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Full Title:
Developing a novel comprehensive framework for the investigation of cellular and whole heart electrophysiology in the in situ human heart: historical perspectives, current progress and future prospects.

Short title: Basic electrophysiology in the in situ human heart

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Abstract
Understanding the mechanisms of fatal ventricular arrhythmias is of great importance. In view of the many electrophysiological differences that exist between animal species and humans, the acquisition of basic electrophysiological data in the intact human heart is essential to drive and complement experimental work in animal and in-silico models. Over the years techniques have been developed to obtain basic electrophysiological signals directly from the patients by incorporating these measurements into routine clinical procedures which access the heart such as cardiac catheterisation and cardiac surgery. Early recordings with monophasic action potentials provided valuable information including normal values for the in vivo human heart, cycle length dependent properties, the effect of ischaemia, autonomic nervous system activity, and mechano-electric interaction. Transmural recordings addressed the controversial issue of the mid myocardial "M" cell.
More recently, the technique of multielectrode mapping (256 electrodes) developed in animal models has been extended to humans, enabling mapping of activation and repolarisation on the entire left and right ventricular epicardium in patients during cardiac surgery. Studies have examined the issue of whether ventricular fibrillation was driven by a "mother" rotor with inhomogeneous and fragmented conduction as in some animal models, or by multiple wavelets as in other animal studies; results showed that both mechanisms are operative in humans**.** The simpler spatial organisation of human VF has important implications for treatment and prevention. To link in-vivo human electrophysiological mapping with cellular biophysics, multielectrode mapping is now being combined with myocardial biopsies. This technique enables region-specific electrophysiology changes to be related to underlying cellular biology, for example: APD alternans, which is a precursor of VF and sudden death. The mechanism is incompletely understood but related to calcium cycling and APD restitution. Multielectrode sock mapping during incremental pacing enables epicardial sites to be identified which exhibit marked APD alternans and sites where APD alternans is absent. Whole heart electrophysiology is assessed by activation repolarisation mapping and analysis is performed immediately on-site in order to guide biopsies to specific myocardial sites. Samples are analysed for ion channel expresion, Ca 2+-handling proteins, gap junctions and extracellular matrix. This new comprehensive approach to bridge cellular and whole heart electrophysiology allowed to identify 20 significant changes in mRNA for ion channels Ca 2^{+}-handling proteins, a gap junction channel, a Na^{+}-K^{+} pump subunit and receptors (particularly K_{ir} 2.1) between the positive and negative alternans sites.

1. Introduction

Understanding the mechanisms of fatal ventricular arrhythmias is important in order to develop strategies to combat the high incidence of sudden arrhythmic cardiac death, amounting to in excess of 50,000 per year in the UK alone (John et al, 2012). Although considerable progress has been made in animal models, extrapolating from these data to humans is not straightforward due to the many electrophysiological differences that exist between animal species and humans (Coronel et al, 1997; Zicha et al, 2003; Akar et al, 2004; O`Hara and Rudy, 2012). Furthermore the cardiac pathology that usually accompanies these arrhythmias is not always possible to exactly reproduce in animal models. The acquisition of basic electrophysiological data in the intact human heart, and from the hearts of patients with cardiac pathology, is therefore essential to complement experimental work in animal and in-silico models.

Ethical considerations require that techniques and protocols designed to achieve this should be free of risk and impose minimal additional burden on patients and clinical personnel. Procedures which access the heart such as cardiac catheterisation and cardiac surgery provide the opportunity to incorporate such measurements into the routine clinical procedure. Seminal in this regard was the early discovery that an electrode opposed to myocardium by suction and referenced locally produced an action potential configuration in which the entire repolarisation course faithfully represented the repolarisation of the intracellular action potentials beneath the electrode (Hoffman et al, 1959). This led to the development of a cardiac catheter which could be introduced into the heart during routine investigative procedures and so obtain measurements of the intracellular cardiac action potential duration (APD) from humans in vivo (Olsson et al, 1971). This provided a direct link with the cellular electrophysiology laboratory and hence with experimental work and theoretical mechanisms of arrhythmogenesis.

From these single site recordings of APD, available since the Eighties, techniques have evolved to enable a wide range of basic electrohysiological measurements to be recorded. At the same time strategies have been developed to enable normal and abnormal electrophysiological processes to be investigated during routine clinical procedures. Here we review the techniques that have been developed for acquiring basic electrophysiological data directly from the human subjects' hearts. We describe the information that has been obtained and how this may impact on our insight into arrhythmogenesis. Numerous and important differences between the human and animal models are highlighted, not only with respect to basic electrophysiological properties but also with regard to arrhythmia mechanisms.

Although the mapping of the electrical activity of the heart is necessary to understand the functioning of the normal and diseased human heart in situ, it is not enough. To achieve the level of understanding vital to develop strategies to combat sudden arrhythmic cardiac death we face at least two challenges. The first one is to link electrophysiological measurements obtained in vivo with cellular biophysics, while the second consists of revealing the interplay between electrophysiology, cellular biophysics and cardio-mechanics.

In the second part of this article, we describe our current multielectrode mapping technique combined with cellular electrophysiology from guided myocardial biopsies. Myocardial biopsies are taken from sites guided by on site analysis of the whole heart electrophysiological measurements. Application to important theoretical and clinical questions such as the mechanism of APD alternans is described together with new data.

