Novel method for modification of vein surface by Fetuin A and its optical characterization

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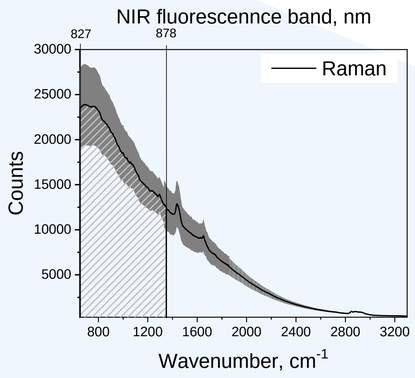
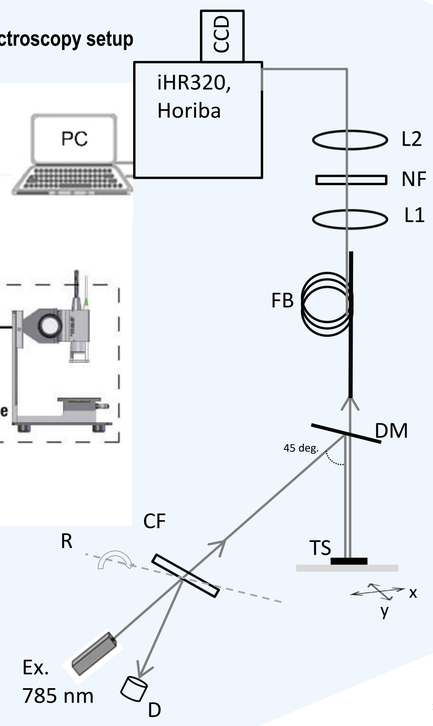
The aim: in the present work, we report on modification of vein samples by Fetuin-A and application of optical methods (Raman spectroscopy, NIR–fluorescence, optical coherence tomography) for characterization of samples to detect the immobilized Fetuin-A

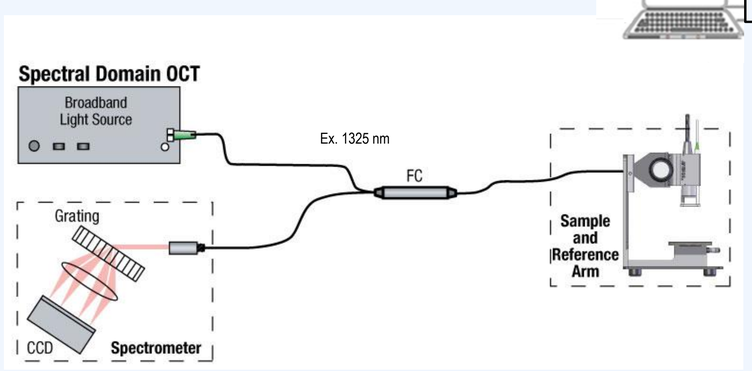
Methods: bovine jugular vein xenogenic material preparation and optical testing

Spectra preprocessing: polynomial fit to separate Raman and NIR fluorescence



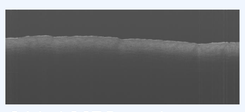
Raman spectroscopy setup





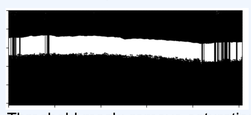
Pipeline of OCT image processing

Step #1



Original OCT B-scan image

Step #2



Threshold mask: remove saturation lines, crop the image, fill voids

Step #3

Overlap threshold mask and original image
Apply sliding window analysis
(15x15 pixels/ 53x53 μm, stride=1)

Step #4

Array is created with corresponding parameter values for each binned pixel

0.055
0.053
0.047
0.054
0.049
0.056
0.55
...

i) coefficient of variation
ii) mean of intensity
iii) standard deviation
iv) range of intensity values

Basic preparation of tissue patches

Removing excess of glutaraldehyde
- chemical treatment
- protein treatment

Treatment with Fetuin-A
- only Fetuin-A
- dopamine coating

Optical characterization of the samples:
- Raman & NIR fluorescence spectroscopy
- optical coherence tomography (OCT)

