*Int. J. Morphol.,*
*41(4)*:1058-1065, 2023.

# Cardiac Corpuscles: A "New" Morphofunctional Entity?

## Corpúsculos Cardíacos: ¿Una "Nueva" Entidad Morfofuncional?

**Adriano Ferreira da Silva[1]; Patrick William Frank[2]; Carlos Augusto Camargo de Souza Baptista[3]; José Antonio Franchini Ramires[4] & Edson Aparecido Liberti[5]**

**DA SILVA, A. F.; FRANK, P. W.; BAPTISTA, C. A. C. S.; RAMIRES, J. A. F. & LIBERTI, J. A. F.** Cardiac corpuscles: a "new" morphofunctional entity? *Int. J. Morphol., 41(4)*:1058-1065, 2023.

**SUMMARY:** The existence of "transitional muscular structures" between subendocardial branches (Purkinje fibers) and ventricular working muscle fibers (WF) was first described by the German anatomist, Kurt Goerttler, in 1964. He designated them as "subendocardial nucleus organs." He supposed such fibers functioned as mechanoreceptors, controlling of the intensity of contraction of the ventricular musculature. Brazilian anatomist Ferraz de Carvalho described similar structures in 1993. A thorough literature search failed to identify any other research articles confirming or denying their existence. The objective of this work was to find such structures in subendocardial ventricular walls in human hearts. We collected fifteen formalin-preserved hearts from the Anatomy Department of São Paulo University and sectioned the apical portions on the right and left ventricles according to method used by Goerttler. We utilized conventional histology (light microscopy- LM), scanning electron microscopy (SEM), and a new preservation method called micro-plastination (MP). At the anterior wall of the right ventricle in the subendocardial region between the interventricular septum and moderator band, we found several bundles of fusiform and helicoidal fibers of similar histology to the WF. The bundles measured between 400 and 1150 μm in length and were separated from adjacent muscular fibers by thin collagen fiber, thus acting as a "pseudo capsule." Some structures seemed to be linked to PF and were appeared to be lymphatic and blood vessels and nerves. We called those structures "cardiac corpuscles" (CC). The observation of the previously "unknown" CC in this initial study confirmed the previous descriptions and its discovery may contribute to new perspectives in the study of cardiac muscle structure and function.

**KEY WORDS: Anatomy; Ventricular myocardium; Subendocardial region; Cardiac corpuscles; Subendocardial branches; Purkinje fibers; Working ventricular muscle fibers.**

## NTRODUCCIÓN

Although described as early as the 19th century, many aspects of the distribution of subendocardial branches or Purkinje fibers (PF) within the ventricles remain unknown. PF connect to the working ventricular muscle fibers (WF). The PF are important in determining the organized and synchronized contraction of the ordinary myocardial working fibers (WF), so that an effective ventricular systole occurs (Testut & Latarjet, 1949; Oosthoek *et al*., 1993; Eliska, 2006). The shape and distribution of PF in mammals are highly variable. In ruminants (sheep, cow) and cetaceans (whales), they are significantly bigger than WF and present as subendocardial and intra myocardial distribution. In primates and carnivores, they are bigger than WF, but their distributions are only subendocardial; and in rodents, they are smaller than WF and continue directly to them in subendocardial region (Ono *et al*., 2009). Also, in dogs (Martinez-Palomo *et al*., 1970) and pigs (Garcia-Bustos *et al*., 2017), a few authors found transition myocardial fibers (TF) between the PF and WF. Such fibers are unconfirmed in humans.

Intrigued by the differences among PF distribution in man and sheep and its electrophysiological implications, the German anatomist Kurt Goerttler (born on May 17, 1898 – Deceased on April 16, 1983) published in 1964 a paper

[1] Radiologist, Hospital Universitário, Universidade de São Paulo, Brazil.
[2] Doctor in Chiropractic. Anatomy assistant Professor. Department of Medical Education/Neurosciences University of Toledo - College of Medicine & Life Sciences, Toledo, Ohio.
[3] Department of Medical Education, College of Medicine and Life Sciences, University of Toledo, Toledo, Ohio 43699, USA.
[4] Head Professor of Cardiology Instituto do Coração-INCOR, Universidade de São Paulo, Brazil.
[5] Head Professor/Senior at Instituto de Ciências Biomédicas, Universidade de São Paulo. Coordinator of the Cultural Development Center of the Butantan Institute.

Received: 2023-04-01 Accepted: 2023-05-22

called 'Über Sonderstrukturen im Verlauf des menschlichen Reizleitungssystems ("RLS")' – "On special structures in the course of the human stimulus conduction system (RLS)". He examined histologically the subendocardial region of ten human hearts, and described, in right ventricle, between interventricular septum and moderator band, groups of coiled or fusiform muscle fibers between the PF and the WF, which he called "organs of the subendocardial nucleus." He implied those fibers as "transitional - TF," involving lymphatic vessels and richly innervated. He supposed these structures would function as mechanoreceptors, so that the degree of compression or distension of the liquid inside the lymphatic vessel would trigger nervous impulses capable of controlling the intensity of muscle contraction which, in turn, would propagate to the rest of the WF. He indicated that "strangely, these structures have never been described" (Fig. 1A-D).

These structures were also observed by Carvalho *et al*. (1993), in 1993, in human hearts, both in serial histological sections and under scanning electron microscopy (SEM) without, however, making any inference to a probable function. This work was presented in a Brazilian conference, but it was never published (Carvalho *et al*., 1993). The present authors could not find any other publication or paper confirming or denying the existence of such structures (Fig. 1E,F).

