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Review

Detailed Insights into Heart Histology and Cardiomyocyte Molecular Architecture

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ABSTRACT: The heart is a unique muscular organ, and understanding its complex ultrastructure is essential for studying the pathophysiology of genetic and iatrogenic diseases. Many recently discovered cells and molecular structures serve as therapeutic targets, making knowledge of normal cardiac structure fundamental for preclinical and clinical research.

This review focuses on recently discovered cells and their roles in health and disease, including valve endothelial cells, multipotent progenitor cells of the pericardium, circulating multipotent stem cells, and telocytes. Telocytes, in particular, play a significant role under physiological and pathological conditions and show promise in treating myocardial infarction.

Various cellular structures and their fundamental relations to cardiac conditions are also explored. These include the structure of titin and cMyBP-C proteins, whose mutations are linked to dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM). Additionally, the normal structure of T-tubules and their dilation in certain forms of cardiotoxicity, the role of RyR2 dysfunction and SERCA leak in atrial fibrillations, mitochondrial biology and its role in cardiac senescence, the role of Cav proteins in signal transduction, and the normal structure of area composita are described. Mutations in area composita contribute to the development of DCM and HCM.

Furthermore, this review covers several experimental drugs and techniques targeting specific cardiac structures. These include myosin modulators for treating heart failure, RyR2 stabilizers for anti-arrhythmia therapy, mitochondrial transfer for treating cardiotoxicity, and 144DG11, a new polyglucosan-reducing compound for treating glycogen storage disease.

Finally, a detailed description of the histology of normal cardiac tissue is provided.

1. INTRODCTION

The heart is a unique muscular organ. It maintains the continuous blood flow keeping us alive. Understanding its complex ultrastructure is now the basis to study the pathophysiology behind many genetic and iatrogenic diseases. Over the last few decades, many key structural and signalling proteins have been identified within cardiomyocytes and linked to various disorders. In addition, many new cell lines were newly discovered and described. Their pivotal role of them has been recognized in health and disease. In this review, the histology of the heart and the ultrastructure of its cardiomyocytes have been illustrated and linked to many known pathologies. New treatment modalities targeting cellular and subcellular structures have been illustrated as well. All data in this article has been mentioned after reviewing recent original and review articles, in addition to textbooks on histology, anatomy, physiology, and molecular biology.

- **Overview of the heart structure**

The heart is an autonomous muscular pump that maintains the unidirectional flow of blood by contracting continuously and rhythmically in a highly coordinated fashion [1]. It lies in the middle mediastinum, and it is covered by the pericardium, which also covers the great vessels entering and leaving the heart. The heart contains four chambers, two atria, and two

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ventricles. Atria and ventricles are separated by cardiac valves, while the right and left sides are separated by interatrial and interventricular septa. The right side of the heart pumps blood through pulmonary circulation but the left one pumps blood through systemic circulation. The heart tissue receives vascular supply from the coronary arteries that originate from the ascending aorta, while its venous drainage is through cardiac veins which drain into the posterior wall of the right atrium [2]. Automaticity of cardiac contractions is caused by the presence of the sinoatrial (SA) and atrioventricular (AV) nodes, while the rate and force of cardiac contractions are regulated by the autonomic nervous system. Parasympathetic nerve fibers carried by the vagus nerve, release acetylcholine which consequently reduces the heart rate and decreases the force of contraction, while sympathetic fibers that originate from the lateral horns of thoracic segments T1-T6 increase the heart rate and the force of contraction by releasing norepinephrine [3, 4].

- **Fibrous skeleton of the heart**

It is the dense fibrous tissue present at the base of the heart. It remains stationary during heart contraction. It acts as an electrical insulator between the atria and ventricles, forms annuli fibrosi; a fibrous annulus at the base of each great vessel and serves as an attachment site to cardiac valves. It also forms the membranous part of the interventricular septum. the latter is also termed “spetum membrancum” and it is formed of dense connective tissue that houses the AV bundle [5-7].

- **Valves of the heart**

There are four heart valves that maintain unidirectional blood flow during the cardiac cycle. Two of them are atrioventricular valves (tricuspid and mitral valves) and the other two are semilunar in shape and present at the great vessels of the heart (pulmonary and aortic valves). Generally, they are composed of connective tissue and a lining endothelium arranged in a trilaminar manner (**Figure 1**):

1. Fibrosa: It is the main layer that forms the core of cardiac valves. It is formed by collagen-rich connective tissue derived from the fibrous skeleton that forms an annulus around each valve, covered by an endothelial layer. The fibrosa strengthens the valve leaflets.
2. Spongiosa: formed of loose connective tissue rich in proteoglycans. Spongiosa is present towards the atrial side of the atrioventricular valves, and at the arterial side of the semilunar valves. This layer gives flexibility to the valves during their motion and acts as a shock absorbent during valve closure.
3. Ventricularis in semilunar valves or atrialis in atrioventricular valves: is the third layer, present at the ventricular side of the valves. It is formed of a dense connective tissue layer rich in radially arranged elastic fibers and covered by endothelium.

From the free edge of the atrioventricular valves, ventricularis/arterialis continues as fibrous cords covered by endothelium called the chordae tendineae, which in turn continue as papillary muscle; muscular projections arise from the wall of ventricles [8].

The three layers are populated by quiescent fibroblasts called valve interstitial cells (VICs). They can differentiate into osteoblasts being stimulated by the transforming growth factor beta -1 (TGF-β1) and form dystrophic calcification of cardiac valves in certain valvular degenerative diseases [9, 10]. Endothelial cells that cover both the fibrosa and ventricularis are called valve endothelial cells (VECs). In response to TGF-β1, they can transform from being epithelial cells to myofibroblasts and acquire protein markers such as α-smooth muscle actin (α-SMA), this process is called endothelial-to-mesenchymal transition (EndoMT). EndoMT is needed during embryonic valvulogenesis and injury repair [11]. Unfortunately, it is activated during the pathogenesis of diseases, such as cardiac fibrosis, cancer, and valvular degeneration. EndoMT is a promising route to be applied in regenerative medicine to construct tissue patches. Also, the EndoMet pathway can be a target in treating or preventing various valvular diseases [12-14].

- **Layers of the heart wall**

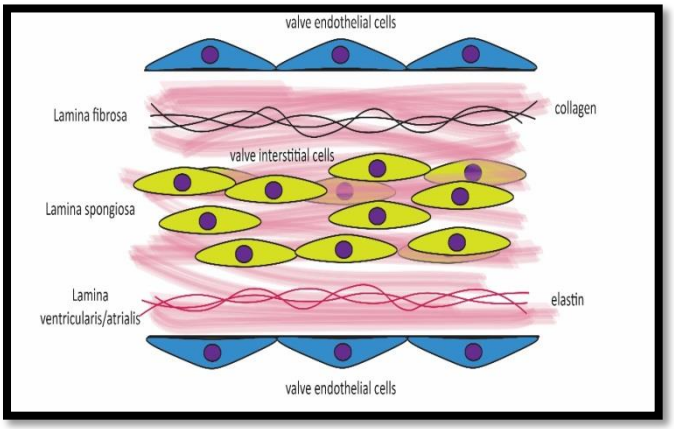

Figure 1. A diagram illustrating the cardiac valve structure.

