Epicardial origin of cardiac arrhythmias: clinical evidences and pathophysiology

Corentin Chaumont, Nadine Suffee, Estelle Gandjbakhch, Elise Balse,
Frédéric Anselme, Stéphane N Hatem

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1   **Epicardial origin of cardiac arrhythmias: clinical evidences and pathophysiology**

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3   Corentin Chaumont[1,2], Nadine Suffee[3], Estelle Gandjbakhch[3], Elise Balse[3], Frédéric
4   Anselme[1,2]*, Stéphane N Hatem[3]*

5

6   [1]Cardiology Department, Rouen University Hospital, Rouen, France

7   [2] FHU REMOD-VHF, UNIROUEN, INSERM U1096, F76000, France

8   [3]INSERM UMRS1166, ICAN - Institute of CardioMetabolism and Nutrition, Sorbonne
9   University, Institute of Cardiology, Pitié-Salpêtrière Hospital, Paris, France
10
11 *Drs. Anselme and Hatem contributed equally to this article.

12

13 **Correspondence:**

14 Stephane N Hatem

15 UMR_S1166, Faculté de médicine

16 91, boulevard de l'hôpital

17 75013 Paris, France

18 Fax: +33 1 40 77 96 49

19 Telephone: +33 1 40 77 95 84

20 stephane.hatem@sorbonne-universite.fr

21

1 **ABSTRACT**

2 Recent developments in imaging, mapping and ablation techniques have shown that the

3 epicardial region of the heart is a key player in the occurrence of ventricular arrhythmic events

4 in several cardiac diseases such as Brugada syndrome, arrhythmogenic cardiomyopathy or

5 dilated cardiomyopathy. At the atrial level as well, the epicardial region has emerged as an

6 important determinant of the substrate of atrial fibrillation, pointing to common underlying

7 pathophysiological mechanisms. Alteration in the gradient of repolarization between

8 myocardial layers favoring the occurrence of re-entry circuits has largely been described. The

9 fibro-fatty infiltration of the subepicardium is another shared substrate between ventricular and

10 atrial arrhythmias. Recent data have emphasized the role of the epicardial reactivation in the

11 formation of this arrhythmogenic substrate. There are new evidences supporting this structural

12 remodeling process to be regulated by the recruitment of epicardial progenitor cells that can

13 differentiate into adipocytes or fibroblasts under various stimuli. In addition, immune-

14 inflammatory processes can also contribute to fibrosis of the subepicardial layer. A better

15 understanding of such "electrical fragility" of the epicardial area will open perspectives for

16 novel biomarkers and therapeutic strategies. In this review article, a pathophysiological scheme

17 of epicardial-driven arrhythmias will be proposed.

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1   **I. INTRODUCTION**

2   Recent developments in imaging, mapping and ablation techniques have shown that the

3   epicardial region of the heart is a key player in the occurrence of ventricular arrhythmic events

4   in several cardiac diseases such as Brugada syndrome[1], arrhythmogenic cardiomyopathy

5   (ACM)[2] or dilated cardiomyopathy.[3] At the atrial level as well, the epicardial region has

6   emerged as an important determinant of the substrate of atrial fibrillation (AF), the most

7   frequent cardiac arrhythmia in clinical practice. Taken together, these observations raise

8   questions whether distinct mechanisms underlie the "tissue fragility" of the epicardial region

9   and whether they could provide new therapeutic targets for cardiac arrhythmias. The present

10 article will describe specific features of the epicardial region that can contribute to activation

11 of arrhythmogenic processes, it will review evidences for an epicardial origin of cardiac

12 arrhythmias, and discuss the role of epicardial reactivation in the formation of the

13 arrhythmogenic substrate.

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15 **II. DEFINITIONS AND SPECIAL FEATURES OF THE EPICARDIAL REGION**

16   **1. <u>Anatomy and histology</u>**

17 The epicardial area is delineated by the **epicardium,** the outer mesothelial layer of the heart.

18 The epicardium contains multipotent progenitors that can undergo epithelial-to-mesenchymal

19 transition (EMT), migrate into the subepicardium and, during cardiac ontogeny, give rise to

20 multipotent mesenchymal epicardium-derived cells (EPDCs).[4] At that point, the EPDCs can

21 differentiate into smooth muscle cells, coronary vessels[5] or myocardial fibroblasts[6] and, less

22 importantly, into coronary endothelial cells[7] and cardiomyocytes.[8] The epicardium has also

23 important signalling functions and exchanges paracrine factors with the neighbouring

1 myocardium. Quiescent in the healthy adult heart, the epicardium can be reactivated, becoming

2 a source of myofibroblasts and of growth and angiogenic factors.[9]

3 The **subepicardium** rapidly increases in volume from embryonic day 6 to day 11 during

4 mesenchymal cell invasion. It is mainly composed of connective tissue, located between the

5 epicardium and the myocardium with a predominance of collagen I and collagen III compared

6 to the rest of the myocardium.[10] It contains also mesenchymal cells such as smooth muscle and

7 Cajal-like cells together with lymphocytes, mast cells, macrophages, fibroblasts, nerves and

8 capillary (Figure 1). Myocardial trabeculations are present within the subepicardium, yet with

9 a distinct spatial organization well visualized by clinical imaging techniques and characterized

10 by a transmural orientation and a rotation of 120° on the axis of myocytes from epicardium to

11 endocardium.[11,12]

12 The adipose tissue localized between the myocardium and the visceral pericardium,

13 referred to as the **epicardial adipose tissue** (EAT), can be considered as another component of

14 the epicardial area. Firstly, EAT is histologically tightly associated with the epicardium and the

15 neighbouring myocardium that it can infiltrate.[13] Secondly, epicardial progenitors are the source

16 of adipocytes that compose EAT.[14] Finally, EAT differs from paracardiac adipose tissue located

17 at the outer surface of the fibrous pericardium by its embryologic origin, its biological

18 properties and its vascularization from coronary arteries. However, the difficulty in

19 distinguishing these two types of cardiac adipose tissue using clinical imaging techniques

20 explains that they are often confused in studies. There is no barrier between EAT and the