The aim of this work is to find these muscular structures in human ventricular walls, confirming or not the findings of Goerttler and Ferraz de Carvalho.

## MATERIAL AND METHOD

**Collection data**. The present study was conducted in accordance with the approval of the Ethics Council of the Institute of Biomedical Sciences of the University of São Paulo (ICB/USP - opinion n.2.519.121), and the Ethics Council of the Faculty of Veterinary Medicine and Zootechnics of the University of São Paulo (CEUAVET - opinion n. 691850817).

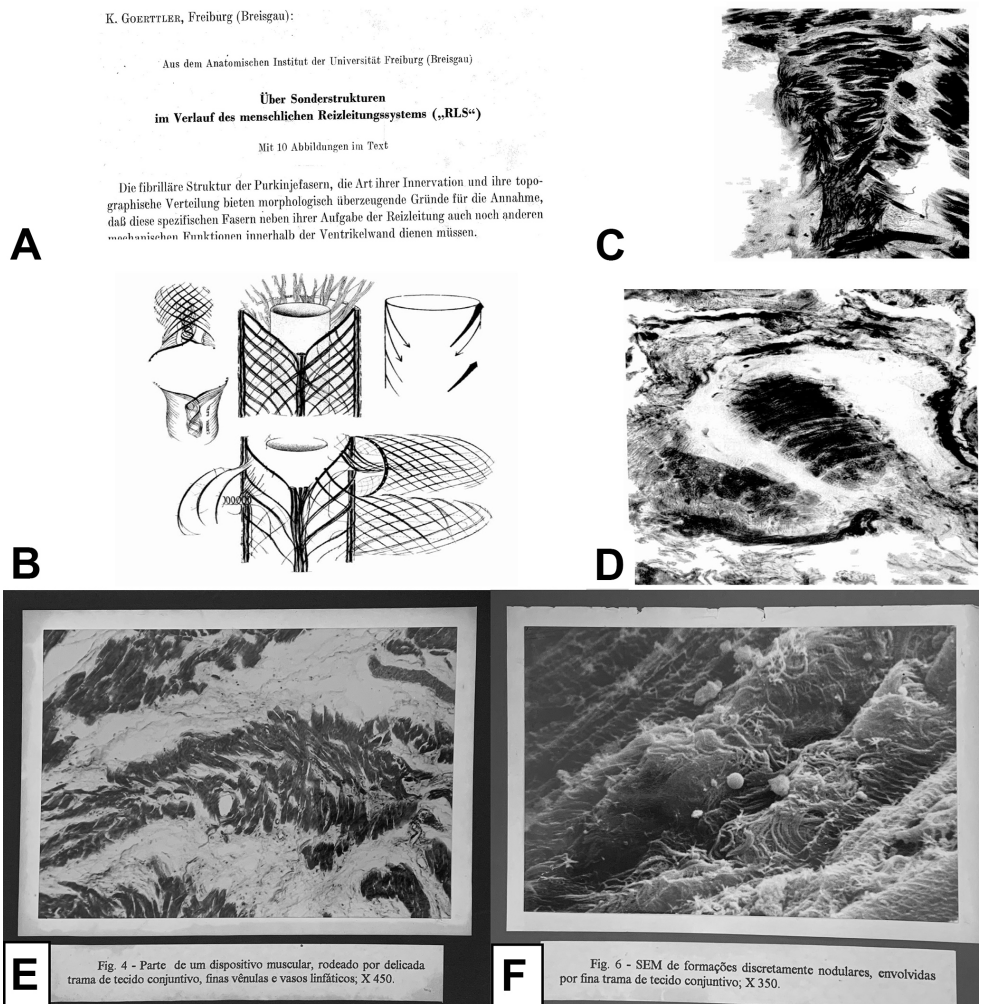


Fig. 1. A, B, C an D : Goerttler´s original images in his 1964 article. A: frontispiece (in German). B. photomicrography of a muscular fusiform bundle (H&E stain). C: hypothetical model made by him about the interactions between muscle fibers and lymphatic vessels. D: nerves found around the muscular fusiform fibers (silver impregnation stain - nerves in black). E and F: Ferraz de Carvalho's original photos (1993). E: Light Microscopy. The subtitles say: "part of a muscular dispositive, surrounded by a delicate net of connective tissue, thin venules, and lymphatic vessels (400 x)". F: SEM. The subtitles say: "a slightly nodular formations surrounded by a thin mesh of connective tissue".

Fifteen human hearts preserved in 10 % formaldehyde were used, from the didactic collection of the Human Anatomy laboratory of the Institute of Biomedical Sciences III of the University of São Paulo (ICB-III). Portions of approximately 2.0 x 2.0 cm of the most apical anterior wall of the right (including the moderator band) – RV and left ventricles (LV) were removed from each specimen (Fig. 2A,B)

**Conventional histology - Light microscopy (ML).** Portions of nine hearts were prepared under routine histological techniques. After being embedded in paraffin, they were submitted to serial histological sections tangential and perpendicular to endocardium wall of 6 μm thickness, and stained with Hematoxylin-Eosin, Masson 's Trichrome and Picro Sirius Red; some of them remained unstained, to be evaluated under polarized light. The images were obtained through an image analysis system (Zeiss Axiovision Rel 4.8™ ), attached to a binocular microscope (Carl Zeiss Microimaging, Axioscope 40™ ).