2. Early work using monophasic action potential (MAP) recordings

Monophasic action potentials (MAPs) are extracellularly recorded wave forms that, under optimal conditions, can reproduce the repolarisation time course of transmembrane action potentials with high fidelity (Franz 1999). Early MAP recordings during cardiac catheterisation procedures were made with a catheter that employed suction onto the endocardium (Olsson et al, 1971). Nowadays they are most commonly recorded by simply applying gentle pressure contact in a bipolar

configuration. One Ag-AgCl electrode is in contact with myocardium which is partially depolarised as a result of the pressure contact and a second Ag-AgCl electrode is in contact with the adjacent non depolarised tissue (see Fig. 1) (Moore and Franz, 2007). The Franz catheter for endocardial studies incorporates a steerable tip and pacing electrodes close to the tip.
Direct comparisons with transmembrane action potential have shown that MAPs provide accurate quantitative estimates of local repolarisation dynamics (Hoffman et al, 1959). A limitation of the MAP is a sensitivity to motion artefacts: the mechanical activity of the beating heart may alter the contact pressure which in turn may alter MAP amplitude. Also, accurate MAPs cannot be recorded transmurally. Nevertheless, the MAPs still represent an extremely valuable source of electrophysiological data.

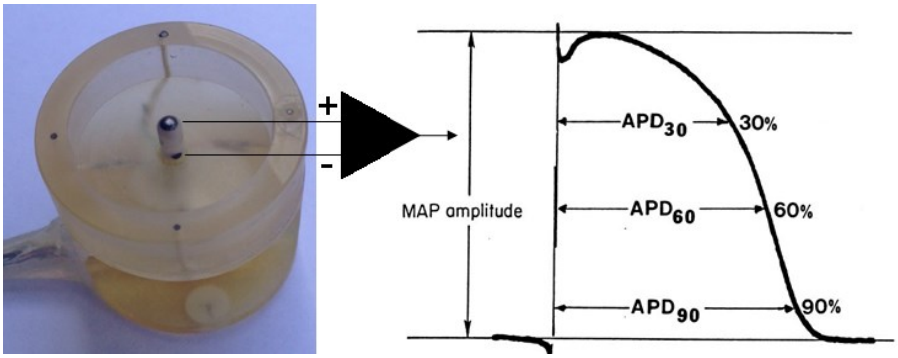

Figure 1. Left panel: Bipolar configuration of a device designed to record epicardial MAP. A suitably shaped sponge is usually incorporated in cup of holder. When the device is gently pushed against the epicardium, a small region underneath the tip is depolarized and provides a stable reference for the potential recorded from the electrode placed few millimetres apart from the tip, where the tissue is still excitable. Right panel: A typical MAP and corresponding APD estimates. Note that the MAP amplitude is measured from baseline to the crest of the plateau. Adapted from (Franz 1999).

Endocardial monophasic action potential. Work in the cardiac catheterisation laboratory using a monophasic action potential (MAP) catheter provided normal values for APD in humans on the endocardium, the effect of ischaemia (John et al, 1992a,1992b; Taggart et al, 1996), the dynamics of APD restitution and rate adaptation (Franz et al, 1988;Taggart et al, 1996, 2003; Bueno-Orovio et al, 2012), mechano-electric interaction (Levine et al, 1988), the effect of drugs (Lee et al, 1992), and classification of fractionated electrograms in human atrial fibrillation (Narayan et al, 2011).

Epicardial monophasic action potential. The technique of MAP recording was extended to the epicardium with the design of special hand held probes for use during cardiac surgery (see Fig. 1) providing basic epicardial data on normal values for APD, the effect of ischaemia (Sutton et al, 2000; Taggart et al, 2000). The relation between action potential repolarisation and refractoriness (post repolarisation refractoriness) (Lee et al, 1992; Sutton et al, 2000), and mechano-electric interaction (Taggart et al, 1988;1992).

3. Activation-recovery intervals and the unipolar electrogram

An electrogram (EGM) is a representation of the time course of the potential difference between two sites during the cardiac cycle. Many different types of EGMs can be recorded during human electrophysiological studies. These differences are based on configuration (unipolar/bipolar), technology of the specific device (contact/non-contact), number of electrodes (from 1 to 256) and site of recording (endocardium, epicardium and transmural).
In the bipolar configuration, an EGM is measured as the difference between the potential of two close sources (typically less than 5 mm), whereas the unipolar EGM is the difference between the

potential measured from an exploring (proximal) catheter and the potential measured from an electrode, called indifferent, placed at a site where there is little or no cardiac activity. Ideally, the indifferent electrode should be placed at an infinite distance from the exploring one. In practice, when the ECG is available, the Wilson central terminal is used as indifferent reference. If the Wilson central terminal is not available or when its use results in a noisy EGM, the indifferent reference can be taken from a distal electrode placed, for example, in the vena cava during catheterization or at the rib retractor during cardiac surgery. The bipolar configuration rejects the far-field potentials, thus providing a representation of local and fast electrical dynamics only. In contrast to the bipolar EGM, the unipolar EGM is less sensitive to the direction of wave front propagation, which in turn can be inferred from its morphology. Importantly, repolarisation dynamics can be reliably estimated only from the unipolar EGM.

The so-called activation-recovery interval (ARI) has been shown to be a reliable estimate of APD (Coronel et al, 2006, Wyatt et al, 1980). As shown in Fig. 2, ARI is usually measured as the difference between repolarisation time, estimated as the instant of maximum up-slope during repolarisation (dV/dt|max), and activation time, estimated as the instant of maximum down-slope during depolarization (dV/dt|min). Although this measurement is widely accepted and supported by rigorous studies, it has been suggested that in EGM exhibiting a positive T-wave it may underestimate the duration of repolarisation, and an alternative method to estimate ARI has been proposed (Yue et al. 2004).

