Atherosclerotic plaque characterization in apoE and LDL-receptor double-knock out mice by FTIR spectroscopy combined with histological and histochemical evaluation

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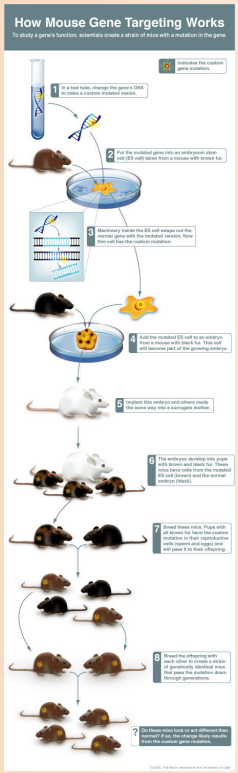
INTRODUCTION

Atherosclerosis is the leading cause of death in the developed world. It is a disease of large and medium-sized muscular arteries and is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall. This buildup results in plaque formation, vascular remodeling, acute and chronic luminal obstruction, abnormalities of blood flow, and diminished oxygen supply to target organs. Pathomechanism of this disease is not entirely understood. It has been proposed that atherosclerosis is a multietiological inflammatory and degenerative disease, related not only to cholesterol overload.

FTIR imaging (microspectroscopy) enables the comparison of chemical images between healthy and atherosclerotic arterial walls based on the absorbance of IR bands that correspond to specific lipids and proteins present in the sample.

Using SSLS' ISMI beamline and the high brilliance of the synchrotron radiation edge effect source we are able to analyze arterial samples in greater spatial and chemical detail.

MATERIALS and METHODS



The Nobel Prize in Physiology or Medicine 2007
Mario R. Capecchi, Martin J. Evans and Oliver Smithies

Gene targeting is a method for modifying the structure of a specific gene without removing it from its natural environment in the chromosome in a living cell. This process involves the construction of a piece of DNA, known as a gene targeting vector, which is then introduced into the cell where it replaces or modifies the normal chromosomal gene through the process of homologous recombination.

With gene targeting it is now possible to produce almost any type of DNA modification in the mouse genome, allowing scientists to establish the roles of individual genes in health and disease. Gene targeting has already produced more than five hundred different mouse models of human disorders, including cardiovascular and neuro-degenerative diseases, diabetes and cancer.

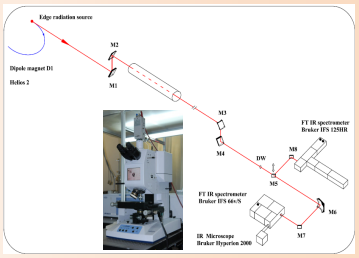
In our research apolipoprotein E and LDL receptor-double knockout (apoE/LDLR -/-) mice model was used that spontaneously develops severe hyperlipidemia and atherosclerotic plaques.

- Hearts with ascending aortas were excised, snap-frozen and 10 μm-thick cryosections were cut using standardized protocol then placed on separate slides.
- Some sections were mounted on 2.5 μm-thick Mylar foil and used for FT-IR analysis.
- Consecutive sections of aortic roots were stained to visualize specific components present in atherosclerotic plaques: **oil red-O** for lipids and **sirius red** for collagen fibers.
- Immunohistochemistry with **CD68** specific antibody was used to demonstrate macrophages distribution.

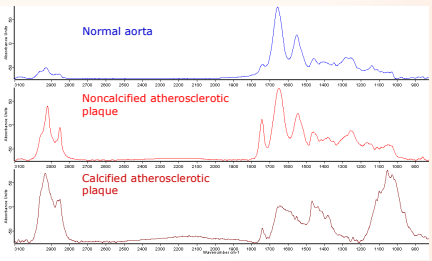
ISMI BEAMLINE at SSLS

FT-IR absorption spectra were recorded from the sections of aorta using 100 scans at each point (collection time about 15 s, spectral resolution 4 cm^{-1}). All spectra were collected in transmittance mode with the aperture area 15 by 15 μm^2.