21 subepicardial myocardium such that peptides and adipokines can freely diffuse between the two

22 tissues. For instance, EAT is an important source of free fatty acids used for myocardial

23 energetic metabolism; it releases twice as much fatty acid as pericardial depots and also protects

24 the heart against toxic levels of fatty acids.[13] EAT is a brown adipose tissue, expressing the

25 uncoupling protein-1 located at the mitochondria inner membrane, a protein regulating heat

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1   production, permitting EAT to protect the heart against hypothermia.[13] Moreover, EAT

2   regulates myocardial oxidative stress by releasing adiponectine that inhibits nicotinamide

3   adenine dinucleotide phosphate oxidase activity.[15]

4   **2. <u>Special electrical features of the epicardial region</u>**

5   The presence of distinct electrophysiological properties of the subepicardial myocardium also

6   call for the individualization of this cardiac region. A repolarization gradient exists between the

7   subepicardial and subendocardial myocardium in the human heart as well as in the hearts of

8   other animal species.[16–18] This gradient prevents retrograde depolarization of subendocardial

9   layers by operating as a secure lock against the occurrence of re-entry circuits.

10   This repolarization gradient is due to the distinct electrical properties of cardiomyoyctes

11   between myocardial layers. Action potential (AP) of subepicardial cardiomyocytes is of shorter

12   duration compared to other cardiomyocytes[19,20], having a more pronounced rate dependency

13   indicated by the persistent suppression of the plateau phase with premature beats, *i.e.* AP

14   duration shortening with decreasing S1S2 interval between S1 steady-state pacing and

15   premature S2.[16,18] Moreover, upon increased extracellular $[K+]_o$, AP shortening is predominant

16   in subepicardial layers resulting in tall positive T waves whereas upon decreased $[K+]_o$,

17   epicardial AP lengthens and T waves flatten.[19]

18   At the cellular level, the repolarization gradient is generated by a regional difference of

19   the fast component of the voltage-dependent outward current, I_{to}, of much higher density in

20   subepicardial than subendocardial layers.[18] This current governs the early repolarization phase[18]

21   of the AP, the notch, and tunes the duration of the plateau phase to heart rate.[21] It is the

22   functional expression of *shaker* voltage-gated potassium Kv 4.2 and Kv 4.3 channels depending

23   on species.[22,23] In human and large mammal hearts, the molecular basis for transmural

24   difference of I_{to} is the expression gradient of gene encoding for Kv4.x channels and for

25   KCHiP2, a β-subunit that chaperones ionic channels at the plasma membrane.[24–29] The small

1 density of delayed potassium currents, Iks and Ikr in M cells, too contributes to the electrical

2 gradient.[30]

3   Other mechanisms underlying epicardial electrical heterogeneity are the left-handed

4 helix to a right-handed[31] arrangement of muscle fibres and the organization of the laminar

5 structure that drives ionic current flow.[32] Whereas in subendocardial and midwall myocardium,

6 myocytes are grouped together by perimysial collagen into branching layers called

7 myolaminae[33], in the subepicardium, perimysial collagen is present only as longitudinal cords.[34]

8   The fiber direction of the right ventricle appears to change suddenly at the level of the

9 subepicardium[35] which may make this area prone to conduction delay and block.

10 Additionally, the embryologic origin of the subepicardium in the right ventricular outflow tract

11 appears to differ from other sites which makes this area prone to conduction slowing in mice.[36]

12 Finally, fatty infiltration in the right ventricular subepicardium is more outspoken than at other

13 sites even in normal subjects.[37]

14

III. CLINICAL EVIDENCE FOR EPICARDIAL DRIVEN ARRHYTHMIAS

1. Arrhythmogenic cardiomyopathy

17 Arrhythmogenic cardiomyopathy (ACM) classically occurs in young patients by 12-lead ECG

18 QRS-T abnormalities and ventricular arrhythmias. Fibro-fatty replacement progresses from the

19 epicardium to the endocardium and leads to areas of slow conduction that constitute the main

20 substrate for reentrant ventricular tachycardia (VT) in ACM.[38] Other arrhythmia mechanisms

21 such as focal epicardial activities have also been described in ACM patients.[39] The epicardial

22 origin of ventricular arrhythmias (VA) associated with ACM has been particularly highlighted

23 by ablation procedures, showing the epicardial predominance of low voltage areas and

24 abnormal electrograms such as fractioned or late potentials, when endocardium may be normal,

25 especially in the earlier stages of the disease.[2] Interestingly, endocardial unipolar low-voltage

6

zones (< 5.5 mV) were shown to predict epicardial substrate in patients with ACM, with respect to size and location.[40,41] This predictive value of endocardial unipolar mapping was also confirmed in a recent study in which RV epicardial scar region significantly correlated with endocardial unipolar low-voltage zones (< 5.5 mV).[42]

Noninvasive epicardial mapping with Electrocardiographic Imaging (ECGI) is also suitable for mapping electrophysiologic substrate on the epicardial surface. Andrews *et al*.[43] performed ECGI and late gadolinium enhancement cardiac magnetic resonance in 20 ACM patients. Compared with controls, ACM patients had significantly longer ventricular activation duration and prolonged mean epicardial activation-recovery intervals. ECGI also showed varied epicardial activation breakthrough locations and regions of nonuniform conduction and fractionated electrograms that colocalized with late gadolinium enhancement scar.

Arrhythmic substrate cannot always be targeted by endocardial ablation, especially as layered and confined epicardial circuits of ventricular tachycardia were found in ACM patients.[44] Given the high rate of VT recurrence after endocardial ablation[45], epicardial ablation was widely tested in ACM patients.[46] Several studies have shown the superiority of a combined endo/epicardial approach to endocardial-only ablation.[47] In a study comparing endocardial-alone ablation to endo-epicardial ablation, freedom from ventricular arrhythmias was observed at 3-year follow-up in 85% of the patients who benefitted from the combined approach and only 53% of the patients with endocardial-alone ablation.[48] Berruezo et al.[49] evaluated a combined endo-epicardial VT ablation approach associated with conducting channel elimination (scar dechanneling) ; freedom from VT recurrence was obtained in 90% of ACM patients.

