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Case report

Histopathology mapping of biochemical changes in myocardial infarction by Fourier transform infrared spectral imaging

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ABSTRACT

Fourier transform infrared (FTIR) imaging and microspectroscopy have been extensively applied in the identification and investigation of both healthy and diseased tissues. FTIR imaging can be used to determine the biodistribution of several molecules of interest (carbohydrates, lipids, proteins) for tissue analysis, without the need for prior staining of these tissues. Molecular structure data, such as protein secondary structure and collagen triple helix exhibits, can also be obtained from the same analysis. Thus, several histopathological lesions, for example myocardial infarction, can be identified from FTIR-analyzed tissue images, the latter which can allow for more accurate discrimination between healthy tissues and pathological lesions. Accordingly, we propose FTIR imaging as a new tool integrating both molecular and histopathological assessment to investigate the degree of pathological changes in tissues. In this study, myocardial infarction is presented as an illustrative example of the wide potential of FTIR imaging for biomedical applications.

1. Introduction

Histopathological detection and characterization rely on the expertise and know-how of pathologists. A wrong histopathological diagnosis by unskilled or novice personnel may lead to inaccurate treatment. However, discrepancies in interpretation often occur, for the latter is subject to the specialist's experience and judgement [1,2]. Commonly used histopathological diagnostic techniques such as hematoxylin–eosin (H&E) staining can show the location, size, and shape of myocardial infarction sites, but information about their chemical composition is often limited. However, recent research has suggested that it is the microscopic morphology and chemical composition of a myocardial infarction, rather than its anatomy, that determines its progression [3]. Thus, there is an urgent need to obtain both precise morphological and chemical information on the distribution of different components within a myocardial infarction site. Therefore, the development of histopathology-derived tools, based on molecular and chemical information, for discriminating between pathologic lesions and healthy tissues is now viewed as a potent diagnostic tool [4].

Contrary to conventional biological methods such as hematoxylin–eosin (H&E) staining, Fourier transform infrared (FTIR) imaging and data analysis can be performed directly on tissues, without the need for staining. Also, data is computer-processed, making this method less prone to human error and chemical alterations. The instrument for spectral imaging is a powerful tool for analyzing heterogeneous materials, such as biological tissues, with respect to the chemical structure of their constituents and spatial distribution. Using this technique, contrast between different spatial areas become apparent due to the inherent chemical differences found within tissue cells and thus, producing molecule-specific vibrational signatures. A 'chemical' image of the tissue section can then be constructed that is similar to the morphological interpretation of a stained image, thus enabling the identification of tissue classes and providing an insight into their molecular composition. An FTIR spectroscopic approach, therefore, has several advantages over conventional histology [5]. One advantage of the FTIR spectroscopy approach is that a spectrum from an intact cell can be recorded within a few seconds. It is a recent technical advance that has provided imaging systems which are able to provide fast FTIR images of tissue sections and requiring only a few minutes to obtain a functional FTIR image of a particular tissue area. Thus, useful diagnostic information can be extracted from IR spectra for different pathologies [6,7].

In this study, we analyzed biochemical parameters using a chemical FTIR mapping of myocardial infarction lesions. Data and FTIR images acquisition is performed directly on human tissue

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samples. We also described how FTIR images can be used to precisely analyze myocardial infarction in cardiac cross-sections.

2. Materials and methods

2.1. Specimen collection and sample preparation

Formalin-fixed, paraffin-embedded, tissue samples from one human hearts with myocardial infarction, were obtained from the Institute of Forensic Science of Key Laboratory of Evidence Science (China University of Political Science and Law). Ethical approval for this study was given by Anatomy Rules (Ministry of Health of the People's Republic of China).

2.1.1. Case

On June 20 of 2009 at around 10 o'clock in the morning, a man was found inanimate, without breathing and heartbeat in his home's bathroom. His parents, who found him in this state, reported that he entered the bathroom and did not come out even after 20 min. Since it was not in his habit to stay so long in the bathroom, they went to check on him, found him on the bathroom floor and then called emergency services at once. The emergency physician reached them after about 10 min, resuscitation attempts were unsuccessful, and the man was declared dead. An autopsy was requested and was carried out on the same day around 4 pm. The decedent's previous hospital records showed that on 02.2005 he had a blood pressure of 160 mmHg (21.3 kPa)/120 mmHg(16 kPa). On April 2008, the deceased ECG showed pathological Q waves.

On the death scene, there were no signs of struggle, presence of drugs of abuse or other poisons. The autopsy was negative for any lethal injuries or signs of mechanical asphyxia.

The heart weighed 620 g. Atheromatous plaques were present in the coronary arteries. Obstruction of the left circumflex branch (LCA) and the right coronary (RCA) was about 75%, respectively. The region consistent with myocardial infarction was whitish on gross appearance and elliptical in shape. The longest diameter was 75 mm and shortest one, 40 mm. Microscopically, the intima of the coronary arteries was thickened and their lumens severely narrowed. Fibrous tissue, cholesterol crystals and calcified deposits were present in the plaques. Multiple and focal fibrotic scars were present in the papillary muscles, right and left parts of the heart and in the interventricular septum. There was extensive proliferation of myocardial interstitial fibrosis. Mild hypertrophy of cardiomyocytes could be seen. Congestion of the interstitium was conspicuous.