**Scanning electron microscopy (SEM).** Portions of five hearts were washed in phosphate buffer solution (PBS, pH 7.4) at 4°C for 24 hours, immersed in liquid nitrogen, and broken (cryofracture). Some fragments were selected and dehydrated in an increasing series of alcohols (from 70 % to absolute). The specimens were then critical-point dried with liquid carbon dioxide in a Balzers CPD-010 apparatus (Balzers Union, Liechtenstein, Germany™ ), gold coated on a Balzers SCD-040 ion sputter and examined in a scanning electron microscope ((Leo 435 VP™ ).

**Micro-plastination (ultra-thin plastination).** RV and LV portions of one heart were sent to Plastination Laboratory at University of Toledo (USA) to perform micro-plastination, a new technique of anatomical preservation, developed by Ottone (2020), derived from sheet plastination, created by the German Anatomy Professor Gunther von Hagens (1979), and modified by Sora *et al*. (2004). We utilized the protocol made by Ottone (2020). In summary, after being dehydrated in 100 % acetone (25 °C) for 10 days and renewed every 4 days, tissue fat was removed by immersing the specimen for 4 days in methylene chloride at room temperature. Polymer impregnation was obtained by immersing the specimen in plastic forms containing epoxy resin E12, hardener E6 and catalyst E600 (BIODUR™ ), which were kept for 5 days between 30 and 65 °C in an oven with a special built-in vacuum chamber. (AccuTemp-09, Across International™ ). Tangential and perpendicular 250 μm-thick sections to endocardial surface were obtained using a low-speed diamond- blade saw with a 0.35 mm (Buehler Ltd. ISOMET). Then, they were allocated between two glass and acetate plates containing a mixture of resin E12 and catalyst E1 (BIODUR™ ), and after being kept at room temperature for 24 hours, they were transferred to an oven at 50 °C, where they remained for the same period. Images were obtained in the same manner as described for light microscopy (Fig. 2C-F).

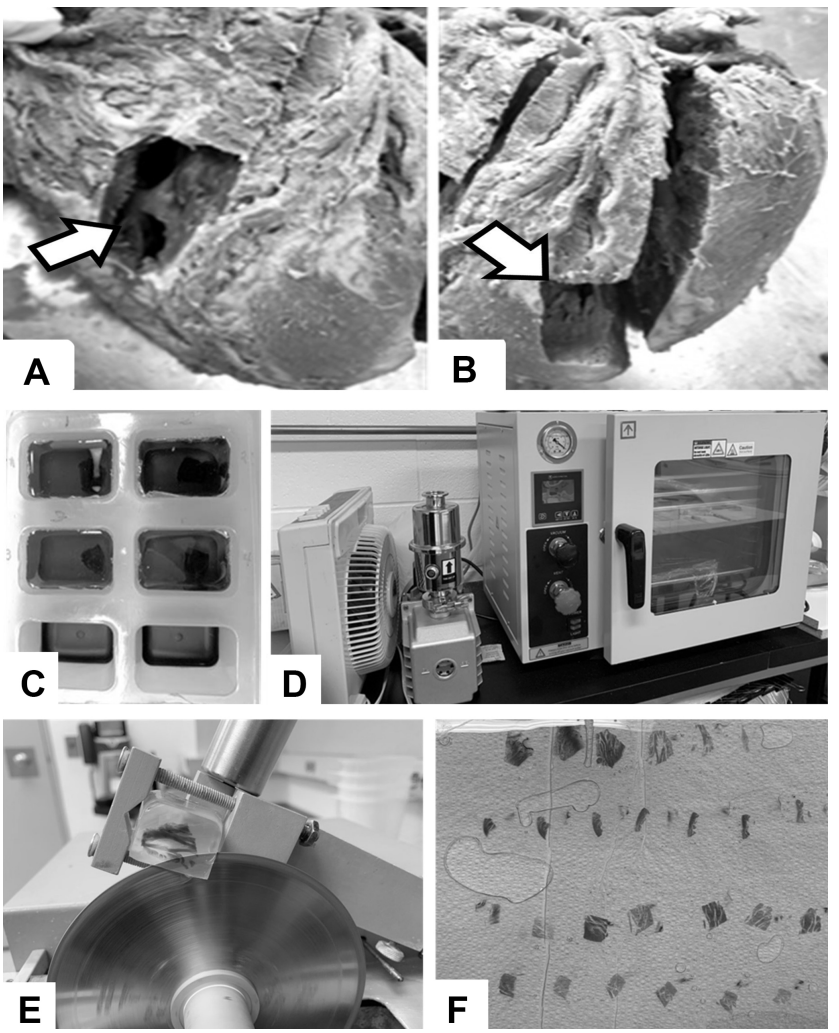


Fig. 2. A and B: removal of portions of anterior apical walls in right ventricle (A) and left ventricle (B). C, D, E and F: steps of the microplastination technique. C; inclusion of samples in the impregnation mixture; D: vacuum chamber attached to an oven; E: myocardial block submitted to sections by a diamond saw; E: resin plate containing the aligned sections. Source: author's archive.

## RESULTS

We found in all hearts, by all methods employed, muscular coiled structures only in right ventricles, interposed between the layers of WF adjacent and parallel to the endocardial surface, from where they are apart between 200 and 500 μm. We called them "cardiac corpuscles" – CC. They are morphologically formed by oblique and helical working-like muscle fibers, some of them helicoidally arranged, and others, with fusiform shape with their longest axis measuring between 400 and 1150 μm, although it may be difficult to measure their total length in section (Figs. 3 and 4).