The cardiac wall is composed of three layers: epicardium (the outermost covering layer), endocardium (the innermost lining layer), and the myocardium in between them (it is the main component of the heart) [15].

2. The Epicardium

It is the visceral layer of the pericardium (serous pericardium). It is widely described in textbooks the hat pericardium is formed of the simple squamous epithelium of mesodermal origin, the mesothelium, resting on a basal lamina and underlined by a submesothelial (subepicardial) layer of connective tissue. It contains blood vessels and nerves that supply the heart. The epicardium is reflected at the root of the great vessels to continue as the parietal layer of the pericardium composed of a superficial cellular part and a deep fibrous part composed of layers of collagen with elastic fibers; hence the name "fibrous pericardium". The pericardial space is present between the two layers of the f pericardium and contains 20 to 60 ml of plasma ultra-filtrate. The main function of the pericardium is to protect the heart and the great vessels and to reduce friction between the heart and the surrounding structures [16]. Several studies have revealed the presence of another cell population forming the mesothelium other than the flat squamous cells. These described

cells are cuboidal, approximately 21 µm in their vertical and horizontal diameter. They are rich in mitochondria, rough endoplasmic reticulum, and Golgi apparatuses. Furthermore, their cytoplasm contains many electron-dense multilamellar vesicles. These dense bodies are believed to produce surfactant-like substances, similar to type II pneumocytes. They also resemble them in their microvilli containing apical surfaces. In addition, tight junctions and desmosomes are present between the mesothelial cells of both types. Cuboidal mesothelial cells actively secrete glycosaminoglycans and lubricant surfactant, to provide a non-adhesive surface to facilitate smooth movement between parietal and visceral serosal sheets. These cuboidal cells are believed to be a predominant cell type in the visceral pericardium, while the flat cells are predominant in the parietal pericardium (**Figure 2**). The flat mesothelial cells are elongated, approximately 9 µm and 25 µm in their vertical and horizontal diameter respectively. They have few mitochondria and poorly developed Golgi and endoplasmic reticulum, suggesting a less metabolically active state. Their organelles are located centrally. Some authors believe that there is a functional adaptation which is seen aa s structural interchange between the flat and the cuboidal types according to physiological needs. Cells of the pericardium are now believed to be multipotent progenitor cells, which can be activated by mediators released during injury of the underlying cardiac tissue, and transform into fibroblasts, endothelial cells, and smooth muscle cells. Also, they can to a lesser extent replace the cardiomyocytes directly or by paracrine signalling [17-21].

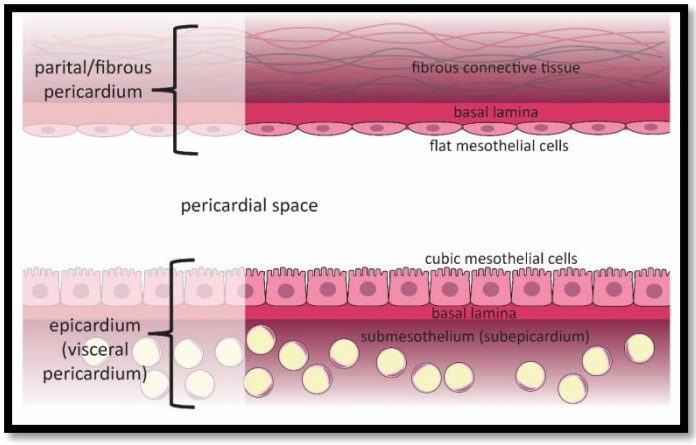

Figure 2. A diagram illustrating the structure of the pericardium.

3. The Endocardium

It is the layer that lines the atria, ventricles, valves, and both sides of the cardiac septa. It is continuous with the endothelial lining of the blood vessels. The endocardium is composed of three sub-layers:

- Endothelium: the innermost layer. It is a simple squamous epithelium.
- Subendothelium: it is a thin layer of connective tissue and smooth muscle fibers. It lies external to the endothelial layer.
- Subendocardium: it is composed of connective tissue that is continuous with the connective tissue of the myocardium. It contains the conductive system of the heart [22].

Recent studies have reported that the endocardium is the origin of the recently discovered circulating multipotent stem cells (CiMS) that have been isolated from human peripheral blood. Yang et al analysed the human leukocyte antigen (HLA) types of CiMS cells that were obtained from the peripheral blood of heart-receiving patients and found that they were matched to the HLA of the hearts of the donors. They also cultured endocardial cells derived from hearts biopsied before the transplantation process. They found that both endocardial cells and the circulating stem cells obtained from recipient blood were positive for both CD31 and Nuclear Factor of Activated T Cells-1gene (NFATc1) which confirms the endothelial origin of these stem cells. They also cultured these circulating stem cells and found their capabilities to differentiate into three germ layers in vitro. Moreover, they confirmed the regenerative capabilities of the CiMS by illustrating their effect in a model of myocardial infarction and hindlimb ischemia. This finding provides a promising strategy in the field of regenerative medicine due to their multipotency and their conveniently accessible sources e [23, 24].

4. The Myocardium

Myocardium is the thickest layer; its thickness is lesser in the atria than in the ventricles due to the difference in the force required to eject blood. Atria eject blood to the ventricles, while ventricles need high pressure to eject the blood to systemic and pulmonary circulations [25]. The myocardium is composed mainly of contractile cardiac muscle fibers (contractile cardiomyocytes) bound together by highly vascularized connective tissue. Collagen and elastic fibers are present between the bundles of cardiac muscle fibers, while a network of reticular fibers surrounds each cardiomyocyte [26].

4.1. Cardiac telocytes

Hinescu and Popescu found these unique stromal cells in 2005 and first referred to them as "cardiac interstitial Cajal-like cells." They were discovered using standard transmission electron microscopy on ultra-thin sections of human atrial tissue, followed by image reconstructions from serial photomicrographs [27]. These cells became known as "cardiac telocytes" in 2010 [28]. Following that, many studies have used transmission electron microscopy (TEM) or immunohistochemistry approaches to visualize telocytes throughout the heart [29-31]. Telocytes are distinguished by their heterochromatic nucleus and tiny spindle-shaped cell bodies with a high nucleocytoplasmic ratio. They have telopodes, which are incredibly thin 2-5 prolongations that can range in length from tens to hundreds of micrometres and are 0.1-0.5 m thick. These telopodes are made up of numerous dilatations called podoms that are linked together by thin segments called podomeres. The arrangement of podoms and podomeres mimics a string of beads, resulting in a "moniliform" pattern [28, 32, 33] (**Figure 3**). Telocytes cannot be seen with standard hematoxylin and eosin staining procedures due to their small size [34]. Telocytes also have lipid droplets, thick bodies (primordial Z-lines), desmosome-like structures (primordial intercalated discs), large mitochondria, numerous caveolae,

and a thin basal lamina. They build networks around cardiomyocytes by wrapping their telopodes around them [35]. Telocytes aid in tissue regeneration by guiding and nurturing myocardial precursors via the release of exosomes (40-100 nm), ectosomes (100-1000 nm vesicles originating from direct budding of the cytoplasmic membrane), and apoptotic bodies (vesicles formed by apoptotic cells' outward blebbing of the cell membrane) [36-38]. Telocyte apoptosis has been linked to poor myocardial state and increased myocardial fibrosis in a variety of illnesses [39, 40]. Furthermore, telocyte-derived exosomes have been shown to be therapeutic in myocardial infarction models [41-44]. Yang et al. discovered that telocyte-derived exosomes have an angiogenic effect [45].

