

Acrylate-based materials for heart valve scaffold engineering

Rosaria Santoro^{a,§}, Seshasailam Venkateswaran^{b,§}, Francesco Amadeo^a, Rong Zhang^{b,c}, Maura Brioschi^d,
Anthony Callanan^e, Marco Agrifoglio^f, Cristina Banfi^d, Mark Bradley^{b,*} and Maurizio Pesce^{a,*}

Supplementary methods

Label-free LC-MSE analysis

Briefly, tryptic digests from coated and uncoated samples were then prepared adding yeast alcohol dehydrogenase (ADH) digest and Hi3 Ecoli standards (Waters Corporation, Milford, MA, USA) at the final concentration of 12.5 fmol/μl, as internal standards for molar amount estimation (Silva 2006) and quality controls. Tryptic peptides separation was conducted with a TRIZAIC nanoTile (Waters Corporation, Milford, MA, USA) using a nano-ACQUITY -UPLC System coupled to a SYNAPT-MS Mass Spectrometer equipped with a TRIZAIC source (Waters Corporation, Milford, MA, USA) ¹. Tryptic digest (4μl) were analyzed in triplicate for each biological sample. Calibration and lockmass correction were performed as previously described. Precursor ion masses and their fragmentation spectra were acquired in MSE mode as previously described ². In order to obtain a qualitative and quantitative analysis of proteins associated with coated and uncoated scaffolds.

Quantitative proteomic analysis

The software Progenesis QI for proteomics (Version 2.0, Nonlinear Dynamics, Newcastle upon Tyne, UK) was used for the quantitative analysis of peptide features and protein identification. A UniProt database (release 2015-3; number of human sequence entries, 20199; number of bovine sequence entries, 6870) was used for database searches. Fold changes in the quantitative expression, p-value and Q-value were calculated with the statistical package included in Progenesis QI for proteomics, using only peptides uniquely associated to the proteins to quantify proteins that were part of a group. A P value <0.05 was considered significant. The significance of the regulation level was determined at a 20% fold change, considering only proteins quantified with at least 2 peptides. Data were further filtered, after manual inspection of the results,

by considering only the proteins with the same modulation in at least two out of three biological replicates.

The data set was also subjected to unsupervised PCA analysis.

Monomer Nomenclature	Name
AAG-H	2-acrylamidoglycolic acid
AES-H	mono-2-(acryloyoxy)ethyl succinate
A-H	acrylic acid
BAEMA	2-(tert-butylamino)ethyl methacrylate
BACOEa	2-[[[(butylamino)carbonyl]oxy]ethyl acrylate
BMA	n-butyl methacrylate
DAAA	diacetone acrylamide
DEAA	Diethylacrylamide
DEAEA	2-(diethylamino)ethyl acrylate
DEAEMA	diethylaminoethyl methacrylate
DEGMEMa	di(ethyleneglycol) methyl ether methacrylate
DMAA	dimethyl acrylamide
DMAPMaA	N-[3-(dimethylamino)propyl] methacrylamide
DMVBA	N,N-dimethylvinylbenzylamine
EMA	ethyl methacrylate
GMA	glycidyl methacrylate
HBMA	hydroxybutyl methacrylate
HEA	2-hydroxyethyl acrylate
HEMA	2-hydroxyethyl methacrylate
HPMA	Hydroxypropylmethacrylate
MA	methyl Acrylate
MA-H	methacrylic acid
MEA	2-methoxyethyl acrylate
MMA	methyl methacrylate
MEMA	methoxyethyl methacrylate
MTEMA	2-(methylthio)ethyl methacrylate
MNPMA	2-methyl-2-nitropropyl methacrylate
PAA	N-isopropyl acrylamide
St	Styrene
THFFA	tetrahydrofurfuryl acrylate
THFFMA	tetrahydrofurfuryl methacrylate
VAA	N-vinylacetamide
VI	1-vinylimidazol
VP-2	2-vinylpyridine
VP-4	4-vinylpyridine

VPNO	1-vinyl-2-pyrrolidinone
------	-------------------------

Table S1. Monomers used to manufacture the polymers used to generate the array.

