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► **To cite this version:**

Valérie Samouillan, Jany Dandurand, Colette Lacabanne, Andréa Stella, Mauro Gargiulo, et al.. Characterization of aneurysmal aortas by biochemical, thermal, and dielectric techniques. *Journal of Biomedical Materials Research Part A*, 2010, 95A (2), pp.611-619. 10.1002/jbm.a.32835 . hal-03474555

**HAL Id: hal-03474555**

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Submitted on 10 Dec 2021

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**To link to this article** : DOI :10.1002/jbm.a.32835

**URL** : <http://dx.doi.org/10.1002/jbm.a.32835>

**To cite this version :**

Samouillan, Valérie and Dandurand, Jany and Lacabanne, Colette and Stella, Andréa and Gargiulo, Mauro and Degani, Annamaria and Gandaglia, Alessandro and Spina, Michel (2010) *Characterization of aneurysmal aortas by biochemical, thermal, and dielectric techniques*. Journal of Biomedical Materials Research Part A, vol. 95A (n° 2). pp. 611-619. ISSN 1549-3296

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# Characterization of aneurysmal aortas by biochemical, thermal, and dielectric techniques

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**Abstract:** Abdominal aortic aneurysms (AAA) are characterized by structural alterations of the aortic wall resulting from the degradation of elastic fibres and an increase of collagen/elastin ratio. In this study we investigated the chain dynamics of AAA tissues by two techniques generally used for the characterization of polymers, Differential scanning calorimetry (DSC) and thermally stimulated currents (TSC), and we correlated the obtained data with biochemical analyses. The thermal denaturation of collagen observed by DSC allowed us to evaluate the thermal stability of the triple helix domain: notable modifications were evidenced between collagen from control tissue and collagen from AAA, particularly con-

cerning the thermal denaturation. The dielectric analysis of pathologic aortic walls by TSC revealed a relevant change of collagen mobility in AAA, with the occurrence of a specific mode of relaxation between  $-60$  and  $-40^{\circ}\text{C}$ . Biochemical, thermal, and dielectric results are compatible with increase of new collagen deposition and/or impairment of the collagen phase stability in the extracellular matrix of AAAs.

**Key Words:** collagen denaturation, relaxation mode, chain dynamics, thermal analysis, aneurysm

## INTRODUCTION

Atherosclerosis is a chronic inflammatory process with complications that are the leading cause of death in western societies. Extensive research has been done to determine the complex pathophysiology of atherosclerosis, although mechanisms for various aspects are still being elucidated. Atherosclerosis is preceded by arteriosclerosis, which can be defined as normal or early ageing of arterial wall, and whose most visible manifestations are a decrease of cross-linked elastin biosynthesis, a fragmentation of elastic fibres and a thickening of the intima.<sup>1</sup>

Accumulation of lipids such as cholesterol and cholesterol esters, which possess a strong affinity for elastin, constitutes one of the main causes of arteriosclerosis;<sup>2</sup> lipid absorption leads to a loss of elasticity and enhances elastase activity.<sup>3</sup> Moreover, calcium fixation on peculiar sequences of elastin, well known as a promoter for elastolysis<sup>4</sup> is favoured by lipids accumulation and vice-versa.<sup>5</sup> All these factors act for arteries ageing.

The advanced atherosclerotic lesion may be characterized by accumulation of extracellular lipids, development of a lipid-rich necrotic core, formation of a fibrous cap and calcification. Atheroma plaques can lead to aneurysms, that is, to a permanent dilatation of vascular lumen and development of fissures, exposing the patient to a double risk of

rupture and embolic migration.<sup>6</sup> Nevertheless, it must be pointed out that atherosclerosis and AAA are two different pathologic entities with different risk profiles.<sup>7,8</sup> Most patients with cardiovascular disease do not develop AAA, and many AAA patients do not have peripheral carotid or coronary disease.<sup>9</sup>

Aortic aneurysm, responsible for 2% of human deaths in industrialized nations, is preferentially located on the infrarenal aorta-iliac artery. This peculiar localisation could be due to the decrease of the elastin/collagen composition from the suprarenal aorta to the infrarenal aorta, which is associated with a thickening of intima and adventitia.<sup>10,11</sup> The pathogenesis of AAA remains uncertain, and the lack of definitive insight suggests a complex, multifactorial process.<sup>12</sup>

Eelectronic microscopy and NMR studies<sup>13,14</sup> confirm that elastic fibre is the primary site of degeneration within the arterial wall, but the role and quantification of the different constituents of the extracellular matrix in aneurysm are not yet elucidated. Abdominal aortic aneurysms are characterized by structural alterations of the aortic wall resulting from the degradation of collagen and elastin, and several studies suggest that aneurysm formation in patients with atherosclerosis is related to the degree of expression of matrix metalloproteinases (MMPs), particularly MMP-2<sup>15</sup>

