Smooth muscle in the human mitral valve: extent and implications for dynamic modelling

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The mitral valve (MV) leaflets have traditionally in education and in clinical practice been regarded as passive flaps. However, MV consists not only of a connective tissue skeleton covered with endothelial cells but also of muscle bundles (1–8), blood vessels and nerves (3, 8–10), suggesting dynamic capabilities (7, 8, 11). The valve is divided in an anterior (aortic) and a posterior (mural) leaflet. The anterior leaflet occupies about a third of the annular circumference and when closed forms the greater part of the atrial floor (12). The atrial surface is smooth in both leaflets, while the ventricular surface is more irregular due to the insertions of the tendinous cords. The connective tissue in the leaflets is described to have a collagenous core (fibrosa) and an elastic layer (spongiosa) prominent at the atrial side (1). Numerous cells are present within the connective tissue. These cells have contractile elements and are often named myofibroblasts or valvular interstitial cells (VICs) (13–16). Others have described three phenotypes of interstitial cells: myofibroblasts, fibroblasts and single smooth muscle cells (17). One study has stated that there is a functional coupling between VICs and the collagen fibres (16).
Nerve fibres are supposed to have a sensory and a regulatory function of the contractile cells, and they are located on the atrial side (9, 10, 18).

The descriptions of muscle bundles within the leaflets of the MV and the relation to muscle tissue in the left atrial wall are inconsistent. It has been found, or referred to, that cross-striated muscles bundles (cardiac muscle) are found in or extend into the valve (2–4, 7, 8), and into the upper two-third of the leaflets in a complex interweaving pattern (4, 8), and that the muscle bundles in the leaflets are in continuity with the annular or atrial cardiac muscle or descend from these (2, 3, 6, 8, 12). Others have stated that muscles bundles in the leaflets are not in direct continuity with the atrial muscles (5), and that the muscles bundles are smooth (1). Single smooth muscle cells have also been observed beneath the endocard at the ventricular surface of the leaflets (6, 8, 19). There seems, however, to be an agreement that the muscle bundles are located beneath the endothelial cell layer at the atrial surface, and primarily in the anterior leaflet.

A limited number of actual studies on the presence of muscle bundles in the MV have been done (1–6). Muscle cells in the muscle bundles in the MV are not properly phenotyped with regard to type of muscle cells. The issue of cardiac muscle vs smooth muscle within the leaflets remains unsettled. Precise knowledge of the histological features of the MV can have implication for interpretation of the dynamic capabilities of the valve. Several finite element simulation studies, employing state-of-art passive material models for the leaflets, show that a non-physiological billowing of the leaflets into the atrium during systole is obtained (20, 21). A first approach on explicit modelling of muscle activation in the leaflets showed that activation reduced the billowing significantly, with shapes approaching the in-vivo shape measured by echocardiography during systole (22). Solving the described inconsistency will provide an improved basis for mathematical modelling of the leaflet material that can lead to more realistic finite element simulations and possible clinical implications.

Understanding the biology, physiology and functional anatomy of the MV are important as it might be relevant to the development of modern valve repair techniques, and evolving new concepts as tissue engineering. Several authors have addressed these topics (23–25).

The aim of this study was, therefore, to further delineate the presence, architecture and phenotype of muscles tissue in the MV.

METHODS

Eligible MV leaflets were collected consecutively early in 2009 from 12 human autopsy cases. These clinical autopsies were performed at the Department of Pathology and Medical Genetics at St. Olavs Hospital. Cases included had to meet the appropriate consent in the autopsy request form. The study was approved by the Regional Committee for Medical and Health Research Ethics (approval 4.2009.124).

The left ventricle was cut open from the atrium through the middle of the posterior leaflet by scissor. The valves, including the tendinous cords and a part of the anteriolateral and posteriomedial papillary muscles, were cut loose from the annulus, mounted with pins on a cork or styrofoam plate with the atrial surface faced up, and fixed in buffered formalin.

The leaflets were, after being photographed from both the atrial and ventricular side, divided in four parts perpendicular to the annulus in middle of and in between each leaflet (Fig. 1). Eight of the valves were then cut in 4–5 mm broad strips parallel to the annulus, and four valves were cut in 4–5 mm broad strips perpendicular to the annulus (Fig. 1). Each strip was wrapped using lens cleaning paper in such a way that the tissue orientation was kept, put in a standard plastic briquette for tissue specimens which was filled with gauze before closed to prevent torsion, and then processed through standard dehydration, clearing and paraffinizing over night. The strips, stiffened due to this last process, were unwrapped, and those strips belonging to the same half leaflet were piled up orderly on the edge and stabilized in a rectangular split of a piece of human liver tissue that had undergone the same process. The split in the liver tissue was formed such that the leaflet sides and orientation could be identified in the microscope. The aggregated strips from the valve leaflets enclosed by liver tissue were then embedded in paraffin. The work hitherto was done by one of the authors (ISN). Each of the 12 cases comprised by now of four paraffin blocks (labelled A, B, C and D), each containing strips of tissue from a half leaflet.

