Lipid Loading of Human Vascular Smooth Muscle Cells Induces Changes in Tropoelastin Protein Levels and Physical Structure

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Supporting Material

Fourier Transform Infrared analysis (FTIR)

Fourier transform infrared spectroscopy/attenuated total reflectance (FTIR/ATR) spectra were collected using a Nicolet 5700 FTIR (THERMO FISHER SCIENTIFIC, Waltham, MA) equipped in ATR device equipped with a KBr beam splitter and a MCT/B detector. Spectra were recorded over the region of 4000–450 cm^{-1} with a spectral resolution of 4 cm^{-1} and 64 accumulations. The ATR accessory used was a Smart Orbit equipped with a type IIA diamond crystal (refractive index 2.4). A single-beam background spectrum was collected from the clean diamond crystal before each experiment and this background was subtracted from the spectra. Fourier-Self-Deconvolution (FSD) of the infrared spectra that allows resolution of several overlapping bands was performed in the amide I/II region using Omnic software (THERMO FISHER SCIENTIFIC, Waltham, MA). The linear baseline correction was made on the FSD trace, and the decomposition of the amide I/II bands on the FSD trace was then performed with a Gaussian curve fitting procedure.

Thermogravimetric Analysis (TGA)

Analyses were performed in alumina pans, with an initial mass of 7mg, between 25 and 650°C at 10°C/min under N_2 atmosphere using a TGA Q50 (TA INSTRUMENTS, New Castle, DE).

Differential Scanning Calorimetry (DSC)

Analyses were performed using a DSC Pyris calorimeter (PERKIN ELMER, Waltham, MA) The calorimeter was calibrated at low temperatures using Hg and In as standards, resulting in a temperature accuracy of ±0.1°C and an enthalpy accuracy of ± 0.2 J/g. Samples, 10 mg in weight, were set into aluminium pans and equilibrated at the initial temperature during 5 minutes before heating at 20°C/min.

Dynamic Dielectric spectrometry (DDS)

The dielectric measurements were performed using a broad-band dielectric spectrometer BDS 4000 system (NOVOCONTROL TECHNOLOGIES GmbH & Co. KG, Hundsangen, Germany). Samples were kept in a special cell usually devoted to biological samples consisting of two stainless steel electrodes surrounded by a Teflon ring. The diameter of the electrodes was 15 mm, and the thickness of the samples was 0.1 mm (powdered sample, gently compressed).

Isothermal measurements of the complex dielectric function $\varepsilon^*=\varepsilon'-i\varepsilon''$were performed at every fifth degree, with an isothermal stability of ± 0.1°C, from -145 to 100°C in the frequency range 10^{-2} to 3.10^6 after a first heating at 100°C to dehydrate the sample. The experimental limit for the loss factor ($\tan\delta = \varepsilon''/\varepsilon'$) was about 10^{-4}.

TABLES

Band position (cm^{-1})		Tentative of assignment
Supernatant from control VSMC	Supernatant from agLDL-VSMC	
3446, 3361, 3197	3347, 3361	amide A H-bonded ν(O-H) and ν(N-H)
2955, 2922, 2850, 2827	2948, 2821	ν(C-H) aliphatic

1651, 1632	1652, 1633	amide Iν(C=O)
1591	1588	ν(C-C) ring Tyr, Phe
1548	1548	amide II ν(CN), δ(NH)
1464-1452	1464-1452	δ(CH_2,CH_3) aliphatic side chains
1408	1408	ν(COO-)
1224	1235	amide III δ_{plan} (N-H)
1206	1205	δ(COH)Tyr
1178	1187	γ(CH_2) Pro, Tyr
1039	1039	ν(C-C), ν(C-O), Phe, Ser
930	930	ν(Cα-C) characteristic of α-helices.
530	530	ν(S-S)

Table S1: FTIR bands assignment of supernatants fractions

Band position (cm^{-1})	assignment
agLDL	
3450-3000	ν(O-H) of bound water
2958, 2927, 2854	asymmetric and symmetric methyl and methylene stretching
1734	ν(C=O) of ester bond
1645	amide I ν(C=O)
1539	amide II ν(CN), δ(NH)
1464	δ(CH_2) (scissoring) in acyl chains
1378	δ_s(CH_3) (bending),δ(CH_2) (wagging)
1241	P=O stretching
1063	ester C-O-C symmetric stretching
969	$N^+(CH_3)_3$ asymmetric stretching from choline group in phospholipids

Table S2: FTIR bands assignment of agLDL

Supernatant	1^{st} stage (25-150°C)			2^{nd} stage (150-450°C)		
	Δm (%)	T_{max} (°C)	T_{max} (°C)	Δm (%)	T_{max} (°C)	T_{max} (°C)
From control VSMC	8.2	56	100-130	26.5	301	335
From agLDL-VSMC	6.4	56	126	29.6	292	332

Table S3: Thermal parameters of purified supernatants (from control and agLDL loaded VSMC).