Chapter 5

An *In Vitro* Model of Early- or No-Reperfusion Scars to Explain How Clinical Reentrant Arrhythmia Characteristics May Relate to Therapeutic Efficacy

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***** Equal contribution

To be submitted

Abstract

Purpose: Early-reperfusion during acute myocardial infarction causes scars with a patchy aspect, whereas no reperfusion causes compact scars. Patchy scars show a lower inducibility and shorter cycle length of arrhythmias compared to compact scars. Despite increasing numbers of early-reperfused patients, little data on arrhythmic mechanisms is available. We therefore developed an *in vitro* model of patchy and compact scar patterns to gain a mechanistic understanding of associated arrhythmia characteristics and how these affect the efficacy of anti-arrhythmic interventions.

Methods: Neonatal rat Ventricular monolayers were locally ablated at day 6-7 of culture by a laser-cut plexiglass stamp that produced an anatomical obstruction to mimic a compact scar or a stamp that produced multiple smaller anatomical obstructions with an equal total outer obstruction diameter to mimic patchy scars. One day later, optical mapping was performed.

Results: Inducibility of reentry was slightly lower in patchy cultures compared to compact cultures (41%, n=34 vs 52%, n=23, $P<0.05$) and cycle length of reentry was shorter (234±52 vs 288±38 ms, $P<0.05$). Reentry was less frequently sustained in patchy cultures (40% vs 88% in compact cultures) while the percentage of complex arrhythmias was higher (31% vs 11%). Meandering was only found in patchy cultures and could result in local polymorphic pseudo-electrograms. A gradient of excitability during reentrant arrhythmias was only detectable in patchy cultures. Termination of reentry by electrical stimulation was more easily achieved in compact cultures (82% vs 20% in patchy cultures) while Nav1.5 blockade by tetrodotoxin only induced meandering in patchy cultures (67% of arrhythmic cultures). In smaller cultures, this resulted in more frequent termination of reentry in patchy cultures.

Conclusions: An *in vitro* model of patchy and compact obstructions reproduces arrhythmic characteristics observed after early- and non-reperfused myocardial infarctions. Furthermore, it may provide mechanistic insights into the efficacy of anti-arrhythmic interventions in different anatomical substrates.

Introduction

Over the past decades, treatment of acute myocardial infarction (AMI) has become increasingly effective by focusing on restoring cardiac blood supply as early as possible. During AMI, time-to-reperfusion is not only a critical determinant of survival, but also ultimately affects scar composition and size.[1-3] By early reperfusion, cardiomyocyte (CMC) survival is improved and as a result, post-infarction scars are smaller and contain surviving myocardial fibers that gives the scars a patchy aspect.[2] This is opposed to the compact, fibrous scars that are rich in fibroblasts and extracellular matrix that do not contain large amounts of CMCs. As early reperfusion is becoming increasingly widespread due to adherence to constantly improving guidelines that focus on early treatment of AMI, the patient population with patchy scars is vastly growing.[4-6] Nevertheless, how scar composition affects arrhythmogenicity and arrhythmic phenotype is not completely clear. Recent clinical evidence suggests that inducibility of arrhythmias in early-reperfused patients was lower compared to non-reperfused patients.[7] Moreover, reentrant cycle length (CL) is shorter in early-reperfused patients. However, the electrophysiological mechanisms for these observations have not been fully elucidated. More importantly, it is there unknown whether these different scar compositions can influence the efficacy of pharmacological or electrical defibrillatory treatment modalities. Therefore, the current study set out to 1) develop a simplified *in vitro* model to reproduce clinical findings of these different scar compositions and 2) utilize this model to investigate the mechanisms behind the different clinical findings to subsequently 3) investigate how these mechanisms influence therapeutic efficacy of electrical or pharmacological defibrillation as this is more difficult to investigate in a clinical setting.

Methods

All animal experiments were approved by the Animal Experiments Committee of the Leiden University Medical Center and conformed to the Guide for the Care and Use of Laboratory Animals as stated by the US National Institutes of Health.

Cell isolation and culture

Neonatal cardiomyocytes were isolated from 2-day old wistar rats as described previously.[8,9] Animals were anesthesized by inhalation of 4-5% isoflurane. After pain reflexes were assured to be absent, hearts were rapidly excised and the ventricular tissue was minced and digested with collagenase I (450 units/ml; Worthington, NJ, USA) under gentle agitation. After a pre-plating step to minimize fibroblast contamination of cardiac cultures, cells were plated out on fibronectin-coated, round glass coverslips (22 mm) at a cell density of $1x10^6$ cells/well in 12-well plates (Corning Life Sciences, Amsterdam, the Netherlands). In another subset of experiments, 15 mm coverslips were used in 24-wells plates (Corning Life Sciences) and cell density was $5x10^5$ cells/well. Proliferation of endogenously present myofibroblasts was inhibited by administration of 10 µg/mL Mitomycin-C (Sigma-Aldrich, St. Louis, MO, USA) at day 1 for 2 hours.[8]

Preparation of anatomical obstructions

To produce pre-defined configurations of cardiac tissue, a method of inducing local cell death using specialized stamps was utilized to mimic scar compositions from early or non-reperfused myocardium. Stamps were designed using CAD-software (Solidworks, Dassault Systèmes, Velizy-Villacoublay, France). Designs were laser-cut into plexiglass using a PLS3.60 Laser (Universal Lasersystems, Scottsdale, AZ, USA). Bottom surfaces were gently polished with 9 µm polishing paper to equalize surfaces. By carefully pressing these stamps onto cardiac cultures at day 6-7 of culture, these stamps formed the desired pattern in the cardiac cultures. Cultures were allowed to adjust for ≥6h after ablation before optical mapping was performed. Stamps that mimicked the compact scar composition had an outer diameter of 11.5 mm and lacked any clefts in order to ablate cells over the entire surface area of the stamp. In contrast, stamps to mimic patchy composition had the same outer diameter of 11.5 mm, but were comprised of smaller circles with a diameter of 1 mm and interpositioned clefts that allowed for the survival of myocardial cells throughout the ablated area.

