Polysaccharide-Coated Thermosets For Orthopaedic Applications: From Material Characterization To *In Vivo* Tests

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SUPPORTING INFORMATION

Contact angles and surface energy

According to the thermodynamics approach followed by Young[3], the wetting of a solid surface by a liquid at the equilibrium is ruled by the equation:

$$\gamma_l \cos \theta = \gamma_s - \gamma_{sl} \quad (1)$$

where θ is the contact angle, γ_l is the surface tension of the liquid, γ_{sl} is the interfacial tension of the solid/liquid interface, and γ_s is the surface tension of solid.

Each tension γ of a solid or liquid is given by the sum of Lifshitz–van der Walls and the Lewis acid-base components:

$$\gamma = \gamma^{LW} + \gamma^{AB} \quad (2)$$

where γ^{LW} designates the Lifshitz–van der Walls contribution due to the long range interactions (i.e. the dispersive interaction, the dipole–dipole interaction, and dipole–induced dipole interaction) and γ^{AB} designates acid–base interactions (hydrogen bonding type) and is given by the following equation:

$\gamma^{AB}=2(\gamma^+ \gamma^-)^{1/2}$ (3)

where γ^+ and γ^- refer to electron-acceptor and electron-donor character, respectively.

The interfacial energy γ_{sl} was defined by van Oss[3]as:

$\gamma_{sl} = [(\gamma_s^{LW})^{1/2} - (\gamma_l^{LW})^{1/2}]^2 + 2[(\gamma_s^- \gamma_s^+)^{1/2} + (\gamma_l^- \gamma_l^+)^{1/2} - (\gamma_l^- \gamma_s^+)^{1/2} - (\gamma_s^- \gamma_l^+)^{1/2}]$ (4)

By combining equations (1-4) the Young-Duprè equation is obtained:

$(1 + \cos\theta)\gamma_l = 2[(\gamma_s^{LW} \gamma_l^{LW})^{1/2} + (\gamma_s^+ \gamma_l^-)^{1/2} + (\gamma_s^- \gamma_l^+)^{1/2}]$

In the acid-base method, the contact angles of at least three liquids with known values of γ^{LW}, γ^+ and γ^- are measured and the values of each experiment are used in the Young-Duprè equation which allows the calculation of all γ_s components solving the three simultaneous equations generated.

Considering the thermodynamic work of adhesion between surface and liquids, the total work is given by $W^T = W^{AB} + W^{LW}$ where: $W^{AB} = (1 + \cos\theta)\gamma_l - 2[(\gamma_s^{LW} \gamma_l^{LW})^{1/2}$ and $W^{LW} = 2[(\gamma_s^{LW} \gamma_l^{LW})^{1/2}$

The detailed theoretical description of this approach can be found in the literature[3]. The contact angle values obtained for the different samples are listed in Table S2.

	Contact angles for liquids θ (°)		
Material	Water ($\gamma^{TOT} = 72.8$)	Ethylene Glycol ($\gamma^{TOT} = 48.0$)	Diiodomethane ($\gamma^{TOT} = 50.8$)
TS	75.9 ± 2.7	40.4 ± 2.7	31.4 ± 1.8
HCl-TS	77.0 ± 1.9	43.4 ± 1.6	32.0 ± 1.8
Chitlac-TS	70.9 ± 2.7a	28.9 ± 2.0a	39.6 ± 1.2a

Table S1. Contact angles of three reference liquids on untreated thermosets (TS), acid-treated thermosets (HCl-TS) and Chitlac-coated thermosets (Chitlac-TS). Apex "a" indicates significant differences of angle values between Chitlac-TS and both TS and HCl-TS obtained by Student's t test (p<0.01).

The surface free energy parameters and work of adhesion for the different samples are listed in Table S2.