In vitro shear stress, biostability vs calcification conditions testing

In vitro biocompatibility and cell culture calcification testing

In vivo calcification studies

Medical device requirements-conformed & industry-oriented biomaterial development

Table 1. Sample preparation protocol

#	Sample type	Glutaraldehyde fixed	Chemical treatment	Protein treatment	Fetuin-A incubation	Treatment condition
1.	Control	Yes	-	-	-	Fat, connective, and muscle tissue surgical removal from a vein; Glutaraldehyde (0,625%) 21 days fixation at room temperature (RT)
2.	Control (reduced glutaraldehyde)	Yes	$NaBH_4$	-	-	0.1M $NaBH_4$ (pH 7.4, RT, 24 h)
3.		Yes	-	Glycine	-	0.1M Glycine (pH 4.5), 37°C, 48 h (or 4°C/ 24 h)
4.		Yes	-	L-glutathione	-	10% Citric acid, RT, 30 min
5.		Yes	$NaBH_4$	Glycine	-	0.1M Glycine (pH 4.5, 37°C, 48 h) + 0.1M $NaBH_4$ (pH 7.4, RT, 24 h)
6.		Yes	$NaBH_4$	L-glutathione	-	10% Citric acid (30 min, RT) + 0.1M $NaBH_4$ (pH 7.4, RT, 24 h)
7.	Fetuin-A only	Yes	-	-	Yes	Incubated in 0.5 mg/ml Fetuin-A solution in $NaHCO_3$ (RT, 24 h) on a rotator (120 rpm)
8.	Control (reduced glutaraldehyde), with Fetuin-A	Yes	-	Glycine	Yes	above-stated
9.		Yes	-	L-glutathione	Yes	above-stated
10.		Yes	$NaBH_4$	-	Yes	above-stated
11.		Yes	$NaBH_4$	Glycine	Yes	above-stated
12.		Yes	$NaBH_4$	L-glutathione	Yes	above-stated
13.	Dopamine coated	Yes	$C_8H_{11}NO_2$	-	-	2 mg/ml Dopamine in 0.1 M Tris (pH 8.5).
14.	Dopamine coated, with Fetuin A	Yes	$C_8H_{11}NO_2$	-	Yes	above-stated

Table 2.Assignment of functionalization impact on Raman spectral bands in vein samples

Wavenuber (cm^{-1})	Mode assignment	Compound assignment	Dopamine treatment *vs* control	Fetuin-A treatment *vs* control	Dopamine & Fetuin-A vs. dopamine (only)
786	ν_{asym}(O - P -O) U, T C	DNA, nucleic acids, aromatic rings vibrations in polydopamine.	↑	↑	NS
805	δ C-H (-CHO)	glutaraldehyde. out-of-plane ring breathing in polydopamine	↑	NS	NS
815	ν_{asym}(O-P-O), RNA phosphate backbone ring br. Tyr	RNA Proteins	↑	↑	↓
903, 909	δ C-H (-CHO)	glutaraldehyde	↑	NS	NS
1031	*ν*C-O *ν*(C-O-C-O/ C-OH)	proteins, GA– protein crosslinking, glutaraldehyde	↓	NS	↓
1099	*ν*(PO_2^-)	nucleic acids	↑	NS	NS
1175	C-H in -plane bend. Tyr& Phe C, G	proteins nucleic acids	NS	↑	NS
1224, 1292	amide III *ν*(C-N),N-H bnd. ν_{asym} (PO_2)	proteins nucleic acids	↑	↑	NS
1448	CH_2 bend. δC-H (-CH_2)	Proteins glutaraldehyde	↓	↑	NS
1528	*ν*C=C	indole ring of polydopamine	↑	NS	NS
1656	*ν*(C=C) amide I *ν*(C=O), *ν*(C-N), N-H bend	lipids, proteins	NS	↑	NS
1946, 1979	*ν* C=O	carbonyl stretching vibrations in Fetuin-A conjugated with polydopamine; vibrations in extended π-systems, due to interaction between polydopamine's aromatic rings and Fetuin-A	↑	NS	↑
2178	ν C≡N ν C≡C	oxidation and polymerization of dopamine into polydopamine	↑	NS	NS
2847	*ν* C-H	conjugation induced CH_3 symmetric stretching loss	NS	↑	NS
2880	ν_{sym} C-H (-CH2)	glutaraldehyde/fetuin-A cross-linking lead to more effective Raman scattering	↓	↑	NS
2939	*ν* (C-H)	glutaraldehyde	↓	↑	↓
3279	*ν* O-H	glutaraldehyde	↑	NS	NS

