Journal of Geriatric Cardiology (2015) 12: 76–82

Review · **Open Access** ·

The role of extracellular matrix in age-related conduction disorders: a forgotten player?

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Abstract

Cardiovascular aging is a physiological process gradually leading to structural degeneration and functional loss of all the cardiac and vascular components. Conduction system is also deeply influenced by the aging process with relevant reflexes in the clinical side. Age-related arrhythmias carry significant morbidity and mortality and represent a clinical and economical burden. An important and unjustly unrecognized actor in the pathophysiology of aging is represented by the extracellular matrix (ECM) that not only structurally supports the heart determining its mechanical and functional properties, but also sends a biological signaling regulating cellular function and maintaining tissue homeostasis. At the biophysical level, cardiac ECM exhibits a peculiar degree of anisotropy, which is among the main determinants of the conductive properties of the specialized electrical conduction system. Age-associated alterations of cardiac ECM are therefore able to profoundly affect the function of the conduction system with striking impact on the patient clinical conditions. This review will focus on the ECM changes that occur during aging in the heart conduction system and on their translation to the clinical scenario. Potential diagnostic and therapeutical perspectives arising from the knowledge on ECM age-associated alterations are further discussed.

J Geriatr Cardiol 2015; 12: 76–82. doi:10.11909/j.issn.1671-5411.2015.01.009

Keywords: Ageing; Arrhythmia; Cardiac; Conduction system; Extracellular matrix

1 Introduction

Aging is currently perceived as an ongoing physiological process that intertwines the patho-biological mechanisms of a "diseased" state, influencing the development of a clinically evident morbidity. The field of cardiovascular disease is thus oriented to consider the aging process as the determinant of a priori structural and functional alterations of cardiac and vascular substrates that are further exposed to superimposed pathogenic noxae. The interaction between age-associated structural and functional changes and the actual biological mechanisms of a disease—along with a plethora of other risk factors—will define threshold, severity, and prognosis of cardiovascular disease occurrence in older persons.[1]

In the cardiovascular clinical practice, the commonest conditions encountered in the elderly are progressive heart failure, arrhythmias, and degeneration of heart valve apparatus. Conduction disorders in this population carries a considerable high morbidity and mortality requiring pacemakers or defibrillators implantation in the majority of the cases.[2] Nodal dysfunction leading to chronotropic insufficiency, or increased susceptibility to reentry phenomena triggering ventricular or supraventricular arrhythmias characterize the clinical picture of these patients. Degeneration of the conduction system and nodal pacemaker is thought to begin after the seventh decade of life,[3] and ion channel alterations, along with beta adrenergic receptor down regulation and signaling impairment, have been reported as physiological substrates for tachyarrhythmia or tachyarrhythmia in the elderly.[4] A reduction to less than 10% of cardiac pacemaker cells has also been reported in respect to young adults,[5] and calcium, potassium and sodium handling systems have been shown to be defective leading to prolonged action potential and repolarization time with further increased susceptibility to reentrant arrhythmias.[6] At the cellular level, a so called electrical remodeling including post

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Received: November 10, 2014 **Revised:** November 21, 2014
Accepted: November 28, 2014 **Published online:** December 28, 2014