**TABLE I. Description of the Pathologic Tissues Sampled from Nonspecific Abdominal Aortic Aneurysms**

Aneurysm	Tissue	Age	Sex	Label <sup>o</sup>
Nonspecific	Aortic wall	53	m	A1
Nonspecific, familial tendency	Aortic wall + atheroma plaque	72	m	A2
Nonspecific	Superior part of atheroma plaque	77	f	A3a
	Fragments of atheroma plaque			A3b
	Aortic wall supporting atheroma plaque			A3c
Nonspecific, polyaneurysm condition	Aortic wall + atheroma plaque	73	m	A4a
	Aortic wall + atheroma plaque			A4b
	Fragments of atheroma plaque			A4c

and MMP-9,<sup>16–18</sup> which show strong elastolytic activity. A high concentration of MMP-8 could represent a potent pathway for collagen degradation in aneurysm.<sup>19</sup> A mixture of matrix metalloproteinases and cysteine collagenases could lead to the degradation of the fibrillar collagens of adventitia and media in AAA, which are highly resistant to proteolysis.<sup>20</sup>

Literature data are divergent on the elastin and collagen composition in aneurysms: first studies reported a significant reduction in the elastin and collagen contents of aneurysms when compared with normal and atherosclerotic aortas,<sup>21</sup> while more recent works bring to the light an important decrease of the relative quantity of elastin.<sup>22</sup> This decrease could be explained by a dilution of the elastin fraction to other components, the absolute amount of elastin remaining the same,<sup>22</sup> or slightly greater.<sup>23</sup> It is noteworthy that most of studies do not evidence a loss of cross-links which would favour a degradation of mature elastin in abdominal aortic aneurysms.<sup>23,24</sup> Most of studies indicate an increase of collagen,<sup>12,22,23</sup> which is considered as the major component of atheroma plaque. However, the evolution of the ratio between type I collagen and type III collagen is variable according to different reports.<sup>25,26</sup>

Moreover, an alteration of mechanical properties of aneurysmal artery has been shown, and associated with modification in collagen organization and deposition.<sup>27</sup> As a matter of fact, mechanical properties depend not only on its quantity and biomechanical properties but also on the fibre architecture. Structural alterations of the adventitial collagen organization have been evidenced in aneurysmal wall (from a three-dimensional organization to a two-dimensional one<sup>12</sup>). Investigation of vascular changes and protein composition by spectrometry techniques have been initiated and led to substantial information on extracellular matrix proteins alterations in aneurysm.<sup>13,14,26</sup>

In this work, we have adapted classical techniques of characterization of synthetic polymers to pathological and unaffected cardiovascular tissues, to bring supplementary information on the possible evolution of collagen and elastin physical properties associated with the heterogeneity of abdominal aortic aneurysmal wall.

## EXPERIMENTAL SECTION

### Preparation of tissues for analysis

Specimens were collected from the anterior aspect of the infrarenal abdominal aorta during operation for nonspecific

abdominal aortic aneurysms (AAA) in 17 patients of both sexes, from 53 to 77 years of age following selection criteria and sampling procedure previously described.<sup>22</sup> Specimens from 10 patients were used for quantification and amino acid analysis of elastin and collagen while other 3 were assayed for time course release of hydroxyproline by pepsin digestion. The remaining 4 cases were dedicated to the characterization by thermal and dielectric techniques and comprised one specimen without evident lesions and three samples exhibiting atheroma plaque lesions. A detailed description of sampling from such specimens is reported in Table I. An aortic valve leaflet from an aortic root (devoid of atherosclerotic lesions) obtained at autopsy (66 years, male) was chosen as control sample to compare AAA with a tissue not affected by evident disorders and having a comparable content of elastin and collagen. Quantification and amino acid composition of elastin and time course release of hydroxyproline by pepsin digestion was also carried out on 4 age-matched control specimens of infrarenal abdominal aorta obtained at autopsy.

Biopsies and control samples washed with ice-cold PBS in presence of protease inhibitors and stored at  $-80^{\circ}\text{C}$ .<sup>22</sup> To remove possibly interfering residual blood, readily soluble fractions, minerals, lipids and cell components the samples investigated by thermal and dielectric techniques were first suspended in a hypotonic, 10 times diluted, PBS pH 7.4 containing protease inhibitors and 10% DMSO (dimethylsulfoxide) and gently extracted at  $4^{\circ}\text{C}$  for 3 h<sup>28</sup> before decalcification with EDTA (EDTA 5M, PBS buffer) and defatting by successive 2-h extractions at  $4^{\circ}\text{C}$  in chloroform:methanol (2:1, vol/vol), chloroform:methanol (3:1, vol/vol), ethanol:distilled water (1:1, vol/vol) and distilled water. Cell remnant removal was completed by replacing DMSO with 1% (w/w) Triton X-100 in PBS buffer and extraction for  $2 \times 10$  h periods at  $4^{\circ}\text{C}$ . In turn Triton X-100 was replaced by 10 mM sodium cholate for  $2 \times 10$  h treatments at room temperature. Samples were successively washed with 10% isopropanol in saline and deionized water before freeze-drying and final weighing after storage under reduced pressure over  $\text{P}_2\text{O}_5$  (dddW, dry defatted decalcified weight).

