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Medical Sciences

Pneumographic Imaging of Potential Cleavage Planes within the Ventricular Myocardium in Histology and Computed Tomography

Klaus Redmann[*], Paul Peter Lunkenheimer[**], Sandra Stöppeler[§], Ulrich Spiegel[§], Morten Smerup[§§], Peter A. Nielsen[§§], Peter Niederer[¶], Stephan Weiss[¶], Randas V. Batista[¶¶]

** Center for Reproductive Medicine and Andrology, University Hospital, Münster, Germany*
*** Foreign Member of the Georgian National Academy of Sciences, Former Dept. of Experimental Thoraco-vascular Surgery, University Hospital, Münster, Germany*
§ Department of Surgical Research, Clinique of General and Visceral Surgery, University Hospital, Münster, Germany
§§ Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital, Skejby, Denmark
¶ Institute for Biomedical Engineering, ETH and University of Zürich, Zürich, Switzerland
¶¶ Batista Heart Foundation, Curitiba, Brazil

ABSTRACT. We distended the relaxed porcine left ventricular myocardium by inflating the coronary arteries with compressed air, using rising pressures between 1 and 3 atmospheres. Computed tomographic analysis revealed a feathered arrangement of the myocardium aggregated together within the walls, with an overall system of spatially netted lamellar structures. Histological examination in orthogonal planes revealed the lamellas themselves to be made up of endless chains of myocytes, which show the well recognised systematic change in helical angle when traced through the thickness of the walls. The pneumatic distension had opened clefts between the lamellas, with sparse and loosely aligned fibrous tissue present within the clefts. The lamellar structure itself is continuous through the walls via a system of interlamellar connections from epicardium to endocardium, and from the ventricular base to the apex. The ventricular walls, therefore, which appear compact when sectioned without pneumatic distension, are shown by distension to be composed of a system of three-dimensionally curved and nested blades, with the individual blades taking their origin from a central circular myocardial collar, Krehl´s Triebwerkzeug, and then extending with opposing curvatures towards the endocardium and epicardium. *© 2011 Bull. Georg. Natl. Acad. Sci.*

Introduction

During contraction thickening of the individual cardiomyocytes is minimal. It is difficult, therefore, to explain the thickening of the wall on the basis of the increase in thickness of the contracting cardiomyocytes [1]. Histological investigations have revealed a mean increase of two-fifths in the number of myocytes packed between the epicardium and endocardium during systole as compared to diastole [1, 2]. Histology and ultrastructural investigations have shown the cardiomyocytes to be tightly anchored within a matrix of connective tissue [3-7]. Over recent decades, several contrasting models have been advanced to explain myocardial regional displacement, albeit that none, to date, have identified any anatomical pathways which would permit the necessary gliding between the postulated myocardial components [1, 8-11]. The aim of our current study, therefore, was to use pneumatic distension [12] to identify spaces that might provide the necessary freedom of motion for the myocytes aggregated together within the ventricular walls.

Methods

We had previously used compressed air to dilate the interstitial spaces within the ventricular mass in an unsuccessful attempt to validate the notion that the myocardial mass was organised on the basis of a rope-like continuum [9,12]. We used computed tomography and histology to investigate the shape, size, and alignment of the myocardial mass aggregated within the ventricular walls. Porcine hearts were imaged using computed tomography, first in the control state, and then while the coronary arteries were inflated by compressed air delivered at pressures up to 3 atmospheres (Fig.1).

Computed tomographic images were acquired on a current dual-source system (Somatom Definition, Siemens Healthcare, Forchheim, Germany), using detector collimation of 16 by 0.3 mm, and rotating the gantry for 1 second at a fixed pitch of 0.5. The effective current time product was 80 milliamperes per rotation, and the tube voltage was 120 kilovolts. The images were reconstructed using a slice thickness of 0.4 millimeters, a reconstruction increment of 0.1 millimeter, and a very sharp-tissue convolution kernel (U75) to achieve high contrast resolution. Secondary multiplanar reformations perpendicular to the short axis of the heart were created using a standard workstation (Syngo MultiModality Workplace; Siemens Healthcare, Forchheim, Germany).