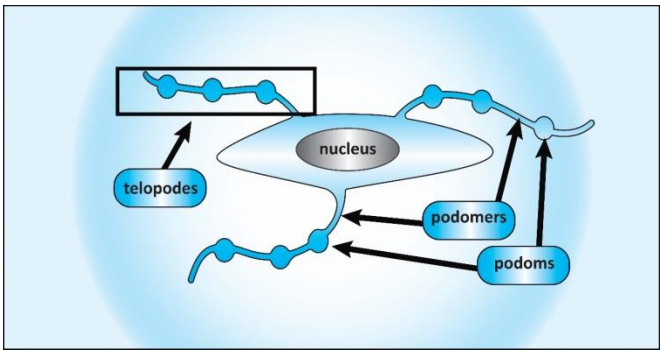

Figure 3. A diagram illustrating the telocyte morphology [46].

4.2. Histology of the contractile cardiomyocytes

4.2.1. The Light microscopic structure of cardiac muscle fibers

In routine H&E longitudinal sections, cardiomyocytes appear as long cylindrical cells arranged end to end to form cardiac muscle fiber. Some of these cells branch and anastomose with two or more other ones, leaving a slit-like space in between. They are joined by a special junctional complex called the intercalated discs (ICDs), which appear as dense linear structures oriented transversely at irregular intervals. Cardiac muscle fibers show faint eosinophilic cross-striations. Such striations are alternating light and dark bands of myofibrils called (I) and (A) bands respectively. An oval large v*esicular nucleus* is present at the center of each cell**.** Some cardiomyocytes are occasionally binucleated. In transverse sections, cardiomyocytes have various sizes and irregular outlines. Shows an abundant amount of granular cytoplasm surrounding the central nuclei. On one field, rounded nuclei appear in most of the cells [47].

By polarizing microscope, the dark bands alter the polarized light in two planes (birefringent) and that property makes them doubly refractive, so they are described as being anisotropic, hence the name the A-band. On the other hand, The light bands do not alter the plane of polarized light (mono-refringent), so, they are described as isotropic and named the I-bands [48].

4.2.2. Ultrastructure of the cardiomyocytes

The cardiomyocytes are 15-30 µm in diameter and 85-120 µm long. They are surrounded by a delicate sheath of endomysium with a rich capillary network.

4.2.2.1. The sarcomere

Cardiomyocytes contain myofibrils which are long cylindrical parallel bundles of myofilaments. Myofilaments run parallel to each other and the long axis of the cardiomyocyte. They are composed of thousands of thick and thin filaments. The ultrastructure of the myofilaments shows end-to-end repeated units called the sarcomeres, which are the contractile units of the myofilaments. [49] Each sarcomere shows an extraordinary complex arrangement formed by A-and I-bands arranged in the following fashion.

Each I-band is composed of hundreds of thin filaments. It is bisected by a dense zigzag line called Z-line, which is perpendicular to the long axis of the myofibril and is composed of a variety of protein molecules. Each A-band is composed of hundreds of thick filaments and overlapping parts of thin filaments. It is bisected by a less dense region called the H-band, which in turn is bisected by a narrow dense line called the M-line. The H-band is purely composed of thick filaments only, while the M-line is the binding protein that holds thick filaments together. The sarcomere is limited at its lateral sides by two successive Z-lines. It measures around 2-3 µm in the relaxed muscle and it can be reduced to 1 µm in the contracted state. Sarcomeres are not in register laterally, which is responsible for the faint striated appearance of the cardiac muscle tissue by the light microscope [50-52].

The thin filaments are formed of filamentous actin (F-actin) units. F-actin is a double-stranded helix of hundreds of polymerized globular actin units (G-protein), and it is 5-6 nm wide and 1.0-1.3 µm in length. Actin filaments are polar; have a plus(barbed) end that is attached to the Z-line by α-actinin protein and a minus (pointed) end that extends to invade the A-band. The actin thin filaments are associated with two groups of proteins; regulatory proteins which are tropomyosin and troponin, and thin filament-associated proteins such as tropomodulin and nubulin [53].

Tropomyosin is a protein that is also composed of a double helix of two polypeptides. It runs in the groove between the filamentous actin molecules. In relaxed muscle, it hides the myosin binding site on the actin molecule [54, 55]. Troponin molecule is composed of a complex of three globular subunits; Troponin-C, Troponin-T and Troponin-I. Troponin-C (TnC) binds to calcium (Ca^{2+}), an important step in initiation of contraction. Troponin-T (TnT) anchors the troponin complex to tropomyosin. Troponin-I (TnI) inhibits actin–myosin interaction. Each tropomyosin molecule contains one troponin complex [56]. Serum level measurement of troponins is a preferred diagnostic method in cardiac injury. When cardiomyocytes are damaged, troponin level rises within 2-4 h later, reaches its peak within 48 h and remains elevated for 7-10 days [57].

Tropomodulin is an actin-capping protein that is attached to the minus end of the actin filament. It maintains the length of the actin filament in the sarcomere by preventing further polymerization or depolymerization [58, 59]. While nebulin is an elongated, non-elastic, protein that is attached to the Z-lines spanning most of the length of the actin filament. It regulates the length of thin filament and augments its stability [53, 56].

Every thick filament is composed of 200-300 myosin II

molecules, which are rod-like motor protein. Myosin molecules generate contractions by cyclic interaction with actin molecules that leads consequently to the sliding of actin filaments over their myosin counterparts. Each myosin II molecule is formed of two identical heavy polypeptide chains and four light polypeptide chains.

The heavy chains have two globular heads each of which is connected to a tail. They are wrapped around each other forming an α-helix. The globular head represents the motor domain that projects at right angle at one end of the myosin molecule. Each head has two specific binding sites, one for adenosine triphosphate (ATP) with ATPase activity and the other for actin. As for the light chains, they are four in number, two for each of the heavy chains [60].

Myosin II molecules are lined in a staggered antiparallel fashion. The middle zone of each thick filament is formed of tail regions only, also called the bare zone, while the two ends are composed of both heads and tails. Myosin filaments are held in position by a family of proteins called the M-line proteins.

Heads of myosin molecules are in register with actin filaments, in order to slide over them during contraction and the following relaxation [53, 56].

Cardiac muscle dysfunction, commonly due to ischemic heart disease leads to systolic heart failure, also called heart failure with reduced ejection fraction (HFrEF), in which the ejection fraction falls below <45%. Recently, myosin modulators such as omecamtiv mecarbil, have shown great progress in treating HFrEF in clinical trials. They show a great ability to enhance myocardial contractility without increasing the amplitude of the Ca^{2+} transient, by direct activation of myosin [61].