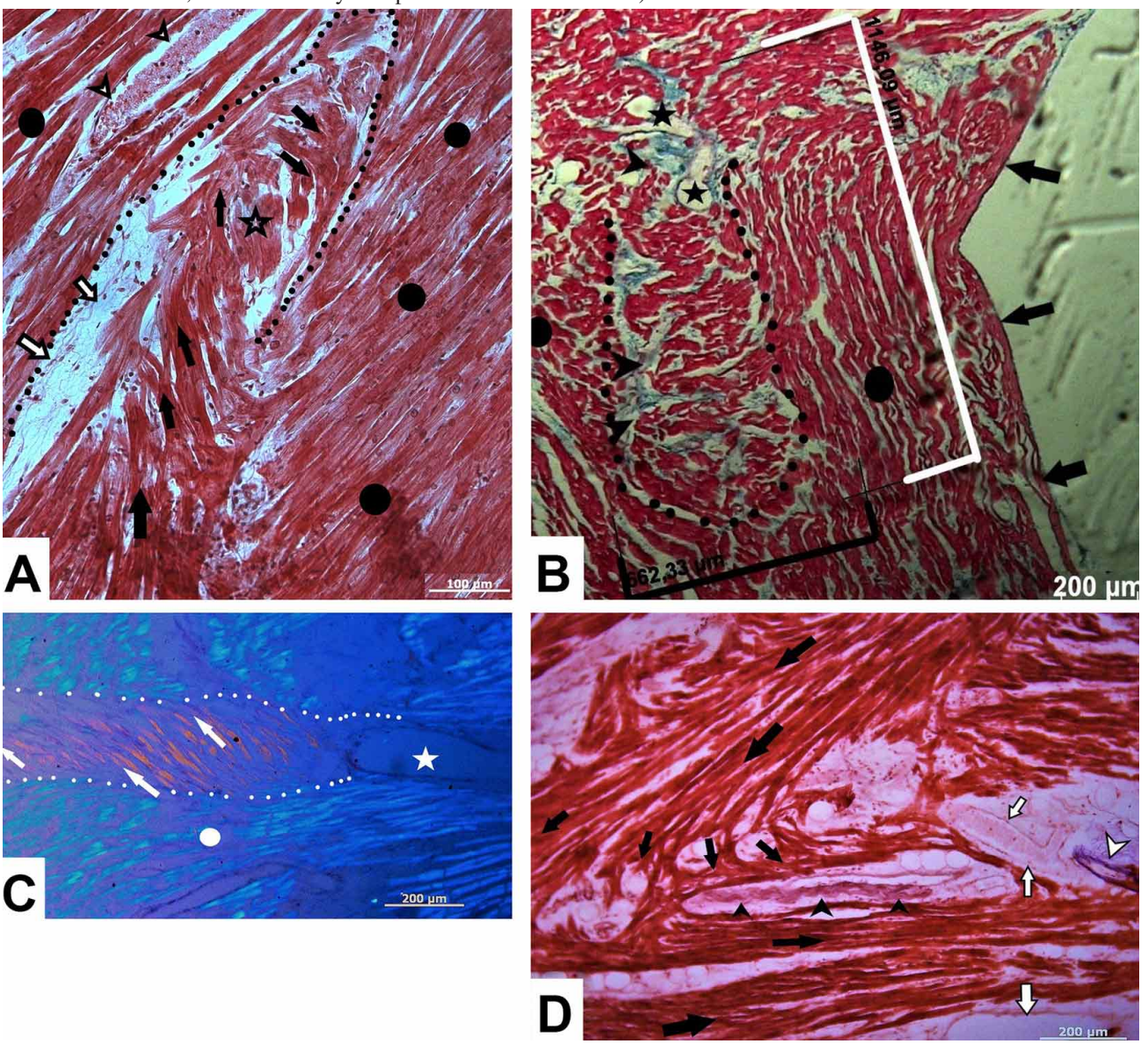


Fig. 3. Photomicrographs. A. Composite tangential section of RV subendocardial region, showing the shifting of fiber direction (black arrows) for form a fusiform bundle (CC dotted line). Star indicates the core of CC. White arrows show collagen fibers surrounding muscle fibers. Arrowheads: blood vessel (probably a venule); circles: working fibers (WF). Technique: Masson's trichrome. Scale bar: 100 μm B. Transversal section of RV subendocardial region. Structure of a CC (dotted lines). Arrows: endocardial surface; black and white square brackets: measurements (white bracket: 1148,09 μm; black bracket: 662,33 μm); arrowheads: endocardial surface; star: vessel (presumably lymphatic); circles: WF. Technique: Masson's trichrome. Scale bar: 200 μm. C. Tangential unstained section of RV subendocardial region under by polarized light, showing the shift of fiber direction (arrows), indicated by change of polarized coloration from blue to red. Dotted line: CC contour. Star: vessel (presumably lymphatic). Scale bar: 200 μm. D. Tangential section of RV subendocardial region, showing the shifting of fiber direction (black arrows). White arrows: probably lymphatics; white arrowheads: venule; black arrowhead: probably nerve. Technique: Masson's trichrome. Scale bar: 200 μm. Source: author's archive.