### Biochemical analyses

Isolation of elastin was carried out by a multi-step extraction based on chaotropic agent followed by treatment with purified collagenase and by trypsin to remove age-related polar proteins deposited on elastin.<sup>22</sup> The content and

composition of collagen and elastin in the resulting insoluble matrix was determined by weight of insoluble elastin and amino acid analysis of the collagenase solubilized fraction.<sup>29</sup> Hydroxyproline was also determined separately according to Woessner.<sup>30</sup> Sample hydrolysis and amino acid analysis was performed as previously described.<sup>29</sup>

Determination of hydroxylation level of insoluble collagen was based on the content of proline and lysine determined in both their nonhydroxylated and hydroxylated form in the material solubilized by collagenase.

Solubilization of collagen by pepsin was evaluated on the basis of time-course release of hydroxyproline as determined on aliquots of the supernatants and related to total hydroxyproline comprising the final insoluble residual pellet. Freeze-dried AAA and control samples of infrarenal aorta obtained at autopsy were minced with scissors, suspended in 0.5 M acetic acid for 48 h at 4°C and then treated by addition of pepsin at 35 mg/g dddW under constant stirring for 24 h at 4°C.<sup>31</sup> Aliquot of supernatant was taken after 6, 16 and 24 h, respectively, and separated from solids by high speed centrifugation. The insoluble material separated by centrifugation was re-suspended in 0.5 M acetic acid and treated with fresh pepsin in the same conditions for additional 24 h before further supernatant separation. Such procedure was successively repeated for two 48 h and one final 120 h period for a total of 264 h.

#### Differential scanning calorimetry (DSC)

The DSC thermograms were recorded with a Perkin Elmer DSC7 Differential Scanning Calorimeter. The calorimeter was calibrated using Indium and Tin as standard. Samples (5–10 mg) were sealed into aluminum pans, and empty pans were used as references. Investigations were performed between 30 and 250°C with 20°C/min heating rates. This heating rate was chosen to obtain well-defined glass transitions and to explore the 20–250°C range in a fast experiment reducing the degradation phenomena.

#### Thermostimulated currents (TSC)

Samples (20–40 mg) were compressed ( $2 \times 10^8$  Pa during 2 min) resulting in disks 1 mm thick, 8 mm diameter. To avoid perturbations linked to residual bound water in freeze-dried tissues, the samples were annealed for 1 h at 110°C in N<sub>2</sub> atmosphere, and then kept in vacuum ( $1.33 \times 10^{-4}$  Pa) for 2 h. Thermogravimetric Analysis checked this procedure to remove bound water without denaturing proteins.

Thermally stimulated currents measurements were carried out with a set up developed in our laboratory and previously described.<sup>32</sup> Samples were placed between two plate stainless steel electrodes and the sample cell was flushed and filled with dry He. The TSC relaxation spectra were recorded with a very sensitive electrometer (Keithley 642,  $10^{-16}$  A accuracy) versus temperature during a linear heating rate (7°C/min, which represents an ideal compromise between signal resolution and temperature regulation) after a static polarization at a given temperature and freezing at liquid nitrogen temperature. In the present study, the best

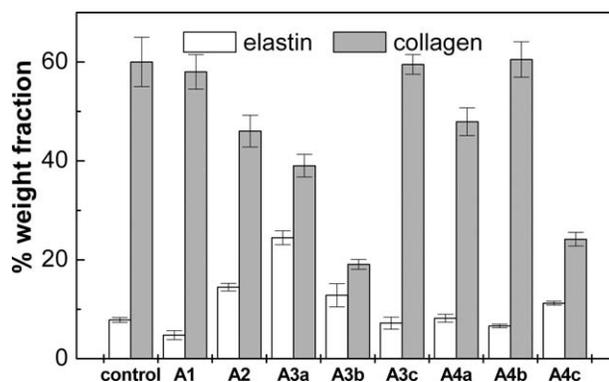


FIGURE 1. Elastin and collagen contents of tissues sampled from non-specific aneurysms (Table I) and of control sample (aortic leaflet).

poling conditions resulting in reproducible dipolar relaxations were as follows: polarizing field  $E_p = 400$  V/mm, polarization time  $t_p = 2$  min, linear heating rate  $q = 7^\circ\text{C}/\text{min}$ .

For each set of samples, DSC and TSC experiments were performed in triplicate and Origin Software was used for statistics.

## RESULTS AND DISCUSSION

### Biochemical analysis

**Elastin and collagen content.** Concentration of elastin and insoluble collagen content in nonspecific AAA ( $n = 10$ ) accounted for  $3.2 \pm 1.3$  and  $41.5 \pm 9.8$ , respectively, while in control infrarenal aorta ( $n = 4$ ) it accounted for  $18.6 \pm 1.9$  and  $40.4 \pm 9.2$ , respectively, (% dry defatted weight). Elastin and collagen contents of the tissues sampled from the 4 AAA specimens selected for thermal and dielectric investigation are given in Figure 1 (dddW). Although there is a great variability of elastin and collagen amounts in the different samples (the plaque fragments being the poor less elastin-collagen tissues), the proportion of collagen was always largely in excess with respect to elastin, as reported in literature,<sup>23</sup> that is the reason why age-matched human valvular leaflet, composed by 50–60 % collagen and 12% elastin,<sup>33</sup> was chosen as reference tissue to follow the evolution of the structural and dynamical parameters of extracellular matrix biopolymers in aneurysms.