2. **Brugada syndrome**

The other cardiac arrhythmia with a well-established epicardial substrate is Brugada syndrome (BrS). In a systematic review, Fernandes *et al*[50] showed that BrS patients undergoing both epicardial and endocardial mapping had exclusive epicardial substrate in 93% of cases.

Simultaneous noninvasive epicardial and endocardial mapping further establishes the epicardial predominance of electrical abnormalities.[51] Noninvasive epicardial mapping with Electrocardiographic Imaging was also conducted in 25 BrS patients and 6 patients with right bundle-branch block (RBBB) for comparison. Unlike patients with RBBB, BrS patients had delayed activation localized to the right ventricular outflow tract, and fractionation, or repolarization abnormalities on RVOT electrograms.[52]

Accordingly, targeting of epicardial rather than endocardial substrate appeared much more effective to prevent VT/VF in BrS. Brugada *et al.*[53] performed right ventricular epicardial mapping of patients with BrS and identifed low-voltage (<1.5 mV) and abnormal electrograms areas with abnormally prolonged fragmented epicardial potentials on the anterior right free wall and RVOT. Radiofrequency ablation of this area resulted in the suppression of the BrS ECG pattern and suppressed VT/VF inducibility. Data on 135 patients with BrS showed that elimination of abnormal epicardial electrograms led to a normalisation of the BrS ECG pattern in all but two patients.[1] In another study in which 28 symptomatic BrS patients underwent RV epicardial mapping, an anterior RVOT abnormal epicardial substrate was found in all patients.[54] Abnormal electrograms covered the entire RVOT epicardium and extended to the RV body in more than half of the patients (53%). The chosen endpoint was to eliminate all abnormal electrograms detected during epicardial mapping after the administration of a sodium channel blocker. Only 3/28 patients had recurrent VF episodes and required reablation.[54]

3. Scar related cardiomyopathy

Following the first description of the technique in 1996[55], transthoracic epicardial catheter ablation was mainly used to treat patients with Chagas disease[55] or with VT related to inferior myocardial infarction.[56] The technique and its indication have expanded over recent decades. In a multicentre study including all patients undergoing VT ablation, epicardial mapping and/or ablation was required in 17% of patients and 35% of those presented with non ischemic

1 cardiomyopathy (NICM).[3] Lateral subepicardial and anteroseptal intramural arrhythmogenic

2 substrate are very common in NICM, related to presence of interstitial fibrosis that is usually

3 more diffuse and patchy than in ICM.[57] In a series of 22 patients with NICM who failed prior

4 endocardial ablation and/or had ECG suggesting an epicardial origin, Cano *et al.*[58] found larger

5 low voltage and dense scar areas within the epicardium compared to the endocardium. Almost

6 half of the patients, showed an abnormal epicardial voltage map in contrast with a normal

7 endocardial map. Despite the expansion of epicardial ablation, long-term outcomes after VT

8 ablation in NICM remain poor when compared to ICM, possibly due to the diffuse and deep

9 location of arrhythmic substrate. In the HELP-VT registry study, VT free-survival at 1-year

10 follow-up was 40.5% in patients with NICM and 57% in patients with ICM, even though

11 ablation procedures were performed with an epicardial access when necessary.[59] These data

12 reflect the highly complex substrate for VT in NICM and the further progress that remains to

13 be made. Myocarditis related ventricular arrhythmia are also likely to be related to an epicardial

14 substrate, as suggested by Dello Russo *et al.*[60] In this case series of 20 patients with drug

15 refractory VT in the context of proven myocarditis, epicardial ablation was necessary in 30%

16 of patients.[60] These findings are consistent with contrast-enhanced cardiac magnetic resonance

17 (CMR) imaging of subepicardial distribution of late enhancement in patients with active

18 myocarditis.[61]

19 **4. Atrial fibrillation and epicardial region**

20 The thin atrial wall is a heterogeneous structure with electrical dissociation between

21 endocardium and epicardium and transmural muscle fibres connecting those distinct layers[62]

22 that can constitute the substrate for re-entries. Indeed, high-density mapping of long standing

23 persistent atrial fibrillation (AF) performed during cardiac surgery[63,64] has recorded epicardial-

24 endocardial breakthroughs (EEB), a source of "focal" fibrillation waves favouring AF

25 persistence, that originate from the epicardial surface. Furthermore, during the progression from

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1   paroxysmal to persistent AF, secondary to fibro-fatty infiltration, endo-epi dissociation[65] and

2   low voltage areas have been shown to increase. After the restoration of sinus rhythm, EEB can

3   still be recorded with epicardial-endocardial asynchrony and the muscular connections between

4   the endo-epicardial layers clearly indicating the presence of an epicardial substrate.[66] In this

5   line, Pak *et al.*[67] showed that epicardial catheter ablation with a pericardial approach was

6   effective in patients with redo-AF ablation procedure at risk for PV stenosis. Another option is

7   the hybrid AF ablation combining minimally invasive surgery and percutaneous

8   electrophysiology study.[68] In a meta-analysis of 563 patients, long-term success rate (sinus

9   rhythm after a mean of 26 months) after hybrid ablation ranged from 60% to 87%.[69]

10   ## 5. <u>Value and limitations of the different epicardial mapping techniques</u>

11   Contact electro-anatomical mapping systems have emerged as essential tools for precise

12   mapping and ablation of cardiac arrhythmias. These magnetic and or impedance-based systems

13   use dedicated mapping catheters that are introduced within the cardiac chamber of interest or

14   the pericardial space for electrophysiological analysis. Once in place, they are able to determine

15   the mechanism and delineate the site of origin of the arrhythmia with a precision of a few

16   millimeters. As discussed above, ventricular endocardial unipolar low-voltage electrograms

17   were shown to predict epicardial substrate location, notably in ACM[40] and nonischemic left

18   ventricular cardiomyopathy.[41] Ventricular epicardial contact mapping using these technologies

19   also clearly demonstrated the epicardial origin of numbers arrhythmias in BrS, ACM and

20   NICM.

21   The inability for catheters to reach the targeted site in order to efficiently suppress the

22   arrhythmia is one of the limitations of this technology. This is particularly true for epicardial

23   substrate for which an epicardial access is required. Due to past medical (pericarditis) or

24   surgical (cardiac surgery) history, the virtual space between the 2 pericardium sheets could be

25   inaccessible via a transcutaneous puncture. Closely located coronary arteries to the site of

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1   interest or surrounding subepicardial adipose tissue may also limit the ability to successfully

2   suppress the arrhythmogenic substrate.