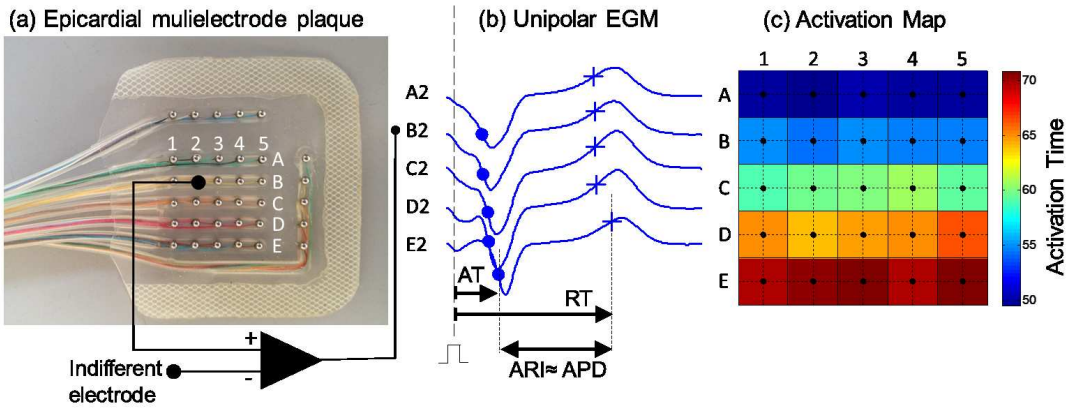

Figure 2. Schematic representation of electrical mapping by means of multielectrode devices. A: Unipolar electrograms are recorded using an epicardial multielectrode plaque. External electrodes are designed for pacing. B: Example of recording. ARI is calculated as the difference between activation time (dV/dt|min) and repolarisation time (dV/dt|max). C: example of activation map.

3.1 Transmural recordings

Work emanating from several laboratories demonstrating different electrophysiological properties in cells isolated from endocardial, mid myocardial and subepicardial regions (Sicouri and Antzelevitch, 1991) with potential implications for arrhythmogenesis, together with the surrounding controversy as to whether these electrophysiological differences persisted in intact hearts which were electrically and mechanically well coupled (Anyukhovsky et al, 1999), prompted the development of a plunge electrode for obtaining transmural recordings in patients during surgery. The plunge electrode which was inserted into the left ventricular free wall had 5 electrodes on the shaft at 1.5mm intervals. These studies quantified the effect of global ischaemia on transmural conduction and repolarisation, and demonstrated an absence of any electrophysiological evidence of mid myocardial “M” cells in humans in the left ventricular wall (Taggart et al, 2001). The contrast between these results and the clear differences between M cells and endocardial and epicardial cells regularly observed in studies in isolated cells, was attributed to electrotonic cancellation of transmural electrical gradients in the intact left ventricle in man (Taggart et al, 2003; Conrath et al,

2004). These issues have important implications for arrhythmogenesis and the generation of the T wave in the electrocardiogram (Janse et al, 2012).

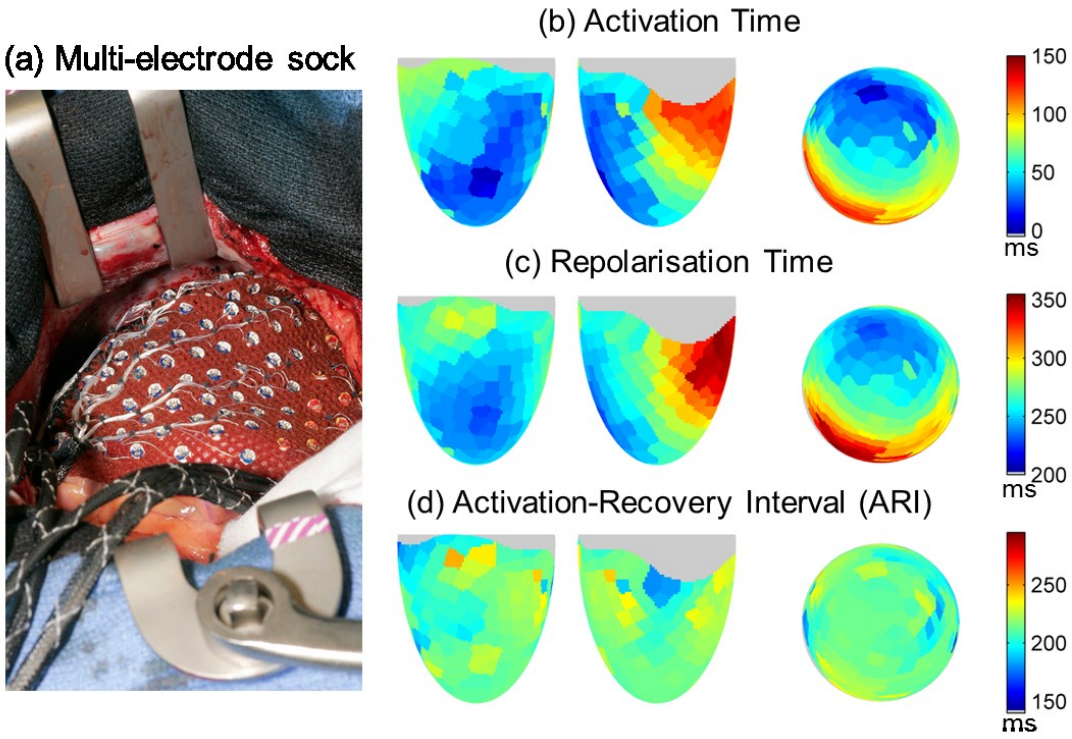

Figure 3. Multielectrode sock and corresponding cardiac mapping. (a) During cardiac surgery, following cannulation, the multielectrode sock is fitted over the epicardium of both ventricles. Only the anterior part of the sock is shown in this picture. Each one of the 256 electrodes has a label to allow the surgeon to collect the biopsy at any specific site. In this configuration the indifferent electrode which provides the potential reference is connected to the rib retractor shown in the picture. Unipolar EGMs as those shown in Fig. 2b are recorded and maps of activation and repolarisation are created. (b)-(d) Colour coded maps representing the patterns of activation (top) and repolarisation (middle), as well as the special distribution of ARI over the epicardium. From left to right, representations are: anterior, posterior and bottom-up view.