translational modification of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA-2), sarcoplasmic reticulum Ca^{2+}-release channel (RYR2) and phospholamban changes,[7,8] coupled with impairment in gap junction function[9] and energy generation at mitochondrial level,[10] has been claimed to constitute an electrophysiological substrate for arrhytmogenicity in the elderly.[11] However, the generation of specific zones of myocardial refractoriness, or areas characterized by heterogeneity in the impulse propagation and conduction anisotropy suggests the role of different mechanisms, other than the described intracellular alterations, in the determinism of arrhytmogenicity in the elderly.[12] Myocyte loss and compensatory hypertrophy together with interstitial focal fibrosis[13] induce the appearance of specific zones of functional conduction block or slowing eventually generating and stabilizing reentry circuits.[14] The description of specific ectopic foci, intracardiac pathways or reentrant circuits —often target of specific therapeutic interventions—further substantiate this point and progressively led to individuate other co-responsible for cardiac arrhythmias in the aged population.[12] In this context, in spite of the interest addressed by the literature to the "aged cardiomyocyte" as the main pathological responsible of age-related conduction disturbances, there are several evidences pointing at changes in the structure and function of the connectival extracellular matrix (ECM) as an important actor.[11] At the biophysical level, cardiac ECM exhibits a peculiar degree of anisotropy, which is responsible for the elastic and compliant properties of the ventricle and for the structural properties of heart valves. However, ECM components and their arrangement are also the main determinants of the conductive properties of the specialized electrical conduction system.[15] Moreover, cardiac ECM is actively sending biological signals regulating cellular function and tissue homeostasis.[1] Alterations of ECM function in the elderly might additionally exert a detrimental effect on the normal function of the conduction system and on overall ventricular function and cardiac performance.[15] Thus, this review will focus on changes of ECM components in the aged myocardium and on their relevance in conduction disorders appearance. Keeping an eye on the clinical side, it will explore the potential implications of ECM changes in the clinical management and on the therapeutic strategies potentially deriving from the scientific knowledge currently acquired on ECM.

2 The clinical scenario

Prevalence of cardiac arrhythmias increases over time during aging, carrying significantly higher morbidity and mortality in the elderly. In particular, the commonest arrhythmic conditions encountered in the elderly regard atrial fibrillation and ventricular tachyarrhythmia, but major ventricular arrhythmic events are the main responsible for sudden cardiac death (SCD) in older population, greatly impacting health care resource utilization.[11] The most recent epidemiological analyses are also remarking a striking incidence of atrial fibrillation or ventricular dysrrhytmias both malignant and benign independently on an underlying cardiac structural disease.[16] On the other side, atrio-ventricular block and asystole are also increasingly frequent with aging and account for up to 20% of sudden cardiac death.[17] In this regard, generation and conduction of the electrical impulse has been reported to be defective in the elderly, generating increased need for pacemaker devices implantation in the clinical management of this population.[18]

The function of the sinoatrial node (SAN) deteriorates with age with an increase in the nodal conduction time and a decrease in the intrinsic heart rate. Collectively, those alterations translate at the clinical side in the so-called sick sinus syndrome, whose manifestations include bradycardia, sinus arrest, and sinus exit block.[19]

Beside sinus node dysfunction, neurally mediated syndromes, acquired atrioventricular block, fascicular blocks, or (supra) ventricular tachyarrhythmias are the commonest indication for pacemaker implantation. Additionally, considering the hemodynamic changes occurring with aging, which are basically constituted by a reduction of ventricular compliance and an increased contribution of atrial contraction to ventricular filling, dual chamber pacemakers maintaining synchrony between atria and ventricles are advantageous in older adults.[20] During the aging process, the described structural and functional changes occurring in the left ventricle are interlaced with malfunction of the conduction system, which in turn results in non-efficient and non-synchronous activation of both ventricles, fostering a vicious circle eventually worsening the detrimental effects on cardiac performance.[21] Therefore, the use of biventricular leads in attempt to resynchronize ventricles activity is becoming a routine practice, usually coupled with a defibrillator system in order to protect the patient from malignant ventricular arrhythmias.[22] Also, with the aim to overcome issues of chronotropic incompetence and weakened response to adrenergic stimulation, the use of rate-responsive ventricular pacing has been proven effective in improving the quality of life in older patients compared to fixed-rate devices.[23]

3 The extracellular matrix

Multiple factors may influence age-related arrhythmic

events and SCD, including structural and electrical changes in the heart at the microscopic level. Aging results in increased fibrosis, reduced cellular coupling in the cardiac muscle,[24] as well as retarded activation and slowed velocity of the specialized conduction system throughout both the ventricle and the His-Purkinje system.[25] Age-related alterations in anisotropic conduction velocity—with a preferentially reduced transverse conduction—provide a substrate for reentrant arrhythmias and exert a pro-arrhythmic effect by decreasing the threshold for ventricular fibrillation.[26,27] This phenomenon is associated to reorientation of myofibrillar and myocardial sheet structures, as occurring during aging[28] and contributes to myocardial wall thickening.[29]