The edges of the leaflet strips at the cutting surface in the paraffin blocks represented the edges most distal from the annulus in the cases cut parallel to the annulus, and the edges most to the 'left' at each strip in the cases cut perpendicular (Fig. 1). Five-micrometre thick sections from each block were cut and stained.
The sections were stained with three histochemical stains: standard haematoxylin-eosin-saffron (HES), elastin and haematoxylin-Masson’s trichrome (HMT) and immunohistochemically examined with four mouse monoclonal antibodies: α-smooth muscle actin (α-SMA, Clone HHF35, Dako M0851, Glostrup, Denmark) in dilution 1:300, the intermediate filament desmin (Desmin, Clone D33, Dako M0760) in dilution 1:100, neurofilament protein (NFP, Clone 2F11, Dako M0762) in dilution 1:100 and CD68 (CD68, Clone KP1, Dako M0814), and one polyclonal rabbit antibody: calcium-binding protein S-100 (Labels S100B, S100A1, S100A6) in dilution 1:400. These sections were incubated with a primary antibody at room temperature, and heat induced epitope retrieval was done prior to staining. Immunohistochemical reactions were in all cases visualised with Dako Cytomation EnVision, and in case H3 (block B and D), H6 (block B-D), H9 (block B-D) and H12 (block A-D) also with Dako Cytomation EnVision FLEX when examined with antibody against desmin.

Elastin stains elastic fibres black, and HMT stains muscle tissue red and collagen fibres blue. The antibodies used identify different cells. Relevant in the context of this study are: α-SMA to identify smooth muscles cells and myofibroblasts, but not to identify cardiac muscle cells; Desmin to most muscles cells, but not smooth muscles cells everywhere; S-100 to cardiac muscle cells, myofibroblasts and myoepithelial cells, but not smooth muscle cells; NFP to nerves and CD68 to histiocytic cells and also fibroblasts. In this article we focus on the results from HES stain, and the expression of α-SMA, Desmin, and S-100.

In addition, from two additional heart sections perpendicular to the annulus were taken of the MV leaflets including the annular base and the juxtapositioned atrial wall to assure the quality of the results from immunophenotyping of the muscle cells and bundles with α-SMA, S-100 and desmin in the study cases. These two cases were also immunophenotyped with the polyclonal antibody myoglobin (Myoglobin, Dako A5–78) in dilution 1:000.

The slides were examined microscopically. Muscle bundles were defined as muscle cells grouped together in elongated structures either they appeared to be cut transversely or alongside in the sections as identified in HES stained sections and confirmed with α-SMA, Desmin or S-100. The findings of muscle bundles were plotted on drawings of the MV enabling three-dimensional positioning (Fig. 1). Muscle bundles with muscle cells without cross striation and expressing α-SMA were regarded as smooth muscle, and muscle bundles with cells with cross striation not expressing α-SMA as cardiac muscle.
RESULTS

The 12 mitral valves came from adult persons 42 to 85 years of age with different heart and body weight (Table 1). Many of the hearts were to some extent hypertrophic. Muscle cells forming bundles were detected in the leaflets. Cross striation was not present. Immunophenotyping also showed that these muscle cells were smooth as they expressed α-SMA, and not desmin and S-100. None expressed CD68.

Figure 2 presents the findings of smooth muscle bundles in the 12 mitral valves. The number of strips of tissue from each half leaflet (labelled A, B, C and D) cut parallel to the annulus was: A = 2–3, B = 4–5, C = 4–5 and D = 2–3. The number of strips from each half leaflet cut perpendicular to the annulus was: A = 4, B = 4–5, C = 4–5 and D = 4–5. The muscle bundles were present beneath the atrial surface of the leaflets, and distinctly more in the anterior leaflet compared with the posterior leaflet. The muscle bundles extended up to two-thirds the distance from the annulus towards the rim of the leaflets. The thickness and density of the bundles decreased markedly with the distance from the central part of the leaflets attachment to the annulus and towards the rim. The muscle bundles ran in various directions, but seemingly more perpendicular than parallel to the annulus.