Optical mapping

Arrhythmogenicity of cultures was evaluated using optical mapping as described previously.[8,9] At day 7-8, cultures were loaded with 6 µmol/L of the voltage-sensitive dye Di-4-ANEPPS (Invitrogen, Breda, the Netherlands) diluted in DMEM/Hams' F12

(Invitrogen) for 10 minutes at 37^{o}C. Next, cultures were refreshed with warm (37^{o}C) DMEM/HAMS F12 and immediately mapped. Cultures were never exposed to mapping conditions for >30 minutes, and cumulative exposure time to excitation light never exceeded 1 minute. Electrical stimulation was delivered for 10 ms at ≥1.5x diastolic threshold using custom-made platinum electrodes spaced either 1 mm (narrow bipolar) or 20 mm (wide bipolar) apart. Narrow bipolar stimulation was used to investigate conduction velocity (CV) and action potential duration (APD), while wide bipolar stimulation was used to induce or terminate reentry. To investigate the inducibility of reentry, burst stimulation was performed by wide bipolar 14 Hz 10 ms pulses. Reentry was defined as at least 3 consecutive circular activations. Reentry was considered not inducible after 6 unsuccessful attempts at induction with burst stimulation. Electrical termination of reentry was performed by wide bipolar burst stimulation. Optical signals were recorded using a 100 by 100 pixels CMOS camera (MiCAM Ultima-L, Scimedia ltd, Costa Mesa, CA, USA) and several parameters were analyzed offline with Brainvision Analyze 1201 (Brainvision, Scimedia, Tokyo, Japan). CV was determined at least 3 times, directly perpendicular to the activational wavefront. Pharmacological interventions (tetrodotoxin (TTX), Alomone Labs, Jerusalem, Israel) were pipetted directly into the middle of the well, after which wells were gently agitated for 5 seconds to enhance distribution of the pharmacological agent. Excitability gradients were determined as the difference between CV at the spiral tip during an arrhythmia and the CV at the periphery.

Statistical analysis

Student t-tests were performed where appropriate. Statistical analyses were performed using SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL, USA). Differences were considered statistically significant if $P<0.05$.

Results

Partial or complete conduction block in cultures with patchy or compact obstructions

Neonatal rat ventricular monolayers were confluent and spontaneously beating by day 2 of culture and contained roughly 15% of non-myocytes as described previously.[8] At day 5, cultures were ablated with custom-made probes to form either patchy or compact obstructions (Figure 1A). These ablations produced local areas of

cell death with smooth, circular edges and often removed the ablated cells altogether (Figure 1B). Cultures are referred to as “patchy” or “compact” cultures depending on the obstruction pattern produced by the ablations. Optical mapping at day 8 of culture showed that APD_{80} did not significantly differ between any of the groups (233±76 ms and 249±31 ms for patchy and compact cultures, respectively, $P>0.05$, Figure 1D). In addition, CV was similar for patchy and compact cultures (21.5±2.1 cm/s and 21.1±1.7 cm/s, respectively, $P>0.05$, Figure 1E). Therefore, the basic electrophysiology of these cultures was considered to be equal. However, the structural differences as produced by the ablations did cause different degrees of dyssynchrony across the ablated areas, as conduction was possible across the ablated area in patchy cultures but not in compact cultures. This resulted in a conduction delay of 70±8 ms in patchy cultures vs 89±6 ms in compact cultures across equal distances ($P<0.05$, Figure 1F). Thereby, partial or complete local conduction block was established in this culture model to mimic scar compositions after early- or no reperfusion of myocardial infarctions, respectively.