### Composition of the elastin fraction in control tissue and aneurysms.

As reported on Table II, the amino acids composition of control and AAA elastin shows some significant differences. AAA elastin exhibits an increase of polar residues with a concomitant decrease of the hydrophobic ones typical of human elastin from nonaneurysmal aortic wall. In fact elastin from AAA results is contaminated by an additional insoluble polar protein fraction. Considering that in human aorta the ageing process is related to the progressive deposition of polar glycoproteins (removed by trypsin treatment), the presence of a protein resistant to trypsin is indicative of a component likely different from the fraction deposited during ageing in nonaneurysmal aortic tissues.<sup>22</sup>

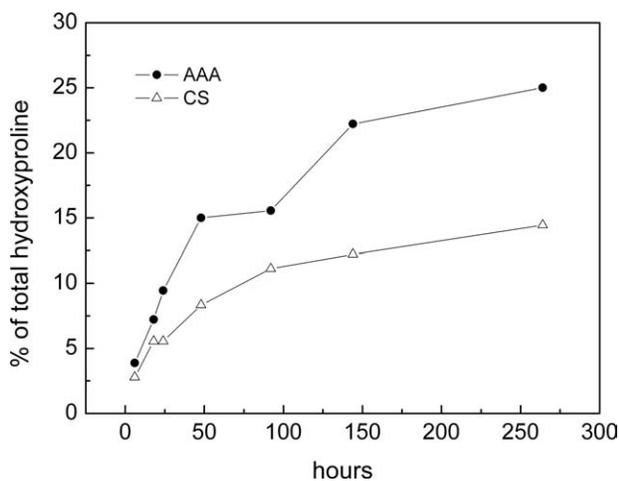
**TABLE II. Amino Acid Composition (mean  $\pm$  SD) of Elastin Isolated From Nonspecific Aneurysms (AAA) and Control Samples (CS)**

	AAA (n = 10)	CS (n = 4)
Lys	15 $\pm$ 6	8 $\pm$ 2
Hys	7 $\pm$ 3	4 $\pm$ 1
Arg	18 $\pm$ 5	13 $\pm$ 2
1/2 Cys	9 $\pm$ 3	5 $\pm$ 1
Hyp	16 $\pm$ 3	15 $\pm$ 3
Asx	29 $\pm$ 11	14 $\pm$ 5
Thr	26 $\pm$ 6	19 $\pm$ 2
Ser	26 $\pm$ 8	15 $\pm$ 3
Glx	46 $\pm$ 9	31 $\pm$ 6
Pro	105 $\pm$ 10	119 $\pm$ 6
Gly	238 $\pm$ 26	261 $\pm$ 14
Ala	181 $\pm$ 17	211 $\pm$ 10
Val	105 $\pm$ 6	121 $\pm$ 5
Met	8 $\pm$ 1	6 $\pm$ 2
Ile	29 $\pm$ 2	27 $\pm$ 1
Leu	71 $\pm$ 7	64 $\pm$ 2
Tyr	28 $\pm$ 1	26 $\pm$ 2
Phe	31 $\pm$ 4	26 $\pm$ 2
Ide+Des	10 $\pm$ 3	13 $\pm$ 3

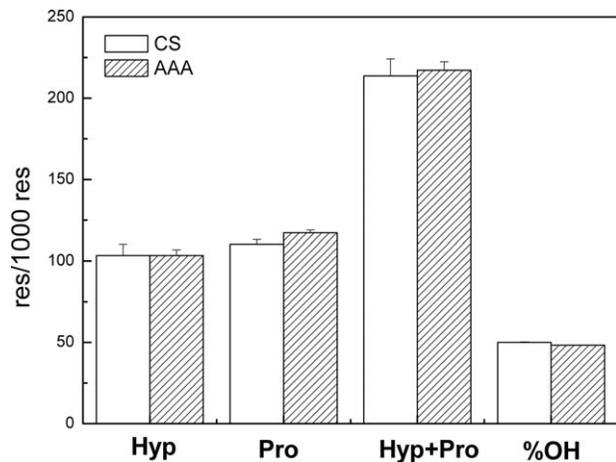
Data are Expressed as Res/1000 Total Res.

**Time course solubilization of insoluble collagen by pepsin.**

Figure 2 shows time-course solubilization of insoluble collagen by pepsin; a larger fraction of collagen is solubilized by pepsin from AAA with respect to age-matched nonaneurysmal abdominal aorta (CS). It is well known that the triple helical domain of collagen is resistant to pepsin attack in contrast to nonordered telopeptide regions; therefore, the difference of solubilization could be seen as expression of a lower order in collagen from aneurysms. Two hypotheses can be driven from this observation. On one hand, the large release of AAA collagen by pepsin is compatible with the presence of less cross-linked neo-synthesized collagen, owing to its recent deposition, or to some impairment of its stable incorporation into the extracellular ma-



**FIGURE 2.** Pepsin solubilization of insoluble collagen: hydroxyproline release. Each value is the mean of the determinations carried out on three control samples (CS) and three nonspecific aneurysms (AAA), respectively.

