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SUPPLEMENTARY MATERIAL TO

A novel PGA/TiO_2 nanocomposite prepared with poly(*γ*-glutamic acid) from the newly isolated *Bacillus subtilis* 17B strain

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MASS SPECTROMETRY OF ISOLATED PGA

The spectra of glutamic acid (Fig. S-2) contain only the signals corresponding to the ions generated by the proton or sodium addition (*m/z* 148.09 or 170.09, respectively). In addition, the ions formed by the proton/sodium replacement or the loss of water molecules were observed, which is in agreement with previously analyzed spectra of amino acids.[1] In the spectrum of a hydrolyzed polymer (Fig. S-2B), the ions detected at low m/z positions arise from inorganic ions present in the carrier fluid, whereas ions in the mass range m/z 140-180 arise from glutamic acid. Because of the high concentration of HCl in the solution, required for hydrolysis, no Na-adducts are detectable in the spectra. These results strongly imply that Glu does not polymerize during the acquisition of mass spectra and that the signals detected in the polymer spectra arise exclusively from the ions present in the isolate (Fig. S-2C). Although ESI mass spectra can contain multiply charged ions, in the spectra acquired under our conditions, we have detected only singly charged ions, and the highest number of units detected is 15. Ions are generated by the loss of CO, and the spectra are in agreement with the literature data.[2]

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Table S-I. Limits of the Studied Parameters

Factor	Symbol	Factor levels		
		Low (-1)	Central (0)	High (+1)
$c_{glucose}$ /g L^{-1}	A	10	45	80
$c_{glycerol}$ /g L^{-1}	B	0	25	50
$c_{glutamate}$ /g L^{-1}	C	0.5	2.75	5

Table S-II. Three-Factor Box-Behnken Experimental Design

Run	A	B	C
1	-1	-1	0
2	1	-1	0
3	-1	1	0
4	1	1	0
5	-1	0	-1
6	1	0	-1
7	-1	0	1
8	1	0	1
9	0	-1	-1
10	0	1	-1
11	0	-1	1
12	0	1	1
13	0	0	0
14	0	0	0

Table S-III. TiO_2 and PGA concentrations (c / μg mL^{-1}) used for nanocomposite formation and cell cytotoxicity assay.

Sample	TiO_2	PGA
NC1	1000	200
NC2	1000	400
NC3	1000	500

Table S-IV. ANOVA

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	593.74	6	98.96	3.88	0.0496	Significant
A-Glucose	55.65	1	55.65	2.18	0.1833	
B-Glycerol	389.21	1	389.21	15.25	0.0059	
C-Glutamate	0.1013	1	0.1013	0.0040	0.9515	
AB	87.42	1	87.42	3.43	0.1066	
AC	14.44	1	14.44	0.5658	0.4764	
BC	46.92	1	46.92	1.84	0.2172	
Residual	178.63	7	25.52			
Lack of Fit	104.21	6	17.37	0.2334	0.9161	Not significant
Pure Error	74.42	1	74.42			
Cor Total	772.38	13				

Table S-V. Position and signal identity of peaks detected in the positive ion ESI mass spectra given in Fig. 5: (A) glutamic acid, (B) hydrolyzed polymer, and (C) isolated PGA is given.

Position, m/z	Signal identity	Position, m/z	Signal identity
130,04	Glu-H2O+H+	306,94	Glu2+H2O+Na+-H+
142,09	Glu-CO+Na+	448,96	Glu3+2Na+-2CO-H++H2O
164,93	Glu+H2O-H+	590,86	Glu4+3Na+-3CO+H2O+H+
148,09	Glu+H+	1150,07	n.i.
170,09	Glu+Na+	1662,81	Glu13-CO+H+
192,09	Glu+2Na+-H+	1793,11	Glu14-CO+H+
214,08	Glu+3Na+-2H+	1957,2	Glu15+H+
226,08	n.i.		