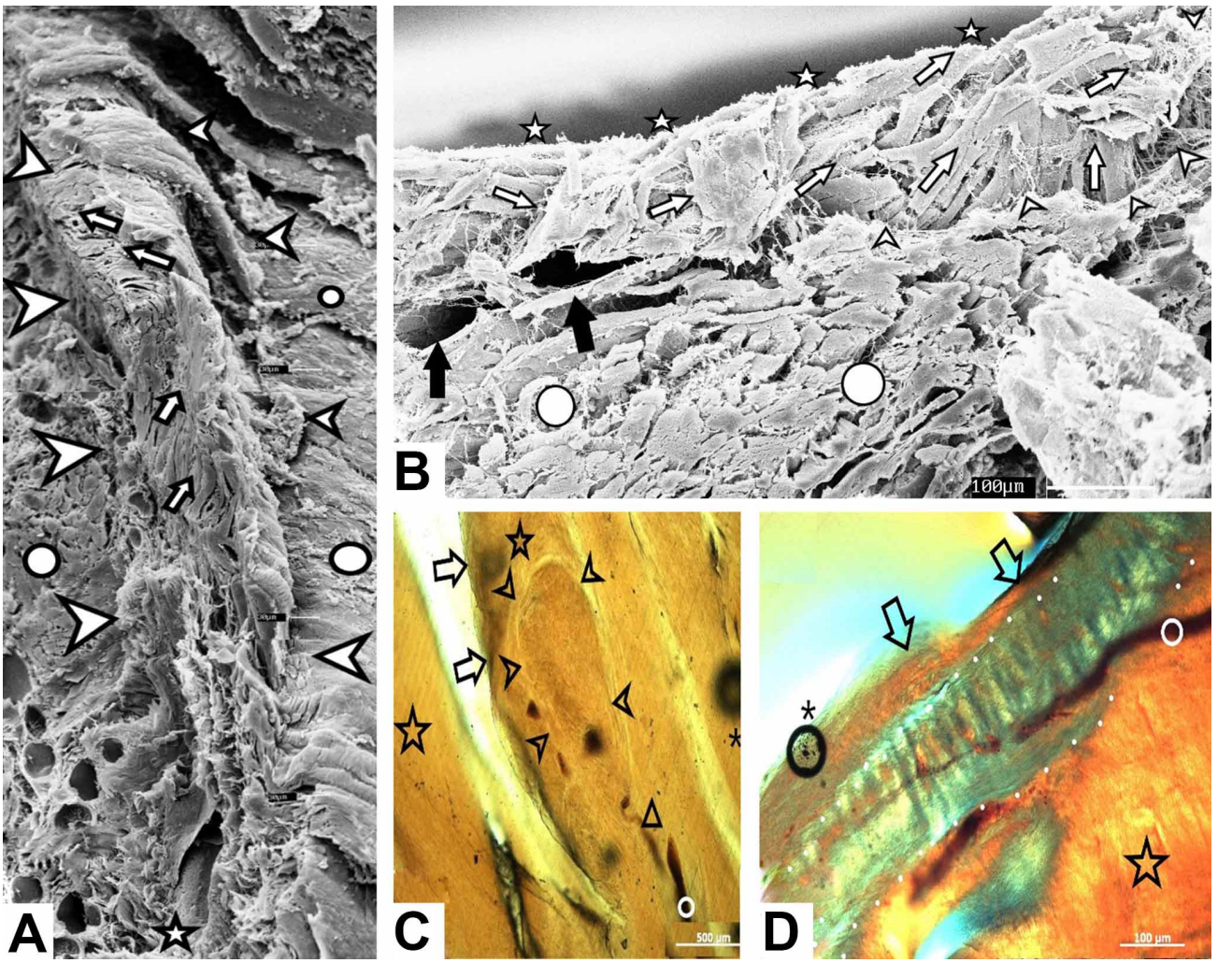


Fig. 4. A and B. SEM. A: tangential section of subendocardial region in RV. A. Composite image showing the helicoidal/ fusiform fibers of CC (arrows). Arrowheads: CC contour; star: blood or lymphatic vessel; circles: WF. Scale bars: 30 µm. B. transversal section of subendocardial region in RV. White arrows show CC fibers; arrowheads indicate the collagen "pseudo capsule"; black arrows: blood or lymphatic vessel; stars: endocardial surface; circles: WF. C and D: micro-plastination. 250 µm-thick non-stained subendocardial RV sections. C. tangential section show the fusiform structure of a CC (arrowheads), surrounded by a thin bright margin. Circle and triangle show a blood vessel entering on the base of CC. Stars: WF; arrows: endocardial surface; asterisks: impregnation artifacts. Scale bars: 500 µm. D. Transversal section under polarized light, showing the change of light color from orange to bluish related to shifting of muscle fibers. Dotted line: CC contour. Arrows: endocardial surface; circle: blood vessel. Star: working fibers; asterisks: impregnation artifacts. Scale bar: 100 µm Source: author's archive.

The CCs are wrapped around by a thin layer of discontinuous collagen fibers, forming a pseudo capsule. Among the vascular and nervous elements associated with CCs, the lymphatic vessels were the ones that stood out the most. Characterized by having thin walls, these vessels were present both in the central and peripheral regions of the corpuscles (Fig. 5). The nervous fibers were less evident by the methodology employed on this study. In some histological sections, The PF were observed in the subendocardial region in continuity with CC by means of we called "transition fibers" (TF), on the narrowest part of the fuse. On this region, we found increased number of blood vessels, lymphatic vessels, and nerves, like a "neurovascular pedicle" (Figs. 3 and 4).