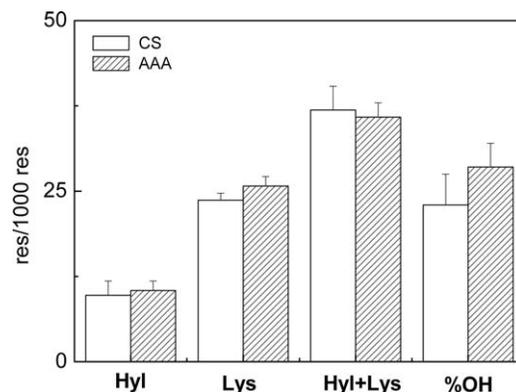


**FIGURE 3.** Proline and hydroxyproline concentration and % of proline hydroxylation in the collagen of AAAs (n = 10) and control samples (CS) (n = 4).

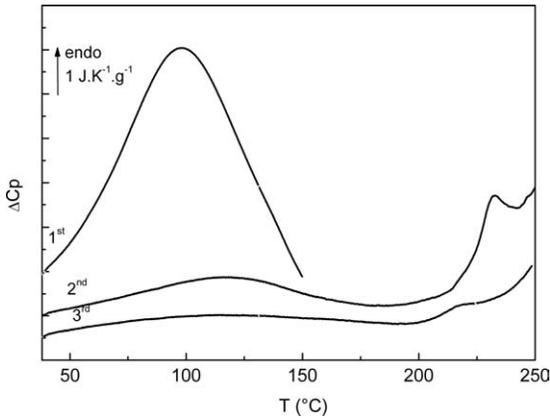
trix. In fact collagen has been reported to become more resistant to pepsin with ageing in nonaneurysmal aortic tissues.<sup>34</sup> On the other hand weakening of the AAA insoluble extracellular matrix cannot be excluded as a result of previous nicking by MMPs enzymatic attack.

**Analysis of insoluble collagen solubilized by collagenase: hydroxylation of proline and lysine.**

As reported on Figure 3, proline hydroxylation in AAA collagen is similar to that of the control one. It is noteworthy that the thermal stability of the collagen triple helix is attributed to the hydrogen bonded networks, mediated by water molecules, which connect the hydroxyl group of hydroxyproline in one strand to the main chain amide carboxyl of another chain.<sup>35</sup> Therefore, difference in hydroxyproline contents is generally correlated to differences in denaturation temperatures. These data will be compared in the following section. As for hydroxyproline few differences are observed between lysine hydroxylation in control and AAA samples (Fig. 4). Nevertheless, a slight increase can be detected in AAA collagens. This could be due to the presence of different types of



**FIGURE 4.** Lysine and hydroxylysine concentration and % of lysine hydroxylation in the collagen of AAA (n = 10) and control samples (CS) (n = 4).



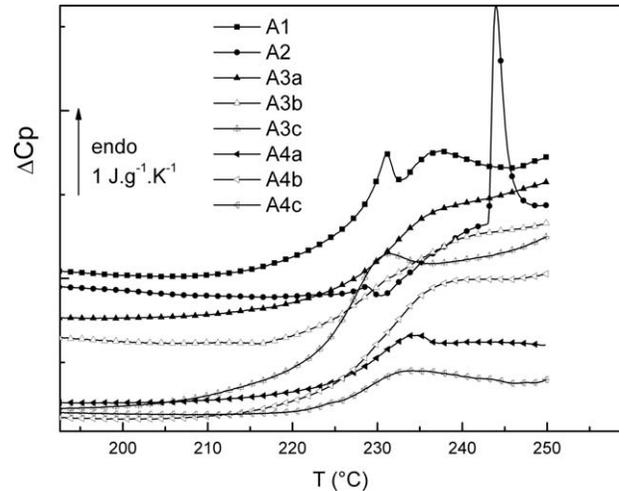
**FIGURE 5.** DSC thermograms of control sample (human aortic valvular leaflet).

collagen (other than type I and III). However, the amount of hydroxylysine varies greatly between collagen types as well as within the same collagen type in the same tissue in different physiological and pathological conditions. It also seems to be age-related, so that hydroxylysine content of collagen from embryonic tissues is higher than that of collagen from adult tissues. The amount of hydroxylysine is also increased in some diseases.<sup>36</sup>

### Thermal transitions

The thermograms of aortic valvular leaflet corresponding to three successive scans between 30 and 150°C and 250°C are reported on Figure 5.

As previously observed in porcine tissues,<sup>28</sup> the first scan is characterized by a large endothermic peak with maximum at 97.7°C associated with the loss of residual bound water. The second scan presents the denaturation endotherm of collagen (i.e., the transition from an ordered state -triple helix arrangement- to a random state -gelatin-) at 231.6°C, with an enthalpy of  $13.2 \pm 1 \text{ J g}^{-1}$  (normalized to the weight of collagen in the tissue). The third scan evidences the glass transition of gelatin at 207.6°C, which can be assimilated to an amorphous polymer. It is noteworthy that the thermal signature of elastin, namely a reversible glass transition that would occur at around 200°C on the second scan for freeze-dried tissues<sup>28</sup> is not detectable, due to the low content of elastin in aortic leaflet.