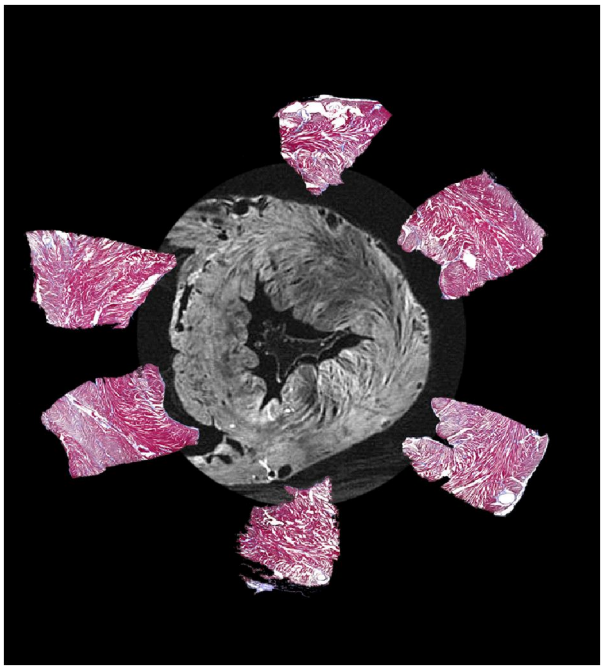

Fig. 1. Cross-section through the porcine heart in computed tomography during pneumatic distension exposing its feathered basic structure. Histological specimens are arranged at corresponding locations around the near-equator cross-section.

In 2 hearts, we catheterized the coronary arteries, rinsed them with saline, inflated them for some minutes with compressed air, and perfused them with 4 % formaldehyde for 2 days, using a pressure of perfusion which maintained them at the size shown to be produced by pneumatic distension at 1 atmosphere. The walls of the left ventricle were then prepared for histological investigation, examining the segments previously identified for the purposes of echocardiographic analysis [13]. Sections in the first heart were cut parallel to the short axis, while in the second heart they were cut parallel to the long axis, each section extending transmurally from endocardium to epicardium (Fig. 2). The sections were stained with Azan.

All procedures for animal care and experimentation followed the guidelines of the American Physiological Society and the German Law of Animal Protection. The Protocol was approved by the University of Muenster Institutional Animal Care and Use Committee and adhered to the guidelines for the use of laboratory animals of the National Institutes of Health and Prevention.

Results

As the coronary arteries are inflated with compressed air, the intramural pressure rises, and the interstitial spaces between the aggregated myocytes become distended (Fig.3). The microvascular pathways and their connec-

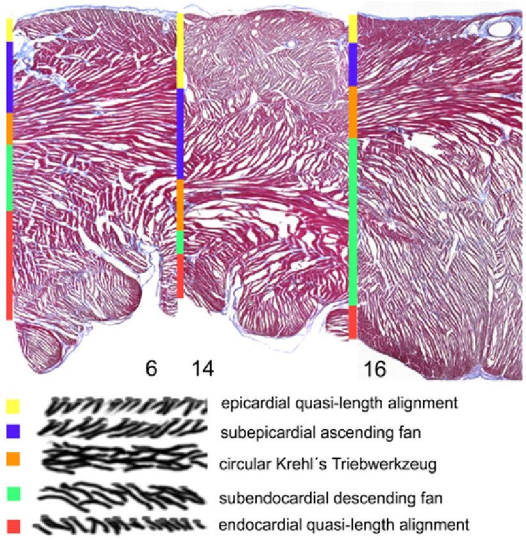

Fig. 2. Histology stained Azan and 10 times amplified, showing the typical feathered structure of left ventricular porcine myocardium which is cross sectioned at the base (area 6) and midway between base and equator (areas 14 and 16), with 5 arbitrarily discriminated layers. There are no demarcating connective tissue membranes between those "layers". Note that the quasi-circular layer, which is Krehl´s Triebwerkzeug, varies markedly in thickness and position between epicardium (upper edge) and endocardium (lower edge).