3.2 Multi-electrode mapping

In view of experimental work and theoretical modelling highlighting the dynamic nature of arrhythmogenesis and the importance of the spatial dispersion of electrophysiological properties in modulating wavefront propagation, the technique of multielectrode mapping developed in animal models was extended to humans. Initially, recordings were made using small epicardial multielectrode plaques (25 electrodes) as that shown in Fig. 2, and provided information on transverse and longitudinal conduction velocity during normoxia and ischaemia.

More recently a multielectrode sock, which was developed initially for use in large animal models (Nash et al, 2003), has been adapted with 256 electrodes for use in patients undergoing cardiac surgery. This technique enables activation and repolarisation times to be recorded over the entire left and right ventricular epicardium. Unipolar EGMs are sampled at 1kHz using either a UnEmap system (Uniservices Ltd New Zealand) or a Bard system with the reference channel connected to the rib retractors (see Fig. 3). The studies are performed in patients having cardiac operations incorporating cardiopulmonary bypass. The cardiopulmonary bypass procedure involves the venous return to the heart being interrupted and siphoned from a canula placed in the right atrium to a pump / oxygenator where it is reoxygenated, carbon dioxide removed and returned to the circulation through a cannula in the ascending aorta. Blood thereby bypasses the heart and lungs while maintaining the systemic circulation. In order to achieve a still operating field, the heart is arrested either by infusion of cardioplegic solution containing 16 mM potassium into the coronary

circulation, or by using the technique of "cross clamp fibrillation". In the latter case the aorta is cross clamped between the input from the pump oxygenator in the ascending aorta and the coronary artery ostia at the root of the aorta thereby interrupting coronary blood flow. The heart is then fibrillated with high frequency electrical stimulation.
An example of high density mapping obtained using our multielectrode sock technique is shown in Fig. 3b-d. From top to bottom, panels represent colour coded maps of activation, repolarisation and ARI, within one heartbeat.

3.2.1 Signal processing

A key practical concern in the use of a multielectrode sock or plaque is the processing of raw EGMs, typically a unipolar signal, to produce maps of activation and recovery, and to identify wavefronts and other dynamic features such as rotors. Raw EGMs are often noisy, and both filtering and de-trending is effective in removing most noise. However, some electrodes may make a high-impedance connection with the epicardium, especially if they are located over a region of fat, and some signals may therefore need to be excluded from the analysis. The quality of the recording should be assessed by evaluating both the amount of broad-band disturbances, typically with signal-to-noise ratio estimates, and morphological distortions. The latter can be quantified by using measurements of consistency between consecutive EGMs recorded at the same site, or between waveforms from adjacent electrodes, or by comparison with a template.
Signals recorded from an array of electrodes represent a measurement of electrical potential close to the electrode. It is often desirable to interpolate these signals, in order to estimate signals that would be recorded between the electrodes. An important development for cardiac mapping, and in particular for the mapping of fibrillation, has been the transformation of excursions of electrode voltage to represent phase in the cycle of activation and recovery (Gray et al, 1998; Nash et al, 2006). When interpolated electrode voltages are transformed into phase, then it is possible to identify both activation wavefronts and points of phase singularity around which rotors rotate. A processing pipeline is illustrated in Fig. 4, which starts with raw EGMs from a multielectrode sock, and ends with wavefronts and phase singularities. This approach enables the complexity of fibrillation in atria (Narayan et al,2012) and ventricles (Nash 2006, Massé et al, 2009) to be quantified in terms of the number of phase singularities and wavefronts that are present. This quantitative approach has been extended in studies in animal hearts, where a list of different indices capable of quantifying activation are compiled (Cheng et al, 2012). An important assumption underlying this approach is that the spacing of electrodes is sufficient to identify all of the activation wavefronts that are actually present, and so the interpolation is justified.

3.2.2 Application to the study of mechanisms of ventricular fibrillation

The global nature of the mapping system makes it possible to map activation and repolarisation wavefronts in humans and thereby study the dynamic properties of ventricular activation during arrhythmias. Understanding the mechanisms underlying ventricular fibrillation is important both for treatment and for prevention as well as identifying individuals at high risk. Two particular mechanisms capable of sustaining VF have been proposed by different groups, each supported by convincing experimental work in animal models. One mechanism proposes that VF is sustained by multiple circulating unstable wavelets perpetuated by a sequence of wavebreak and self-regenerating re-entry, similar to that originally described by Moe in atrium (Moe 1962). This mechanism was shown to be facilitated by steep APD restitution (Garfinkel et al, 2000). An alternative mechanism proposed that VF is driven by a single rapid periodic source of excitation, a "mother rotor", that is too fast to sustain 1:1 conduction through the myocardium, resulting in intermittent conduction block and multiple irregular activation patterns (Jalife 2000). The presence of mother rotors has been reported in VF experiments in guinea pig (Samie et al, 2001) and rabbit hearts (Gray et al, 1998; Chen et al, 2000; Zaitsev et al, 2000; Wu et al, 2004). However, multiple attempts to find evidence for mother rotor VF in porcine hearts have not proved successful (Valderrabano et al, 2002; Huang et al, 2005; Kay et al, 2006). Whether VF in animal models is

driven by multiple wavelets or is driven by a mother rotor has been the source of much debate (Berenfeld et al, 2001; Choi et al, 2001)

