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ELECTRONIC SUPPLEMENTARY INFORMATION

Poly(D,L-Lactide)-Grafted Cu-doped Bioglass Microspheres as Core-Shell Building Blocks for Biomaterials: From Grafting to Early-Stage *in vitro* Behaviour

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1 ANALYTICAL METHODS

1.1 EVALUATION OF PRIMARY AMINES GRAFTED ON THE MICROSPHERES SURFACE

To evaluate the amount of primary amines on the surface of nano- and microparticles the method described by Lagarrigue *et al.* was applied [1]. The quantification of the amino-functionalisation was determined starting from the TGA thermograms of the samples. Each thermogram was divided in 3 temperature domains: i) 25-150°C, ii) 150-350°C, iii) 350-800°C. The weight loss belonging to the organic aminopropyl group was approximately identified with the zone III, in the temperature range 350-800°C. Lagarrigue *et al.* realised for the purpose a calibration curve by measuring the weight loss in zone III of silica NPs/condensed APTES mixtures at different mass ratios used as standards, thus having a predictable aminopropyl molar amount (**Figure S1**). Therefore, the calibration curve and its equation allow associating the percentage of mass loss in zone III of any amino-functionalised sample with a molar value of aminopropyl (CHN) groups grafted.

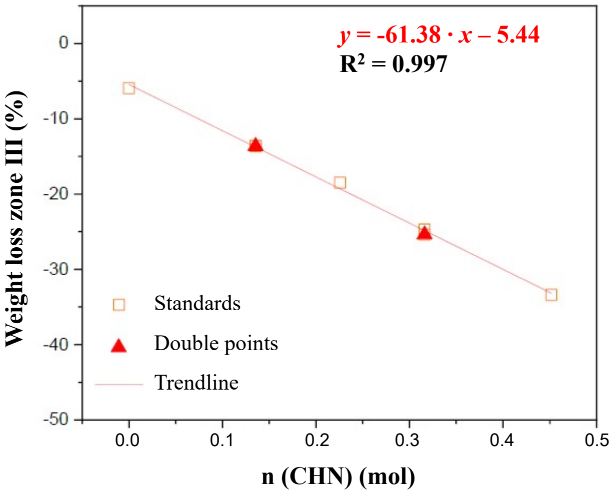

Figure S1. *Calibration curve of standard mixtures of silica NPs/condensed APTES obtained from the weight loss of their thermograms in zone III (adapted from P. Lagarrigue PhD dissertation [2]).*

However, this calibration curve is valid only if the sample analysed contains silica NPs. For bioglass nanoparticles or microparticles the amount of superficial silanol groups lost in the temperature range 150-350°C (zone II) and that of internal silanol groups lost in zone III is decreased with respect to bare silica nanoparticles, as it can be observed from **Figure S2**. The reasons may be due to the lower surface area of the spray-dried MPs and to the integration of cations in MPs, which reduces the amount of silanol groups by reorganising the silica network during the thermal treatment.

Therefore, in order to adapt the above-mentioned model to the synthesised bioglass samples (amino-functionalised and PDLLA-grafted MPs), a correction factor must be applied on the non-functionalised samples as follows:

$$F_{corr}^{\ i} = \Delta m(SiO_2\,NPs)^i - \Delta m(BG\,MPs)^i \qquad \text{(S.1)}$$

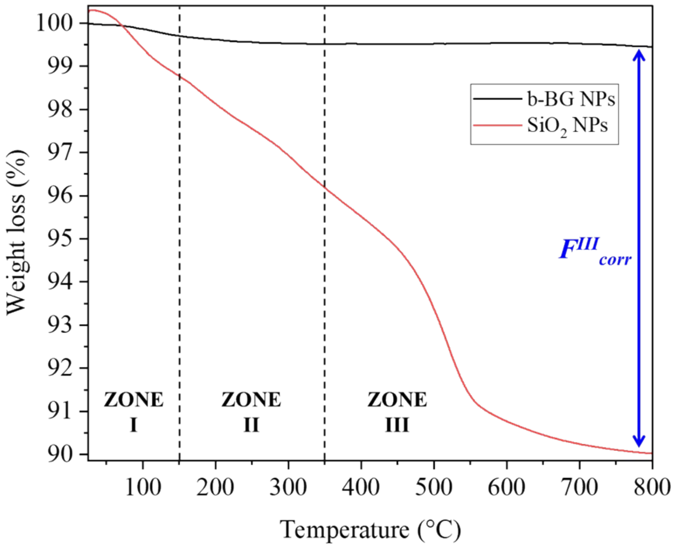

Figure S2. *Thermogram of bare BG NPs (black line) compared with that of model SiO₂ NPs (red line), highlighting the weight loss difference in the zone III (adapted from P. Lagarrigue PhD dissertation [2]).*