3   Finally, noninvasive epicardial mapping with Electrocardiographic Imaging (ECGI) is another

4   effective option for mapping electrophysiologic substrate on the epicardial surface. Despite

5   considerable recent development, validation of ECGI is still challenging[70] as some important

6   discrepancies have been found between ECGi and epicardial contact mapping which should

7   theoretically produce identical maps.

8

IV. FIBRO FATTY INFILTRATION AND EPICARDIAL ARRHYTHMIAS

10 Alteration of repolarization gradient and action potential heterogeneity between the

11 subepicardial and subendocardial myocardium are well established arrhythmogenic

12 mechanisms. Brugada syndrome is an archetypal example with two main models, repolarization

13 and depolarization[71,72], with a key role of the epicardium layer in both of them. The fibro-fatty

14 infiltration of myocardial layer appears as another major shared pathophysiological mechanism

15 between several epicardial driven arrhythmias.

16 *a- Adipose tissue infiltration at the ventricular level : the paradigm of ACM.*

17 Both post-mortem studies and surgical ablation procedures have revealed the presence of adipose

18 depots in the right ventricle of ACM patients with a progressive replacement of myocardium by

19 fibro-fatty infiltrates and the progressive loss of right ventricle (RV) muscle fibres[73] starting from

20 the subepicardium and then progressively extending to the subendocardium thereby become

21 transmural (Figure 2).[73,74] Clusters of mononuclear cells in the fatty infiltrates corresponding to

22 immune cells have been observed in fibro-fatty infiltrates leading to the notion of lymphocytic

23 myocarditis[75] further supported by the recent observation of clinical myocarditis in patients with

24 ACM.[76]

1   Some degree of fibro-fatty remodelling has also been observed in the subepicardial layer

2   of the right ventricle outflow track in BrS patients. In a post-mortem study, RVOT histological

3   sections of BrS patients showed an increased epicardial surface collagen that was thicker than

4   that in control hearts. This epicardial fibrosis infiltrated into the underlying epicardial

5   myocardium, admixed with fat. BrS cases also had reduced Cx43 expression in the RVOT when

6   compared with controls.[77]Ohkubo et al.[78] also performed endomyocardial biopsy in 25 patients

7   with BrS. Moderate to severe fatty infiltration was observed in 5 patients and significant fibrosis

8   infiltration in 4. Noteworthy, right ventricular cardiomyopathy with fibro-fatty replacement was

9   observed in patients with Brugada like-ST segment elevation.[79] This histological feature

10   together with the delayed activation of the RVOT points to a phenotypic overlap between ACM

11   and BrS.[80,81]

12   *b- Fibro-fatty infiltration of the atrial myocardium and the substrate of AF*

13   Strikingly similar fibro-fatty remodelling of the subepicardium resembling the histology of the

14   ACM heart has been described in the atria of patients suffering from AF (Figure 2).[82,83] In

15   healthy adult atria, the epicardium is mainly a cell monolayer with thin, extracellular matrix in

16   contact with the myocardium or adipose tissue. However, with ageing or in patients with

17   hypertension[84], mitral valve diseases[85] or AF, the epicardium can become thick and adipose

18   tissue fibrotic, resulting in fibro-fatty infiltrates.[83] In a model of persistent AF in sheep, the

19   degree of fibrosis of the subepicardial adipose tissue follows the progression of AF from

20   paroxysmal to permanent.[83] As in ventricle, clusters of inflammatory cells can be observed at

21   the epicardial site in the transition zone between adipocytes and fibrosis in both human and

22   sheep atria. Immuno-histochemistry analysis of these cell clusters revealed a predominance of

23   CD3+ T lymphocytes, with the vast majority of them CD8+ cytotoxic T cells, displaying

24   functional cytotoxic activity through granzyme B against adipocytes.[83] Indeed, such an immune

25   component mediated by CD8+ cytotoxic T cells has been described for other visceral adipose

1 tissues notably in obese patients and is considered as a major mechanism underlying the fibrosis

2 of adipose tissue.[86]

3 *c- Evidences for the arrhythmogenicity of fibro-fatty infiltration of myocardial layers*

4 By comparing histology with electrical mapping of the right ventricular outflow tract, it has

5 been possible to establish a relationship between arrhythmogenicity and the different

6 distributions of subepicardial fibro-fatty infiltrations. For instance, 80 consecutive ACM

7 patients in sinus rhythm were classified into three groups according to the type of fibro-fatty

8 infiltration, referred to in this study as scar gradient (<10%: transmural, 10-20%: intermediate,

9 >20%: horizontal).[42] Patients with horizontal scars experienced significantly more syncope,

10 sustained VT and fatal VA when compared to patients with transmural and intermediate scar

11 gradient, independently of the right ventricular volume. Horizontal extension of epicardial scars

12 was an independent predictor of life-threatening VA whereas patients with transmural scars had

13 a greater number of clinical and EP induced PVCs that was not correlated with fatal VA.[42] A

14 heterogeneous distribution of scars between subepicardial and subendocardial layers was well

15 described in ACM patients, with a predominance of scar area and late abnormal ventricular

16 potentials within the subepicardium layer compared to the endocardium.[2,44] Moreover, the

17 dense fibrotic infiltrates, characteristic of ACM, may electrically isolate the epicardium from

18 the endocardium. When studying the transmural right ventricular activation pattern, it was

19 observed that the epicardium is activated with a major delay in ACM patients without direct

20 transmural spread from the endocardium but with a laminar activation pattern from the border

21 to the central scar favouring re-entry circuits.[44]

22 Pieroni et al.[87] described the relationship between electroanatomic abnormalities and

23 pathological substrate in BrS patients. 3-dimensional electroanatomic mapping-guided RVOT

24 biopsies were performed in 20 patients and histopathological abnormalities including fibrosis

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1   were found in 15 of them and could provide an explanation for the areas of low voltage recorded

2   in those patients.