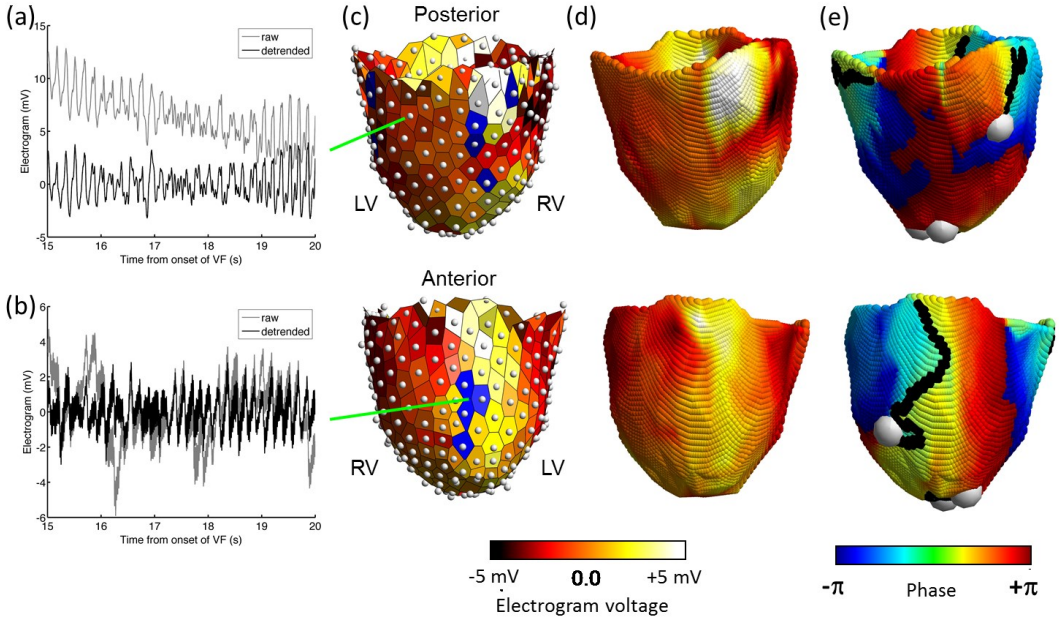

Figure 4. Processing pipeline for recordings from mulitelectrode sock (for details see Nash et al 2006, Bradley et al 2011). (a) Example of good quality electrogram before (grey) and after (black) detrending. (b) Example of noisy electrogam before (grey) and after (black) detrending. (c) Snapshot of epicardial electrogram voltages, 20 s after onset of VF. Approximate locations of electrodes are indicated by grey spheres, and the region around each electrode is coloured according to the instantaneous voltage. Blue regions indicate electrodes that were rejected from the analysis. (d) Snapshot of epicardial electrode voltage obtained by interpolating the data in (c). (d) Corresponding snapshot of phase, with wavefronts (lines of zero phase) shown as black lines, and phase singularities as gray spheres.

One group performed mapping over a 4.5 x 4.5cm area of the left ventricular epicardium in humans and found evidence compatible with either wavelets or mother rotors and concluded that multielectrode mapping was necessary to resolve the issue (Nanthakumar et al, 2004). We studied 10 patients using global multielectrode mapping of the entire left and right ventricular epicardium (see Fig. 4) and showed that early human VF was sustained by large coherent wavefronts on the epicardium punctuated by periods of disorganised wavelet behaviour (Nash et al, 2006). While this methodology has limitations, that include data limited to the epicardium, the presence of anaesthetic and spatial resolution of the electrodes, it was nevertheless possible to reveal that in humans both mechanisms of VF appear to co-exist. These findings were supported by modelling studies (Keldermann et al, 2008) in which the spatial heterogeneity of APD restitution previously shown to be present in the hearts of patients with coronary artery disease (Nash et al, 2006b) was embedded in a detailed multiscale model of human ventricular electrophysiology. The model tested the hypothesis that the observed heterogeneity in APD restitution could serve as a substrate for mother rotor VF. Based on these data, the results showed that mother rotor VF could occur in the human heart and that both mother rotor and multiple wavelet VF could occur depending on the initial conditions.

The finding that in humans both types of VF may co-exist raises the question as to why some animal models appear to be driven by a mother rotor and others by multiple wavelets. One suggestion is that heart size is a factor (Panfilov 2006) with the number of re-entrant sources increasing as a function of heart size. In rabbit hearts, for example, VF may be driven by 1 or 2 sources, (Gray et al, 1998; Jalife 1996) whereas in the larger sheep heart there are an estimated 20