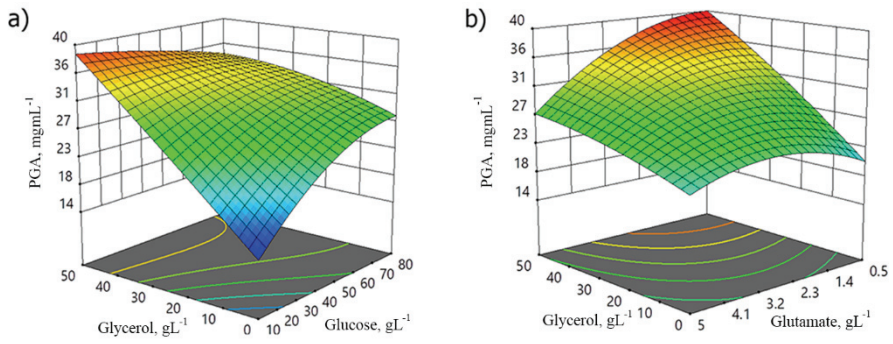

Fig. S-1. 3D response surface graph for the effects of a) concentrations of glycerol and glucose and b) concentrations of glycerol and glutamate on PGA yield. The third variable was held constant at the center point.

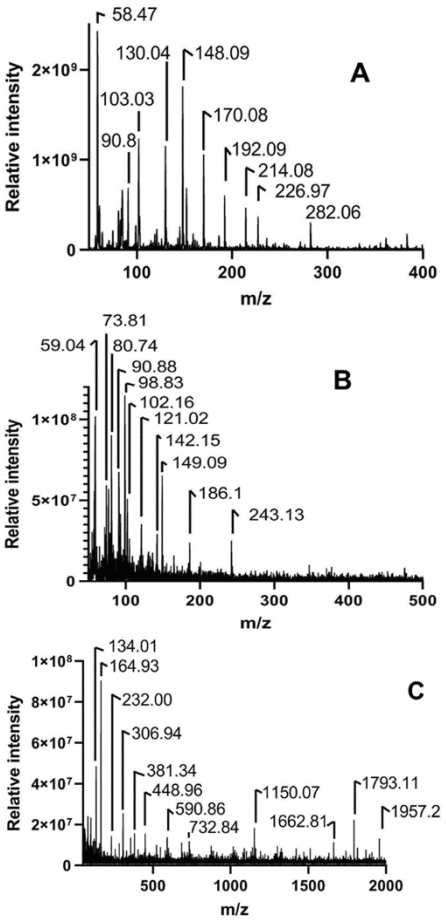

Fig. S-2. Positive ion ESI mass spectra of glutamic acid (A), hydrolyzed isolated PGA (B) and isolated PGA (C).

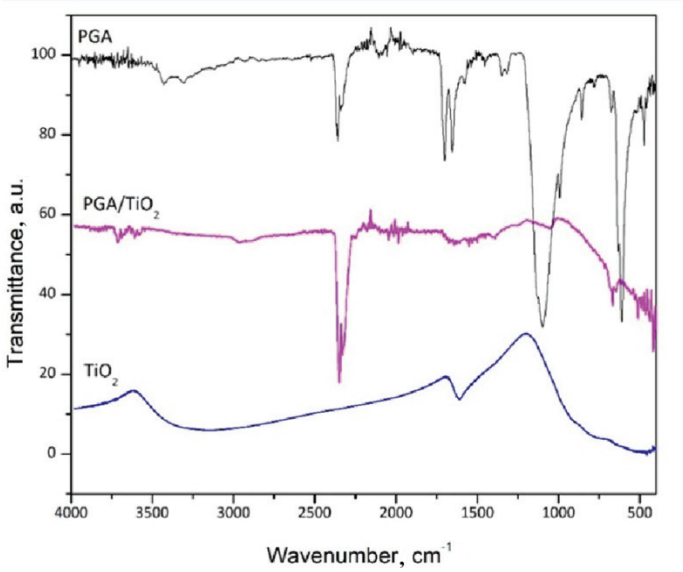

Fig. S-3. FTIR spectra of purified PGA, PGA/TiO_2 nanocomposite, and TiO_2.

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2. I. Kwiecień, I. Radecka, M. Kowalczuk, K. Jelonek, A. Orchel, & G. Adamus, *J. Am. Soc. Mass Spectrom.* **28** (2017) 2223 (https://doi.org/10.1007/s13361-017-1731-y).