Amine Nomenclature	Name
BnMA	N-benzylmethylaniline
cHMA	Cyclohexanemethylaniline
DBnA	Dibenzylaniline
DcHA	Dicyclohexylaniline
DnHA	di-n-hexylaniline
DEMEDA	N,N-diethyl-N'-methylethylenediamine
DnBA	di-n-butylaniline
MAEPy	2-(2-methylaminoethyl)pyridine
MAn	N-methylaniline
MnHA	N-methylhexylaniline
Mpi	1-methylpiperazine
Pyrle	Pyrrole
TEDETA	N,N,N',N'-tetraethyldiethylenetriamine
TMEDA	N,N,N-trimethylethylenediamine
TMPDA	N,N,N'-trimethyl-1,3-propanediamine

Table S2. Amines used for GMA polymer functionalization.

Gene	Direction	Sequence
hALP	Forward	TCACTCTCCGAGATGGTGGT
hALP	Reverse	GTGCCCCGTGGTCAATTCT
hRunx2	Forward	TCTGGCCTTCCACTCTCAGT
hRunx2	Reverse	GACTGGCGGGGTGTAAGTAA
hOPN	Forward	GAGGGCTTGGTTGTCAGC
hOPN	Reverse	CAATTCTCATGGTAGTGAGTTTTCC
hBMP2	Forward	TGTATCGCAGGCACTCAGGTC
hBMP2	Reverse	TTCCCACTCGTTTCTGGTAGTTCTT

Table S3. 5'-3' sequences of the primers used to screen human VICs phenotype in real time RT-PCR experiments.

Monomer (1)	Monomer (2)	Monomer (3)	Ratio (mol)			Functionalization
			M (1)	M (2)	M (3)	
MEMA	DEAEA		50	50		
MEMA	BAEMA		90	10		
MEMA	BAEMA		70	30		
MEMA	VP-4		50	50		
HEMA	BAEMA		50	50		
HEMA	DMVBA		50	50		
HEMA	VI		70	30		
HEMA	VP-4		70	30		
MEMA	GMA		90	10		DnBA
MEMA	GMA		90	10		MAn
MEMA	GMA		70	30		TMPDA
MEMA	GMA		50	50		TMPDA
MMA	GMA		90	10		MnHA
MMA	GMA		70	30		MnHA
MMA	GMA		90	10		BnMA
MMA	GMA		50	50		BnMA
MMA	GMA		50	50		MAEPy
MMA	DEAEMA		90	10		
MMA	DMAEA		70	30		
MEMA	DEAEA	DAAA	40	30	30	
MEMA	DEAEMA	MMA	60	30	10	
MEMA	DEAEA	MMA	60	30	10	
MEMA	DEAEMA		55	45		

Table S4. Chemical compositions, molar ratios and functionalization of the polymers that demonstrated a VICs adhesion score = 2.