Propagation of the electrical impulse is well orchestrated within the heart and relies on a complex interplay between excitability, cell-to-cell coupling, and architecture of myocardial tissue. Myocardial interstitium is emerging as playing a pivotal role in this context: collagen fibers constitute the main component of extracellular matrix cardiac architecture and, in association with Connexin-43 (Cx43), determine and modulate cell-to-cell coupling in ventricular myocardium.[30] Under normal physiological conditions, myocardial collagen between cardiomyocytes is organized in a delicate network constituting less than 1% of total tissue volume. The peculiar distribution of type I collagen confers the particular conduction anisotropy and modulate electrical cell-to-cell coupling by determining the distance among myocytes.[30] During aging, a 200% increase in collagen content, together with a 50% decrease in Cx43 expression, has been reported.[26] Increased collagen content, together with the enhanced interstitial and reactive fibrosis seen during aging, mechanistically leads to separation of the cardiomyocytes, with subsequent reduction of gap junction plaques and impairment in cell coupling.[31] As a result of these changes, an increase in the vulnerability to tachyarrhythmias occurs, as the augmented anisotropic ratio and heterogeneity of conduction dramatically impair the conduction velocity, predisposing to reentrant arrhythmias.[32] The clinical reflex of this condition lies in the increased risk for fatal arrhythmias, that also partially accounts for the reported high incidence of SCD in patients with age-related remodeled hearts.[33] The relation between myocardial interstitium and Cx43 has been stressed by studies in which long-term inhibition of the renin-angiotensin-aldosterone system (RAAS) of aging mice to blunt excessive intramyocardial fibrosis preserved the normal Cx43 expression and reduced arrhythmia vulnerability.[27,34] Interestingly, if treatment is administered in a model of age-associated ventricular hypertrophy, in which gap junction downregulation and fibrosis formation is well established, it is possible to obtain a reversal of Cx43 expression but not to rescue the fibrosis process.[35] To clarify this mechanism, a recent work by Jansen, *et al*.[36] demonstrated that Cx43 decrease in aged hearts precedes the process of interstium remodeling, and that antifibrotic treatment determines primarily an increase in gap junction expression, just as if Cx43 could have a permissive action towards collagen deposition. Explanation to this phenomenon is thought to lie in the fact that reduced Cx43 levels may alter cardiomyocyte-fibroblast and fibroblast-fibroblast communication, leading to increased fibroblast proliferation and/or activity. In this regard, Jansen and colleagues demonstrated that gap junction remodeling, i.e., Cx43 age-related downregulation, induces an increase in fibroblast activation rather than proliferation. In their study on aged mice ($Cx43^{fl/fl}$ *vs*. $Cx43^{Cre\text{-}ER(T)/fl}$) following transverse aortic constriction, the authors demonstrated that the expression of discoidin domain receptor 2, a fibroblast marker, remained unchanged, while the expression of procollagen peptide (type I and III) and of collagen type I significantly increased in the Cx43-impaired group.[36] This study also demonstrates how both gap junction negative remodeling and collagen deposition (fibrosis) are required for slowing electrical conduction. The exact mechanism underlying the Cx43 mediated activation of cardiac fibroblast still needs to be unraveled, but a recent work by Bowers, *et al*.[37] demonstrated that communication between cardiomyocytes and fibroblasts via Cx43 channel exerts a potent influence on cytokine production. In support of these findings, Pedrotty, *et al*.[38] underlined the role of paracrine fibroblast activity in influencing myocyte electrophysiology favoring conductance slowing. However, it is interesting to notice that the most recent literature is increasingly pointing at the role of cardiac ECM as a culprit of age-related cardiac dysfunction. In this context, the proliferative and secretory activity of fibroblasts and myofibroblasts mediating ECM deposition and intramyocardial fibrosis have been recently elucidated,[15] and four different patterns of fibrosis, compact, patchy, interstitial and diffuse have been described.[39] Areas of patchy fibrosis with collagen bundles separating cardiac cells carry an important arrhythmogenic potential altering source–sink relationships between myocytes and therefore favoring reentry phenomena or triggering ectopic electric activity.[15] Several reports describe a shift in the ratio between collagen type I and III at the ventricular side and the accumulation of exuberant quantity of collagen seems to occur in association with fibronectin deposition, contributing to interstitial fibrosis and dramatically affecting conduction anisotropy.[40] Stein, *et al*.[26] while remarking the link between increased collagen deposition and reduced expression of Cx43 and of $Na_v1.5$