## DISCUSSION

Research on the functional anatomy of the ventricular myocardium has concentrated on the architecture of the LV, probably due to its role in the

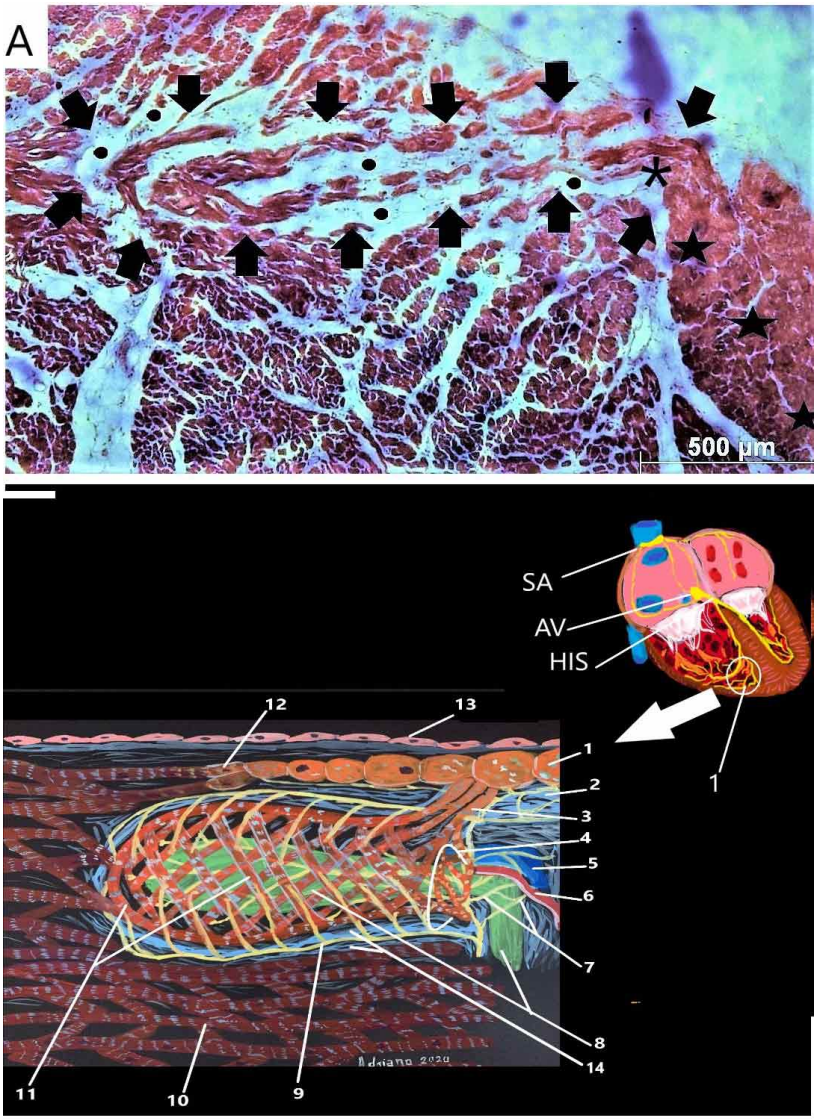


Fig. 5. A. Photomicrography of a transversal section of subendocardial region in RV, showing the connection between conduction tissue (Purkinje fibers, stars) and the base of CC (arrows) by we called "transition fibers" (asterisk). Circles show (presumably lymphatics) entering on the base of CC and surrounding it. Technique: Masson's trichrome. Scale bar: 500 µm. B. Hypothetical model of a CC, based on Goerttler, Ferraz de Carvalho and the present authors. SA: sinoatrial node; AV: atrioventricular node; HIS (atrioventricular bundle); 1. Purkinje fibers; 2: Connective sheath of PF, presumably forming the "capsule" surrounding the CC; 3: "transition fibers"; 4: base of CC; 5: venule; 6: arteriole; 7, 9: nerve fibers; 8: lymphatic vessel; 10: working fibers; 11: CC fibers; 12: PF-WF junction; 13: endocardium. Source: author's archive; "collagen pseudo capsule". Source: author's archive.

greater circulation (Mall, 1911; Torrent Guasp, 1980; Greenbaum *et al*., 1981; Gouda, 2015). Failure to pay attention to a functional approach to the structure of the RV myocardium would be justified by the reduced thickness of its walls, a fact that would not make it essential for establishing cardiac output (Buckberg & Hoffman, 2014).

Paradoxically, CCs with their characteristic helical or fusiform (claviform) shapes were present only in the RV myocardium, as reported by Goerttler (1964). He believed there were two kinds of CC: the helicoidally ones or "open cuffs," and fusiform or "closed cuffs" interrelated one another. Here we found fusiform and helicoidal fibers, but their interrelationship was not definitively proven in this paper. Regarding the PF, these were observed in the subendocardial region of the ventricles surrounded by a connective tissue and characterized by their cells with a globular or elongated appearance, with larger dimensions than WF, and presenting vacuoles in their interior (Testut & Latarjet, 1949; Eliska, 2006).

Goerttler (1964) believed that CC fibers are continuous to PF and were constituted to TF. However, with the methodology employed, it was not possible to accurately establish the real nature of the CCs. Thus, complementary studies should be conducted under transmission electronic microscopy (Martinez-Palomo *et al*., 1970) or using specific methods such as connexins, especially Cx40 for gap junctions of conduction fibers, and Cx43, present in myocardial fibers in general (Bastide *et al*., 1993; Atkinson *et al*., 2011).