Description	Peptide count	Peptides used for quantitation	Confidence score
40S ribosomal protein S10 OS=Homo sapiens GN=RPS10 PE=1 SV=1	2	2	16.9692
40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4	6	6	38.7609
60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2	6	6	35.2543
78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2	22	3	181.5422
Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1	40	9	360.1381
Actin-related protein 3 OS=Homo sapiens GN=ACTR3 PE=1 SV=3	2	2	12.3792
ADP-ribosylation factor 3 OS=Homo sapiens GN=ARF3 PE=1 SV=2	9	4	63.0735
ADP-ribosylation factor 4 OS=Homo sapiens GN=ARF4 PE=1 SV=3	9	2	59.2381
Aldo-keto reductase family 1 member C3 OS=Homo sapiens GN=AKR1C3 PE=1 SV=4	4	3	23.9868
Alpha-actinin-1 OS=Homo sapiens GN=ACTN1 PE=1 SV=2	2	2	10.687
Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2	3	2	17.1712
Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	13	4	98.6375
Annexin A1 OS=Homo sapiens GN=ANXA1 PE=1 SV=2	14	13	116.0018
Annexin A2 OS=Homo sapiens GN=ANXA2 PE=1 SV=2	22	4	210.9559
Annexin A6 OS=Homo sapiens GN=ANXA6 PE=1 SV=3	3	2	20.8679
ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3	9	7	66.0634
ATP-citrate synthase OS=Homo sapiens GN=ACLY PE=1 SV=3	2	2	12.5667
Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2	3	3	18.6303
Calreticulin OS=Homo sapiens GN=CALR PE=1 SV=1	5	2	36.0071
Cathepsin B OS=Homo sapiens GN=CTSB PE=1 SV=3	4	3	39.4488
Cell division control protein 42 homolog OS=Homo sapiens GN=CDC42 PE=1 SV=2	2	2	13.0404
Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5	18	2	111.8109
Clathrin heavy chain 2 OS=Homo sapiens GN=CLTCL1 PE=1 SV=2	4	2	23.8112
Coagulation factor V OS=Homo sapiens GN=F5 PE=1 SV=4	13	3	101.8484
Cofilin-1 OS=Homo sapiens	3	3	24.3757

GN=CFL1 PE=1 SV=3			
Collagen alpha-1(VI) chain OS=Homo sapiens GN=COL6A1 PE=1 SV=3	9	7	83.5377
Collagen alpha-2(VI) chain OS=Homo sapiens GN=COL6A2 PE=1 SV=4	7	5	45.5679
Collagen alpha-3(VI) chain OS=Homo sapiens GN=COL6A3 PE=1 SV=5	62	54	568.9731
Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=2	9	3	52.2116
Cytoskeleton-associated protein 4 OS=Homo sapiens GN=CKAP4 PE=1 SV=2	20	15	142.6946
D-3-phosphoglycerate dehydrogenase OS=Homo sapiens GN=PHGDH PE=1 SV=4	5	5	25.4388
Desmin OS=Homo sapiens GN=DES PE=1 SV=3	9	3	55.5057
Dolichyl-diphosphooligosaccharide- -protein glycosyltransferase subunit 1 OS=Homo sapiens GN=RPN1 PE=1 SV=1	3	3	18.3067
Dolichyl-diphosphooligosaccharide- -protein glycosyltransferase subunit 2 OS=Homo sapiens GN=RPN2 PE=1 SV=3	5	4	25.9487
Elongation factor 1-gamma OS=Homo sapiens GN=EEF1G PE=1 SV=3	7	5	45.0429
Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4	4	4	23.3417
EMILIN-1 OS=Homo sapiens GN=EMILIN1 PE=1 SV=2	20	18	155.6316
Endoplasmic reticulum chaperone GN=HSP90B1 PE=1 SV=1	18	5	135.0901
Extended synaptotagmin-1 OS=Homo sapiens GN=ESYT1 PE=1 SV=1	3	2	16.4926
Fibronectin OS=Homo sapiens GN=FN1 PE=1 SV=4	77	31	786.6938
Fibulin-2 OS=Homo sapiens GN=FBLN2 PE=1 SV=2	11	11	85.8633
Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4	37	29	229.4901
Filamin-C OS=Homo sapiens GN=FLNC PE=1 SV=3	5	3	29.5847
Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2	3	3	24.3995
Glucose-6-phosphate 1- dehydrogenase OS=Homo sapiens GN=G6PD PE=1 SV=4	2	2	12.7813
Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3	20	8	205.7743
Guanine nucleotide-binding protein subunit beta-2-like 1 OS=Homo sapiens GN=GNB2L1 PE=1 SV=3	5	5	31.9078

Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5	11	5	67.62
Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1	15	8	111.2648
Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2	4	3	33.7317
Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4	9	5	49.0821
Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=1 SV=4	3	2	18.3377
Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2	4	4	25.4431
Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2	2	2	13.3738
Histone H3.1 OS=Homo sapiens GN=HIST1H3A PE=1 SV=2	7	6	32.4605
Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2	10	10	73.5387
Hypoxia up-regulated protein 1 OS=Homo sapiens GN=HYOU1 PE=1 SV=1	3	3	16.4668
Integrin-linked protein kinase OS=Homo sapiens GN=ILK PE=1 SV=2	2	2	12.1267
Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens GN=ITIH2 PE=1 SV=2	5	4	45.0825
Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6	7	4	50.1221
Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3	6	4	37.0856
Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6	11	7	76.71
Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2	5	2	31.7762
Keratin, type II cytoskeletal 7 OS=Homo sapiens GN=KRT7 PE=1 SV=5	11	5	69.9343
Lamin-B1 OS=Homo sapiens GN=LMNB1 PE=1 SV=2	5	2	31.6538
L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2	4	2	26.3436
Malate dehydrogenase, mitochondrial OS=Homo sapiens GN=MDH2 PE=1 SV=3	7	3	59.7112
Microfibril-associated glycoprotein 4 OS=Homo sapiens GN=MFAP4 PE=1 SV=2	3	3	32.8357

Myosin light polypeptide 6 OS=Homo sapiens GN=MYL6 PE=1 SV=2	6	6	59.2082
Myosin-10 OS=Homo sapiens GN=MYH10 PE=1 SV=3	63	14	498.9244
Myosin-11 OS=Homo sapiens GN=MYH11 PE=1 SV=3	25	8	163.744
Myosin-14 OS=Homo sapiens GN=MYH14 PE=1 SV=2	9	4	65.4783
Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4	73	47	614.2162
NADH-cytochrome b5 reductase 3 OS=Homo sapiens GN=CYB5R3 PE=1 SV=3	3	3	18.8524
Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3	5	4	34.6812
Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2	7	4	48.924
Phosphoglycerate mutase 1 OS=Homo sapiens GN=PGAM1 PE=1 SV=2	4	4	36.1484
Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1	42	36	384.8055
Profilin-1 OS=Homo sapiens GN=PFN1 PE=1 SV=2	4	3	29.832
Prostacyclin synthase OS=Homo sapiens GN=PTGIS PE=1 SV=1	2	2	12.3836
Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4	7	7	48.0058
Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3	14	12	117.4338
Protein-glutamine gamma- glutamyltransferase 2 OS=Homo sapiens GN=TGM2 PE=1 SV=2	22	20	183.6525
Putative elongation factor 1-alpha- like 3 OS=Homo sapiens GN=EEF1A1P5 PE=5 SV=1	13	13	105.1561
Pyruvate kinase PKM OS=Homo sapiens GN=PKM PE=1 SV=4	25	20	211.3167
Ras-related protein Rab-10 OS=Homo sapiens GN=RAB10 PE=1 SV=1	4	2	27.1474
Ras-related protein Rab-1B OS=Homo sapiens GN=RAB1B PE=1 SV=1	7	2	56.1081
Ras-related protein Rab-7a OS=Homo sapiens GN=RAB7A PE=1 SV=1	2	2	12.5688
Ras-related protein Rap-1b OS=Homo sapiens GN=RAP1B PE=1 SV=1	5	5	36.4749
Sentatin OS=Homo sapiens GN=SNTN PE=2 SV=1	2	2	9.8974
Serine protease HTRA1 OS=Homo sapiens GN=HTRA1 PE=1 SV=1	3	2	20.3164
Serpin H1 OS=Homo sapiens GN=SERPINH1 PE=1 SV=2	17	7	134.7397

Surfeit locus protein 4 OS=Homo sapiens GN=SURF4 PE=1 SV=3	2	2	12.2203
Talin-1 OS=Homo sapiens GN=TLN1 PE=1 SV=3	2	2	10.4772
Transgelin-2 OS=Homo sapiens GN=TAGLN2 PE=1 SV=3	3	3	19.3578
Tubulin beta-1 chain OS=Homo sapiens GN=TUBB1 PE=1 SV=1	5	2	36.1512
Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens GN=RPS27A PE=1 SV=2	5	4	37.274
Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens GN=UBA1 PE=1 SV=3	7	3	43.523
Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4	39	8	349.7115
Vitronectin OS=Homo sapiens GN=VTN PE=1 SV=1	3	3	24.5187
Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens GN=VDAC1 PE=1 SV=2	6	6	41.9636

Table S5. List (in alphabetical order) of the 100 proteins identified by the Mass-spectrometry analysis of the PCL scaffolds (coated and uncoated) seeded with human VICs and cultured for 14 days. In blue are indicated the proteins with a documented relevance in valve ECM composition. Indicated are the peptide counts, the number of peptides used for identification and the confidence score.