Figure 3 presents examples of photomicrographs of smooth muscle bundles and connective tissue composition in the anterior leaflet. The smooth muscle are located near the atrial surface within a layer of connective tissue, which is mainly composed of thin elastic fibres, more scattered towards the rim of the leaflets, and thick and dense in the central part. The thickness of the muscle bundles could be up to 25% of the leaflet thickness near the annular base with muscle bundles running both parallel and perpendicular to the annulus.

Regarding heart weight, it was not considered feasible to compare the amount of smooth muscle detected in the sections of the valves with the weight of the heart due to the small number of cases.

The immunophenotype of cardiac muscle, which sometimes were visible at the annular base in cases cut perpendicular to the annulus (H3: block B and D, H6: block B–D, H9: block B–D, H12: block A–D), and likewise in the two additional test cases, were opposite to the smooth muscle in the leaflets. The cardiac muscle in these cases expressed desmin and S-100, but not α-SMA. The two test cases were also investigated with antibody to myoglobin. Positive reaction was present in the cardiac muscle, but not in the smooth muscle in the leaflets. Cardiac muscles in the juxtapositioned atrial wall were present in very close proximity to the smooth muscle in the leaflet (Fig. 4). Picture of positive reaction to antibody against S-100, instead of desmin, is used in the figure due to more intense immunoreactivity. Figure 4 also

Table 1. Basic autopsy data and mitral valve cutting direction

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex and age</th>
<th>Heart valve pathology</th>
<th>Heart weight (g)</th>
<th>Body weight (kg)</th>
<th>Myocardial infarct (MI) or stated pathology that affect the myocardium</th>
<th>Mitral valve cutting direction related to annulus</th>
</tr>
</thead>
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<tr>
<td>H1</td>
<td>F 59</td>
<td>No</td>
<td>335</td>
<td>70</td>
<td>Fresh and old MI</td>
<td>Parallel</td>
</tr>
<tr>
<td>H2</td>
<td>M 47</td>
<td>No</td>
<td>510</td>
<td>100</td>
<td>Old MI</td>
<td>Parallel</td>
</tr>
<tr>
<td>H3</td>
<td>F 85</td>
<td>Aortic valve</td>
<td>380</td>
<td>38</td>
<td>Old MI</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>H4</td>
<td>M 61</td>
<td>No</td>
<td>750</td>
<td>113</td>
<td>Cor pulmonale and acute myocarditis</td>
<td>Parallel</td>
</tr>
<tr>
<td>H5</td>
<td>M 43</td>
<td>No</td>
<td>524</td>
<td>97</td>
<td>Hypertension</td>
<td>Parallel</td>
</tr>
<tr>
<td>H6</td>
<td>M 53</td>
<td>Missing</td>
<td>580</td>
<td>117</td>
<td>Old MI</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>H7</td>
<td>M 58</td>
<td>No</td>
<td>470</td>
<td>86</td>
<td>Fresh MI with rupture</td>
<td>Parallel</td>
</tr>
<tr>
<td>H8</td>
<td>F 42</td>
<td>No</td>
<td>340</td>
<td>57</td>
<td>None stated</td>
<td>Parallel</td>
</tr>
<tr>
<td>H91</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H10</td>
<td>F 77</td>
<td>No</td>
<td>305</td>
<td>54</td>
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<tr>
<td>H11</td>
<td>M 74</td>
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<td>635</td>
<td>96</td>
<td>Hypertension</td>
<td>Parallel</td>
</tr>
<tr>
<td>H12</td>
<td>M 41</td>
<td>No</td>
<td>290</td>
<td>54</td>
<td>None stated</td>
<td>Perpendicular</td>
</tr>
</tbody>
</table>

1The journal ID was lost during processing.
Fig. 2. This figure shows the presence of smooth muscle bundles in the mitral valves in each case (H1–12). These bundles are marked as thin and thick dots and lines on the drawings of the leaflets reflecting both the density and thickness, but not the direction, of the bundles in each case. (The numbers below each valve are the actual numbers of strips in each case. These numbers are framed with lines or dots indicating a very good or not so good quality of the sections, respectively. Numbers with no frame represent section with good quality. Grey areas are strips excluded due to poor quality. The number to left, right or above the cases, together with the letters A–D, identifies each strip of tissue).
shows that smooth muscle in case H6 strip B3 extended two-thirds from the annular base to the rim.