sodium channel, observed a difference in the fibrosis distribution between the right ventricle (RV) and left ventricle (LV). Areas of fibrosis were found in the entire thickness of the RV wall, while only in the endocardium and mid-myocardium in the LV. The subepicardic zone of the ventricle, the region form where ventricles are normally stimulated, was found to be affected by fibrosis degeneration only in the RV and this might partially explain the increased vulnerability to arrhythmias of this side of the heart in the elderly.[26] Along with collagen and fibronectin, also accumulation of α1 and α5 integrin have been reported,[41] and an imbalance in the metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) has been claimed to be at the root of the profibrotic shift occurring in aged ventricles. Specifically, Bonnema, *et al*.[42] found a decrease in the MMP-9/TIMP-1, MMP-9/TIMP-4 and MMP-2/TIMP-4 ratios during aging suggesting that a reduced ECM degradative ability might be at the basis of interstitial fibrosis. Additionally, a recent clinical study has shown that serum markers of ECM degradation positively correlate with malignant ventricular arrhythmias.[43] In particular, procollagen type I carboxyterminal peptide (PICP) and procollagen type III aminoterminal peptide (PIIINP), representative markers of collagen I and III synthesis, and MMP9/TIMP-1 ratio were found to be associated to tachyarrhytmic episodes.[43]

Beside intraventricular conduction, impulse generation at the level of SAN and atrioventricular node (AVN) is thought to be defective in the elderly. At the microscopic level, a recent autoptic study on human tissue showed fatty infiltration of the SAN, with observable signs of calcification and general inflammation, but no amyloid accumulation was detected.[44] Again, the recent literature is revealing the importance of changes in the structural component of SAN in aging-related nodal dysfunction. A study of Yanni, *et al*.[45] demonstrated that an interstitium remodeling occurs also in the SAN of aged animals beside the known nodal enlargement and nodal cells hypertrophy. The Authors correlated nodal dysfunction to both cellular abnormalities concerning reduced expression of $Na_v1.5$ sodium channel and to SAN connective tissue reorganization during the aging process. Interestingly, they did not report an increase in fibroblast number, but modifications in ECM component of SAN. Decreased protein collagen density and mRNA expression was reported and, unexpectedly, the ratio between the stiff collagen I, which is abnormally high in diseased heart, and the more elastic collagen III, did not change with age in SAN.[45] Conversely, elastin, the other main structural component of cardiac ECM, was decreased in expression, but the ratio (at the mRNA level) between collagen I and elastin did not vary significantly within nodal region or with age.[45] Those changes were substantiated by alteration in the balance between fibrotic modulators and degrading enzymes (cardiac MMPs). Transforming growth factor β1 (TGF-β1) and tumor necrosis factor α (TNFα) were significantly upregulated in aged animals at nodal level, while MMP-2 showed an age-dependent decrease.[45] This picture converges toward a pro-fibrotic activity, but this biological movement does not translate into an actual increase of structural matrix proteins levels in the SAN of old hearts. However, these data find a correlate in a recent clinical study demonstrating an association between MMP-9 and the risk of developing atrial fibrillation in a cohort of aged patients.[46]

Interestingly, age-dependent changes in other ECM components seen at the ventricular muscle side during aging could not be detected at the SAN level. No significant changes at the mRNA level of fibronectin α1 (adhesive protein), decorin (anti-fibrotic proteoglycan), connective tissue growth factor (CTGF, a cysteine-rich protein induced by TGF-β1 and shown to trigger many cellular processes underlying fibrosis), and integrins α1, α5, and β1 were reported. However, the finding of changes in the expression of MMP-1, MMP-9, and MMP-13 further underpins the involvement of ECM in this context. (Table 1 summarizes all the ECM changes described).