3   In a goat model of AF, EEB incidence and degree of endo-epicardial dissociation

4   increased with increasing AF substrate complexity.[65] EED and breakthroughs also correlated

5   with the degree of epicardial fibrosis[88] as evidenced using a 3D computational model of human

6   atria integrating MR images and histo-anatomical data. Slow electrical conduction was recorded

7   in the region of the human right atrial myocardium infiltrated by fibro-fatty tissue and has been

8   attributed to the deleterious effect of adipose tissue on normal myocyte-myocyte coupling.[89]

9   Several mechanisms can underlie the arrhythmogenicity of the fibro-fatty infiltration of

10   subepicardial myocardium.[90] First, by infiltrating subepicardial myocardial layers, adipocytes

11   and fibrosis disrupt normal myocyte-myocyte coupling leading to local conduction slowing /

12   block and to the formation of myocardial area of low voltage.[89] These altered conduction

13   properties pave the way for reentry circuits within this remodelled area (Graphical Abstract).

14   The adipose tissue can also secrete a myriad of cytokines and adipokines such as leptine,

15   but also small extracellular vesicles[91] that can regulate directly the cardiac electrical properties[92]

16   or indirectly, for instance, by modulating the oxidative stress of the myocardium.[93] Finally, the

17   altered electrical properties caused by fibro-fatty infiltrates subepicardial myocardial layers

18   could disturb the normal endo-epi repolarization gradient.

19   However, the difficulty to reproduce experimentally the precise composition of cardiac

20   tissue with controlled texture of fibrosis and adipose tissues explains the lack of direct evidences

21   for the arrhythmogenicity of fibro-fatty infiltration. To overcome this limitation, computational

22   model of myocardial tissue have been used providing arguments for reduction in conduction

23   velocity, enhanced spiral wave periodicity and increased break-up with the degree of fibro fatty

24   infiltration.[90]

1 **V. EPICARDIAL REACTIVATION AND FIBRO-FATTY INFILTRATION OF THE**

2 **SUBEPICARDIUM**

3 Several explanations have been proposed for the apparent replacement of myocardium by

4 adipose tissue notably in the context of ACM. This includes transdifferentiation of

5 cardiomyocytes[94], proliferation and differentiation of cardiac progenitors[95], or a mesenchymal

6 origin.[96] For instance, in explanted ACM hearts, mesenchymal stromal cells were found to

7 contribute to fibro-fatty infiltrates suggesting an epicardial EMT origin.[96] The link between

8 genetic defects underlying ACM and fibro-fatty remodelling of right ventricle myocardium is

9 still actively investigated and the description of "the connexome" had provided some clues.

10 Connexome is a network of proteins localized at the intercalated disks (ID) that includes

11 desmosome proteins, gap junction and ionic channels. It plays a crucial role in the normal

12 electrical and mechanical coupling between myocytes and also in the maintenance of

13 differentiated myocardium through various signalling pathways that depend on the integrity of

14 myocyte-myocyte contacts including Wnt/β-catenin or Hippo-Yap pathways. It is to be noted

15 that the Hippo-Yap pathway was reported to be activated during ACM as the result of changes

16 of the expression of several ID proteins.[97]

17 *a-Epicardial reactivation and fibro fatty infiltration of the atrial myocardium*

18 The study of the origin of EAT and of fibro-fatty infiltrates in the atria has provided strong

19 evidence for the role played by the epicardium in this remodelling process (Graphical Abstract).

20 Firstly, cells expressing markers of an epicardial origin such as Wilm's tumor (Wt-1) and Tbx18

21 have been detected in the fibro- fatty infiltrates of subepicardium of human atria. Secondly,

22 using a genetic lineage tracing as Wt-1-$^{CreERT2+/-}$Rosa$^{tdT+/-}$ mouse model, it has been possible to

23 track EPDCs in situ and to show their migration into the subepicardium and their differentiation

24 into adipocytes or fibroblasts during various atrial remodelling.[14,98] Single cell RNA-

25 sequencing analysis revealed the heterogeneity of atrial epicardial cells with at least 8 clusters.[98]

1 The analysis of inference trajectory of atrial EPDCs identifies, at least, two differentiation

2 pathways, one towards adipocyte and another towards myofibroblast with an apparent

3 progression from fat accumulation to fibrosis. Atrial natriuretic peptide (ANP) and angiotensin-

4 II (Ang-II) have been shown to be important regulators of the epicardial reactivation and to

5 control the signalling pathways that regulate differentiation lineages of atrial EPDCs

6 (aEPDCs).[14,98] They operate as a switch to induce differentiation of aEPDCs into fibroblasts or

7 adipocytes, respectively. For instance, the atrial natriuretic peptide, at low concentration,

8 activates cGMP-dependent protein kinase (PKG) that regulates the expression of transcription

9 factors C/EBPα and PPARγ.[14]

10 These peptides are locally secreted explaining why areas with a thin epicardial layer co-

11 exist with thick and fibrotic epicardial areas.[98] This could contribute to focal structural

12 reactivation of the epicardium and accumulation of fat or fibrosis. Finally, both Ang-II and

13 ANP up-regulated their own receptors indicating a positive feedback of the agonists on their

14 signalling pathways to secure a long-lasting response. Numerous factors can trigger epicardial

15 reactivation and EMT such as transforming growth factor-β, fibrobast growth factors, platelet

16 derived growth factors, Notch, retinoic acid or thymosin-beta-4 (Graphical Abstract).[100,101]

17 *b- Are their evidences for reactivation of the epicardium at the ventricle level?*

18 An epicardial reactivation has been also reported at the ventricle level in adult heart. For

19 instance, following acute myocardial infarction an intense reactivation of the epicardium with

20 recruitment of progenitor cells contributes to the scar formation and to the fibrotic remodeling

21 of the ventricle myocardium. In this line, the inhibition of embryonic Wt1-Cre lineage

22 mobilization is associated with the reduction in the number of epicardium-derived fibroblasts

23 colonizing the post-myocardial injury scar.[101]

24 Epicardial progenitor cells are also a source of adipocytes that compose the fat depot of

25 atrio-ventricular groove. Furthermore, depending on the activation PPARγ signalling pathway,

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1   epicardial progenitors can differentiate into adipocytes and colonize too the post-myocardial

2   infarction scar.[102] An epicardial reactivation due to local mechanical stretch is likely to explain

3   fibro-fatty scars development in NICM patients and should be investigated.

