

Biophysical methods to characterize cardiovascular tissues

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Abstract

The main constitutive macromolecules of cardio-vascular tissues, essential to the cohesion and resiliency, are also active components which evolve with the physiology and pathology. Their functionality is connected to their internal dynamics over various scales of time and length, in close correlation with water. Vibrational, thermal and dielectric techniques, which have showed their ability to characterize synthetic polymers, deserve to be adapted to the study of proteins and biological tissues. Through some examples, we will show how these techniques are concretely used to answer cardio-vascular problematics.

Introduction

Fourier Transform Infrared (FTIR) spectroscopy is a powerful technique that has provided accurate results with a high reproducibility in many areas including cardiovascular research [1-3]. Fast analyses can be performed directly on tissues, without the need of staining. Another suitable technique to characterize biological tissues at the submicronic scale is Differential Scanning calorimetry (DSC). It allows to evaluate the hydric organization of tissues [4], as well as proteins thermal stability [3,5,6] directly in aortic tissues [7] or biomaterials [8,9]. It has been also successfully applied to characterize protein components of muscle tissues, such as myosin, actin and sarcoplasmic proteins [10-12].

Due to the polar character of biological tissues, dielectric techniques are well suited to the analysis of their various levels of organization in interaction with water or in the freeze-dried stat tissues [13]. The molecular mobility of proteins in tissues can be scanned from the nanometer range to some tens of nanometers. In the very low frequency region, Thermo Stimulated Current (TSC) gives information on the molecular origin of the relaxation modes, while Dynamic Dielectric Spectroscopy (DDS) follows protein/water dynamics over a very broad frequency range.

The combination of FTIR, DSC, TSC and DDS on the pure components of cardio-vascular tissues has allowed us to establish a set of structural, dynamic and chemical indicators on the main macromolecules constitutive of these tissues [14,15]. These vibrational, thermal and dielectric markers were then successfully used to determine the evolution of these main proteins produced by lipid-loading smooth vascular cells [16] and cardiomyocytes in culture [3].

In the next section, we will focus on the application of these biophysical techniques to characterize cardio-vascular tissues themselves.

Characterization of cardio-vascular tissues in animal models

Left ventricle remodeling post-myocardial infarction

Adverse cardiac remodeling after Myocardial Infarction (MI) causes impaired ventricular function and heart failure. If

histopathology is commonly used to detect the location, size and shape of MI sites, information about chemical composition and physical structure of infarct zones post-MI is limited. FTIR/ATR can evidence alteration of key cardiac components post-MI in a mice model [17]. As shown in figure 1, the FTIR spectra of freeze-dried mice left ventricles shows amide I and II as major absorptions bands. Moreover, collagen possesses a specific band at 1338 cm^{-1} [18] that can be used to compile a collagen/proteins indicator. Finally, the sur-resolution of FTIR spectra by the Fourier Self Deconvolution (FSD) method in the amide I/II zone is useful to determine the secondary structure of proteins [19]. The increase of collagen indicator associated with the predominance of triple helical conformation of proteins in infarcted myocardium evidences the deep remodeling of post-MI tissues (collagen deposition in the scar maturation of infarcted zones).

Ventricular remodeling in tachycardia-induced dilated cardiomyopathy

In non-ischemic Dilated Cardiomyopathy (DCM), the main clinical manifestations are progressive heart failure, ventricular and supraventricular arrhythmias, thromboembolisms and sudden death. It also constitutes the most common cause of heart failure reported for cardiac transplantation. Biophysical markers can be used to identify molecular and conformational alterations of cardiac remodeling in a pig model of DCM [20]. Using FTIR, it is shown that the myofiber/collagen ratio is reduced in ventricles from dilated hearts, while the carbohydrate/lipid ratio is upregulated in left and right ventricles (in a greater extent in the right ventricle). Additional information is provided from calorimetric analysis; in peculiar the depression of the onset melting temperature of freezable water, is indicative of an alteration in the tissue architecture of dilated ventricles, while the shift of the protein denaturation temperature evidences newly synthesized

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collagen and protein of lower stability. The combination of these data supports that both accumulation of collagen and thermal instability of myofibers and ECM proteins contribute to the imbalance in the myofiber/collagen ratio and to the accumulation of unorganized and agglomerated collagen in dilated ventricles.