There are Accessory proteins that maintain attachment and alignment of actin and myosin filaments:

- **Titin:** sarcomere compliance is attributed to titin, the largest protein in human body. It is an elastic sarcomeric protein encoded by the largest gene in the human genome (364 exons) [62]. Titin extends from the Z-line to the M-line spanning more than half of the sarcomere and overlaps with titin protein from adjacent half-sarcomeres, it has a spring-like structure at its beginning. Titin molecules form an elastic filament system that prevents overstretching of the sarcomere, regulating the passive myocardial tension [62-64] Variation in titin proteins causes up to 25% of familial dilated cardiomyopathy (DCM); inherited autosomal-dominant cardiac disease , and up to 12–18% of cases of sporadic DCM [65, 66].
- **α-Actinin:** rod-shaped protein that binds titin and actin filaments to the Z-line. α-actinin is a member of the dystrophin proteins superfamily, and it is a marker for Z-disc [67, 68].
- **M-line proteins:** a **network** of protein family of myosin-binding proteins includes myomesin and obscurin. They hold myosin filaments at M-line and act as shock absorber during severe muscle contraction. Another major component of the M-line protein is the creatine kinase-myocardial band (CK-MB) enzyme. It is suggested that CK-MB is the cause of the electron density of the M-lines shown by the electron microscope [69]. CK-MB transfers phosphate moiety from creatine-phosphate to adenosine diphosphate (ADP) to generate ATP needed in the actomyosin cross-bridge cycle. Disruption of the sarcolemma causes CK-MB to be released from the sarcoplasm to the systemic circulation. Therefore, measurement of its serum level can be used as a sensitive and specific indicator of myocardial damage. Its level rises within 2-4 h after cardiac injury, reaches its peak from 24-48 h and returns to normal within 72 h [57, 70].
- **Cardiac myosin-binding protein C (cMyBP-C)**: it is a multi-domain protein that aids in regulation of actin and myosin interaction. Its molecules form several transverse stripes on either side of the M-line**.** Mutation of this protein causes up to 70% of hypertrophic cardiomyopathy (HCM) cases, which is an inherited disease associated with sudden cardiac death, characterized by left ventricular hypertrophy despite absence of any predisposing cardiovascular condition [71-73].

Several sarcomeric protein quality control (PQC) mechanisms have been recently described within the cardiomyocyte, these mechanisms relay on variety of chaperones, a group of intracellular rescue proteins. These chaperones, also called heat-shock proteins (HSP), ensure proper disposal and replacement of the misfolded sarcomeric proteins without compromising the mechanical function of the rhythmically contracting muscle.[52] Enhancing the expression of these chaperones by exercise or caloric restriction may has cardioprotective effect in animal models of heart failure [74]. In addition, some drugs are being studied due to their ability to enhance certain HSP and protect the heart in cases of ischemia/reperfusion injury, dilated cardiomyopathy and arrhythmias [75].

4.2.2.2. Transverse Tubules (T-Tubules)

T-tubules are invaginations of the sarcolemma and their attached glycocalyx, they invaginate into cardiac muscle cells and ring around every myofibril at the level of Z-line, Their mean diameter ranges from 180 to 280 nm. T-tubules form a network of tubules running perpendicular to the cell surface, and they also have longitudinal (axial) components running from one Z-line to the next, so they present at intervals of around 2 μm along the longitudinal axis of the ventricular cardiomyocyte, hence the name "transverse-axial tubular system: (TATS). This unique structure ensures rabid propagation of the excitation wave throughout the cell interior (**Figure 4**) [76, 77]. There is one T-tubule per sarcomere**.** They are wide and well-developed in ventricular muscle fibers but sparse in human atrial cardiomyocytes. Unlike the smaller mammals, in which they are absent [67, 78]. T-tubules increases the surface area holding the calcium channels, the percentage of cell membrane forming the t-tubules was estimated to range from 21% to 61% from total cellular membrane [79]. T-tubules are rich in L-type calcium channels (up to 9 folds more than the surface sarcolemma) through which extracellular Ca^{2+} accesses the sarcoplasm at the time of depolarization to initiate excitation-contraction coupling [79, 80]. Dilatation of the T-tubules has been observed in many conditions of failing human heart such as ischemic heart disease and DOX-induced cardiotoxicity [81].

4.2.2.3. Sarcoplasmic reticulum (SR)

Endoplasmic reticulum (ER) is the largest membranous organelle in the cytoplasm of eukaryotic cells. Within its lumen,

synthesis, folding, and maturation of many proteins occur. Additionally, ER has a detoxification function. In skeletal and cardiac muscle, it is usually called "the sarcoplasmic reticulum" and its main function in these tissues is to regulate intracytoplasmic Ca^{2+} concentration needed for excitation–contraction coupling. Although, some authors suggest that ER and SR are functionally distinct compartments within cardiomyocytes [82-84].

SR is divided into two compartments which differ structurally and functionally: longitudinal and junctional domains. Longitudinal SR forms tubular network around each myofibril. It is rich in Sarcoplasmic/Endoplasmic Reticulum Ca^{2+}-transport ATPase (SERCA) which rapidly removes Ca^{2+} ions after muscle contraction to initiate its relaxation [85]. At Z-line level, longitudinal SR expands and ends by small feet-like terminals, the junctional SR, which in turn is attached to the T-tubules forming diads (**Figure 4**) [86].

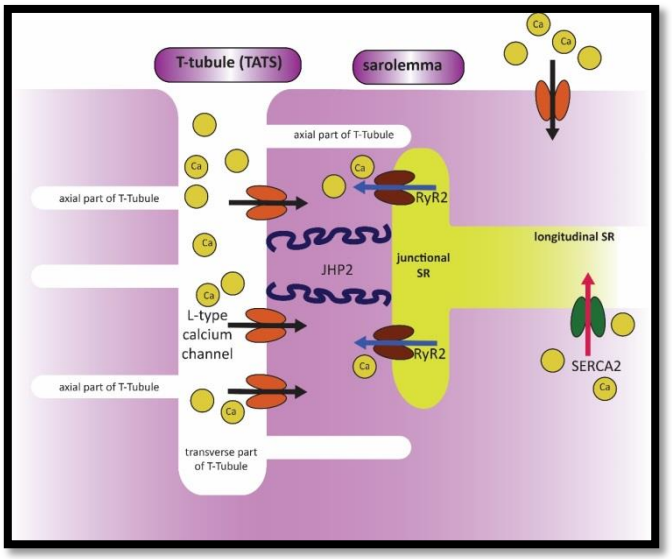

Figure 4. Structure of the TATS and SR.