tive tissue suspension are obviously quantitatively eliminated from the interstitial space by force of the pneumatic distension. During the pneumatic distension, the colour of the epicardial surface turns from deep red to clear red, and the weight of the heart drops within the first hour to around 80% of the control value. The resistance to flow through the myocardium drops during pneumatic distension, with a concomitant fall noted in the driving pressure during inflation.

Computed tomography

Figure 1 shows selected cross-sections of the overall walls, illustrating their feathered appearance. When scrolling through the overall sequence of cross-sectional slices from base to apex, and from apex to base, both the inner and outer fans of the feather (see Fig. 2) seem to turn in opposite direction, the orientation of rotation being reversed when traced from base to apex, or from apex to base. In that pseudo-animation the rotation upon the ventricular cavity is most pronounced in the fans that take their origin from a central circular myocardial mass which is Krehl´s Triebwerkzeug, albeit that some degree of pseudo-motion is noted in all areas of the left ventricular walls. The extent of feathering varies markedly at different levels in the equatorial quadrants of the ventricular mass, while being much less obvious at the base and the apex.

Histological findings

In the hearts distended for 48 hours by high-pressure flow with formaldehyde subsequent to pneumatic distension, lamellar aggregates of myocytes are seen (Fig. 3), separated by wide interstitial spaces, but with marked variation in length, shape and orientation noted within the various ventricular segments. Examination in the sections taken in the short axis reveals the quasi-circular profiles, or "triebwerkzeug", as initially identified by Krehl [14]. This circular mass varies markedly in its thickness, and in its position between the epicardium and endocardium (Fig. 2). Examination of the segments adjacent to the ventricular equator confirms the feathered arrangement, as initially noted by Feneis [8], with opposing aggregates spreading endocardially and epicardially from the central circular aggregates. Beyond the inner fan of the feather, which runs centripetally from the circular mass, a densely aggregated layer of cardiomyocytes forms the sub-endocardial region of the ventricular wall, with a similar layer, of varied thickness, extending beyond the outer fan to produce a sub-epicardial layer (Fig. 2). These five arbitrary layers are exaggerated throughout the ventricular walls by the pneumatic distension, but no strict

Fig. 3. Transmission of light-microscopy at 10 times amplification of a thick slice of myocardium which was taken from the left ventricular wall after pneumatic distension. It exposes its basic lamellar structure.

anatomic boundaries formed by connective tissue are found between them.

The chains of myocytes aggregated to lamellae (Fig. 3) and forming the inner as opposed to the outer fan are angulated one relative to the other. They show a progressive change in their angulation relative to the ventricular equator when traced through the depth of the walls (Fig. 4). The myocytes making up the innermost sub-endocardial, and the outermost sub-epicardial compartments are cut almost crosswise, indicating their almost longitudinal orientation from base to apex. All thus identified wall structures show interweaving.

The histology shows that connective tissue is particularly sparse between the distended aggregates, while collageneous structures are preserved within the lamellae. Examination in the long axis shows that the clefts between the chains of myocytes aggregated together within lamellas in the basal part impart an accordion-like configuration to the wall [15], with multiply interrupted pathways extending from epicardium to endocardium, as well as in longitudinal direction.

Examination at higher power reveals that the extent of the clefts depends on the extent of myocardial bridges between adjacent aggregates, such bridges being more frequent in sections cut in the long than in the short axis. The bridges themselves are thinner than the aggregates of myocytes from which they take origin. Having run for a short distance, they merge with off-springs of long chains of myocytes which belong to an adjacent lamella.