Goerttler (1964) also observed "unusual relationships of the CC with the subendocardial vessels, especially the lymphatics, which would be important for the theory of mechanoreceptor function of the CC established by him. Such relationships were observed by Carvalho *et al*. (1993) and named as "Muscular lymphatic corpuscular system." In the present study, the vessels detected both in the central part and in the periphery of the CC are suggestive of being lymphatic vessels since they have a larger diameter than adjacent blood vessels and no evident muscular layer. Complementary studies using immunohistochemical or immunofluorescence techniques can confirm these findings (Brakenhielm & Alitalo, 2019).

Goerttler (1964), using the silver impregnation method observed nerve endings on peri lymphatic space, and nerve fibers related to the musculature of the corpuscles. Although the staining used here was not specific for nervous tissue, structures compatible with nerve fibers were detected both in the periphery and at the base of the CC, constituting, together with the blood vessels present at the base, a kind of "vascular-nervous pedicle." However, the real nature of these fibers (adrenergic, cholinergic, or sensitive) can only be determined with studies that use specific techniques for nerve fibers (Kawano *et al*., 2003; Yokoyama, 2017). The structural aspects presented in this work, and which corroborate the findings of Goerttler and Ferraz de Carvalho allow establishing the following CC model, as well as its relationships with the adjacent structures of the subendocardial region (Fig. 5).

The possibility of the existence of these corpuscles raises several questions: 1) Is it just only one CC with helical and fusiform components (tree-like) or are there are several ones? 2) Are they located only in the apical region of the RV or in other regions of the myocardium? 3) What are the physiological and pathophysiological implications that could be involved, especially in the cardiac conduction mechanism between the PF and the WF and the diseases related to their dysfunction?

In conclusion, this initial study shows CCs as muscular formations structurally organized, vaguely resembling neuromuscular spindles of striated musculature, located in apical portion of VD, unlike what occurs in several cardiomyopathies such as the hypertrophic one (Hensley *et al*., 2015). It is not enough to affirm that is an initial study, by reactivating Goerttler's line of research, and it is imperative the use of more sophisticated methodologies may contribute to the elucidation of the morphophysiological, and pathological implications related to this myocardial region.

## ACKNOWLEDGMENTS

To Claudio Antonio Ferraz de Carvalho M.D, PhD (1932-). He is Professor assistant at the Department of Anatomy at FMUSP, since 1959 (retired). By studying with Professor Alfred Benninghoff and Kurt Goerttler in 1964 at Institute of Anatomy at the University of Mainz, Germany, he acquired solid foundations for the development of Functional Anatomy. As a result of this experience, he developed several works about this subject, including the study of Cardiac Corpuscles, presented at XIV Colóquio da Sociedade Brasileira de Microscopia Eletrônica, in1993, but it was not published. The authors dedicate this paper to him.

To Rose Eli Grassi Rici, PhD. from Department of Anatomy of Domestic and Wild Animals, Faculty of Veterinary Medicine and Animal Science, Universidade de São Paulo, for her assistance in the acquisition and interpretation of scanning electron microscopy images.

To the laboratory technicians of the Insituto de Ciências Biomédicas III from Universidade de SãoPaulo: Sebastião Aparecido Boleta and Marta Maria da Silva Righetti, who contributed their knowledge to prepare light and electronic microscopy specimens.

**DA SILVA, A. F.; FRANK, P. W.; BAPTISTA, C. A. C. S.; RAMIRES, J. A. F. & X LIBERTI, J. A. F.** Corpúsculos cardíacos: ¿una "nueva" entidad morfofuncional? *Int. J. Morphol., 41(4)*:1058-1065, 2023.

**RESUMEN:** La existencia de "estructuras musculares de transición" entre los ramos subendocárdicos (fibras de Purkinje) y las fibras musculares ventriculares activas(FMV) fue descrita por primera vez por el anatomista alemán Kurt Goerttler en 1964, quien las denominó "órganos del núcleo subendocárdico". Supuso que tales fibras funcionaban como mecanoreceptores, controlando la intensidad de la contracción de la musculatura ventricular. El anatomista brasileño Ferraz de Carvalho describió estructuras similares en 1993. Una búsqueda bibliográfica exhaustiva no logró identificar ningún otro artículo de investigación que confirmara o negara su existencia. El objetivo de este trabajo fue encontrar dichas estructuras en las paredes ventriculares subendocárdicas de corazones humanos. Recolectamos 15 corazones conservados en formalina del Departamento de Anatomía de la Universidad de São Paulo y seccionamos las porciones apicales de los ventrículos derecho e izquierdo según el método utilizado por Goerttler. Utilizamos histología convencional (microscopía de luz-LM), microscopía electrónica de barrido (SEM) y un nuevo método de conservación llamado microplastinación (MP). En la pared anterior del ventrículo derecho en la región subendocárdica entre el tabique

interventricular y la banda moderadora, encontramos varios haces de fibras fusiformes y helicoidales de histología similar a la FMV. Los haces medían entre 400 y 1150 μm de longitud y estaban separados de las fibras musculares adyacentes por una fina fibra de colágeno, actuando así como una "pseudocápsula". Algunas estructuras parecían estar vinculadas a la fibras de purkinje y parecían ser vasos linfáticos, sanguíneos y nerviosos. Llamamos a esas estructuras "corpúsculos cardíacos" (CC). La observación del CC previamente "desconocido" en este estudio inicial confirmó las descripciones anteriores y su descubrimiento puede contribuir a nuevas perspectivas en el estudio de la estructura y función del músculo cardíaco.