Table 1. ECM changes in the electrical conduction system and their functional consequences.

ECM component modification	Effect	Reference
↑ Collagen Type I	Gap junction remodelling	[26]
↓ Connexin-43	Altered cell-cell communication and increased fibroblasts activity	[26]
↑ Fibronectin	Fibrosis	[40]
↑ α1 and α5 integrin	Fibrosis	[41]
↓ Elastin	Sinus atrial node fibrosis	[45]
↑TGFβ1		
↑TNFα	Interstitial fibrosis	[45]
↓ MMP-2		
Fatty infiltration and flogistic infiltrate	Sinus atrial node dysfunction	[44]

TGFβ1: transforming growth factor β1; TNFα: tumor necrosis factor α; MMP-2: metalloproteinases-2.

4 The clinical perspectives

Prevalence of cardiac arrhythmias increases over time during aging, carrying significantly higher morbidity and mortality in the elderly. Beside atrial fibrillation, ventricular tachyarrhythmias and major ventricular arrhythmic events

are the main responsible for SCD in older population, greatly impacting health care management.[11] Defective impulse generation and conduction and ECM disarray with augmented intramyocardial fibrosis during aging are considered the main biological responsible of these disturbances. Complex cellular interplay and paracrine biological signaling underlie this phenomenon and targeting fibrosis generation and its pathological characteristics might be a promising therapeutical approach for age-related arrhythmic disease. The knowledge obtained over the electrophysiological significance of intramyocardial fibrosis distribution fueled an interesting piece of research concentrating on the spatial precise resolution of fibrotic strands and bundles within the ventricle by means of MRI-based imaging techniques.[47] Gadolinium enhanced MRI combined with computer-aided image processing could describe patterns and amount of fibrosis at the submillimeter scale, potentially allowing for tailored ablation interventions or device implantations.[48] Another important clinical translational remark that could be inferred by the mentioned paracrine interaction between cardiac myocytes and fibroblasts regards the fact that pharmachological stabilization of cell-to-cell coupling could exert a positive role not only on the immediate electrical homeostasis, but also on the prevention of collagen deposition and intramyocardial fibrosis. Cellular interaction via gap junction has been shown to be associated to a paracrine signaling activation which in turns reflects in to enhanced matrix production and deposition by fibroblasts.[37] Compounds able to optimize cell electrical coupling might therefore induce a regulation of ECM production. Alternatively, targeting directly the fibrosis process could represent a valid approach, and, despite both ACE inhibitors[49] and aldosterone antagonists[50] have been shown to reduce fibrosis and decrease sudden death in aged decompensated patients, exploration of novel biological therapeutical approaches might deserve further investigations in this context. Inhibitors of AT-1 receptors for angiotensin have been shown to be a promising option,[51] and inhibition of collagen synthesis through blockers of geranylgeranyl transferase and farnesyl transferase,[52] or antagonist of prenyl chain biosynthesis as statins,[53] is under investigation. However, targeting the complex paracrine signaling established in the delicate network between cellular and extracellular component of the heart might potentially enlighten new avenues in the treatment of age-related disease.

5 Conclusions

Despite the interest lavished in the current research on the cellular cardiac component, ECM plays an active and pivotal role during the aging process, influencing several aspects of cardiac biology and conditioning myocardial structural properties and function. Conduction disturbances are frequent among the elderly and carry significant morbidity and mortality representing a clinical and economical burden. From this analysis of the literature appears that ECM alterations are important physiopathological substrates of age-related arrhythmias. Deepening knowledge on ECM age-associated alterations might be important in the development of novel therapeutical approaches in the widespread panorama of age-related disease.

Acknowledgements

This work was supported in part by MIUR-PRIN "Engineering physiologically and pathologically relevant organ models for the investigation of age related diseases" (grant # 2010J8RYS7). The authors declare no conflicts of interest.

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