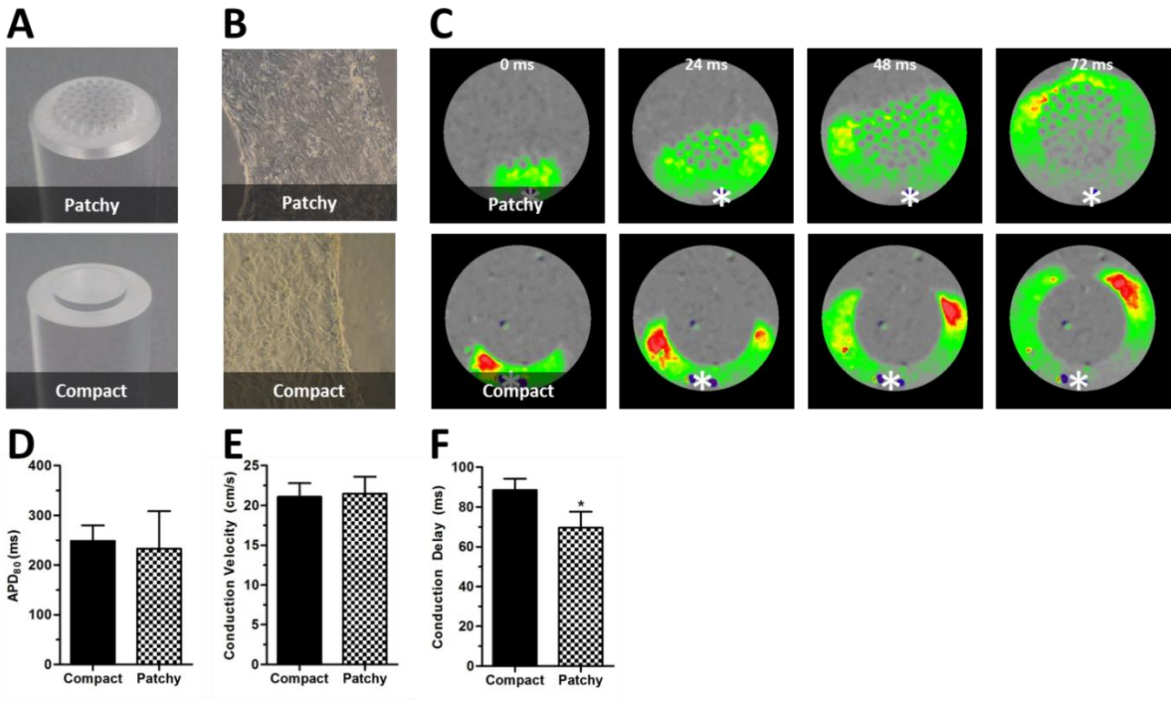

Figure 1. Local ablations in neonatal rat monolayers cause anatomical obstructions of conduction that do not affect basic electrophysiological parameters. (**A**) Pictures of the laser-cut plexiglass stamp that are used to perform (**B**) ablations of neonatal rat ventricular cardiomyocyte monolayers that produce surviving strands of cardiomyocytes and (**C**) non-conducting local anatomical obstructions as visualized by optical mapping. The (*) marks the site of electrical stimulation. Green, yellow and red signify activating tissue while gray marks inactive tissue. (**D**) APD_{80} and (**E**) CV do not significantly differ between cultures treated with the compact probe and cultures treated with the patchy probe. (**F**) Conduction delay between edges across the ablated area is significantly higher in the cultures treated with the compact probe. *: $P<0.05$ vs patchy cultures.

Patchy or compact obstructions reproduce clinical findings of differing inducibility, reentrant cycle length and complexity

In vivo, reentry in early reperfused hearts with patchy scars is less frequently inducible but has a shorter cycle. To investigate reproducibility of these findings in the current *in vitro* model, we performed burst stimulation during optical mapping experiments to induce reentrant tachyarrhythmias (Figure 2A, B). Inducibility of reentry was 41% in patchy (n=34) and 52% in compact scar cultures (n=23, Figure 2C). Moreover, while most of the reentry episodes in compact scar cultures were sustained (7 out of 8 reentry induction episodes), reentry in patchy scars was less frequently sustained (6 out of 15 episodes). Reentrant CL was shorter in patchy scar

cultures compared to compact scar cultures (234±52 ms vs 288±38 ms, *P*<0.05, Figure 2D). Of these CLs, 62% of reentry in patchy cultures was <250 ms, whereas in compact cultures, 24% was faster than 250 ms. Furthermore, CL adaptation was most pronounced in patchy cultures (decrease in CL within 1 minute of 50±29 ms versus 24±17 ms in compact scar cultures, *P*<0.05).