sources (Moreno et al, 2005) and in the much larger dog and pig heart approximately 50 sources (Huang et al, 2004; Valderrabano et al, 2003). Using a detailed electrophysiological model for human ventricular cells, 3D human ventricular geometry and fibre direction anisotropy together with the epicardial VF mapping data of Nanthakumar et al (2004) and Nash et al (2006), Ten Tusscher et al (2007) estimated that human VF is driven by approximately 10 re-entrant sources. Human VF therefore appears to be much more organised than animal VF in hearts of comparable size, ie 50 sources in dog and pig hearts. The computational model used in this study enabled the striking difference in complexity between human VF and large animal VF (dog and pig) to be examined mechanistically. The model was adapted to dissect out the effects of anisotropy, increased excitability (sodium conductance), APD restitution and the minimal APD attained at short cycle lengths (Ten Tussscher et al, 2007; 2008). The minimum APD provided the most likely explanation for the differences in complexity between large animal and human VF. Minimum APD in humans is between 140 and 200ms (Misier et al, 1995; Taggart et al, 2003) in contrast to between 70 and 110ms in dog (Koller et al,1998) and 90 and 110ms in pig (Huang et al, 2004). These values for minimum APD match the VF activation frequency for the different species, which is approximately 10Hz for dog and pig, and approximately 5 Hz for human VF. Given that minimum APD during VF probably relates to refractoriness (Opthof et al, 1991), and assuming a roughly comparable conduction velocity between species, the longer minimum APD in humans implies a longer wavelength and lower dominant frequency, hence less rotors can be sustained.

3.2.3 Ventricular fibrillation: Early effects of ischaemia

The foregoing studies of ventricular fibrillation described above relate mainly to models in which the heart is perfused during VF. In the case of human VF the coronary artery and systemic circulation are maintained by the cardiopulmonary bypass pump oxygenator. While this provides invaluable information by avoiding the added complexity of the electrophysiological effects of ischaemia, real life VF is inevitably associated with circulatory arrest with the absence of coronary artery perfusion and the onset of progressive myocardial ischaemia. Studies of ischaemic VF in dog and pig hearts have shown that VF evolves through a series of stages with changing activation frequency, increasing organisation and a reduction in the number of waves (Huizar et al, 2007; Huang et al, 2004). In contrast, studies in isolated rabbit hearts (Liu et al, 2004) and isolated human hearts from patients with cardiomyopathy (Masse et al, 2007) and simulation studies (Xie et al, 2001) have shown the opposite with an increase in activation waves as ischaemia evolves. Using the multielectrode sock described above, we mapped epicardial activation during VF in patients undergoing cardiac surgery with the systemic circulation maintained with cardiopulmonary bypass. We recorded electrical activity throughout a two and a half minute period of ischaemic VF and reflow (Bradley et al, 2011). We observed an increase in the number and complexity of wavefronts as ischaemia developed in contrast to the observations in pig and dog hearts (Huizar et al, 2007; Huang et al, 2004), but in keeping with the results in rabbit (Liu et al, 2004), myopathic human hearts (Masse et al, 2007) and modelling studies (Xie et al, 2001). We proposed an explanation for these species differences whereby molecular mechanisms with opposite effects compete during VF in the globally ischaemic human heart (Bradley et al, 2011) . In particular we proposed a significant role for post repolarisation refractoriness (PRR), which we have previously observed to develop rapidly within this time frame in humans in a similar model of global ischaemia (Sutton et al, 2000). For example if PRR is slow to develop and refractoriness remains largely dependent on APD, then ischaemia-induced flattening of APD restitution (Taggart et al, 1996) may dominate and tend to prevent wavebreak, and encourage stable rotors. On the other hand, if PRR develops early then regions of prolonged refractoriness may dominate and promote wavebreak.

3.2.4 Ventricular fibrillation: Longer duration ischaemia

Since the average time from cardiac arrest to attempted resuscitation in the community is in the region of 4 to 10 minutes, an understanding of the mechanisms of VF and the electrophysiological changes that operate during this longer time frame of VF are of great importance in the clinical

management of cardiac arrest victims (Cobb et al, 1999; Barton et al, 2000; Wik et al, 2003;). We therefore extended our multielectrode-sock mapping technique in the human heart using the 256 electrode epicardial sock to enable recordings to be made from patients with coronary artery disease throughout a period of ischaemic VF for up to 10 to 12 minutes. This was achieved by modifying the sock by creating a slit, which may be aligned along a coronary artery such that the surgeon is able to operate on the artery and anastomose a graft to the vessel with the sock in place. The surgical technique of "cross clamp fibrillation" described earlier involves the use of cardiopulmonary bypass to sustain the systemic circulation and electrical fibrillation of the heart to create a still operating field. The coronary circulation is interrupted during the time that the graft is being anastomosed to the coronary artery, usually between 8 to 12 minutes during which time the myocardium is ischaemic. This is well within the time at which the onset of myocardial damage would be expected. By this means it is possible to record global epicardial activation maps in patients from the onset of VF and ischaemia for up to 12 minutes followed by reflow.

4. Combining whole heart electrophysiology with cellular electrophysiology in humans

The techniques described so far for acquiring basic electrophysiological data directly from the hearts of patients have been focussed on whole heart electrophysiology. A logical progression in bridging the gap between experimental electrophysiology and the clinic is to acquire simultaneous whole heart and cellular electrophysiology data. This would provide the unique possibility to relate the local electrical activity of the in-situ human heart to its underlying causes, i.e. cellular biophysics. To this end, we have extended our epicardial multielectrode sock mapping technique by combining it with myocardial biopsies for cellular electrophysiology. The biopsies are taken from selected sites guided by the on-site analysis of the whole heart electrophysiology recordings. This approach is enhanced by the fact that a characteristic feature of whole heart electrophysiology is spatial inhomogeneity, particularly in the presence of disease or pathological processes such as ischaemia. This enables region-specific electrophysiology differences to be related to underlying cellular biology, for example: APD alternans, which is a precursor of VF and sudden death. The mechanism is incompletely understood but currently related to intracellular Ca^{2+} handling and APD restitution.