4   *c-Role of desmosome proteins in the differentiation of cardiac progenitors into adipocytes*

5   Several evidences indicate a role of desmosomal proteins in fatty infiltration of the venticule

6   myocardium. First, desmosome proteins are present in fibro-adipogenic progenitors whereas

7   some mutations of desmosomal proteins favour their differentiation into adipocytes.[103] Second,

8   in a population of non-excitable cardiac-resident cells, plakophiline has been shown to regulate

9   intracellular lipid accumuation. Finally, epicardial progenitor cells derived from iPS cells,

10   generated from skin fibroblasts obtained in patients suffering from ACM and harboring

11   Plakophilin-2 mutations, spontaneously differentiated into adipocytes. The activating enhancer

12   binding protein 2 alpha (AP2α) could be the transcription factor involved in the adipogenic

13   differentiation of EPDCs.[104] Further studies using epicardial progenitor lineage models

14   harboring desmosomal protein gene mutations are necessary to establish firmly the role played

15   by epicardial reactivation in the ventricle subepicardial remodeling during ACM.

16   Despite solid arguments for an epicardial origin of fibro-fatty infiltration of myocardium

17   and several evidences for the arrhythmogenicity of such fibro-fatty infiltrates, a direct causal

18   role of epicardial reactivation in the occurrence of cardiac arrhythmias remains to be

19   established. Furthermore, there is no evidence for a reactivation of the epicardium during BrS.

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VI. CONCLUSION

Progress in the mapping of cardiac arrhythmias, notably ventricular tachycardia, has revealed the crucial role played by the epicardial region in the activation of arrhythmogenic mechanisms. This has led to important practical implications with the development of noninvasive and percutaneous catheter based epicardial mapping and ablation procedures. It has also given rise to intense research activity to better understand why the epicardial region can be a source of arrhythmias. It turns out that this region is characterised by distinct electrical and histological characteritics that under some circumstances can become a risk factor for arrhythmias. For instance, the epicardial origin of adipose tissue explains likely the propensity of the infiltration of subepicardial myocardial layers by fibro-fatty tissue as observed during several epicardial-driven arrhythmias and that has been shown to be potentially arrhythmogenic. In this line, the reactivation of the epicardium and the capacity of progenitor cells to differentiate into adipocytes or fibroblasts could be an early event in the pathophysiology of certain cardiac arrhythmias. Promising anti-arrhythmic strategies emerge from these novel pathological insights , one of them could target the accumulation of epicardial adipose tissue and its replacement by fibrosis. Progress will come also from the development of biological and imaging biomarkers that could detect the electrical fragility of the epicardial region.

1 **DISCLOSURE**

2 Drs Chaumont, Suffee, Gandjbakhch, Balse, Hatem: none. Dr Anselme is consultant for and

3 has received lecture fees from Boston Scientific, Medtronic, Microport CRM.

4

5 **LIST OF ABBREVIATIONS**

6 AF : atrial fibrillation

7 APD : action potential duration

8 ACM : arrhythmogenic cardiomyopathy

9 BRs : Brugada Syndrome

10 EAT : epicardial adipose tissue

11 EEB : epicardial-endocardial breakthroughs

12 EMT : epithelial-to-mesenchymal transition

13 EPDCs : epicardial derived cells

14 EP : electrophysiological

15 ID : intercalated disks

16 MMPs : Matrix metalloproteinases

17 NICM : non ischemic cardiomyopathy

18 RV : right ventricule

19 RVOT : right ventricular outflow tract

20 VT : ventricular tachycardias

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1   **FIGURE LEGENDS**

2   **Figure 1. Histology and structural organization of the epicardial area.** **(A)** Schematic

3   representation of a cross section of left atrium showing the interaction between epicardium,

4   adipose tissue and myocardium with distinct orientations of fibers between layers (cross section

5   vs longitudinal myocytes). **(B)** Masson's trichrome staining of human atrial tissue (1) showing

6   the epicardium in blue, myocardium in red and subepicardial adipose tissue in white (Scale bar,

7   100 µm). At high magnification, immunofluorescence staining of human atrial section reveals

8   the presence of adipocytes expressing perilipin-1 (2) and progenitor cells expressing c-Kit (3)

9   and WT-1 (4) in both epicardium and subepicardium (Scale bars, 10 µm), from Suffee *et al.*[14]

10

11   **Figure 2. Fibro-fatty infiltration of subepicardial layers of ventricle and atrial**

12   **myocardium.** **(A)** Hematoxylin–phloxin–safran staining of the right ventricle free wall of a

13   patient with arrhythmogenic cardiomyopathy showing a large amount of adipose tissue

14   occupying subepicardial layers (1, ×10) and isolated strands of myocardium bordered by or

15   embedded in fibrous tissue (2, ×100), from Mallat Z *et al.* [105]   **(B)** Red sirius staining of a

16   human right atrial section showing an example of non-fibrotic remodelled epicardium (1) either

17   without subepicardial adipose tissue or with subepicardial adipose tissue and (2) fibrotic

18   remodelled epicardium with various degree of fibro-fatty infiltration, from Haemers *et al.* [83]

19

20   **Graphical abstract.** **From the reactivation of the epicardium to the formation of an**

21   **arrhythmogenic substrate.** Transition from a quiescent epicardium (panel A) to epicardial

22   reactivation followed by fibro-fatty infiltrations of subepicardial myocardial layers (panel B).