During systole, the change in cardiac action potential opens voltage-gated L- type Ca^{2+} channels on both the sarcolemma and the T-tubules. Calcium influx stimulates ryanodine receptor (RyR2) found on the junctional SR to release their calcium content into the sarcoplasm; Ca^{2+}-induced Ca^{2+} release. The transient rise in intracellular Ca^{2+} concentration leads to binding of calcium ions to troponin C, which initiates the actin-myosin interactions. To start the diastole, calcium ions sequestered back into the SR by being pumped in by SERCA to allow sarcomere relaxation.Calsequestrin-2 (CSQ2), a major Ca^{2+}-buffering protein is also present in junctional SR. Recently, disturbances in intracellular Ca^{2+} handling due to inherited mutation or acquired defects lead to RyR2 dysfunction and/or SRCA leak, have been linked to development of atrial fibrillation (AF) and heart failure [87]. Targeting RyR2 by the new stabilizing agents has been recently under the preclinical trials in order to selectively normalize cardiac rhythm and improve the overall cardiac function [88].

Junctional SR is anchored to the sarcolemma of T-tubules by Junctophilin-2 (JPH2), which is essential to ensure stability of the diad by providing a structural bridge between their membrane [89] . The estimated distance between the T-tubule and the junctional SR is 10-20 nm [90]. In various types of heart failure, reduction in JPH2 expression leads to increase this distance, and thus impairs the excitation-contraction coupling. Recent studies suggest JPH2 as a potential target in treating the failing heart [91, 92].

Many cardiac conditions such as acute or chronic tissue ischemia or cardiotoxicity can cause "ER stress"; in which ER protein folding capacity is overwhelmed, and thus leads to accumulation of unfolded or misfolded proteins within its lumen, which in turn stimulates the unfolded protein response (UPR) pathway [93-95]. Activation of the UPR initiates a reduction in protein synthesis, enhancement of misfolded proteins degradation, and up-regulation of genes involved in cellular proteostasis pathways. Furthermore, UPR can promotes apoptosis as a protective response [96, 97]. Many studies have revealed that ER stress and the UPR can be a promising target for many therapies in treating cardiovascular diseases [98-100].

4.2.2.4. Mitochondria

Compared to other cell types, mitochondria occupy up to 40% of the cardiomyocyte volume. They are electron dense and have densely packed cristae. Cardiac mitochondria are classified according to their location into perinuclear, interfibrillar, and subsarcolemmal. Perinuclear mitochondria (**Figure 5**) are present at the nuclear poles. They are spherical, and their lengths range from 0.8 to 1.4 µm. These mitochondria contain well developed curved cristae with relatively little matrix area. On the other hand, interfibrillar mitochondria (IFM) are elongated and present between the myofibrils forming longitudinal rows. IFM occupies the entire spaces between Z-lines, usually one mitochondrion exists per sarcomere. Lengths of IFM range from 1.5 to 2.0 µm. Also, they have tubular cristae. Lastly, subsarcolemmal mitochondria (SSM) are lamelliform and present beneath the sarcolemma, they are variable in length (0.4 –3.0 µm). SSM also vary in their shapes, they can show oval, spherical, polygonal, or horse-shoe patterns [101, 102].

Normal mitochondrial function depends on a balance between fission and fusion events, a process known as mitochondrial dynamics. Four proteins are responsible for this balance; mitofusin-1(Mfn1), mitofusin-2 (Mfn2) and optic atrophy-1(OPA1) proteins are responsible for fusion, while dynamin related protein-1(Drp1) is responsible for fission. Many drugs such as doxorubicin, a tricyclene anticancer, cause an oxidative imbalance and thus inhibits genes responsible for production of Mfn1, Mfn2 and OPA1, while it increases the expression of Drp1, resulting in unopposed fission and ultimately mitochondrial fragmentation, which is hinders mitochondrial function [103-108].

Kleele et al described two distinct types of fission occur in the mitochondria of cardiomyocytes, the peripheral and the mid-zone fission (**Figure 6**). In the peripheral type, fission occurs near the tip of the mitochondria, resulting in small daughter mitochondria. This small globular daughter contains damaged material and marked to be autophagocytosed. They observed a relationship between the peripheral fission events and increased intracellular calcium and reactive oxygen species. On the other hand, midzone fission occur at the center of the mitochondria,

leading to production of two equal daughter mitochondria. Kleele et al linked the mid-zone fission to cell proliferation, in which production of new mitochondria is highly needed [109].

Mitochondria have a pivotal role in heart pathology, they can trigger apoptosis if their mitochondrial DNA, inner mitochondrial membrane (IMM)[110-112], or electron transport chain (ETC)[113]have been damaged by various drugs or toxins [114-116].

Wang et al suggest that mitochondrial transfer from cultured mesenchymal stem cells into cardiomyocyte of an in vitro anthracycline induced cardiotoxicity model, could improve their function. Mitochondrial transfer must be extensively studied to find and effective yet safe intervention to salvage the hearts of oncology patients receiving cancer therapy [117].

Surprisingly, mitochondria; the power house of the cell are accused of being the cause of its damage and the subsequent tissue senescence, due to their production of reactive oxygen species (ROS) during the oxidative phosphorylation. Unfortunately, beside its limited replicative capacity, cardiac tissue rich in mitochondria, and very poor in the internal antioxidants [113, 118-121]**.**

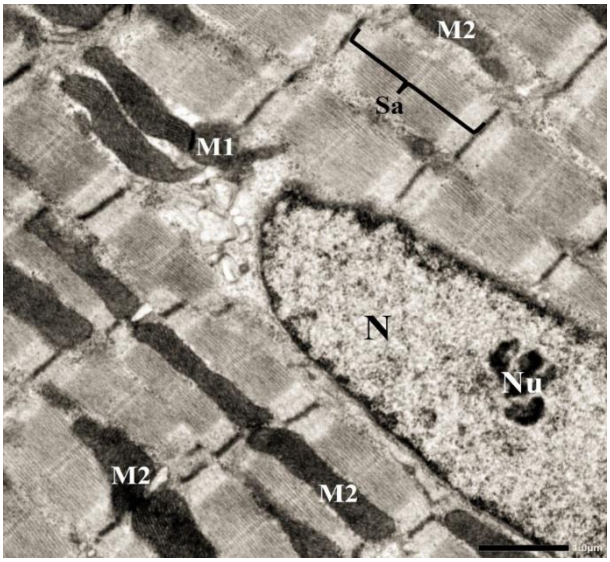

Figure 5. An electron micrograph of the myocardium from an adult male Wistar rat a cardiomyocyte with regularly arranged myofibrils and a central elongated euchromatic nucleus (N) with prominent nucleolus (Nu). M1; mitochondria at the nuclear pole, M2; intermyofibrillar mitochondria, Sa; sarcomere. (Mic. Mag. × 5000).

4.2.2.5. Nucleus

Each cardiomyocyte contains an euchromatic oval central nucleus, parallel to the long axis of the cell (**Figure 5**). It has been reported that mutation of lamin A/C gene (LMNA gene) that encodes for proteins of intermediate filaments A- and C-type lamins, which are essential component of the nuclear envelope, accounts for 10% of DCM [122, 123].

4.2.2.6. Other organelles and inclusions

Myofibrils diverge and pass around the nucleus leaving biconical juxtanuclear area in which cell organelles such as mitochondria and Golgi complex are present. Myoglobin molecules and lipid droplets are abundant throughout the cell [80].