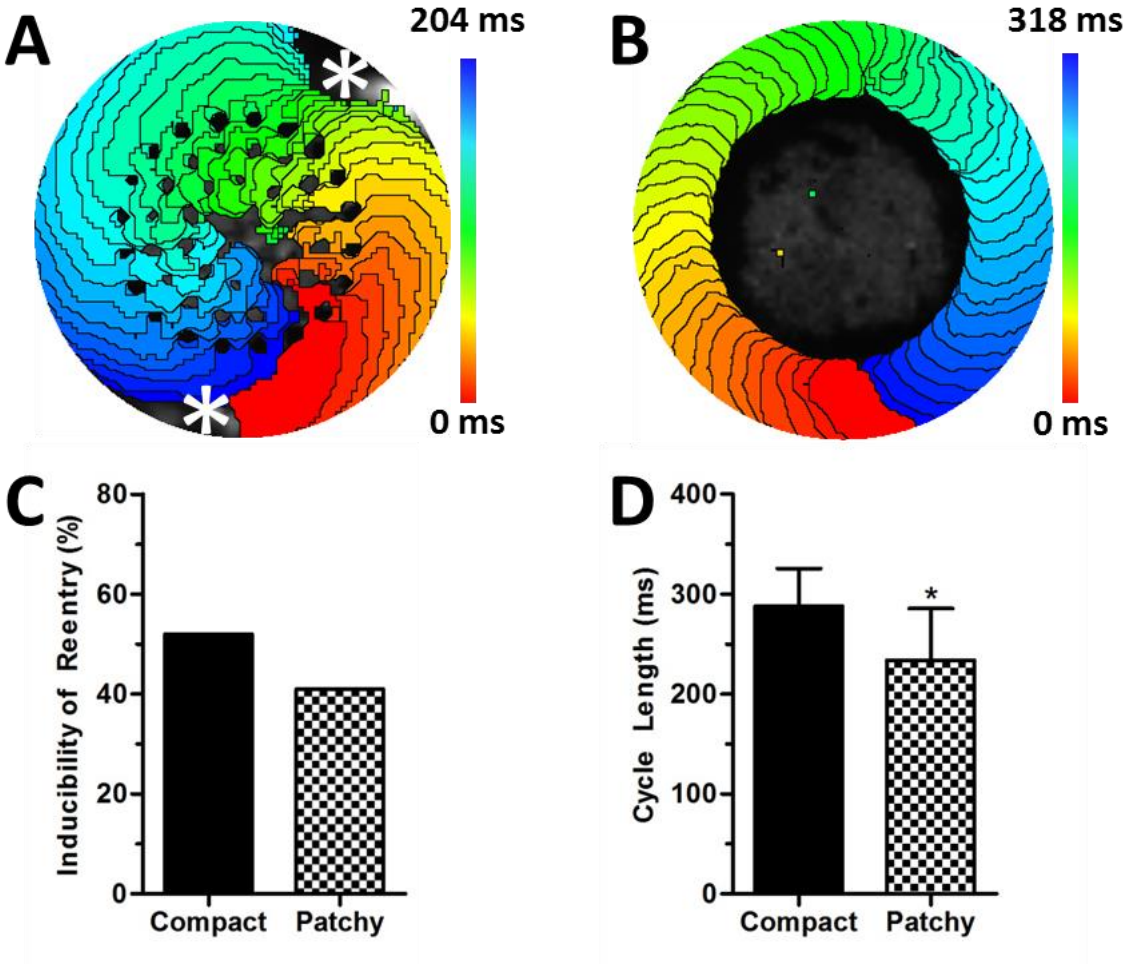

Figure 2. Reentrant tachyarrhythmias in cultures with patchy or compact obstructions. (**A**) Typical activation map of induced reentry in a culture with patchy obstructions. Isochronal spacing = 6 ms. The (*) marks the positions of the electrodes used to induce reentry by burst stimulation. (**B**) Typical activation map of induced reentry in a culture with a compact obstruction. Isochronal spacing = 6 ms. (**C**) Inducibility of reentry was slightly lower in patchy cultures. (**D**) Reentrant cycle length is shorter during reentry in cultures with patchy obstructions. *: *P*<0.05.

Excitability gradient from core to periphery is only present during arrhythmias in patchy cultures

Next, to utilize the model and investigate electrophysiological parameters that are difficult to measure *in vivo*, more in-depth analyses of arrhythmias were performed. Complexity of arrhythmias was assessed within the first minute of induction. Interestingly, complexity of observed reentry in compact cultures rarely consisted of more than one activating wavefront (1 out of 9 reentry episodes, Figure 3A). In contrast, reentrant arrhythmias in patchy cultures could consist of multiple wavefronts (5 out of 16 reentry episodes, Figure 3B, C). Moreover, reentrant arrhythmias within patchy cultures were shown to meander following induction (15 reentry induction episodes with meandering found in 11 episodes, Figure 4A) while none out of 8 reentry induction episodes in compact cultures revealed any meandering as reentrant arrhythmias immediately stabilized. During meandering in patchy cultures, local pseudo-electrograms had a polymorphic appearance (Figure 4B). In contrast, all observed reentrant arrhythmias in compact cultures showed a monomorphic pseudo-electrogram (Figure 4C). Out of 9 non-sustained episodes of reentry induction in patchy cultures, 7 showed meandering which implied a role of meandering in the termination of these arrhythmias.

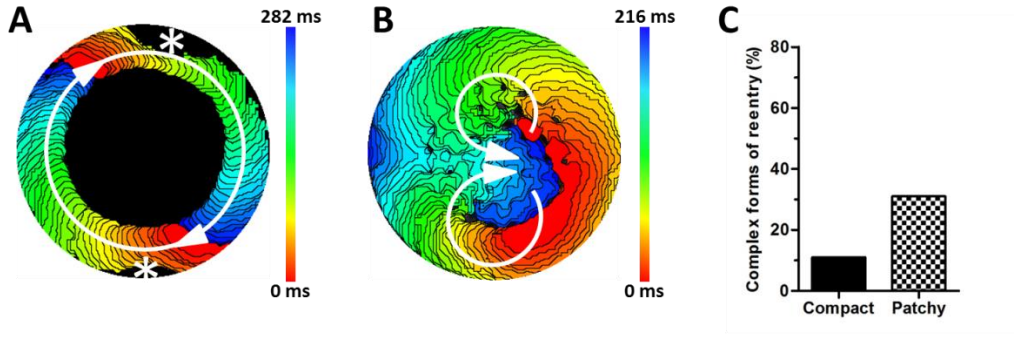

Figure 3. Complexity of reentrant arrhythmias. (**A**) Typical example of a complex reentrant arrhythmia in a culture with a compact obstruction showing multiple activational fronts. The (*) marks the locations of the electrodes that were used to induced reentry. (**B**) Typical activation map of a complex reentrant arrhythmia in a culture with patchy obstructions. Isochronal spacing = 6 ms. (**C**) Incidence of complex forms of reentrant arrhythmias was higher in cultures with patchy obstructions.