The relation between cardiac muscle in the atrium and the findings of subendocardial located smooth muscle bundles in the atrial wall, and smooth muscle bundles in the atrial side of a posterior leaflet from one of the two test cases is shown in Fig. 5. The smooth muscle bundles in the atrium and those in the leaflet did not seem to interconnect, and the cardiac muscle extended only into the annular base of the leaflet as in case H3, 6, 9 and 12. In the interstitium of the leaflets many single cells expressed S-100, and a number of cells expressed α-SMA and CD68, indicating myofibroblastic qualities.

**DISCUSSION**

The present study establish in our opinion that the muscle bundles in the MV leaflets are composed of smooth muscle cells because no cross striation was visible and because the cells showed immunoreactivity against antibody to α-SMA, but not desmin and S-100. This is a profile that is opposite to cardiac muscle.

Figure 6 illustrates a proposed interpretation of the distribution of muscle tissue in the anterior leaflet based upon the results in this study: the smooth muscle bundles near the atrial side form a meshwork running in various directions up to two-thirds from the base to the rim, mainly perpendicular to the annular base, with decreasing thickness and density towards the leaflet rim. The amount of muscle bundles was most prominent in the anterior leaflet. The interpretation is based on muscle tissue visible in the cut edge of each of the 4–5 mm broad tissue strips from the leaflets of 12 human MV. This meshwork seems to form a separate leaflet muscle, not in continuity with cardiac muscle or subendocardial smooth muscle bundles in the left atrium.
Five identified studies on the histology of the MV have examined: 1000 human MV (1), 20 dog MV (2), 40 human MV (3), 35 human MV (2 serial sectioned) (5), and 1 human MV (6) respectively. These studies do not always describe in enough detail how the valves have been cut and processed to allow proper critical review or to allow reproducibility studies to be done. Also, the findings of muscle bundles are typically presented in brief. These studies were published in the period 1931–1979. The oldest one is a quite informative study on the anatomy and histology of the valves in the human heart based on 1000 hearts (1). The method of investigation in that study is, however, only described in a very small paragraph, and the results summarized. Published papers after 1979 are basically review articles or articles referring to these studies, sometimes with photomicrographs that illustrate the histology (7, 8, 15, 18, 19). Our findings, and the findings in the above quoted studies, seem to agree upon the presence of a meshwork of muscle bundles especially in the anterior leaflet of the MV. This muscle tissue has been named differently in the literature, and only one study so far has interpreted it as smooth muscle (1).

Only one study has actually shown that the muscle bundles in the MV are not in continuity with those in the left atrium (5). That result was based on longitudinal serial sections of two tissue blocks from a MV with the juxtaposed left atrial wall. The type of muscle was not specified. Although our study was not primarily designed to investigate the relation between muscle tissue in the left atrium and in the MV, sections perpendicular to the annulus revealed cardiac muscle in the annular base of the leaflet strips. Perpendicular sections from two additional test cases also showed that atrial cardiac muscle reached only into the very annular base of the MV, and in addition we found smooth muscle bundles just beneath the endocard in the juxtaposed atrial wall as a separate thin layer. These smooth muscle bundles were not seen to be interconnected with those in the MV. A separate layer of smooth muscle bundles in the atrium beneath the endocardial surface, in addition to the cardiac muscle deeper in the wall has formerly, to our knowledge, only been presented in a drawing (3) and mentioned in a textbook (26). This subendocardial muscle layer was said to be bundles of striated muscle continuing into the mitral leaflets and was not detailed.
further in the text in that study, which is according to our study not correct. Figure 7 might be a fairly relevant delineation of the presence and relationship between muscle tissue in the left atrial wall and the MV.

The smooth leaflet muscle might act as a contractile unit. Itoh et al. (8) have demonstrated two types of contractile activity in anterior leaflets from sheep, raising the possibility of the tissue in the leaflet being neurally controlled. Swanson et al. (11) have investigated this further using 10 sheep. They discuss annular, belly and edge stiffening of the anterior leaflet, and propose at least two contractile systems in the leaflet; one being the cardiac myocytes in the annular third (as they say) of the leaflet responsible for the transient stiffening, and another involving VICs and single smooth muscle cells.

Based upon our histological findings there are two separate muscles that can act dynamically upon the function of MV. One is the cardiac muscle in the atrium that extends into the very
annular base of the leaflet, and the other is the smooth muscle in the leaflet. In addition, a third functional unit (contractile or stiffening system) involving VICs and collagen may be present (16).