Characterization of human cardio-vascular tissues in clinical cases

Abdominal aortic aneurysms (AAA)

The pathogenesis of AAA, responsible for 2% of human deaths in industrialized nations, remains uncertain, and the lack of definitive insight suggests a complex, multifactorial process. For this thermal/dielectric study [21], specimens were collected from the anterior aspect of the infrarenal abdominal aorta during operation for non-specific Abdominal Aortic Aneurysms (AAA) in 4 patients. DSC thermograms of freeze-dried atheroma plaques are plotted in figure 3A. It is noteworthy that in contrast with safe aortic wall which is characterized by both elastin and collagen thermal answers (elastin glass transition at 205°C and collagen denaturation at 230°C), in atheroma plaques the

collagen signal is predominant. Moreover, the collagen denaturation signal is multiple in 3/4 of pathologic tissues, what can be explained by the accumulation of neo synthesized collagen as evidenced by Gargiulo et al. [22] and sharp increase of C-telopeptide fragments in AAA wall [23] associated with collagen degradation by cysteine proteases.

To get insight the dynamics of atheroma plaque, the TSC spectra of rich control tissue and aneurysmal tissue have been compared (Figure 3B): an enhanced molecular mobility is evidenced for pathological tissue, corroborating the accumulation of neo synthesized or fragmented collagen.

Cardiomyopathies

In this last case biophysical techniques are used to characterize right and left ventricles from patients suffering from cardiomyopathy. This current study involves 16 patients, and the objective of this study is to compare thermal/vibrational indicators according to the localization (right/left ventricles, remote or infarcted zone) and the pathology (control/non-ischemic/ischemic cardiomyopathy). The feasibility of vibrational and thermal techniques to analyse such human cardiac tissues has been demonstrated and relevant markers can be extracted.

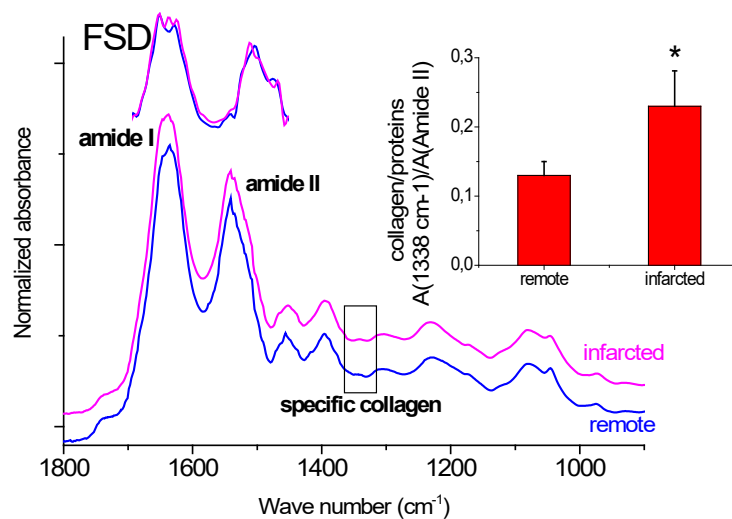


Figure 1. Vibrational characterization (FTIR/ATR) of remote and infarcted zones in mice ventricles