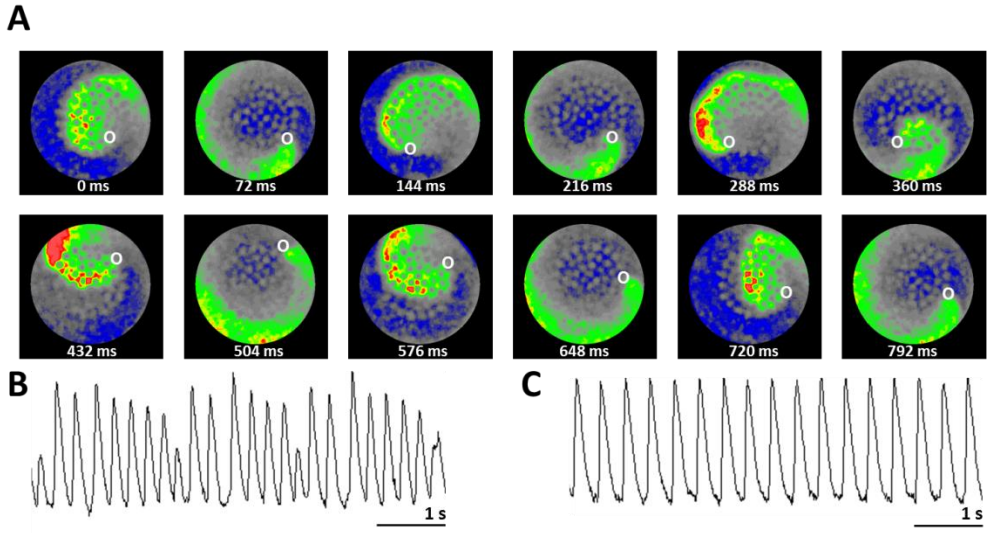

Figure 4. Rotor meandering in cultures with patchy obstructions gives rise to polymorphic pseudo-electrograms. (**A**) High-pass filtered pseudo-voltage map sequence showing meandering of a reentrant arrhythmia through the culture. The (O) marks the pivot point of reentry. Green, yellow and red signify activating tissue while gray signifies inactive tissue. Blue marks depolarizing tissue. (**B**) High-pass filtered pseudo-electrogram during meandering reentry in a culture with patchy obstructions shows a polymorphic appearance. (**C**) High-pass filtered pseudo-electrogram during non-meandering reentry, the only type of sustained reentry in compact cultures. These pseudo-electrograms were typically monomorphic.

During reentrant arrhythmias in patchy cultures, it was observed that the amplitude of the optical pseudo-voltage differed throughout the culture (Figure 5A). As changes in fluorescence of the used voltage-sensitive dye are an indication of changes in membrane potential, it appeared there was an excitability gradient during reentry in patchy cultures that was absent during reentry in compact cultures (Figure 5B).[10] To substantiate this claim, CV was determined at locations nearest to the pivot point of reentry and peripheral to the pivot point in reentry, as CV is highly dependent on the excitability of cardiac tissue. A significant difference between these values was only detected for reentrant arrhythmias in patchy cultures. Moreover, values of these differences were (5.8±0.3 cm/s) in patchy cultures vs (0.8±1.1 cm/s) in compact cultures (P<0.05, Figure 5C).

