heartbeat can be obtained from ultrasound measurements from about five weeks gestational
age, and from the RR intervals of the fECG from about 10 weeks. The fECG is a small signal (approx. 100 µV) with a low signal-to-noise ratio, and so the T wave of the fECG can only be detected consistently from about 15 weeks.

Figure 6 collects data from the literature on the period of the human foetal heartbeat, obtained from ultrasonography [23,24], and RR intervals and QT intervals, obtained from fECG [25,26] or foetal magnetocardiographic recordings [27]. The period and RR intervals (represented by black squares in figure 6) decrease from approximately 640 ms (at four weeks) to a minimum of 350 ms (at eight weeks) and then drift upward to around 400 ms for the remaining period of gestation. These periods are produced by the pacemaking system, which develops into the sinoatrial node, and any model of foetal cardiac electrophysiology needs to be able to reproduce these rates. The mean QT interval is an index of approximate ventricular action potential duration and is constant at around 270 ms. It, however, shows great variability (as illustrated in figure 6 by the black triangles) as it depends on the foetal heart rate.

3.1. Human foetal electrocardiogram recordings

Non-invasive fECG recordings were obtained with a portable, 5-electrode Monica AN24 device (Monica Healthcare Ltd) from the maternal abdomen during rest and normal activity, in volunteers who had given informed consent, and under clinical supervision in the prenatal unit. The recordings during maternal rest lasted for 30 min. For one volunteer, recordings were obtained weekly throughout gestation, from week 28 until 2 days before a normal, full term delivery.

RR, PR and QR intervals, and QT intervals and their dispersion were extracted from the fECG. Figure 7 shows a sample of raw recordings of the fECG and maternal ECG (figure 7a), and an extracted average fECG (figure 7b) from which the QT, PR and QR intervals can be obtained at different stages of gestation (figure 7c). The PR and QR intervals represent the times between atrial and ventricular excitation, and the spread of excitation through the ventricular tissue, i.e. propagation times. These propagation times do not change systematically from week 18 of gestation to full term [27] (figure 7c); and during this time there is an increase in cardiac volumes [28] and a more than twofold increase in the linear dimensions of the heart. This implies a more than twofold increase in conduction velocity, which from the fECG timings and the foetal cardiac geometry is of the order of 1–10 cm s⁻¹. For a ventricular action potential duration (APD) of 200 ms the wavelength of a re-entrant wave would be approximately 2 cm, and so the ventricle is large enough for a re-entry to be possible.

3.2. Foetal cellular electrophysiology

Electrophysiological models for human cardiac cells are based on experiments on adult cells and tissues, and on patch clamp experiments on human ion channels expressed in Xenopus eggs or mammalian expression systems. Human foetal and adult cardiac cells have the same genes and so the same channels, but different isoforms are expressed in the foetus and the channel microenvironment may be different. Thus, although a quantitatively accurate electrophysiological description of a foetal cell will have the same channels as in the model for an adult cell, it will have different maximal conductances and perhaps different kinetics.

There are few data on the electrophysiology of human foetal cardiac cells, so we inform models of human adult cardiac cells with interval data from fECG, to obtain the appropriate periods (from RR intervals) and APDs. Figure 8a shows human foetal ventricular APDs [29] and QT intervals plotted as a restitution curve, together with the dynamic restitution curve computed for a model of an adult human endocardial ventricular cell [7]. The computed restitution curve provides a reasonable approximation to the observed QT interval dependence on the RR interval.

Physiological viable human atrial and ventricular cells have been studied and modelled, and so their models are reasonably well founded and validated. Viable adult human sinoatrial tissue has not been extensively investigated, but the mRNA expression in post-mortem sinoatrial nodal tissue has been mapped, and used to modify the parameters of a human atrial cell model [5,6]. Increasing the hyperpolarization-activated ‘funny’ current Iₖᵣ and decreasing the potassium inward rectifier current Iᵣᵣᵣ can produce a pace-maker cell model with the appropriate rate for a second and third trimester human foetal heart—figure 8.

These foetal cell models are no more than an informed and plausible speculation. However, it is quantitatively testable, as when incorporated into a simplified one-dimensional [30] or three-dimensional detailed foetal heart model will generate a pseudo-ECG, which can be validated by the timings of the fECG response to maternally administered pharmacological agents.