Mapping during incremental pacing enables epicardial sites to be identified which exhibit marked APD alternans and sites where APD alternans is absent (see Fig. 5). Biopsies taken from each site are being analysed using mRNA profiling and Western blot. Bringing this procedure into practice requires the active participation of a multidisciplinary team. Following cannulation for cardiopulmonary bypass (but prior to its commencement) the surgeon fits the multielectrode sock over the epicardium of both ventricles. If needed, contact between the epicardium and the sock is improved by increasing the filling of the heart with the cardiopulmonary pump. Pacing pulses are delivered by a Micropace device which is connected to the acquisition system and PC. Duration and amplitude of the pacing pulses are set by an experienced user and can be modified to ensure capture and contraction. Importantly, stimulation can be delivered from virtually any of the 256 electrodes which cover the entire heart, thus offering the possibility of selectively activating epicardial regions of interest. Pacing is established over a range of 6 cycle lengths, from 600ms to 350ms, in steps of 50ms. Immediately after the pacing protocol, signals are analysed on-site to produce maps of spatial distribution of repolarisation alternans magnitude within a couple of minutes (Orini et al, 2014). The analysis includes noise reduction, quantitative assessment of the recording from each electrode both in terms of noise and morphological consistency between consecutive waveforms, localization of activation and repolarisation markers, calculation of ARI and quantification of repolarisation alternans and variability. Customized algorithms developed within our group highlight the electrode of maximum alternans and minimum variability, and provides the user with the possibility to visually inspect the recording and to modify the selection of positive and negative alternans sites in a semi-automatic way. When the maximal positive or negative alternans site is identified, the

surgeon collects two core biopsies underneath the corresponding electrodes. The tissue is immediately stored in liquid nitrogen and send to a laboratory for mRNA and Western blot analysis.

At the molecular level, phenomena such as alternans are likely to involve changes to the action potential waveform (and therefore ion channels and Ca^{2+}-handling proteins) as well as its conduction characteristics (and therefore gap junctions and extracellular matrix). Epicardial biopsies taken from the alternans positive and negative sites are analysed for ion channel, Ca^{2+}-handling proteins, gap junctions and extracellular matrix molecular profiling. Total RNA is isolated from the biopsies and reverse transcribed to produce cDNA, which is then used for quantitative PCR (qPCR). For qPCR, TaqMan Low Density Array (TLDA) cards, which enable 384 genes to be assessed at the same time, are used to measure the expression of ion channels, Ca^{2+}-handling proteins, gap junctions and extracellular matrix constituents at the mRNA level. For qPCR, the abundance of a transcript (mRNA) of interest is normalised to the abundance of a 'housekeeper' transcript, the level of which should be constant from sample to sample. Included on the TLDA cards is a number of potential housekeepers including 18S, GAPDH and HPRT.

In a first set of samples, RealTime StatMiner software was used to analyse the data from the TLDA cards and the GeNorm stability score method was used to examine the suitability of the potential housekeepers for use individually and also in combination. This suggested that the best housekeeper was 18S+HPRT followed by 18S followed by HPRT followed by GAPDH. Following this, the abundance of the target transcripts (normalised to the chosen housekeeper) was calculated. As an example, Fig. 6 shows the abundance of mRNA for $K_{ir}2.1$ (an inwardly rectifying potassium channels) normalised to different housekeepers. With 18S+HPRT, 18S and 18S+GAPDH as the housekeeper, there was a significant upregulation of $K_{ir}2.1$ mRNA from the negative to positive alternans samples. Could $K_{ir}2.1$ be involved in alternans? An upregulation of $K_{ir}2.1$ is expected to shorten APD and the refractory period – this could favour alternans. In preliminary analysis, qPCR with 18S as the housekeeper identified 20 significant changes in mRNA for ion channels, Ca^{2+}-handling proteins, a gap junction channel, a Na^{+}-K^{+} pump subunit and receptors between the positive and negative alternans samples. In our experience a change in mRNA is usually translated to a change in protein, but this is not necessarily the case. Therefore, for some targets, Western blot is being used to investigate the protein level. In the future, there is scope for increasing the amount of data that can be obtained from such heart biopsies. Rather than qPCR, RNA-Seq (next generation sequencing) could be used to measure the expression of all ~24,000 transcripts in the human; complementary MiSeq could be used to measure the expression of all regulatory micro-RNAs; proteomics could be used to measure the expression of many proteins; and computer modelling could be used to predict the arrhythmogenic consequences of the changes in expression (Chandler et al, 2009).

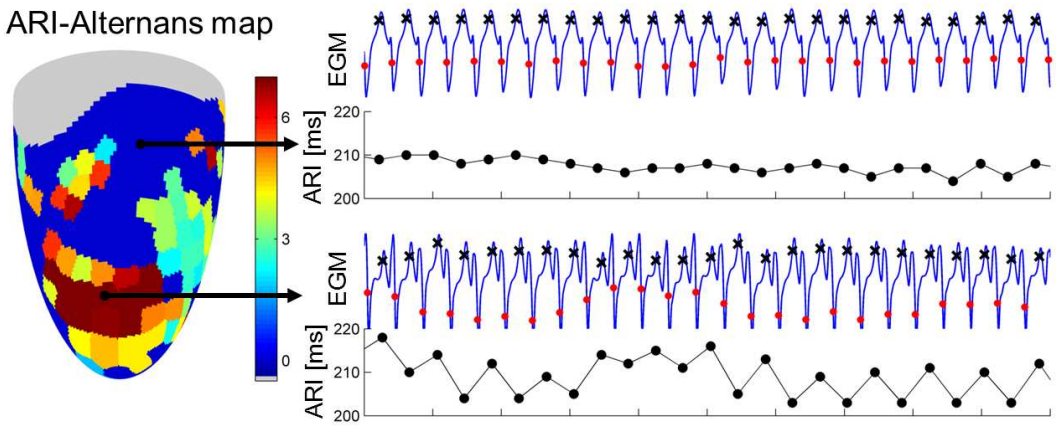

Figure 5. Left: Map of the spatial distribution of ARI alternans. Right: Unipolar EGM from a site exhibiting repolarisation alternans (bottom) and with stable repolarisation dynamic (top).