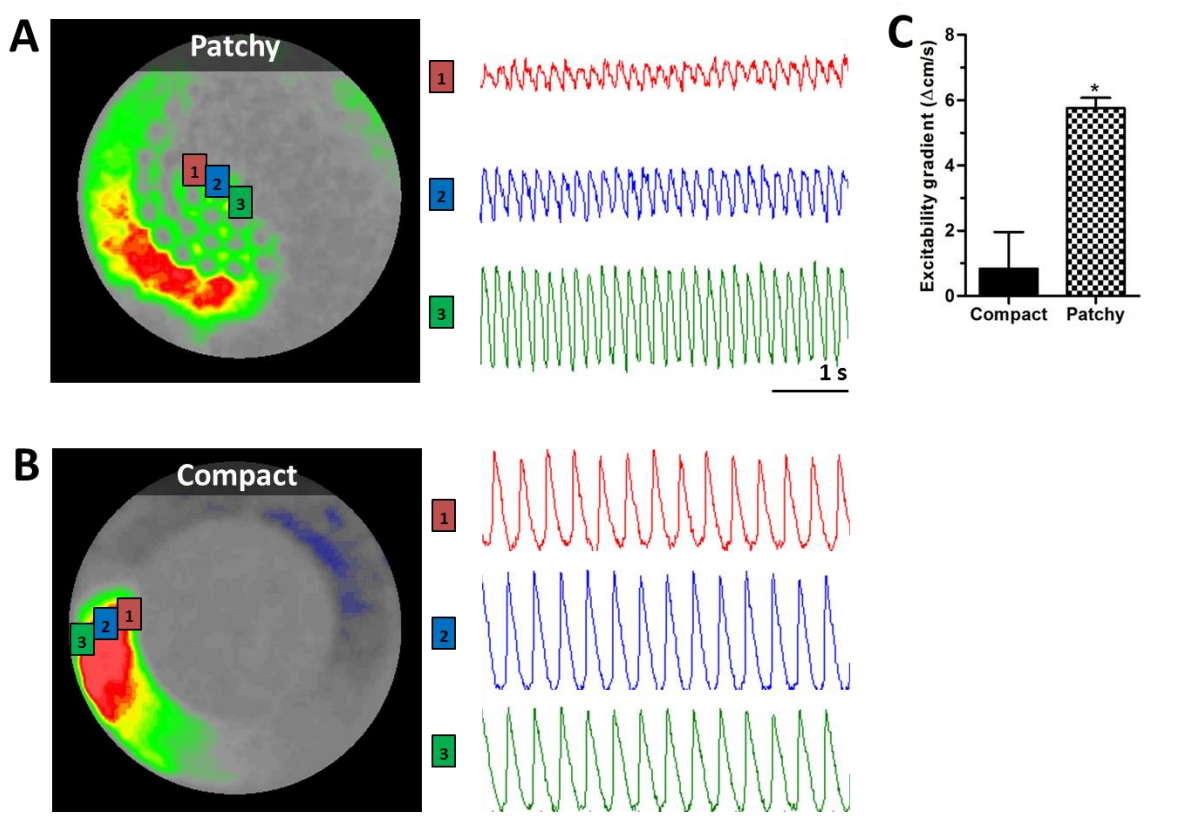

Figure 5. An excitability gradient during reentrant arrhythmias is only found in cultures with patchy obstructions. (**A**) Typical example of a high-pass filtered pseudo-voltage map with corresponding optical signals shows that optical signal amplitude increases from core to periphery during reentry in a patchy culture. Green, yellow and red signify activating tissue while gray marks inactive tissue. (**B**) Typical example of a high-pass filtered pseudo-voltage map with corresponding pseudo-voltage signals show that optical signal amplitude does not differ between the tissue nearest to the core of reentry or outer ring of tissue during reentry in a compact culture. Green, yellow and red signify activating tissue while gray marks inactive tissue. (**C**) During reentry, only patchy cultures showed a significant difference between the CV at the tissue nearest to the core of reentry and CV at the periphery. **P*: <0.05.

Anti-arrhythmic efficacy of fast-sodium channel blockade and electrical stimulation depends on the anatomical configuration

To investigate the efficacy of fast sodium channel blockade, 20 µM TTX was administered during arrhythmias. After arrhythmias were confirmed to be stable and sustained for at least 30 seconds, TTX was directly pipetted in the middle of the culture. After such an intervention in patchy cultures, reentry started to wander

throughout the culture in 67% of 12 cultures, whereas 14 compact cultures did not show such an activity (Figure 6A). Interestingly, only 1 out of 12 arrhythmias terminated in patchy cultures despite strong meandering. Moreover, 2 out of 14 arrhythmias terminated in compact cultures after TTX administration. To investigate whether this may have been due to the size of the culture and multitude of small obstructions in these cultures that provide re-anchoring points for these arrhythmias, we performed the same intervention in smaller cultures (15 mm in diameter) with smaller and less obstructions (6 mm outer diameter). In these smaller patchy cultures, TTX administration often resulted in termination (88%, n=8 arrhythmias, Figure 6B). In contrast, TTX was less effective in compact cultures (25% terminated arrhythmias, n=8 arrhythmias).