Results

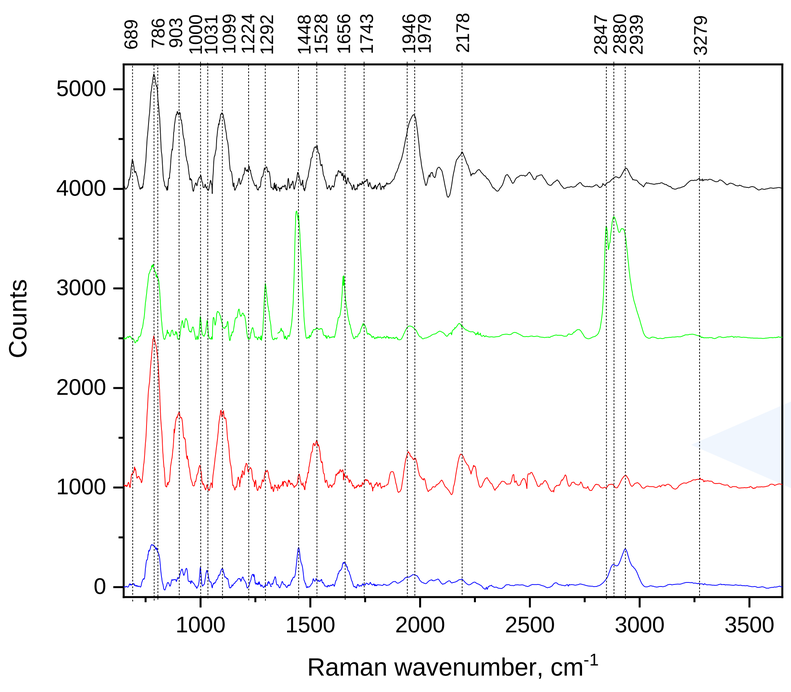


Fig. 1 Immobilization of Fetuin-A on vein tissue patches was carried out using two approaches: (i) by incubating the patches in a Fetuin-A solution, and (ii) by pre-treating the patches with dopamine; the latter demonstrated enhanced binding affinity/interaction of Fetuin-A to bioprosthetic material

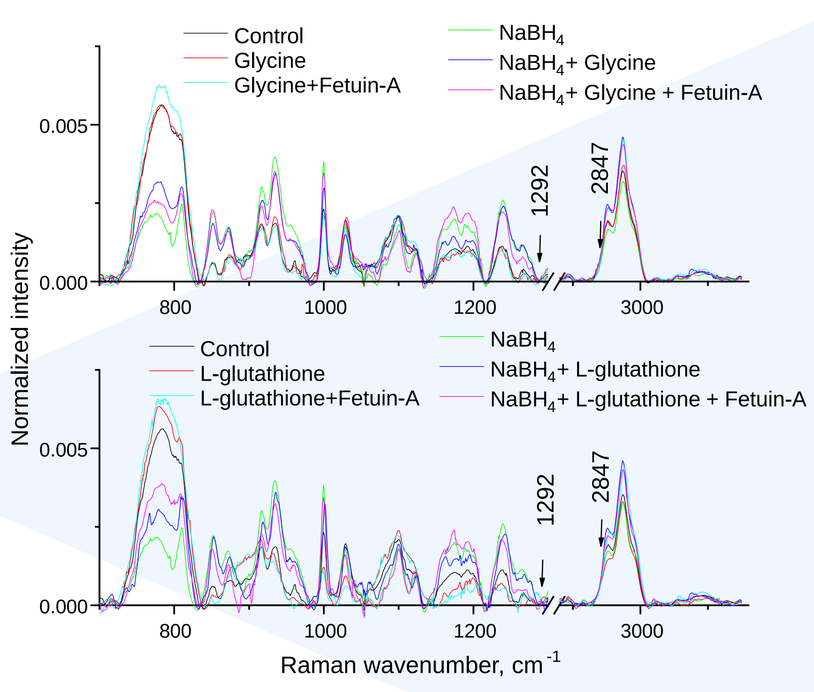


Fig. 2 Excess of glutaraldehyde was removed through protein treatment and/or chemical treatment with $NaBH_4$, which led to a reduction in Fetuin-A binding affinity to the inner surface of the veins. The Raman spectra have been normalized by area under the curve