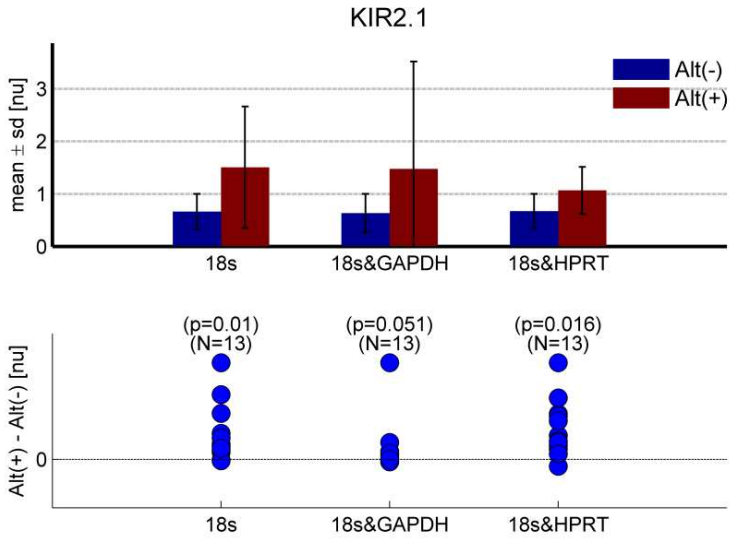

Figure 6. Abundance of mRNA for Kir2.1 (an inwardly rectifying potassium channels) normalised to housekeepers 18s, 18S+GAPDH and 18S+HPRT. Upper panel shows mean and standard deviation of Kir2.1 across patients, for both positive and negative alternans sites. Bottom panel shows the difference between Kir2.1 abundance in positive minus negative alternans sites. Each marker corresponds to a patient. Results suggest that Kir2.1 is overexpressed in tissue exhibiting ARI-alternans. *N* and p represent the number of pairs of samples studied and statistical significance of the difference in Kir2.1 abundance between positive and negative sites.

5. Future applications

In addition to these further developments at the molecular level, the techniques described in this article pave the way for future studies including combining multielectrode mapping (+/- biopsy) with simultaneous measurements of regional ventricular wall motion obtained from trans-oesophageal echocardiography, often routinely used during cardiac surgery. Combination of multielectrode mapping with imaging of scar regions is also an exciting possibility.

The acquisition of this whole heart tissue level data has important implications for biophysical modelling especially in terms of identifying novel drug targets and predicting the effects of specific agents. There is increasing interest in utilising in silico modelling to screen new anti-arrhythmic agents on the basis of their individual ion channel modulating effects to minimise the ethical and financial expense of animal studies (Mirams et al, 2012). Current in silico models are based upon animal and cellular data derived from multiple species and hence risk being inaccurate when attempting to model normal and pathological human electrophysiology (Fink et al, 2011). These in vivo studies will help ensure that such models are optimised. In addition, this gap is being closed through a rapid evolution in cell biology techniques which has meant that it is now possible to create induced pluripotent cell lines (iPSCs) derived from patient skin biopsies (Thomson et al, 2008, Takahashi, 2011). These cells can be programmed to become atrial, ventricular and AV nodal cardiomyocytes. However, a significant challenge includes ensuring that these cells recapitulate normal adult cardiomyocyte electrophysiology in terms of their ion currents and action potential characteristics as opposed to a fetal phenotype (Hoekstra, et al 2012). Current research is focussed on creating homogeneous cardiac cell lines with a common genetic background to provide consistent experimental systems. Data derived from mapping and tissue biopsy studies will serve to inform how these derived cells match normal and pathological electrophysiological behaviour to ensure that basic and pharmacological studies will be aligned with measured human electrophysiological parameters. This will close the translational loop when attempting to predict the effects of specific drugs at a cellular level & subsequently the whole heart as the models will be based on verified real human data. It will add considerable power to selecting optimal anti-

dysrhythmic drug properties and enable more accurate prediction of pro-arrhythmia in diseased myocardium. This will pave the way to a pipeline of in silico drug testing utilising refined screening through cellular and whole, heart biophysical models with effects verified in human iPSC derived cardiomyocyte cell lines before proceeding to expensive animal testing and eventual phase 1 human trials.

Another potential application of whole heart mapping studies will be to facilitate the development of anti-VF initiation strategies in patients who have received Internal Cardiac Defibrilltors (ICDs). At present ICDs purely function reactively to pace-terminate VT and defibrillate VF. What is required is a strategy to recognise electrical instability in the myocardium before the onset of VF eg. alternans and develop pacing strategies to electrically stabilise the heart eg. by promoting more homogenous repolarisation and prevent the onset of ventricular arrhythmias. Therefore, through utilising whole heart data it may be possible to develop sensing and pacing algorthms to avoid shocks in such patients which have been associated with increased mortality as reported in MADIT RIT (Moss et al, 2012).

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