Material	Surface free energy parameters (mJ/m²)					Polarity (%)	Work of adhesion			
	γ^{LW}	γ^+	γ^-	γ^{AB}	γ^{TOT}		water		ethylene glycol	
							W^{AB} (mJ/m²)	W^{AB} (%)	W^{AB} (mJ/m²)	W^{AB} (%)
TS	43.6± 0.8	0.26± 0.01	5.53± 1.23	2.4± 0.26	46.00± 0.84	5.21± 0.57	28.8	31.8	13.4	15.9
HCl-TS	43.4± 0.8	0.16± 0.02	5.47± 0.87	1.87± 0.19	45.27± 0.19	4.13± 0.42	27.8	31.1	12.1	14.6
Chitlac-TS	39.8± 0.6	1.16± 0.09	7.07± 1.57	5.73± 0.67	45.53± 0.90	12.58± 1.49	37.9	39.1	22	24.5

Table S2: Free energy parameters and work of adhesion of untreated thermosets (TS), acid-treated thermosets (HCl-TS) and Chitlac-coated thermosets (Chitlac-TS). γ^{LW} is the apolar term of the specimens surface free energy; γ^+ and γ^-, are respectively the acid and base terms of the specimens surface free energy; γ^{AB} is the polar term of the specimens surface free energy. γ^{TOT} is the total surface free energy of the specimen and is given by the sum of γ^{LW} and γ^{AB}. The polarity is the ratio between γ^{AB} and γ^{TOT}. Work of adhesion is due to acid-base interactions between polar liquids and surfaces.

ATR/FTIR

ATR/FTIR analysis was performed at 700–4000 cm^{-1} using a Perkin-Elmer Spectro One-B spectrometer equipped with a golden gate MK II single reflection diamond ATR systems (Specac). The samples were analyzed directly on an ATR platform. For all samples, 16 spectra were collected and the mean curve had background correction and normalization to a factor 15%.

ATR-FTIR method was used to investigate changes in functional groups of the thermosets upon surface modifications. Figure S3 shows the infrared spectra of surface modified thermosets in comparison with the two controls, namely the unmodified thermoset (TS) and lyophilized Chitlac, while Table S3 reports the values of the main peaks of both compounds and their assignments.
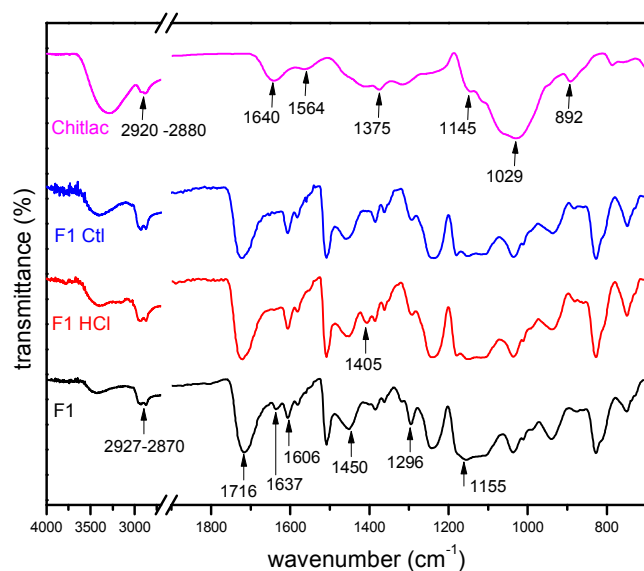
Figure S3: ATR-FTIR spectra of modified thermosets with polysaccharide-based coatings: the unmodified thermoset (TS, black), HCl treated thermoset (HCl-TS, red), Chitlac coated (Chitlac-TS, blue) and the lyophilized polysaccharide (Chitlac, purple).

Chitlac (lactose-modified chitosan)		BisGMA/TEGDMA	
Peak	Assignment	Peak	Assignment
3290	ν OH – ν NH	3426	ν OH
2920	ν CH of CH_2 (asymmetrical)	2927	ν CH (asymmetrical)
2880	ν CH of CH_2 (symmetrical)	2870	ν CH (symmetrical)
1640	ν C=O (amide I) or NH_3^+	1716	ν C=O
1564	δ N-H	1637	ν C=C monomers (aliphatic)
1375	ν CH in methyl group	1606	ν C=C (aromatic)
1145	ν C-O-C (glycosydic linkage)	1450	ν (O-CH_3)
1029	ν C_6-O	1296	ν C-O monomers
892	CH rocking and bending of glycosydic linkage	1155	vibration of ester groups

Table S3. ATR-FTIR peak assignation of the polysaccharide (Chitlac) and BisGMA/TEGDMA thermoset (TS).