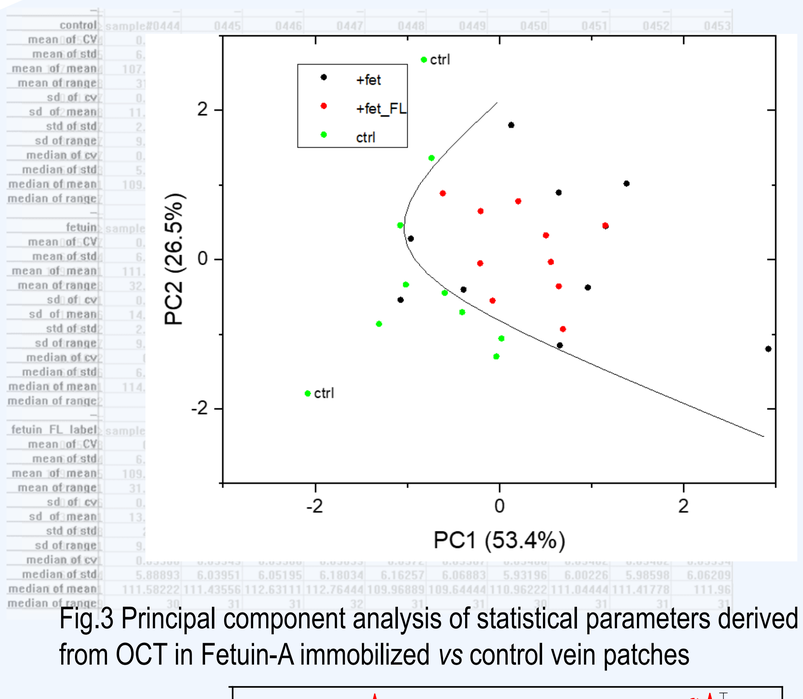


Fig.3 Principal component analysis of statistical parameters derived from OCT in Fetuin-A immobilized *vs* control vein patches

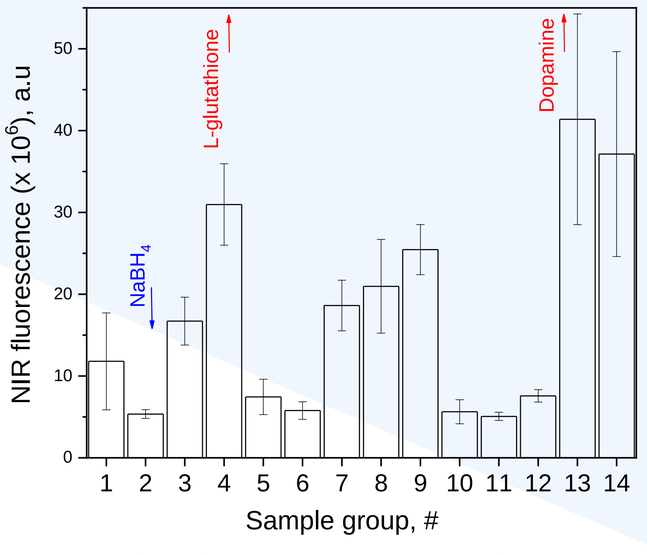


Fig.4 Analysis of NIR-fluorescence intensity data from the samples

Conclusion: different strategies to immobilize Fetuin-A onto elastin-rich tissue patches were optically evaluated *in vitro*, and the optimal method (applying polydopamine coating before Fetuin-A functionalization) was identified for subsequent anticalcification properties testing

Latvian Council of Science

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