**FIGURE 6.** DSC thermograms of aneurysms (second scans, denaturation zone).

For this study, we will mainly focus on the denaturation parameters of collagen, which constitute a good evaluation of triple helix stability.

DSC thermograms of aneurysms were recorded according to the same protocol as control tissue; in all the cases, second scans are characterized by the denaturation peak of collagen; we have plotted in Figure 6 this peculiar zone for all the tissues.

Although the thermograms of pathologic tissues have a similar aspect than the thermogram of normal tissues, some of them can be distinguished by the broadness, the multiplicity and the intensity of endothermic events observed above 200°C and the occurrence of a shoulder at around 170/180°C in two sets of samples<sup>37</sup> (not shown here).

The thermal parameters of collagen denaturation are reported on Table III. Other thermal events are also noticed and reported in Table III.

Various modifications are observed on DSC thermograms; as a matter of fact, the collagen denaturation signal is multiple in some pathologic tissues; this feature is never observed in safe aortic tissues. In literature data, the multiplicity of collagen denaturation peak has been reported for different kinds of tissues in solution. Most of the authors attribute this sequential melting to a distribution of collagen molecules with a distinct degree of thermal stability.<sup>38</sup> In some cases, this multiplicity would arise from the

**TABLE III. Characteristic Thermal Transition Parameters of Tissues Sampled From AAA as in Table I**

	Nature	Tpeak (°C)	$\Delta H$ peak (J g <sup>-1</sup> )	Other transitions
A1	doublet	231.1 ± 0.6; 236 ± 0.4	8.1	Shoulder at 170°C
A2	triplet	229 ± 0.5; 239 ± 0.3; 244 ± 0.5	7.6	Shoulder at 180°C
A3a	singlet	236.9 ± 0.4	3.7	
A3b	singlet	239.2 ± 0.4	3.8	
A3c	singlet	230.4 ± 0.5	8.6	
A4a	singlet	234 ± 0.3	8.2	
A4b	singlet	236.5 ± 0.5	7.2	
A4c	singlet	232.8 ± 0.5	4.3	

**TABLE IV. Denaturation Enthalpy of Collagen From AAAs and Control Tissue (Aortic Leaflet) (in J/g of Collagen)**

Leaflet	A1	A2	A3a	A3b	A3c	A4a	A4b	A4c
13.2 ± 1	14.0 ± 0.5	16.5 ± 1.2	9.5 ± 0.6	19.9 ± 1.0	14.5 ± 0.5	17 ± 1	11.9 ± 0.7	17.8 ± 1

heterogeneity of samples;<sup>39</sup> it was also interpreted as the melting, in a same triple helix, of domains of distinct cooperativity.<sup>40</sup> For Flandin et al.,<sup>41</sup> who studied the evolution of collagen denaturation peaks with ageing, there is a correlation between the multiplicity of this denaturation and the presence of different cross-links (labile cross-links and thermally stable cross-links) in the protein.

Finally, complementary studies on lyophilized collagen from immature rat skin have allowed us to bring to the fore such a doublet of denaturation.<sup>42</sup>

Our thermal results on aneurysms are consistent with biochemical and literature data; different factors can explain the multiplicity of collagen denaturation peaks in some AAAs: accumulation of neo synthesized collagen (evidenced by Gargiulo et al.<sup>22</sup>) and sharp increase of C-telopeptide fragments in AAA wall<sup>8</sup> associated with collagen degradation by cysteine proteases. The different pepsin solubilization and thermal stability of collagen in the aneurysms is likely to reflect an abnormality in regulation of collagen turnover, as suggested by different studies.<sup>23,25</sup> In the present study, difference of denaturation temperature cannot be correlated with difference of proline hydroxylation as generally observed. In its greatest part the AAA collagen phase is not subject to type I-type III deregulation but rather to other chemical and/or physical changes (increased neosynthesized collagen, collagen fragmented by enzymatic attacks).

Moreover, an endothermic peak was evidenced between 170 and 180°C in some thermograms. Even if the identification of this peak is not straightforward, this thermal event shows the existence of structures of weak stability in AAA walls. These results must be correlated with thermogravimetric studies performed on unaffected and pathologic arterial tissues, which reveal an alteration of the thermal stability of elastin and collagen in the atheroma plaque<sup>43</sup> and could be related to the degree of MMPs expressions.<sup>15,19</sup>

A further comparative study of denaturation parameters can be performed by normalizing the denaturation enthalpy to the weight of collagen in the dry and delipided tissue. These results are reported on Table IV. The denaturation enthalpies are comprised between 9.5 and 20 J g<sup>-1</sup>. The values that strongly deviate from the reference value (13.2 ± 1 J g<sup>-1</sup>) correspond to fragments of atheroma plaque, in which collagen alterations are certainly the most pronounced.