4. Discussion and conclusions

Foetal human hearts are not readily available for structural or functional studies, as they are only available, after informed maternal consent, after miscarriage, abortion or stillbirths, and in practice only after abortion. In the UK in 2011, almost 80 per cent of abortions were before-10-week gestational age, and 1 per cent was after more than 20 weeks of gestation. MRI and EFIC datasets of the geometry of human foetal hearts up to 10 weeks gestation are available through [20], the MRI and DT-MRI datasets for hearts from 100 to 143 DGA illustrated here are available at http://vtea.leeds.ac.uk.

The developing foetal heart can be studied in utero within a clinical environment by non-invasive methods. Ultrasound is the main clinical method for monitoring foetal
development, and can provide structural and functional information on the developing heart [31]. If a developmental abnormality is indicated, higher resolution imaging may be obtained by clinical MRI. However, ultrasound and clinical MRI do not provide sufficient resolution for use in research or in computer simulations. A problem with clinical foetal MRI is the rapid beating of the foetal heart. ECG gating allows cine cardiac MRI but foetal gating is not yet reliably developed.

The limited availability of human foetal material combined with the availability of clinical recordings designed to answer practical questions relating to maternal and foetal care allows the development of cardiac foetal computational modelling to add value to clinical datasets, to aid their interpretation and add information. Computational models need to be well based on anatomical and physiological data, and their quantitative predictions validated by experimental and clinical data. When validated, these models can be used, as in adult cardiology, to quantitatively reproduce and explain physiological and pathophysiological activity. They may also be used as an experimental method to quantitatively investigate the mechanisms of arrhythmia and their pharmacological control, and to pre-screen the actions of pharmacological agents on the electrical activity of the heart. Coupled with high-resolution fECG or f-magnetocardiography [32], computational modelling of the electrophysiology of the developing human foetal heart could contribute to the development of a foetal cardiology based on fECGs, which can be recorded from the eighth week of pregnancy and their intervals can be extracted from week 12. Here, we have used these fECG intervals to inform preliminary models of foetal cell electrophysiology.

Foetal hearts obtained from abortions can be perfused using the Langendorff method, and have provided physiologically viable cells and tissues for in vivo electrophysiology experiments, and for producing cell cultures of foetal cardiomyocytes [33]. Langendorff-perfused rat hearts have been used for optical mapping of surface and sub-surface electrical activity of the ventricular myocardium, providing sub-epicardial action potentials, their duration, restitution and conduction velocity throughout the surface of the heart [34]. In principle, these methods could be applied to human foetal hearts, and provide a means of validating the heart models constructed from the cellular electrophysiology of §3.2 and the DT-MRI data of §2.4. Further DT-MRI has been applied to Langendorff perfused rat hearts, to quantify the architecture of the heart in low/high \([Ca^{2+}]\), approximating diastole and systole [35]. If this methodology is extended to human foetal hearts it would allow electromechanical modelling that can be related to ultrasound imaging of the beating heart. Since the foetal hearts are obtained in a hospital environment, and imaged in Fomblin days to weeks later, after storage in 4 per cent formalin, there may be systematic changes in the myocardial structure and gap-junctional connections following the isolation of the heart. Such post-mortem changes in the DT-MRI visualized structure have been shown in rat [36], but amount to approximately 3°

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**Figure 7.** Human foetal ECG characteristics. (a) Sample ECG recording from one pregnant volunteer using the Monica AN24 (Monica Healthcare Ltd) indicating the foetal signal in black ellipses. (b) Average foetal ECG morphology as extracted from the ECG recording of the same pregnant volunteer. (c) QT intervals (squares), PR intervals (circles) and QR intervals (triangles) as extracted from the pregnant volunteer.

**Figure 8.** (a) Foetal QT intervals and APD against cycle length; circles represent computed APD_{90} for the Ten Tusscher human ventricular cell model, triangles represent foetal QT intervals, the squares represent foetal APD_{90} and the single cross shows the position of the average fECG from figure 7b. (b) Preliminary simulations of human foetal cardiac activity using modified computational models of the human adult ventricular (dashed) and sinoatrial node (space-dashed) cells.
change in myofibre helix angle, less than the signal-to-noise ratio of figure 5.

In conclusion, the combination of MRI, non-invasive ECGs and cell models has provided the initial components of a family of three-dimensional computational models of foetal cardiac electrophysiology. These computational datasets and models may contribute to the emerging field of foetal cardiology and will give a quantitative insight into the normal development and activity of the human foetal heart.

References