Description	UniProt Accession	Peptides used for quantitation	Score	Anova <i>P</i> -value	Max fold change	fold change CV (%)
Microfibril-associated glycoprotein 4	P55083	3/3	32.8	1.1 ⁻¹⁶	15.36	10.0
Annexin A1	P04083	13/14	116.0	2.19 ⁻¹⁰	1.48	6.0
Cathepsin B	P07858	3/4	39.44	1.33 ⁻⁸	1.92	28.6
Neutral alpha-glucosidase AB	Q14697	4/5	34.7	5.62 ⁻⁶	1.39	50.5
Actin-related protein 3	P61158	2/2	12.4	3.82 ⁻⁶	1.93	34.2
Extended synaptotagmin-1	Q9BSJ8	2/3	16.5	0.000106	3.39	86.1
Pyruvate kinase PKM	P14618	20/25	211.3	0.000951	1.42	14.2
Clathrin heavy chain 1	Q00610	2/18	111.8	0.01708	1.22	47.9
Guanine nucleotide-binding protein subunit beta-2-like 1	P63244	5/5	31.9	0.004361	1.30	23.1
ATP-citrate synthase	P53396	2/2	12.6	0.005665	1.51	16.1
Ubiquitin-like modifier-activating enzyme 1	P22314	3/7	43.55	0.007769	2.61	18.6
Calreticulin	P27797	2/5	36.04	0.019216	1.65	61.6

Table S6. List of the human proteins significantly upregulated in PCL-PA scaffolds ordered according to decreasing ANOVA *P* values. These data are included in Figure 7B.

Description	UniProt Accession	Peptides used for quantitation	Score	Anova <i>P</i> -value	Max fold change	fold change CV (%)
Lamin-B1	P20700	2/5	31.6	2.46 ⁻¹¹	2.25	10.9
Ubiquitin-40S ribosomal protein S27a	P62979	4/5	37.3	1.42 ⁻⁹	1.63	10.4
Annexin A6	P08133	2/3	20.9	3.81 ⁻⁷	2.89	49.1
D-3-phosphoglycerate dehydrogenase	O43175	5/5	25.4	1.7 ⁻⁷	1.43	16.0
ADP-ribosylation factor 3	P61204	4/9	63.1	0.000132	1.64	25.3
Cell division control protein 42 homolog	P60953	2/2	13.0	0.000519	1.51	9.6
Histone H1.4	P10412	2/2	13.4	0.00854	1.70	27.8
Histone H3.1	P68431	6/7	32.5	0.017908	2.30	54.4
Coagulation factor V	P12259	3/13	101.8	0.030755	1.37	19.7
L-lactate dehydrogenase A chain	P00338	2/4	26.3	0.042941	1.27	3.3
Histone H4	P62805	10/10	73.6	0.047149	2.38	83.3
Heterogeneous nuclear ribonucleoproteins A2/B1	P22626	4/4	25.4	0.048413	1.81	38.5

Table S7. List of the human proteins expressed at higher level in uncoated PCL scaffolds ordered according to decreasing ANOVA *P* values. These data are included in Figure 7B.

References

1. M. Roverso, M. Brioschi, C. Banfi, S. Visentin, S. Burlina, R. Seraglia, P. Traldi and A. Lapolla, *European journal of mass spectrometry (Chichester, England)*, 2016, **22**, 71-82.
2. M. Brioschi, S. Eligini, M. Crisci, S. Fiorelli, E. Tremoli, S. Colli and C. Banfi, *Analytical and Bioanalytical Chemistry*, 2014, **406**, 2817-2825.