Glycogen granules are also present juxtanuclear and between the myofibrils. In glycogen storage diseases (GSD), mutation of a gene encoding acid alpha-glucosidase, a lysosomal enzyme, leads to accumulation of its substrate, the glycogen, in the lysosomes of the liver, skeletal muscle and myocardium. GSD manifest themselves as progressive myopathy and heart failure [124]. Recently, many studies found the alleviating effect of 144DG11, a new polyglucosan-reducing lead compound. 144DG11 enhances lysosomal degradation of glycogen and mitochondrial activity, leading to reduction of glycogen accumulation in various tissues [125, 126]. In addition, gene therapy to replace the defective gene and correct the systemic metabolic abnormalities is now being under studies [127].

Lysosomes are another organelles with a great role within cardiomyocytes, they carry out the process of autophagy, which maintain cellular homeostasis. Autophagy recycles cytoplasmic material and damaged and/or aged organelles. Special type of autophagy is called "mitophagy", in which damaged mitochondria are recycled. Mitophagy decreases with aging, resulting in the accumulation of damaged mitochondria, which in turn results in increased ROS. Oxidative damage of cardiac tissue is directly linked to its senescence, and to further augmentation of mitochondrial damage " a vicious cycle". Cardiac tissue aging manifests itself histologically by the accumulation of autophagic vacuoles and lipofuscin deposits, and functionally by decreased contractility of the heart [119, 128, 129].

Lipofuscin; yellowish-brown auto-fluorescent pigment granule, may be present at the perinuclear region. It is composed of an aggregation of oxidized lipids, cross-linked proteins, and oligosaccharides. By TEM, lipofuscin granules are membrane-bound with electron-dense vacuolar, granular, or lamellar organization, they result in incomplete digestion of the engulfed material. It is widely accepted that.

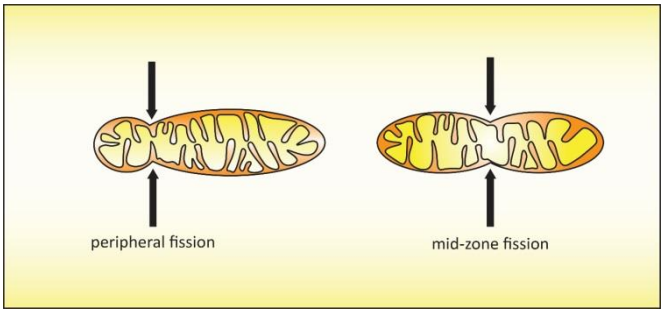

Figure 6. types of mitochondrial fission.

lipofuscin accumulates with the aging process due to the deterioration of autophagy, which in turn contributes to the development of decreased cardiac contractility as a part of cardiac senescence [130, 131]. Many recent studies suggest that

enhancement of autophagy by rapamycin, simvastatin, or caloric restriction, may lead to lesser lipofuscin accumulation and could delay the aging of the myocardium [131, 132].

4.2.2.7. Intercalated disc (ICD)

As mentioned previously, the intercalated disc is the junction site between two adjacent cardiomyocytes at the level of Z-line, forming a space of 15 to 20 nm. When ICDs are viewed by scanning electron microscope (SEM), they appear as finger-like projections which are complementary on each side of the connected cardiomyocytes. By TEM, every intercalated disc has two portions; the first is the transverse component, which contains fascia adherens and desmosomes. The second is the lateral component which is rich in gap junctions [133].

5. The transverse component

The transverse component is the structure responsible for the transverse density that appears in the light microscopic view. Junctions of the transverse component prevent the separation of cardiomyocytes during repetitive contractions. The junctions present in the transverse domain are:

- Fascia adherens: It is the main block of the transverse component. It holds the cardiomyocytes at their ends to form the cardiac muscle fiber and transduces mechanical force among them. It is formed of a transmembrane glycoprotein called N-cadherin (N-cad), which is a member of the cadherin superfamily. This protein mediates calcium-dependent cell-cell interaction. It forms homologous dimers with N-cad from adjacent cells. This interaction results in tissue specificity during development by allowing developing cardiac cells to interact only with cells expressing the homologous N-cad. The intracytoplasmic domain of N-cad interacts with a protein called β-catenin, which in turn associates with α-catenin and vinculin, which bind it to the actin component of the sarcomere and terminal web intracellularly. So, it is the functional analog to the zonula adherents of the epithelium. It extends only over the contact areas of the cells not circumferentially (**Figures 7&8**) [134, 135].

Figure 7: An electron micrograph of the myocardium from an adult male Wistar rat reveals stepladder course of the intercalated discs (black arrows) between adjacent cardiomyocytes. Yellow arrows; straight sarcolemma of the adjacent cardiomyocytes. (Mic. Mag. × 5000).

- Maculae adherents (Desmosome): It is a disc-shaped protein structure at the surface of one of the cardiomyocytes, which is identical to a similar structure on the surface of the opposite cell. It is formed of two Ca^{2+}-dependent cadherin-type transmembrane adhesion molecules; desmoglein-2 and desmocollin-2. Such adhesion molecules are attached to intermediate filaments (desmin) intracellularly via adaptor proteins; plakoglobin, plakophilin, and desmoplakin. Myozap is an interacting protein which is recently been identified as one of the components of the ICD. It is highly expressed in the heart. Mutation of myozap genes results in contractile dysfunction. Generally, desmosomes add extra strength to fascia adherens by their attachment to intermediate filaments (**Figure 8**) [136, 137]**.**

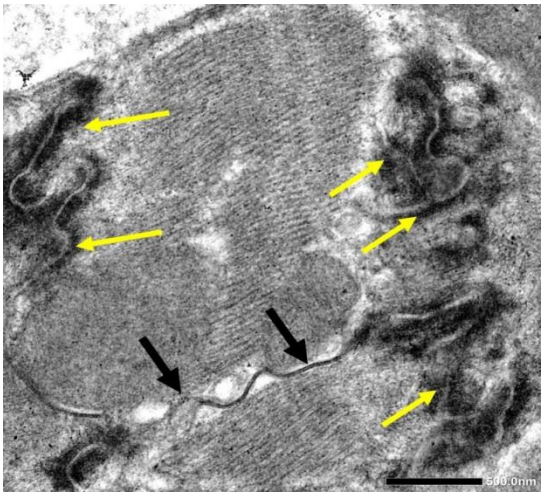

Figure 8: An electron micrograph of the myocardium from an adult male Wistar rat reveals transverse (yellow arrows) and longitudinal components (black arrows) of the intercalated disc. (Mic. Mag. × 12000).

6. The lateral component

Gap junctions are dynamic intercellular channels formed by transcellular structural proteins called connexins (**Figure 8**) [138]. The most predominant connexin form found in contractile cardiomyocytes is connexin 43 (Cx43), mainly in the ventricles. Two other forms are present to a lesser extent: connexin 40 (Cx40) and connexin 45 (Cx45). Gap junctions are made up of various combinations of connexin proteins across the myocardium. They allow the intercellular communication by transportation of ions and small molecules (<1 KDa) across the sarcolemma of the adjacent cardiomyocytes following their concentration gradient. These channels are considered as electrical synapses that allow cardiomyocytes to respond and contract synchronously, forming a functional syncytium**.** Conduction velocity across the heart is controlled by difference in gap junction distribution, size and connexins combination through different myocardial regions [139, 140]. Surprisingly, Cx43 has been recently identified on the membrane of the subsarcolemmal mitochondria and exosomes of cardiac origin forming hemichannels. It is believed that Cx43 participate greatly in

ischemia/reperfusion injury of myocardium after the onset of myocardial ischemia [140, 141].