On selected areas of interest 2D maps were carried out.



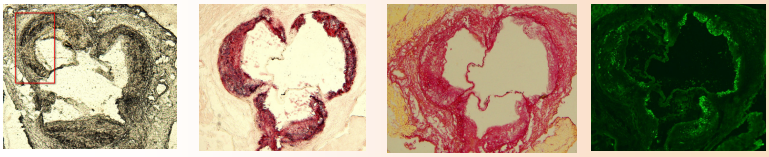
RESULTS



Frequency cm^{-1}	Assignment	Component	Normal aorta	Noncalcified atherosclerotic plaque	Calcified atherosclerotic plaque
2956	CH_3 asymmetric stretching	lipids		vs	
2928	CH_2 asymmetric stretching	lipids		vs	
2870	CH_3 symmetric stretching	mainly proteins		vs	
2853	CH_2 symmetric stretching	lipids		vs	
1735	C=O stretching	ester functional groups in lipids		vs	w
1655	Amide I (mainly protein C=O stretching)	α-helical structure	vs	vs	vs
1550	Amide II (protein N-H bending, C-N stretching)	α-helical structure	vs	vs	s
1468	CH_2 scissoring	lipids		s	
1463	CO_3^{2-}	carbonated apatites			s
1453	C-H bending mode	proteins	m		s
1412	CO_3^{2-}	carbonated apatites			s
1243	Amide III	proteins	m	m	m
1094	PO_4^{3-} antisymmetric stretching	calcium hydroxyapatite			vs
1040	PO_4^{3-} antisymmetric stretching	calcium hydroxyapatite			vs
962	PO_4^{3-} symmetric stretching	calcium hydroxyapatite			m
872	CO_3^{2-}	carbonated apatites			m

vs - very strong s - strong m - medium w - weak

Stainings on consecutive sections of aortic roots



Histological View

Oil red-O
Lipids - red

Sirius red
Collagen – red
Cytoplasm – yellow

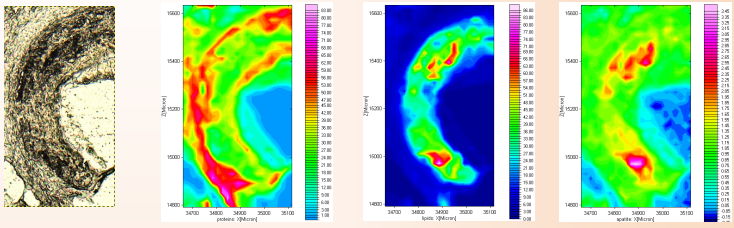
Immunofluorescence
CD68
Macrophages – Green

Histological view (ROI)

Proteins Distribution

Lipids Distribution

Calcium Hydroxyapatite Distribution



CONCLUSIONS

Our results demonstrate that IR microspectroscopy allows identification of the molecular nature of different constituents *in situ* in aorta even when chemical isolation and purification may be difficult or may modify the tissue components. The sensitivity of this method should permit accurate biochemical analysis of unstained specimens, supplementing the subjective judgments of current histological diagnostic methods.

In this study we examined the possibility of developing IR microspectroscopy as an optical tool for the examination of tissues. Histological examination requires histochemical staining of tissue section and identification of various microstructures often require the use of several different staining methods which are expensive and time-consuming.

The excellent sensitivity and spatial resolution of the IR microscope allow to obtain detailed biochemical information from extremely small unstained tissue sections thus provides an optical method for biochemical evaluation of unstained artery specimens. This technique also allows mapping of the spatial distribution of tissue molecular constituents in atherosclerotic artery. In addition the ability of IR microspectroscopy to characterize macromolecules *in situ* suggests that this technique can be a powerful diagnostic tool for other diseases as well.

Acknowledgment

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