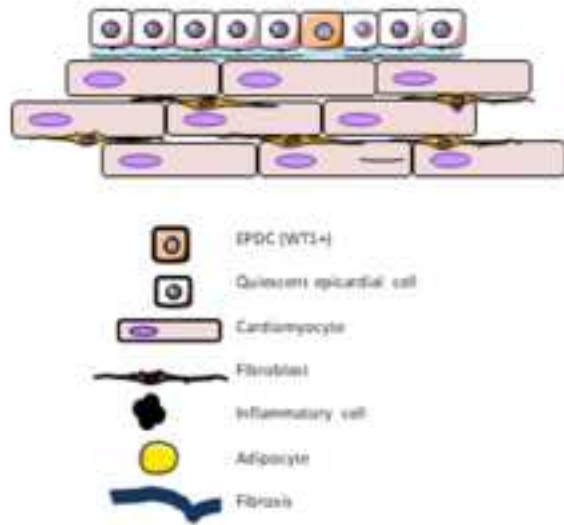23   Potential arrhythmogenic mechanisms (panel C) include i- at the tissue level, conduction

1   slowing or block and low voltage area favoring formation of electrical reentry circuit within the

2   myocardial wall and ii- at the cellular level, altered myocyte coupling and adipokine- and

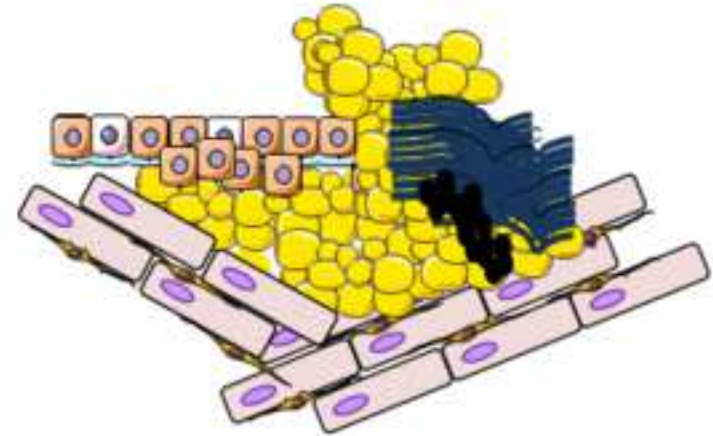3   cytokine- induced abnormal excitation contraction coupling and oxidative stress.