Discussion

Explanations of cardiodynamics during the 20th century have been biased by the erroneous assumption that all the myocytes aggregated together within the ventricu-

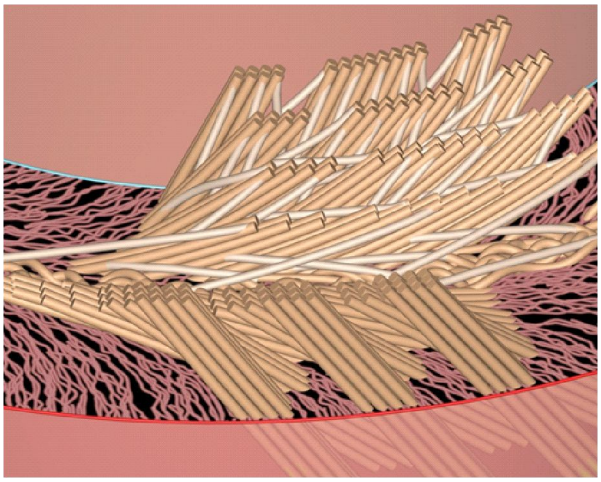

Fig. 4. The cartoon shows schematically the alignment of long chains of myocytes emerging out of the plane of the cross sectioned left ventricular wall with their helix angle rotating systematically between epicardium (lower surface) and the endocardium (upper edge). The chains of myocytes are aggregated, thus forming a lamellar structure. Within the lamellae the long chains are densely interlinked, while linkages are sparse between lamellae.

lar walls are aligned in tangential fashion, with the long axes parallel to the epicardial surface. This concept, initially published in 1901 by Frank [16], was seemingly confirmed by the investigations of Streeter and colleagues. Thus, for decades, mathematical models have similarly been based on the simplistic doctrine that all forces generated by the cardiomyocytes contribute exclusively to ventricular constriction and emptying [16, 18]. Little attention has been paid by modelers, however, to the multiple morphological [1-8, 12, 15, 20-22, 24-26, 29-31] and functional [1, 15, 17, 28, 32] studies showing the ventricular walls to be composed of a three-dimensionally netted continuum with not only tangential, but also intruding epicardially to endocardially aligned components. In compliance with the argument of Frank [16], this three-dimensional network is able to generate simultaneously both constricting and dilating forces [28, 32].

Our findings need also to be considered within the knowledge that, over the centuries, accounts of the arrangement of the myocytes making up the ventricular walls have been replete with descriptions of subdivisions, with various authors accounting for features such as the so-called bulbo-spiral muscle [3], nested toroidal shells [7], a unique myocardial band [9], or radial sheets of myocytes extending transmurally from epicardium to endocardium [10]. Those adapting the anatomical descriptions to their own purposes [19], however, ignored the fact that independent investigators had been uniformly unable to identify any arrangements of the supporting collagenous matrix that permitted division of the ventricular myocardial mass into the purported subunits. Indeed, as long ago as 1864, Pettigrew [20] had made the crucial observation that the ventricular myocardial mass is no more than a modified blood vessel, with each individual myocyte being anchored only within the collagenous matrix, and connected only to its immediate neighbours.

The extent of the population of cardiomyocytes aligned in intruding fashion has recently been measured by histology [24] with these findings confirmed by magnetic resonance diffusion tensor vector imaging [25, 26], For these studies, circular knives had been used to remove blocks from the ventricular walls, the semicircular format of the subsequent sections compensating for the progressive turn in the known helical angle of the aggregated cardiomyocytes in the tangential plane. The curved sections exposed chains of myocytes of sufficient length to permit assessment of their angulation relative to the epicardial surface plane. Pope and co-authors [27] have then implemented the notion of using a curved sectioning technique so as to compensate for the transmural rotation of the helical angle into their method of confocal laser scanning microscopy. Their recent findings, as we interpret their images, provide further confirmation of the notion put forward by Pettigrew [20], namely that the ventricular walls are aggregated together in the form of a three-dimensional mesh, in which the basic anatomical subunit remains the individual cardiomyocyte [24-26]. It is the intruding population of cardiomyocytes found within the three-dimensional mesh that provides auxotonic rather than unloading forces, a fact demonstrated by insertion of force probes into the ventricular mass during cardiac surgery, and confirmed histologically by experiments in porcine hearts [28].