The three complexes of intercalated disc communicate with each other and do not function independently. So, they are collectively renamed "area composita". Mutations of proteins forming the area composita can lead to arrhythmogenic cardiomyopathy, DCM, and HCM (**Figure 9**) [142, 143].

7. Types of cardiomyocytes

In addition to the contractile cardiomyocytes described before, there are two other types of cells found in specific sites within the heart:

- Endocrine cardiomyocytes (atrial cardiomyocytes):

  These are cardiomyocytes found in the atria, mainly the right atrium. They have an endocrine function. The atrial cardiomyocytes are smaller than the contractile cells, contain sparse T-tubules, and prominent perinuclear Golgi complexes which are associated with numerous electron dense secretory granules, which are account up to 600 granule per cell. Each of them measures from 0.3 to 0.4µm in diameter**.** They contain atrial natriuretic peptide (ANP) which aids in blood pressure control and electrolyte balance [144, 145]. Atrial cardiomyocytes show caveolae similar to smooth muscle, they can be visualized by TEM as sarcolemma flask-shaped**.**

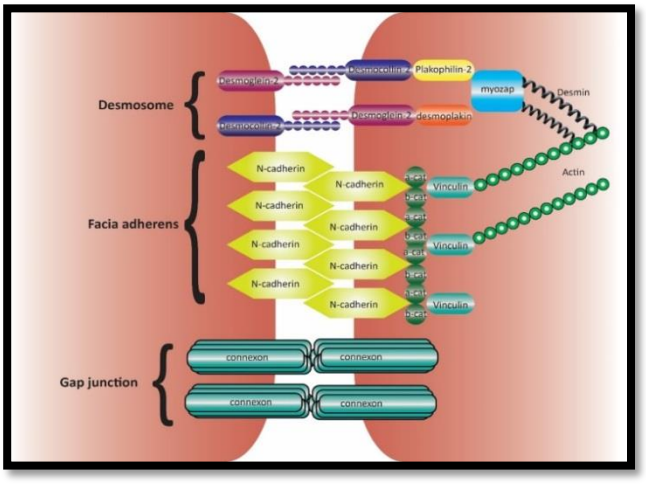

Figure 9. A diagram showing protein components of different junctions of the ICD.

Invaginations [146]. They have cell surface receptors that participate in cell signaling. Caveolin is a muscle-specific protein family that plays an important role in normal heart physiology, and they are potein markers of the caveolae [147]. In normal hearts, caveolin-1 (cav-1) regulates of the TGFβ pathway and thus controls extracellular matrix deposition. Any mutations in cav-1 gene lead to increase interstitial fibrosis and eventually heart failure due to myocardial stiffness [148, 149]. Caveolin-3 (cav-3) play a pivotal role in signal transduction [150]. Surprisingly. Cav-3 level rise in peripheral blood sample in patients of AF has been documented and correlated to the clinical condition of those patients [151].

- Cardiomyocytes of the conduction system:

  They are modified cardiomyocytes, most of them are located in the subendocardial layer and adjacent myocardium. Conductive cardiomyocytes initiate and propagate the waves of depolarization to harmonize the rhythmic contractions of the heart. The conduction system of the heart consists of two nodes of specialized myocardial tissue found in the right atrium: the SA node and the AV node, connected by the internodal pathway. Followed by the AV bundle of His and its right and left branches, and lastly the subendocardial conducting network formed of Purkinje fibers [152].

SA node located in the subepicardial layer of the superior posterolateral wall of the right atrium it extends from the superior vena cava towards the inferior vena cava along the sinus node artery, a branch of the right coronary artery. Histological and immunohistochemical studies on the SA node of ex-vivo human heart showed that SA node is crescent shaped and entwined around the sinus node (nodal) artery tissue and embedded within large amount fibro-fatty connective tissue, and richly innervated by branches sympathetic and parasympathetic nervous systems. It is not covered by capsule, so it has an indistinct and irregular margin with the surrounding atrial myocardium. Its dimensions are around 29.5 mm in its length, 1.8 mm in thickness, and 6.4 mm in width [153-155]. It is composed of two types of cells; nodal cells (P-cells) and transitional cells (T-cells). P-cells surround the nodal artery, they are small (3-10 µm), rounded, pale cells, with a central single, rounded dark nucleus, and they contain fewer mitochondria and few poorly organized myofibrils than the contractile cardiomyocytes. P-cells are grouped forming interconnecting fascicles surrounded by cords of connective. On the other hand, T-cells are pale, elongated, and larger than the P-cells but smaller than atrial cardiomyocytes, owing their intermediate characteristics between them. Their nuclei are central, dark, and oval. They are present at the periphery of the node and linearly organize together forming fiber-like structure [156, 157]. Over the last decades, SA was thought to lack t-tubules, Petkova et al have proven the presence of few sporadic t-tubules in the SA node of the human hearts by staining them with wheat germ agglutinin (WGA), specific marker of t-tubules. unlike in the rodent hearts which have no T-tubules [67]. Impulses generated from the SA node travel through the internodal pathways via gap junctions. The AV node is an oval mass measuring 5 mm in its length, 3 mm in width, and 1 mm in thickness. It is present adjacent to the fibrous skeleton of the heart and separated from the rest of the atrial myocardium by thin irregular dense connective tissue capsule. Human AV node composed of four types of cells; P-cells, T-cells, myocardial cells and Purkinje cells [156]. Purkinje cells (PC) (**Figure 10**) (not to be confused with purkinje cells of the cerebellum) are non-branching, fast-conducting cells joined end-to-end to form the bundle of His which originates from the AV node. This bundle branches repeatedly forming network of Purkinje fibers in the subendocardium of the ventricles [158]. They run in groups of two or more. They are larger than the contractile cardiomyocytes and have clear perinuclear and pale cytoplasm due to their numerous glycogen granules and the peripherally located fewer myofibrils [159, 160]. PC are rich in mitochondria, and their nuclei are often binucleated. (**Figure 10**). These cells are rich in gap junction joined together by desmosomes, which described as "atypical ICD" [161].

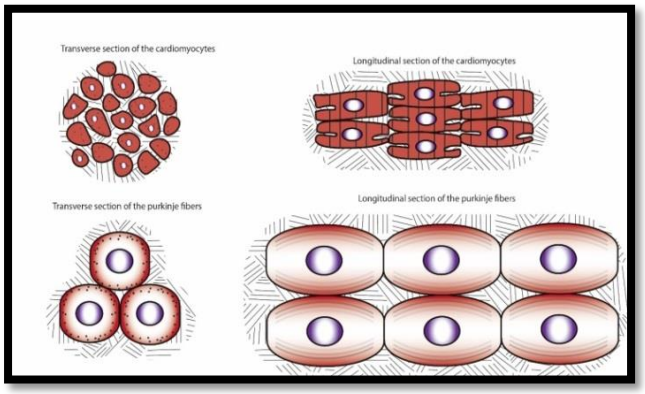

Figure 10. diagram of the structure of Purkinje cells as compared to the ordinary contractile cardiomyocyte.