As summed up in Table S3, both materials display a wide peak between 3500 and 3200 cm^{-1} assigned to OH and NH (in the case of Chitlac) stretching vibrations and display two CH stretching peaks in the wavelength interval 2930-2870 cm^{-1}, which are overlapping in the coated samples Chitlac-TS. In the HCl treated sample, the appearance of the peak at 1405 cm^{-1} can be ascribed to the stretching of the carboxylate anion (vs(C-O) [COO$^-$]) formed upon hydrolysis of the thermoset surface[1]. In general, after UV reticulation, BisGMA/TEGDMA thermosets typically display a degree of conversion (DG) in the range of 50-85 % [2], which is responsible for the presence of residual monomers. This presence was verified in the non-treated thermoset (TS): the peak at 1637 cm^{-1} due to the stretching of aliphatic C=C in unreacted monomers was clearly found. Notably, after the treatment with HCl the peak disappears which is ascribable to HCl reaction with insaturations. This fact points out the double role of HCl which is involved in the surface hydrolysis through the formation of carboxylic moieties, as well as in the removal of insaturations associated with unreacted monomers on the surface of the material. This finding suggests the use of HCl as a possible mean for removal of unreacted monomer in methacrylate-based thermosets.

As to the coated sample, the overlapping of the main characteristic peaks of the methacrylate substrate with the peaks of the polysaccharide Chitlac does not allow to separate its contribution in the infrared spectra.

Chitosan Effects On MG63 Osteoblast-Like Cell Phenotype

Figure S2 shows the effect on MG63 osteoblasts of the polysaccharide chitosan in solution at different concentrations. The graph points out that no significant effects of chitosan on cell growth were observed.
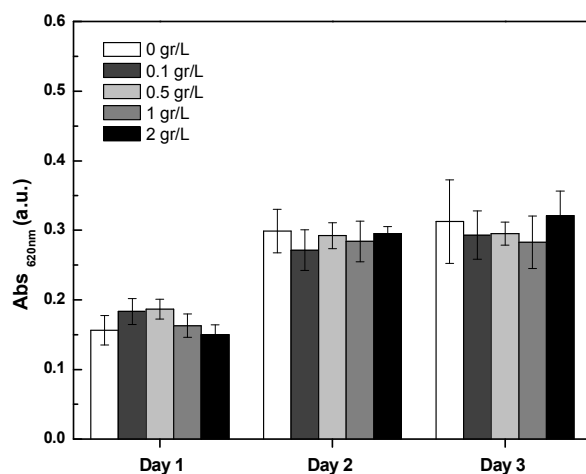
Figure S2: MTT assay performed on adherent MG63 cells in absence (0 gr/L) and in presence of different concentrations (0.1 gr/L, 0.5 gr/L; 1 gr/L; 2 gr/L) of chitosan after 1, 2 and 3 days of culture. Data are expressed as mean optical density ± S.D. (n=5) compared to cell growth in absence of chitosan

Chitlac Adsorption On Activated Thermosets As A Function of pH

As discussed in the manuscript, the activated thermosets were immersed in Chitlac-fluo solutions at various pH values and the amount of polysaccharide adsorbed on the thermoset was estimated as a function of pH by means of fluorescence measurements. The numbers are listed in Table S4.

pH	Chitlac adsorption [$\mu g/mm^2$]	Std. Deviation
2.00	6.56	1.15
2.41	7.84	0.11
2.72	6.80	0.27
4.39	11.60	0.21
4.50	13.66	0.12
6.95	9.67	0.28
7.83	10.35	0.76
7.98	9.34	0.45
8.97	7.58	0.29
9.06	9.43	0.35

Table S4: Chitlac adsorption on activated BisGMA/TEGDMA thermosets as a function of the pH of polysaccharide solution

In vivo Tests in Minipigs

In vivo experiments were designed to perform basic screening of Chitlac-coated thermoset implants for bone incorporation. The comparison was made with control implants made of titanium Ti6Al4V alloy. The bone-implant interfaces were compared with both qualitative and quantitative analysis according to a standardized grading scale[4] and the results are also shown in Table S5.

Implant	Histology		
	Bone reaction, semi-quantitavely assessed (μm)	Bone reaction, qualitatively assessed (score)	Interface, qualitatively assessed (score)
Ti6Al4V	14 (11-15)	3.2 (3.0-3.5)	3.2 (3.0-3.5)
Chitlac-TS	2 (0-3)	3.8 (3.5-4.0)	3.9 (3.7-4.0)

Table S5: Results of the *in vivo* experiment. Values in parentheses represent range limits.

References

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