No gross abnormalities were visible in the brain, spleen and kidneys. On microscopy, arteriolar sclerosis was present in the brain and spleen. Glomerular fibrosis and hyaline degeneration was noticed in part of the renal tissue. No significant pathologies were detected in the lungs, liver, and pancreas.

Toxicology analysis was negative for lethal drugs and poisons.

According to the case history, autopsy notes, pathological findings and the absence of fatal injuries, death due to mechanical trauma, mechanical asphyxia, intoxication by drugs or poisons can be excluded. The deceased had severe coronary artery disease, old myocardial infarction, cardiomegaly and sclerosis of basal cerebral arteries and of small splenic arterioles. Hence, this is a case of sudden death due to coronary artery disease.

Table 1
General band assignments of the FTIR spectrum [2,16].

Wave no. (cm^{-1})	Definition of the spectral assignments
3290	Amide A: mainly N–H stretching of proteins
2837	CH_3 symmetric stretch: mainly proteins
1638	Collagen triple helix exhibits
1081	PO_2^- symmetric stretch: nucleic acids and phospholipids C–O stretch: glycogen

Table 2
FTIR data (Absorbance, Abs).

Definition of the spectral assignments	Red region (Abs)	Green region (Abs)
Amide A: mainly N–H stretching of proteins ($3290\,cm^{-1}$)	1.52	0.61
CH_3 symmetric stretch: mainly proteins ($2873\,cm^{-1}$)	0.88	0.44
Collagen triple helix exhibits ($1638\,cm^{-1}$)	1.34	0.60
PO_2^- symmetric stretch: nucleic acids and phospholipids C–O stretch: glycogen ($1081\,cm^{-1}$)	0.78	0.25

2.1.2. Sample prparation

Specimens were stored as paraffin-embedded tissue blocks and were cut, using a microtome, to provide tissue sections of 6 μm thickness and subsequently deparaffinised. These sections were mounted on slides made of glass coated with a thin Au/Ag layer that allow spectra to be recorded in transflection mode [8].

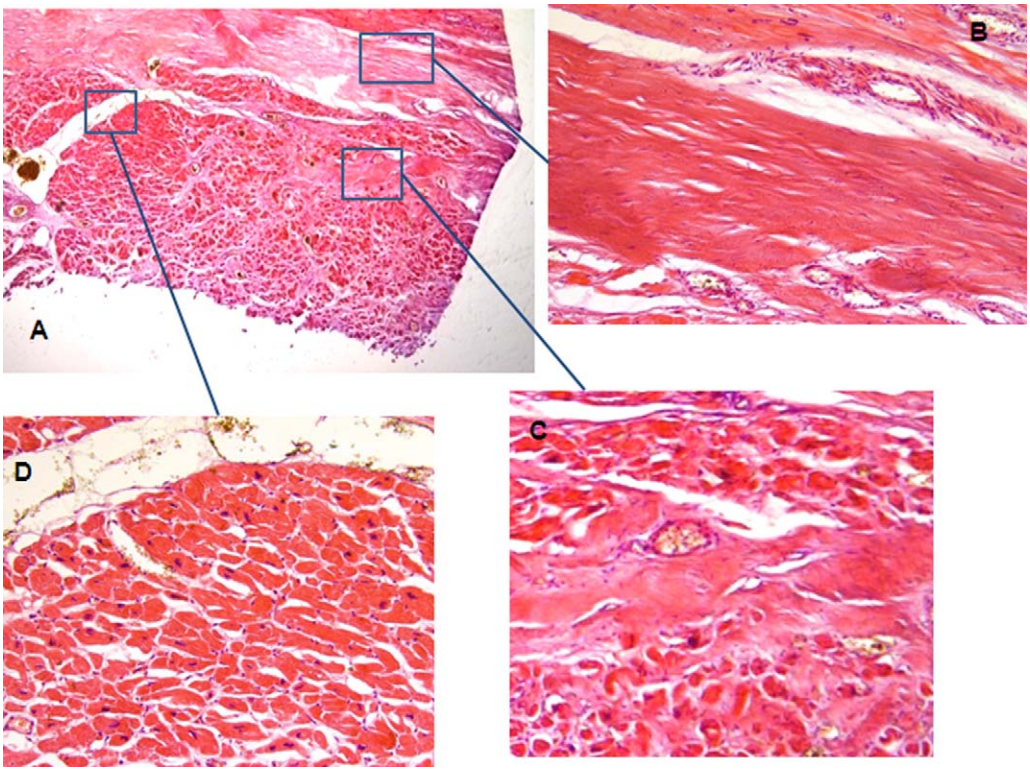

Fig. 1. H&E stain of myocardial infarction: (A) myocardial infarction in left ventricle(40×). The small black boxes indicate the approximate positions of the HE images. (B) Myocardium with coagulation necrosis, myocardial infarct with replacement of the necrotic fibers (200×). (C) Nearly complete removal of necrotic myocytes by loose collagen, a few residual cardiac muscle cells are present (200×). (D) Normal myocardium.