Numerical simulations of MV response offer a possibility to enhance our understanding of the functioning valve, both physiologically and pathophysiologically. However, no numerical simulation is better than the quality of input parameters and models. Therefore, to derive material models representative to MV, enhanced knowledge of the microstructure is required. Improved knowledge emerges from the present study. Knowledge of the phenotype of the muscle bundles in the leaflets has been established, and gives input to muscle bundle modelling. Furthermore, more information on the in-plane distribution of the bundles has been obtained. Also some information on how large part of the leaflet thickness that consists of smooth muscle has been established. This provides a better basis for numerical modelling in that the thickness can be modelled with different layers, e.g. with a layer close to atrial surface mainly consisting of smooth muscle, a central layer consisting of mainly connective tissue, and a layer close to the ventricular surface that consists of connective tissue and a perhaps very small amount of smooth muscle (22). An important step for further studies will be to link heart electrophysiology to the activation of the mitral leaflets smooth muscle. Knowledge of muscle activation as functions of time and maximum activation levels will enhance prediction of the dynamic response of the MV during the heart cycle significantly. In one study, both the activation time functions and maximum activation levels were input parameters that were based on physiology, but the simulation results should be considered as qualitative (22). However, the results presented there clearly showed that accounting for muscle activation is necessary to obtain simulation results that resemble the systolic mitral shape measured by echocardiography.

The method used to investigate muscle tissue in the MV in this study gives an overall impression. It was not possible to detail further the metric thickness and density, or the precise distribution, of the smooth muscle bundles in the MV. The reasons were that the MV attachment to the annulus is not precisely linear, that tissue shrink during dehydration processing, that stiffening of the strips of leaflet tissue due to paraffinization sometimes hampered optimal edge orientating due to e.g. some degree of torsion, and that only the edge of each strip was sectioned for microscopy leaving most of the tissue unexamined.

The anterior and posterior leaflet is divided in segments (scallops). The posterior identified as P1, P2 and P3, with relevance to degenerative diseases in the valve and treatment concepts (23). How muscle tissue is distributed in these segments might therefore be of interest. These segments are not anatomical evident in an unfolded MV mounted on a plate. We decided therefore to cut both leaflets in two parts instead of three metric equal parts that would not reflect exactly the more functional segmental division.

Human hearts eligible to study can typically be recruited from autopsied deaths among hospitalized persons that satisfy specific formal criteria regarding tissue retention. These hearts are, as in this study, often not normal and also

Fig. 7. The relation between muscle tissue in the anterior leaflet of the mitral valve and in the atrium. The red area is cardiac muscles in the atrial wall reaching down into the annular base of the leaflet. The red dots just beneath the surface in the atrium, and in the leaflet, are smooth muscle bundles. The zone A exemplifies the close topographic relation between cardiac muscle and smooth muscle in the annular base of the valve. Actually, they might overlap, and both muscle tissues can therefore be present in the same sections from leaflets cut parallel to annulus near the annular base.
from elderly persons. A more normal heart population could be obtainable among forensic autopsied deaths, but formal and legal issues might be an obstacle.

Hypertrophic heart and MV pathology might affect the dimensions of the smooth muscle in the MV. In one study, a positive correlation between the degree of left ventricular hypertrophy and the thickness of muscle bundles in the MV was suggested (3). The muscle bundles were up to 200-µm thick in the anterior leaflet in the hypertrophic heart, which was up to twice the maximum thickness of the normal controls. However, that study did not describe accurately the degree of decreasing bundle thickness from the central part of the leaflets at the annular base towards the rim of the leaflets. It is unascertained to which degree cardiac hypertrophy could have affected the amount of smooth muscle tissue in the MV in the current study.

Some studies (16, 22) have been performed on porcine hearts. Therefore, it is necessary to be aware of differences between species. Microscopically, the smooth muscle bundles in the anterior leaflet of the porcine MV seem to be thinner and more dispersed than that found in the current study (22).

CONCLUSION

This histological study of the MV has provided enhanced knowledge of the presence of muscle tissue in the valve, and contributes to clarify the former ambiguity on both what type of muscle cells that are present in the muscle bundles in the MV and the relation to muscle tissue in the juxtapositioned atrial wall. The valve, especially the anterior leaflet, seems to have a meshwork of smooth muscle bundles close to the atrial surface running in various directions, but seemingly mainly perpendicular to the annular base, with marked decreasing thickness and density towards the leaflets rim. This smooth muscle is probably not in continuity with the muscle tissue in the atrial wall. Cardiac muscle in the atrial wall extends into the annular base of the leaflets in close proximity to the leaflet smooth muscle. The results provide an improved basis for mathematical modelling of the leaflet that can lead to more realistic finite element simulations that will enhance the understanding of the dynamic capacity of the valve which in turn might have clinical significance.

DISCLOSURES

None.

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REFERENCES