8. Comparative anatomy and histology of the rat

The structure of the human and rat hearts shows overall similarities, yet there are notable distinctions between them. In rats, the heart weight ranges from 0.5-2.5 g, representing 0.20-0.50% of their total body weight. In comparison, the human heart weighs approximately 200-350 g, which corresponds to 0.4-0.45% of lean body weight. Additionally, the rat heart differs in its position within the pericardial sac, as it is not in contact with the diaphragm and has the freedom to move, resulting in an oval to spherical shape. Conversely, the human heart has a conical shape and a flat inferior surface due to its contact with the diaphragm. Moreover, there are dissimilarities in the composition of cardiac tissues. The epicardium in rats is thin and primarily composed of a mesothelial layer with a small amount of fibrous connective tissue beneath it. Conversely, the human epicardium contains a thicker layer of fibroadipose tissue that can measure several millimeters in thickness. When it comes to cardiac valves, rats have a continuous curtain-like structure with less distinct internal features, while human cardiac valves are divided into distinct leaflets and possess a trilaminar architecture internally. Furthermore, the fibrous skeleton of the rat heart is notably thinner compared to the human heart. Another distinction lies in the abundance of binucleated cardiomyocytes. Rat myocardium contains a higher proportion of binucleated cardiomyocytes, making up approximately 75% of the total cardiomyocyte population, whereas in the human heart, binucleated cardiomyocytes account for around 25% of the total cardiomyocyte population.

In summary, while the structure of human and rat hearts shares similarities, there are significant variations in terms of heart weight, shape, epicardial composition, valve structure, fibrous skeleton, and the abundance of binucleated cardiomyocytes. Understanding these differences enhances our knowledge of cardiac anatomy and highlights the unique characteristics of each species [162].

9. Regeneration of heart tissue

Due to absence of mitotic figures in the histological sections, the cardiac tissue is considered terminally differentiated, with no capacity to regenerate. Recently it has been postulated that the cardiac tissue has a minimal capacity to repair minimal damage and maintain normal tissue turnover. It was estimated that under physiological conditions, adult cardiomyocytes undergo cell renewal at a rate of ~1% per year in young individuals and decreases to 0.3% per year in elderly. At this rate, most of the cardiomyocytes will never be replaced in an average human life span [163]. Recent studies have shown that cardiomyocyte cell renewal results either by division of non-myocyte progenitors; circulating or resident endothelial stem cells and epicardial stem cells, or by division of the recently discovered cardiac stem cells [164-166]. Such discoveries open new paths for regenerative medicine to consider. But the heart, like any other organ, does not regenerate large infarcted areas under unfavorable circumstances of vascularity and tissue oxygenation, but it replaces them by non-contractile scar tissue which hinders functionality of the heart leading to its failure [167-169].

Conclusions

In this work, we have described the recently discovered cells and emphasizing their role in health and disease: valve endothelial cells and their role in valve stiffness, multipotent progenitor cells of the pericardium and their limited ability to replace cardiomyocytes in certain conditions, circulating multipotent stem cells and their regenerative capabilities, and the important role of telocytes under physiological and pathological condition, and their promising role in treating myocardial infarction.

In addition, we have described a cellular structure and described their fundamental relation to some cardiac conditions: structure of titin and cMyBP-C proteins and the importance their mutation in development of DCM and HCM, T-tubule detailed normal structure, and its dilatation in some forms of cardiotoxicity, role of RyR2 dysfunction and SERCA leak in development of atrial fibrillations, mitochondrial biology and its role in cardiac senescence, role of Cav proteins in signal transduction, and normal structure of area composita. Mutation of this area share in development of DCM and HCM

Furthermore, we have mentioned many currently experimented drugs and techniques that targets specific cardiac structure: myosin modulator in treating heart failure, RyR2 stabilizers as anti-arrythmia, mitochondrial transfer to treat cardiotoxicity, and 144DG11, the new polyglucosan-reducing lead compound to treat GSD.

In this article we have concentrated our efforts to provide an overview of the latest discoveries and potential therapeutic strategies along with normal cardiac histology.

List of abbreviations

α-SMA: α-smooth muscle actin

A-band: anisotropic band

ADP: adenosine diphosphate

ATP: adenosine triphosphate

AF: atrial fibrillation

ANP: atrial natriuretic peptide

AV: atrioventricular node

Ca2+: calcium

CSQ2: Calsequestrin-2

cMyBP-C: Cardiac myosin-binding protein C

Cav: caveolin

CiMS: circulating multipotent stem cells
Cx: connexin
CK-MB: creatine kinase-myocardial band
DCM: dilated cardiomyopathy
Drp1: dynamin related protein-1
ETC: electron transport chain
EndoMT: endothelial-to-mesenchymal transition
F-actin: filamentous actin
GSD: glycogen storage diseases
H&E: hematoxylin and eosin
HFrEF: heart failure with reduced ejection fraction
HSP: heat-shock proteins
HLA: human leukocyte antigen
HCM: hypertrophic cardiomyopathy
I-band: isotropic band
IMM: inner mitochondrial membrane
ICD: intercalated discs
IFM: interfibrillar mitochondria
JPH2: Junctophilin-2
Mfn1: mitofusin-1
Mfn2: mitofusin-2
N-cad: N-cadherin
NFATc1: nuclear Factor of Activated T Cells-1gene
P-cells: nodal cells
OPA1: optic atrophy-1
PQC: protein quality control
PC Purkinje cells
ROS: reactive oxygen species
RyRs: ryanodine receptor
SERCA: Sarcoplasmic/Endoplasmic Reticulum calcium transport ATPase
SA: Sinoatrial node
SR: Sarcoplasmic reticulum
SEM: scanning electron microscope
SSM: subsarcolemmal mitochondria
TGF-β1: transforming growth factor beta -1
T-cells: transitional cells
TEM: transmission electron microscope
T-Tubules: Transverse Tubules
TATS: transverse-axial tubular system
TnC: Troponin-C
TnI: Troponin-I
TnT: Troponin-T
UPR: unfolded protein response
VECs: valve endothelial cells
VICs: valve interstitial cells

Declarations

Compliance with Ethical Standards

We were strictly adherent to the ethical guidelines established by the Faculty of Medicine, University of Alexandria.

Informed consent

Informed consent is not applicable since this is a review article that did not involve the use of any human subjects. The article is based solely on the review and collection of data from already published articles. As a result, no individuals were directly involved or affected by the research process, eliminating the need for informed consent.

Availability of data and materials

The sources for the information discussed in this review can be obtained from the papers cited in the references.

Competing interests

The authors declare no conflicts of interest

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Author contributions

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Wrote the manuscript: MHH

Drew the scientific illustration: MHH

Revised the manuscript: MHH, LAH, HMA, SAM, NAS

All authors have read and agreed to the final draft of the manuscript.

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