### Dielectric properties

The global TSC spectrum of dehydrated valvular leaflet was recorded between -180 and 150°C, after a polarization at 150°C and is reported on Figure 7.

This spectrum is characterized by a main peak at 129°C ± 3°C, with an intensity equal to 1.5 ± 0.2 pA. Previous

studies performed on porcine aortic walls, porcine valvular leaflets and pure proteins (elastin and collagen) have allowed us to associate this mode with the delocalized motion –in the order of ten nanometers- of dipolar entities along the backbone of collagen and elastin.<sup>44,45</sup>

The other relaxation modes visible as shoulders at around 15 and 70°C are characteristic of the collagenic phase and were attributed in previous works to the reorientation of cross-link zones, preferentially located in telopeptides.<sup>46</sup>

No relaxation mode is observed between -160 and 0°C, as previously described for porcine valvular leaflets in the dehydrated state; as a matter of fact, we showed in a previous study<sup>47</sup> that localized motions along the polypeptidic chains which generally occur in this zone for proteins are only visible when tissues contain a certain amount of water.

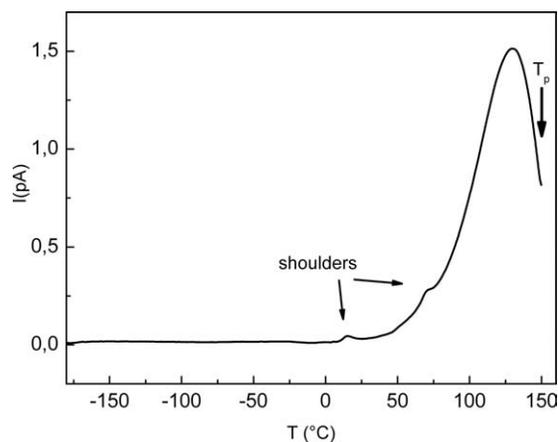
TSC spectra of aneurysms were recorded according to the same protocol as control tissue.

In the case of the aortic wall broken by aneurysm (sample A1), the TSC spectrum reported on Figure 8 is characterized by three relaxation modes respectively located at -47, 21, and 121°C.

The TSC spectrum corresponding to the non specific polyaneurysm (sample A2) reported on Figure 9 is particularly complex: 6 relaxation modes are observed as shoulders (at -60 and 75°C) or peaks (at -22, 33, 47 and 136°C). Moreover, the intensity of the relaxation modes is multiplied by 5 when compared with other samples.

The TSC spectra of samples from the same atheromatous plaque (samples A4a, A4b and A4c) are superimposed on Figure 10.

In the three cases, the spectra are composed of multiple modes: for the sample A4a (aortic wall + plaque), 5 modes are evidenced at -60, 23, 46, 56 and 139°C. For the sample



**FIGURE 7.** TSC spectrum of dehydrated control sample (human valvular leaflet).

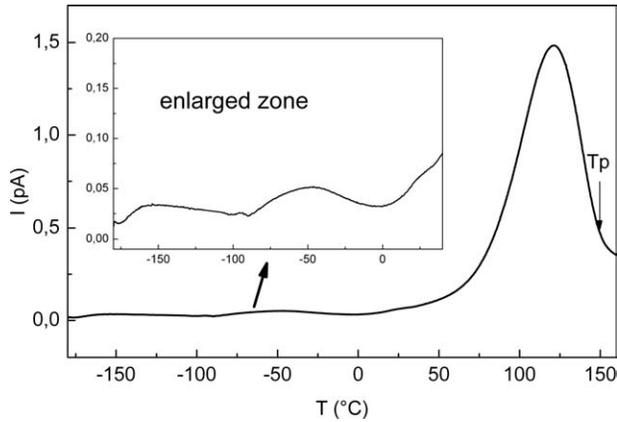


FIGURE 8. TSC spectrum of aneurysm A1.

A4b (wall + plaque, other localization than A4a), only two modes are revealed at  $-60$  and  $123^\circ\text{C}$ . As for the sample A4c (fragments of aortic wall), four relaxation modes are observed on the TSC spectrum at  $-40$ ,  $10$ ,  $55$  and  $110^\circ\text{C}$ .

The most systematic event concerns the existence of a secondary relaxation mode between  $-60$  and  $-40^\circ\text{C}$  in all the aneurysms spectra, well-defined in the systematic analysis of the TSC-derivative curves.

It is noteworthy that a mode located at  $-50^\circ\text{C}$  has been evidenced for immature collagen and associated with cooperative motion of ionic lateral chains, inhibited during maturation by the blocking of involved active sites.<sup>48,49</sup> Moreover, works on collagen molecular mobility in rat skin with ageing<sup>50</sup> showed that such a mode strongly increased for mature specimens.

Therefore the mode we observe between  $-60$  and  $-40^\circ\text{C}$  is the marker of a structural modification of collagen in aneurysms, that can be explained either by an increase of collagen synthesis or by an alteration of cross-links like in ageing process.