**PALABRAS CLAVE: Anatomía; Miocardio ventricular; Región subendocárdica; Corpúsculos cardíacos, Fibras de Purkinje; Fibras musculares ventriculares activas.**

## REFERENCES

Atkinson, C.; Inada, S.; Li, J.; Tellez, J. O.; Yanni, J.; Sleiman, R.; Allah, E. A.; Anderson, R. H.; Zhang, H.; Boyett, M. R.; *et al*. Anatomical and molecular mapping of the left and right ventricular His-Purkinje conduction networks. *J. Mol. Cell. Cardiol., 51(1)*:689-701, 2011.

Bastide, B.; Neyses, L.; Ganten, D.; Paul, M.; Willecke, K. & Traub, O. Gap junction protein connexin40 is preferentially expressed in vascular endothelium and conductive bundles of rat myocardium and is increased under hypertensive conditions. *Circ. Res., 73(6)*:1138-49, 1993.

Brakenhielm, E. &Alitalo, K. Cardiac lymphatics in health and disease. *Nat. Rev. Cardiol., 16(1)*:56-68, 2019.

Buckberg, G. & Hoffman, J. I. E. Right ventricular architecture responsible for mechanical performance: unifying role of ventricular septum. *J. Thorac. Cardiovasc. Surg., 148(6)*:3166-71.e1-4, 2014.

Carvalho, C. F.; Liberti, E. A. & Martin, Z. O. S*obre um dispositivo intercalado entre as musculaturas de trabalho e de condução de estímulos do coração humano*. Caxambu, Instituto de Ciências Biomédicas, Universidade de São Paulo, 1993.

Eliska, O. Purkynje fibers of the heart conduction system--history and the present time. *Cas. Lek. Cesk., 145(4)*:329-35, 2006.

Garcia-Bustos, V.; Sebastian, R.; Izquierdo, M.; Molina, P.; Chorro, F. J. & Ruiz-Sauri, A. A quantitative structural and morphometric analysis of the Purkinje network and the Purkinje-myocardial junctions in pig hearts. *J. Anat., 230(5)*:664-78, 2017.

Goertller, K. Über Sonderstrukturen im Verlauf des menschlichen Reizleitungssystems ("RLS"). *Anat. Anz., 113*:284-294, 1964.

Gouda, Z. A.; Elewa, Y. H. A. & Selim, A. O. Histological architecture of cardiac myofibers composing the left ventricle of murine heart. *J. Histol. Histopathol., 2(1)*:2, 2015.

Greenbaum, R. A.; Ho, S. Y.; Gibson, D. G.; Becker, A. E. & Anderson, R. H. Left ventricular fiber architecture in man. *Br. Heart J., 45(3)*:248-63, 1981.

Hensley, N.; Dietrich, J.; Nyhan, D.; Mitter, N.; Yee, M. S. & Brady, M. B. Hypertrophic cardiomyopathy: a review. *Anesth. Analg., 120(3)*:554-69, 2015.

Kawano, H.; Okada, R. & Yano, K. Histological study on the distribution of autonomic nerves in the human heart. *Heart Vessels, 18(1)*:32-9, 2003.

Mall, F. P. On the muscular architecture of the ventricles of the human heart. *Am. J. Anat., 11(3)*:211-66, 1911.

Martinez-Palomo, A.; Alanis, J. & Benitez, D. Transitional cardiac cells of the conductive system of the dog heart. *J. Cell Biol., 47(1)*:1-17, 1970.

Ono, N.; Yamaguchi, T.; Ishikawa, H.; Arakawa, M.; Takahashi, N.; Saikawa, T. & Shimada, T. Morphological varieties of the Purkinje fiber network in mammalian hearts, as revealed by light and electron microscopy. *Arch. Histol. Cytol.,72(3)*:139-49, 2009.

Oosthoek, P. W.; Virágh, S.; Lamers, W. H. & Moorman, A. F. Immunohistochemical delineation of the conduction system. II: The atrioventricular node and Purkinje fibers. *Circ. Res., 73(3)*:482-91, 1993.

Ottone, N. E. Micro-plastination. Technique for obtaining slices below 250 μm for the visualization of microanatomy in morphological and pathological morphology protocols. *Int. J. Morphol., 38(2)*:389-91, 2020.

Sora, M. C.; Strobl, B. & Radu, J. *High temperature E12 plastination to procedure ultra-thin sheets*. J. Int. Soc. Plastination, 19:22-5, 2004.

Testut, L. & Latarjet, A. *Traité d'anatomie humaine*. Paris, Doin, 1949.

Torrent Guasp, F. La estructuración macroscópica del miocardio. *Rev. Esp. Cardiol., 33(3)*:265-87, 1980.

von Hagens, G. Impregnation of soft biological specimens with thermosetting resins and elastomers. *Anat. Rec., 194(2)*:247-55, 1979.

Yokoyama, T.; Lee, J.; Miwa, K.; Opthof, T.; Tomoyama, S.; Nakanishi, H.; Yoshida, A.; Yasui, H.; Iida, T.; Miyagawa, S.; *et al*. Quantification of sympathetic hyperinnervation and denervation after myocardial infarction by three-dimensional assessment of the cardiac sympathetic network in cleared transparent murine hearts. *PLoS One, 12(7)*:e0182072, 2017.

Corresponding author:
Adriano Ferreira da Silva
Alameda Jaú 511, Ap 13
Jardim Paulista
São Paulo- SP
CEP 01420-000
BRAZIL

E-mail: adrimedsilva@gmail.com