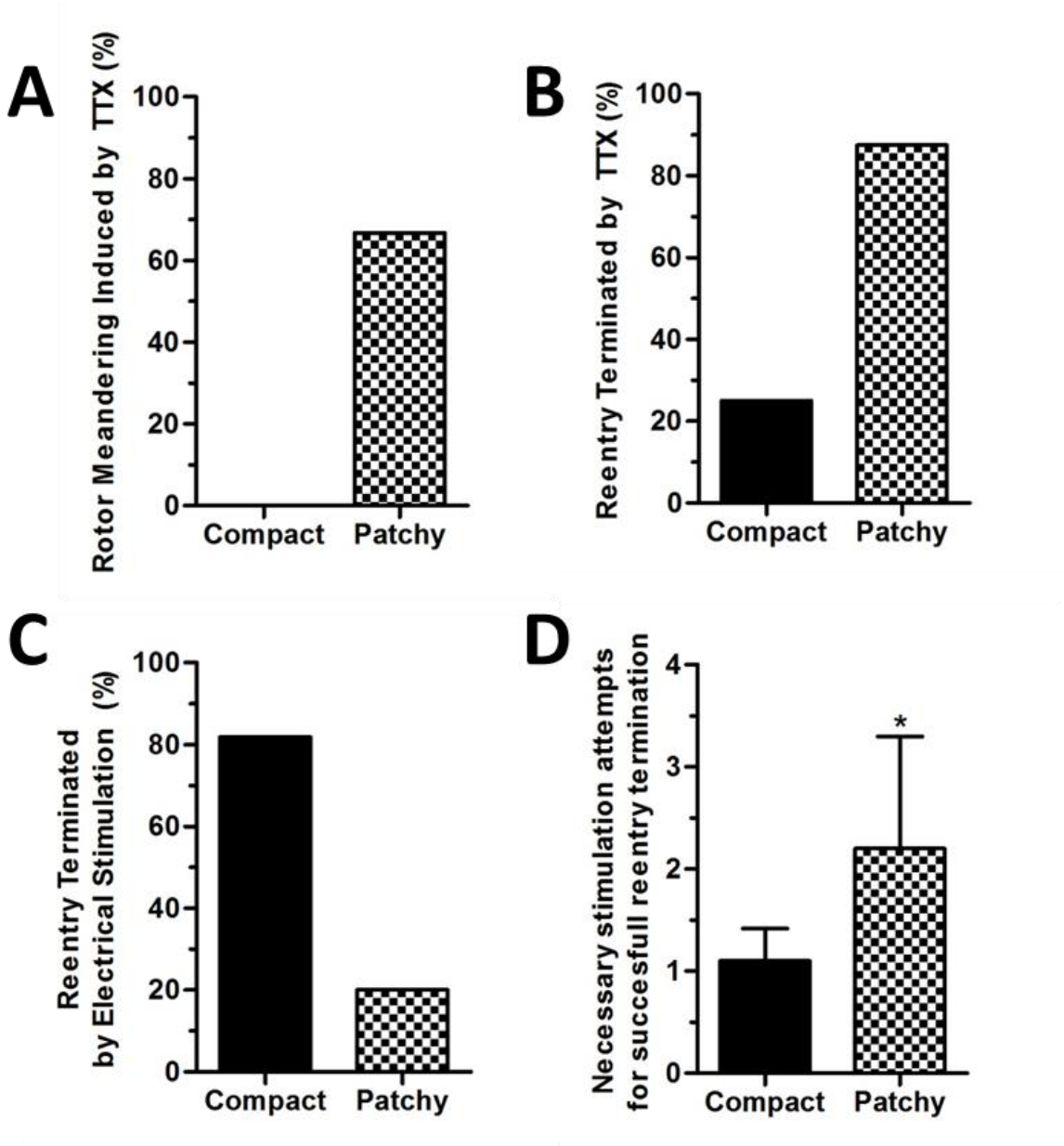

Figure 6. Effect of obstruction pattern on termination of reentry by sodium channel blockade or electrical stimulation. (**A**) Quantification of the percentage of arrhythmias that meander after TTX administration. (**B**) Quantification of the percentage of arrhythmias that terminate after administration of TTX in smaller cultures (total diameter of 15 mm and 6 mm outer diameter of the area of ablation) which shows that the induced meandering by TTX can be important for terminating arrhythmias. (**C**) Quantification of the percentage of reentrant arrhythmias that could be terminated by application of a bipolar 14 Hz electrical stimulation protocol. (**D**) Quantification of the number of attempts necessary to terminate reentry. *: $P<0.05$.

Alternatively, electrical stimulation was investigated for its anti-arrhythmic efficacy. In compact cultures, reentry was terminated by burst stimulation in 9 out of 11 cultures (Figure 6C). In patchy cultures, 1 out of 5 reentrant arrhythmias terminated by a single attempt at burst stimulation. Overall, 2.2±1 attempts of reentry termination by burst stimulation were necessary for successful reentry termination in patchy cultures, whereas 1.1±0.3 attempts were necessary in compact cultures (P<0.05, Figure 6D).

Discussion

Key findings of this study are 1) patchy or compact obstructions in a 2D cardiac model can reproduce clinical arrhythmic findings of early or non-reperfused myocardial scars and 2) reveal an excitability gradient that is only present during reentrant arrhythmias in patchy scar cultures and 3) the absence or presence of this excitability gradient is responsible for differences in anti-arrhythmic outcome of electrical and pharmacological defibrillation.

Reproducibility of clinical findings in a simplified in vitro model

Over the past decades, timely reperfusion during AMI has been proven to be crucial in improving acute but also chronic survival.[1,3] Duration of coronary artery occlusion is directly related to myocardial cell death, the transmural extent and size of the myocardial scar that develops.[11,12] As early reperfusion strongly limits myocardial damage, the subsequent myocardial scar contains a lower collagen density than non-reperfused scars. Importantly, early reperfusion also increases the amount of surviving cardiomyocytes within the infarction area which results in a patchy aspect of the scar.[2] Moreover, the pro-arrhythmic substrate that is formed by post-MI scars is altered in such a way that different arrhythmic characteristics are present in early- or non-reperfused patients.[7] However, it is unknown how early reperfusion affects the efficacy of electrical and pharmacological anti-arrhythmic interventions, despite a growing patient population due to better care and management of AMI. In the current study, an *in vitro* model of these patchy or compact scar compositions was developed to gain more insight into the mechanisms of differing arrhythmogenicity. By locally inducing cell death, anatomical obstructions were prepared in 2D neonatal rat cultures that allowed for surviving myocardial strands capable of conduction

through the scar in the patchy group but formed a solid, compact obstruction that blocked conduction in the compact scar group. As a result, dyssynchrony across the scar was higher in the compact group than in the patchy group. In the clinical setting, dyssynchrony also corresponds to obstruction size.[13] Moreover, this model reproduced the difference in reentrant CL and due to the use of optical mapping, showed that this difference in CL can be explained by differing path lengths.[7] While non-reperfused and early-reperfused patients without prior arrhythmias do not show a difference in the inducibility of reentry, inducibility is lower for early-reperfused patients that had prior arrhythmic episodes compared to non-reperfused patients with prior arrhythmic episodes.[7] Remarkably, the inducibility of reentry in the current study can be positioned in between these findings as the inducibility of reentry in patchy cultures was slightly lower than in compact cultures. However, the electrophysiological equality of both groups in our model regarding APD and CV at 1Hz activation rate, and the observation that arrhythmic episodes can modify the electrical behavior of myocardial tissue to favor arrhythmias[14] are arguments that suggest our model may be most relevant to patients without prior arrhythmias. Though the current model is based on neonatal rat cardiomyocytes and any direct comparison of parameters may appear arbitrary, it is peculiar that the clinically established criterion of “fast VT” with a CL below 250 ms can be applied to our model with similar outcomes.[7] Besides the high degree of reproducibility of clinical findings in this *in vitro* model, the most promising implications of such a model lie in the potential for in-depth investigation of arrhythmic mechanisms.