In a general trend, there is an intensification of the different relaxation modes, with a peculiar exaltation of the modes located between  $0$  and  $80^\circ\text{C}$ , and specifically attributed to collagenic phase. This evolution can be correlated to the aspect of TSC spectra of safe tissues that were subjected

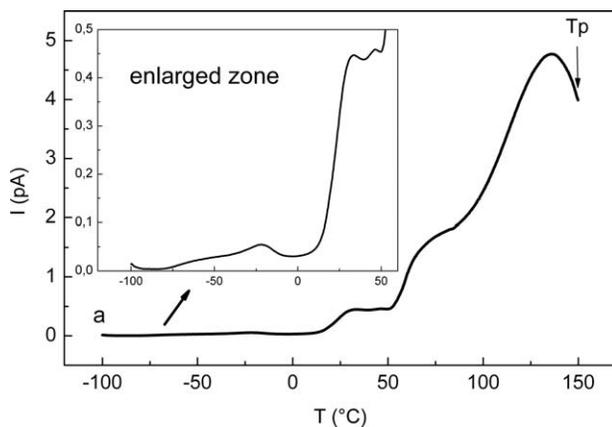


FIGURE 9. TSC spectrum of aneurysm A2.

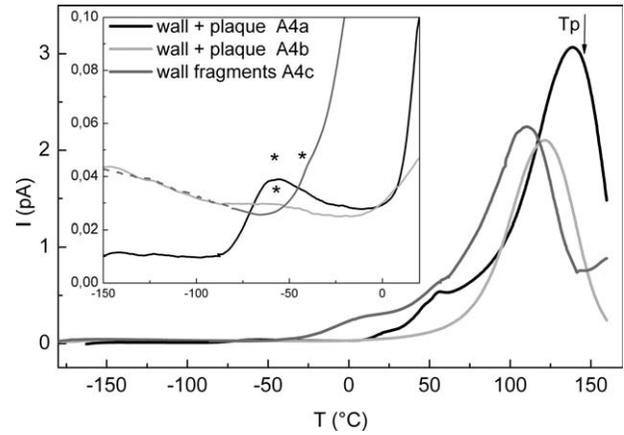


FIGURE 10. TSC spectra of aneurysm tissues A4a, A4b and A4c (\* incidence of a specific mode close to  $-50^\circ\text{C}$ ).

to an annealing temperature of  $230^\circ\text{C}$ <sup>50</sup> (in the denaturation temperature zone). This analogy makes us suppose that the intensification of these modes is due to an increase of collagen mobility or a partial denaturation of collagenic phase in AAA walls.

The global evolution of the characteristic parameters confirms an increase of the molecular mobility of collagen in aneurysms, at different scales of mobility.

This assumption is in good agreement with the alteration of mechanical properties of collagen reported for AAA.<sup>2</sup> In the aneurysmal aorta, the total unfolding of collagen fibers is reached for a weaker deformation when compared with control aorta, and leads to a diminution of the mechanical strength of the wall. This behavior which is attributed to an imperfect waviness of collagen fibers results of structural modifications in quarter-stagger arrangement and triple helix domains of collagen. This result at the nanometer scale is in good agreement with recent observations in polarized light microscopy, typically at the micrometric scale, showing an evolution from a three-dimensional helical waviness of collagen in wild type samples to a bidimensional one in induced aneurysms samples, probably to accommodate the collagen increase observed in this case.<sup>12</sup>

Finally it is noteworthy that the sample which possesses thermal and dielectric characteristics very distinct—multiple collagen denaturation, drastic modification of relaxation spectrum—from the unaffected tissue, belongs to the patient affected by polyaneurysm (genetic basis or general predisposition). These changes illustrate a profound alteration of the collagenic phase, in spite of its content similar to that of other aneurysmal tissues.

## CONCLUSION

The first concern is the great variability of pathologic tissues in their biological composition (fragments of atheromatous plaque exhibit a lower elastin-collagen fraction with respect to samples comprising plaque and aneurysm wall) as well as in their thermal and dielectric properties. This general trend underlies the heterogeneous character of AAA wall when compared with controls. However one cannot

exclude that heterogeneity at the structural level could result at least in part from the coexistence of the early with the more recently deposited extracellular matrix.

Nevertheless, some points common to the whole set of aneurysm samples have been shown by thermal and dielectric techniques, as far as peculiar alterations of collagen phase are concerned. This study shows the ability of such techniques, usually reserved to synthetic polymers, to get an insight into the chain dynamics of biopolymers in pathologic cardiovascular tissues. It must be compared to the recent thermal-dielectric study<sup>51</sup> that show the alteration of elastin network in arteries cultured with homocysteine, an aminoacid responsible of histological impairments in human arteries. Thermal and dielectric studies on collagen type I, type III and gelatin in the hydrated state are also in progress to investigate the formation and rupture of hydrogen bonds network and so to distinguish between the mobility of neosynthesized collagen or partially denatured collagen. These results will be coupled to NMR ones to propose a micro-macro relation for dipolar relaxation, as it emerges in recent literature data.<sup>52</sup> In a next step, we project to complete this work by mechanical analyses, to determine the structure-properties relationship of collagen and elastin in pathologic tissues and to contribute to the understanding of mechanisms that lead to aneurysm burst.

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