When describing the results of our pneumatic distension, we have employed the notion of quasi-layered subdivisions within the ventricular walls. There are, however, no discrete anatomic boundaries to be found between our described sub-divisions. Furthermore, the arrangement and extent of those layers varies markedly from segment to segment within the ventricular mass. The revealed subdivision almost certainly reflects specific local resistances to the forces used for pneumatic distension, and likely is related to the degree of side-to-side coupling between the chains of myocytes aggregated together within lamellas. One functional implication of the loose coherence between the lamellae might be a particular propensity to cyclic realignment.

Our pneumatic distension has shown that inter-lamellar clefts were well distended, whereas the lamellae themselves remained dense and compact. This, we suggest, reflects the influence of the endomysial connec-

tive tissue [4, 5], which provides the supporting matrix for the myocardial mesh. Fibrous tissue is flexible, yet within the dimensions of contractile forces, is practically non-distensible [6]. It is well established that each sarcomere of a myocyte is suspended by a circular crown of struts, which themselves are anchored in the supporting endomysial connective tissue matrix [4, 5, 31]. The arrangement of this endomysial connective tissue is likely to be the key to the rearrangement of the individual cardiomyocytes during each cardiac cycle. They seem to make use of the double domain of mechanical behaviour of the connective tissue, the endomysial one being designed to join chains of myocytes to aggregates as small lamellar actuators, the perimysial [4, 5] one permitting these actuators to glide one against the other. The first domain requires a particularly rigid coupling to each myocyte. The second quality is established by the particularly loose alignment of collagen which is housed between the lamellae. Distinction of these two qualities of fibrous tissue can reconcile the enigma of the strong coupling of each myocyte to collagen via struts, a feature which, at first sight, seems to exclude any major freedom of motion of the myocytes. Morbid alteration of the lubricating function of the perimysial connective tissue, in contrast, such as occurs in myocardial fibrosis, eventually results in myocardial fettering, which disturbs ventricular pump function prior to the onset of any proper myocardial disease [6, 32]

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სამედიცინო მეცნიერებანი

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კ. რედმანი[*], პ. ლუნკენჰაიმერი[**], ს. შტოპელერი[§], უ. შპიგელი[§], მ. სმერაპი[§§], პ. ნილსენი[§§], პ. ნიდერერი[¶], ს. ვაისი[¶], რ. ბატისტა[¶¶]

[*] *რეპროდუქციული მედიცინისა და ანდროლოგიის ცენტრი, საუნივერსიტეტო საავადმყოფო, მიუნსტერი, გერმანია*
[**] *აკადემიის უცხოელი წევრი, ფოფლი გულსისხლძარღვთა ექსპერიმენტული ქირურგიის დეპარტამენტი, საუნივერსიტეტო საავადმყოფო, მიუნსტერი, გერმანია*
[§] *ქირურგიული კვლევების დეპარტამენტი, ზოგადი და შინაგანი ქირურგიის კლინიკა, საუნივერსიტეტო საავადმყოფო, მიუნსტერი, გერმანია*
[§§] *გულმკერდისა და სისხლძარღვთა ქირურგიის დეპარტამენტი, ორჰუსის საუნივერსიტეტო საავადმყოფო, სკეჯბი, დანია*
[¶] *ბიოსამედიცინო საინჟინრო ინსტიტუტი, ფედერალური ტექნოლოგიური ინსტიტუტი და უნივერსიტეტი, ციურიხი, შვეიცარია*
[¶¶] *ბატისტას გულის ფონდი, კურიტიბა, ბრაზილია*

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