Anatomical versus functional reentry and the relevance of an excitability gradient

Using the optical mapping technique, arrhythmic dynamics can be studied in detail with a high spatial and temporal resolution.[15,16] Therefore, this technique was perfectly suited to further investigate arrhythmic mechanisms of reentry in cultures with patchy or compact obstructions in a manner that is currently difficult to realize *in vivo*. By visualizing conduction, it was found that reentry in patchy cultures exhibited features of a functional type of reentry, as wandering of the arrhythmia throughout the culture was observed in a relatively large portion of the cultures.[17,18] In contrast, reentry in compact cultures did not show meandering and adhered more to the specifications of anatomical reentry.[19] These differences can be explained by the differences in minimal obstacle size to which these arrhythmias pin to in patchy or in

compact cultures.[20] Therefore, the distinction between functional and anatomical reentry is based on a gradient rather than strictly separate phenomena.[21] The smaller the object, the higher the degree of head-to-tail wave interaction and the more the arrhythmia resembles functional reentry. Moreover, the degree of head-to-tail wave interaction determines the excitability of the affected tissue by interfering with recovering ion channel dynamics. This also explains the presence of the excitability gradient in patchy cultures and its absence in compact cultures, as during functional reentry, the head-to-tail wave interaction is maximal at the pivot point of reentry and decreases towards the periphery. The relevance of such a gradient was demonstrated in the current study, as Nav1.5 blockade using TTX altered culture excitability and increased the probability of meandering in patchy cultures, but did not strongly affect arrhythmias in compact cultures apart from increasing the CL. The mechanism behind this destabilization depends on the altered source-sink relationships due to decreased excitability of the tissue by Nav1.5 blockade, which forces an increase in the minimal pivoting radius of the reentrant arrhythmia and detachment of the anatomical obstruction.[22-25] By inducing meandering, reentry may be terminated as the core encounters inexcitable boundaries.[20,26] Indeed, this was confirmed in the current study by repeating the same experiment in smaller cultures that made it more likely for the arrhythmia to encounter inexcitable boundaries. In addition, the excitability gradient also determines the excitable state of a culture. Due to the absence of a fully excitable gap during functional reentry, electrical stimulation during reentry in patchy cultures was less successful at termination than in compact cultures.[19] This electrical stimulation was delivered with bipolar electrodes at a high frequency, so that it bears resemblance to anti-tachycardia pacing and defibrillation protocols. Taken together, this model may provide a useful tool to improve our current understanding of reentrant arrhythmia dynamics and to know how they influence therapeutic efficacy in early or non-reperfused patients.

Future perspectives and limitations

Despite the high reproducibility of clinical findings with the currently presented *in vitro* model, the authors recognize that its relevance to clinical situations needs to be substantially increased. While the current study made a clear distinction between the patchy anatomical composition after early reperfusion or the clear-cut compact scar composition without reperfusion for standardization purposes, the *in vivo* setting is

more complex, with for example borderzones that may exhibit features of both patchy and compact fibrosis. By gradually increasing the complexity of the anatomical configurations in this model, the model can be more tailored to such specific clinical situations in the future. Currently, ventricular cardiomyocytes from neonatal rats have been used to create a 2D monolayer of cells in which arrhythmic dynamics can be more easily interpreted than in 3D tissue preparations. These cells were utilized due to their ability to stay in culture for extended periods of time, to form confluent beating monolayers and their availability, as human adult CMCs cannot, despite their superior clinical relevance. However, with the recent discovery of induced pluripotent stem cells (iPS cells) that allow for the genetic reprogramming of somatic cells into cell types of different lineages such as cardiomyocytes, future studies will benefit from utilizing iPS-derived CMCs for their confluent monolayers.[27] Despite reported impurities and inefficiencies of cardiac differentiation of such reprogrammed cells,[28] very recent technological advancements[29] make it likely that purified human cardiac monolayers can be utilized to increase the relevance of *in vitro* models for the clinical setting in the near future. As these iPS-derived human cardiomyocytes may more accurately represent the patient-specific ion channel expression profile, such cells have already been used to demonstrate the feasibility of patient-specific drug-screening techniques.[30] The combination of human cardiomyocytes in monolayers with the anatomical compositions of their respective pro-arrhythmic substrate may therefore not only elucidate pro-arrhythmic mechanisms, but also provide a very powerful patient-specific anti-arrhythmic drug-screening tool in the near future.

Conclusions

An *in vitro* model of early- or non-reperfused post-myocardial infarction scars reproduces clinical arrhythmic findings. Importantly, this model allows for the investigation of arrhythmic mechanisms such as meandering and complexity of arrhythmias that is more difficult to realize in a clinical setting. Finally, this model may provide mechanistic insight in how these arrhythmic mechanisms influence the therapeutic efficacy of anti-arrhythmic interventions.

Acknowledgements

We wish to thank H. van der Stadt and R. van Leeuwen for excellent technical support.

Sources of Funding

This work was supported by the Dutch Heart Foundation (2008/B119) and the Netherlands Organization for Scientific Research (NWO; VENI grant (91611070), D.A.P).

Conflicts of Interest

None declared.

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