A Schematic representation of left atria

B

A

1　　2

B

1　　2

Graphical Abstract

(A) QUIESCENT EPICARDIAL AREA
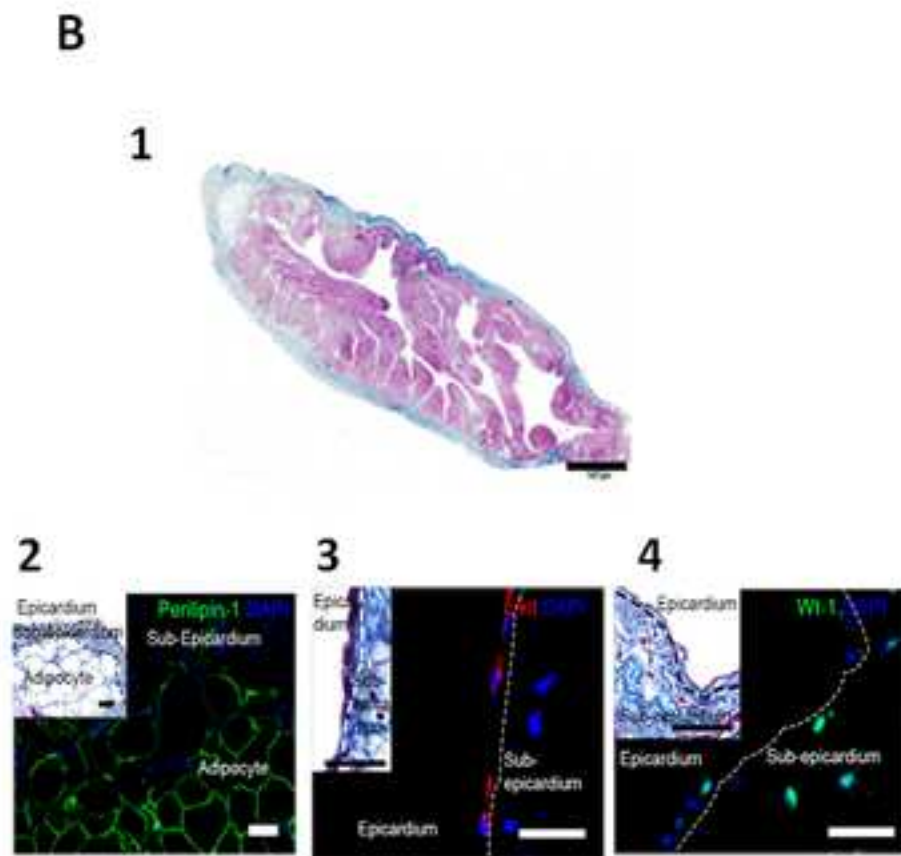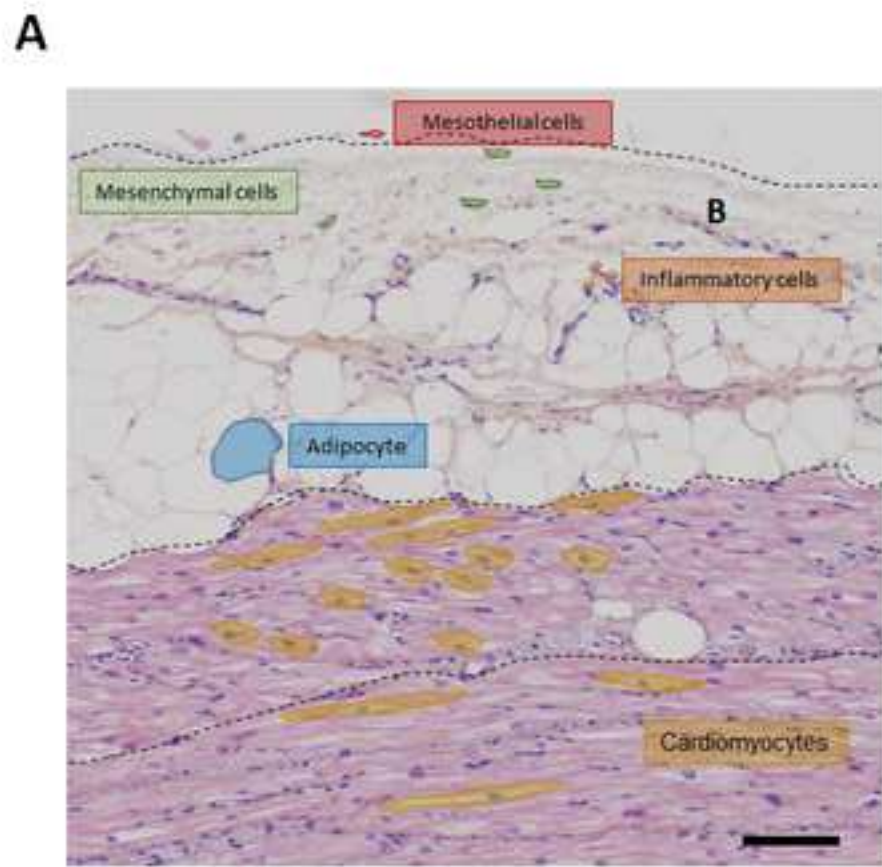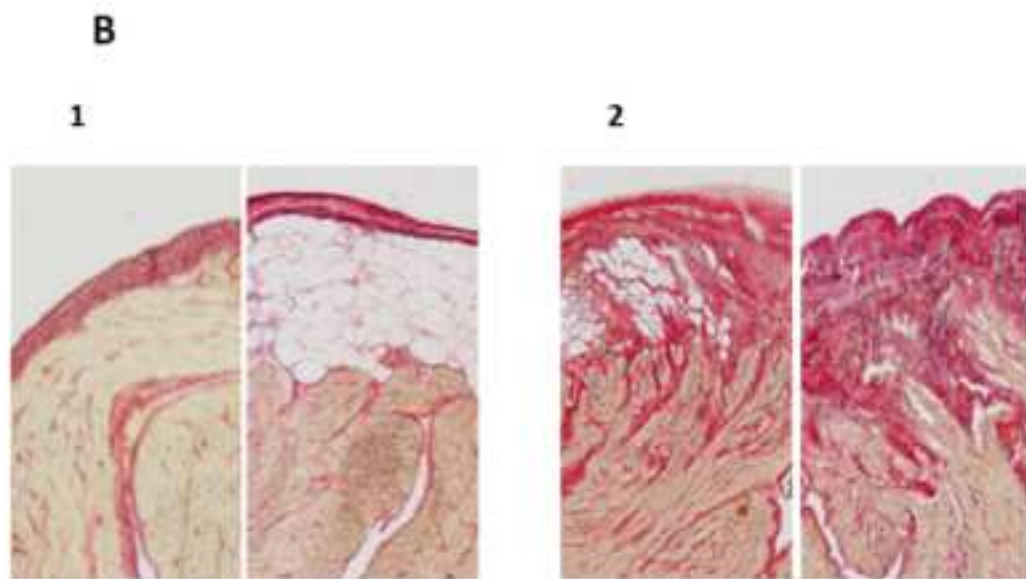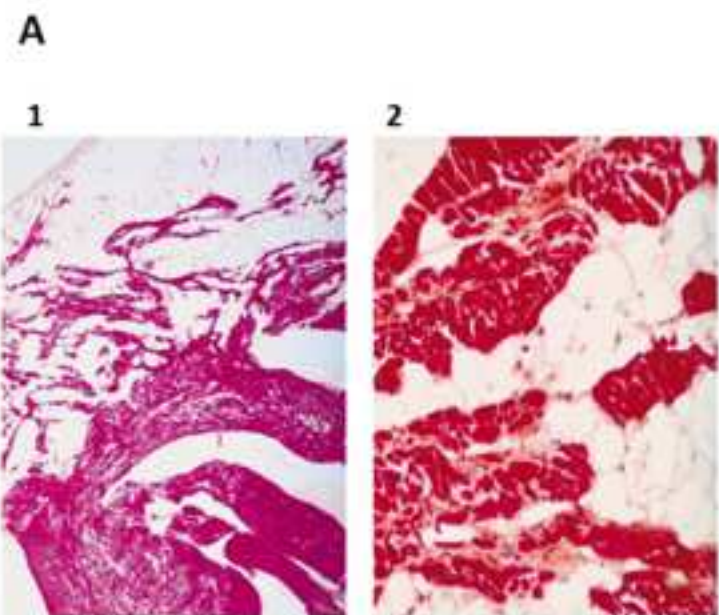
(B) FIBRO-FATTY REMODELLING OF THE EPICARDIAL AREA

REACTIVATION

- TGF-β, bFGF, PDGF, HGF, IGF, HIF-1α, Thymosin β-4
- Abnormal cell-cell contacts
- Mechanical stretch

EPDC (WT1+)

Quiescent epicardial cell

Cardiomyocyte

Fibroblast

Inflammatory cell

Adipocyte

Fibrosis

(C) ARRHYTHMOGENIC MECHANISMS SECONDARY TO FIBRO-FATTY REMODELLING

Tissular level : reentrant arrhythmia secondary to fibro-fatty remodelling

Cellular level

Potential electrical phenomenon observed during mapping of remodelled myocardium

Slow conduction area

Macroreentrant circuit

Low voltage area
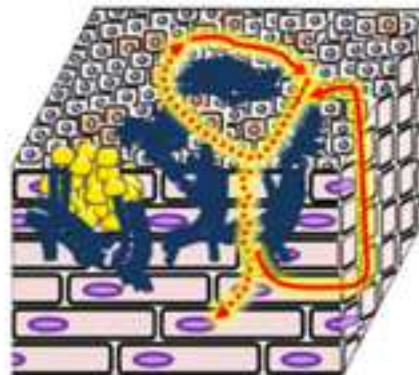
- Altered myocyte-myocyte electrical coupling
- Direct effect of adipokines and cytokines on ionic channels and Ca2+ signaling
- Oxidative stress