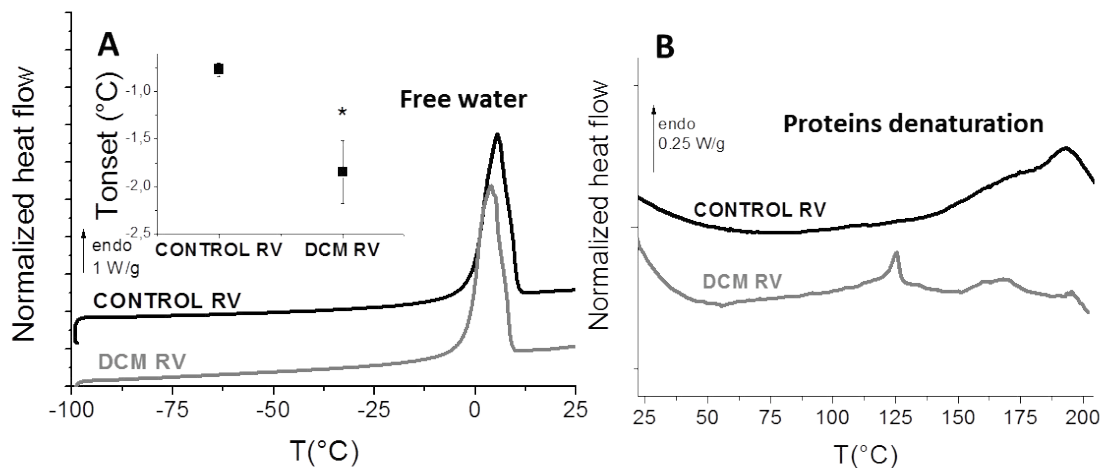


Figure 2. Thermal characterization (DSC) of healthy and dilated pig ventricles (A: hydrated state; B: freeze-dried state)

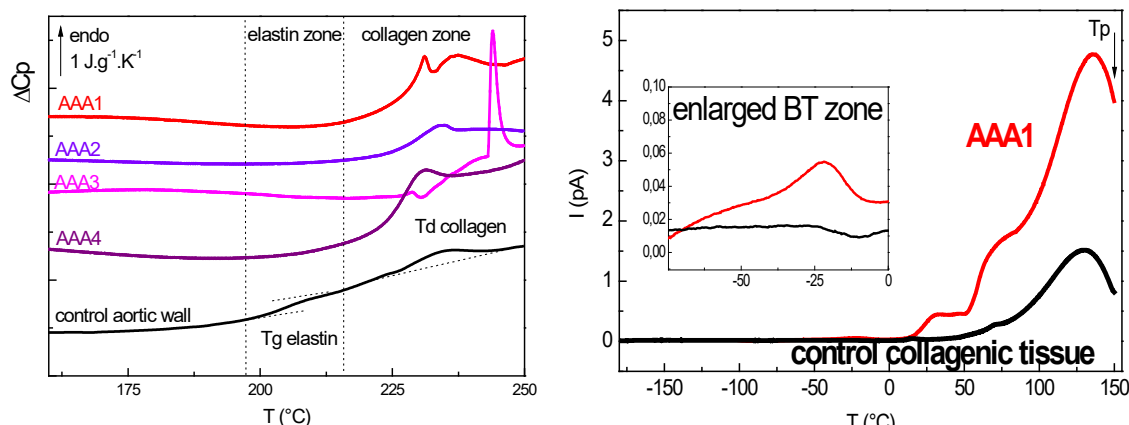


Figure 3. Thermal (A: DSC) and dielectric (B: TSC) characterization of human atheroma plaques

Multivariate data analysis is now in progress to correlate biochemical and biophysical markers in this clinical study.

Conclusion

The molecular, conformational and physical characterization of the cardio-vascular tissues (aortic wall, pericardium, myocardium) has emerged as a novel approach to study remodeling in diseased tissues. The vibrational FTIR spectra of cardiovascular tissues can be correlated to the vibrational answer of the different components, allowing to quantify them and to discriminate the different secondary structures of proteins. Using calorimetry and dielectric techniques, the thermal transitions and dynamics of free water, elastin, collagens or muscle proteins can be detected in cardiovascular tissues, evidencing a strong correlation between chain architecture and mesophase organization. The evolution of these indicators with pathology (cardiomyopathy, infarction, atherosclerosis) completes biochemical analysis and contributes to a better knowledge of the involved mechanisms. It is also promising to optimize the conception of substitutive biomaterials in the cardiovascular research.

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