Where F_{corr}^{i} is the correction factor in the considered temperature range of the zone *i* (II or III) of the thermograms, while $\Delta m(SiO_2\ NPs)^i$ and $\Delta m(BG\ MPs)^i$ correspond to the percentage of mass loss in the zone *i* (II or III) of model silica NPs and BG MPs, respectively. Thus, the corrected mass loss of amino-functionalised or PDLLA-grafted samples in zone *i* was calculated by adding the correction factor to the mass loss of the zone *i*:

$$\Delta m((X)BG\,MPs)_{corr}^{\ i} = \Delta m((X)BG\,MPs)^i + F_{corr}^{\ i} \qquad \text{(S.2)}$$

Where *(X)* stands for amino-functionalised (NH_2-) or PDLLA-grafted (PDLLA-BG samples, and $\Delta m((X)BG\ /MPs)^i$ corresponds to the percentage of mass loss of NH_2- or PDLLA-BG in the zone *i* (II or III) found from the thermograms.

By introducing the corrected weight losses of NH_2-BG MPs samples in zone III to the equation of the calibration curve illustrated in **Figure S1** the molar amount of primary amines grafted could be finally estimated. The grafted density of primary amines, expressed as number of NH_2 groups per square nanometres, was determined by finding the actual mass of BG MPs after functionalisation, taking into account that every APTES molecule grafted provides an additional -Si-O_3 group that increases the particles mass and it is not degraded during TGA analysis. Thus, for 100 g of functionalised particles:

$$m(BG\,MPs)\ (g) = 100 - \left(M_{C_3NH_8} \cdot n_{C_3NH_8}\right) - \left(M_{SiO_3} \cdot n_{SiO_3}\right) \quad (S.3)$$

Where M_{C3NH8} and M_{SiO3} are the molecular weights of the aminopropyl (58 g·mol^{-1}) and SiO_3 (76 g·mol^{-1}) groups, and n_{C3NH8} are the moles of APTES grafted determined through the calibration curve ($n_{C3NH8} = n_{SiO3}$). Once the actual mass of functionalised MPs was found, the density of primary amines grafted is determined by the following formula:

$$\rho_{NH_2}\ ((\#NH_2) \cdot nm^{-2}) = \frac{(n_{C_3NH_8} \cdot N_A)}{m(BG\,MPs) \cdot SSA \cdot 10^{18}} \quad (S.4)$$

Where N_A and *SSA* are the Avogadro's number (6.022·10^{23} atoms·mol^{-1}) and the specific surface area of the considered sample (expressed in m^2·g^{-1}), respectively. Finally, the molar amount of primary amines per gram of MPs was calculated as:

$$\rho_{NH_2}\ (mol_{NH_2} \cdot g_{MPs}^{-1}) = \frac{n_{C_3NH_8}}{m(BG\,MPs)} \quad (S.5)$$

This final value was used to determine the monomer/initiator molar ratio to predict the theoretical molecular weight of the PDLLA chains grafted on the particles surface.

1.2 POLYMERISATION CONDITIONS AND DETERMINATION OF THEORETICAL MOLECULAR WEIGHTS

To determine the theoretical molecular weight of the grafted chains it was supposed that all the primary amine groups on the BG microparticles surface effectively initiated the ring opening polymerisation. By considering the molar amount of primary amines grafted on the surface of the BG microspheres (**Table S2**) and the amount of D,L lactide added in the reaction mixture ($f_{MPs} = 0.2$, **Table S1**), the theoretical degree of polymerisation (DP_{th}), expressed as lactic acid repeating units, was determined as follows:

$$DP_{th} = \frac{n_{D,L\,LA} \cdot 2}{n_{NH_2}} \quad (S.6)$$

Where $n_{D,L\ LA}$ represents the moles of D,L lactide added in solution, and n_{NH2} is the molar amount of primary amines found in the quantity of BG microspheres involved, obtained by multiplying this value with the amino-grafted density ρ_{NH2}. Consequently, the theoretical molecular weight ($M_{n,th}$) of the grafted chains was calculated by the formula:

$$M_{n,th} = DP_{th} \cdot M_{D,L\,LA} \quad (S.7)$$

Where $M_{D,L\ LA}$ is the lactide molecular weight (144.13 g·mol^{-1}). ^{1}H NMR spectra of the four samples at the end of polymerisation (24 hours, crude products) were recorder and reported in **Figure S3**. The monomer conversion was calculated from the ^{1}H NMR spectrum of the crude product at the end of the polymerisation, by considering the integration of the monomer and polymer backbone signals as follows:

$$Conv._{D,L\,LA} = \frac{I_j}{I_j + I_{D,L\,LA}} \tag{S.8}$$

Where I_j is the integration of the PDLLA signal at δ 5.16 ppm, I_{DLLA} is the integration of the monomer signal at δ 5.05 ppm. The monomer conversion of all the samples were reported in **Table S2**.

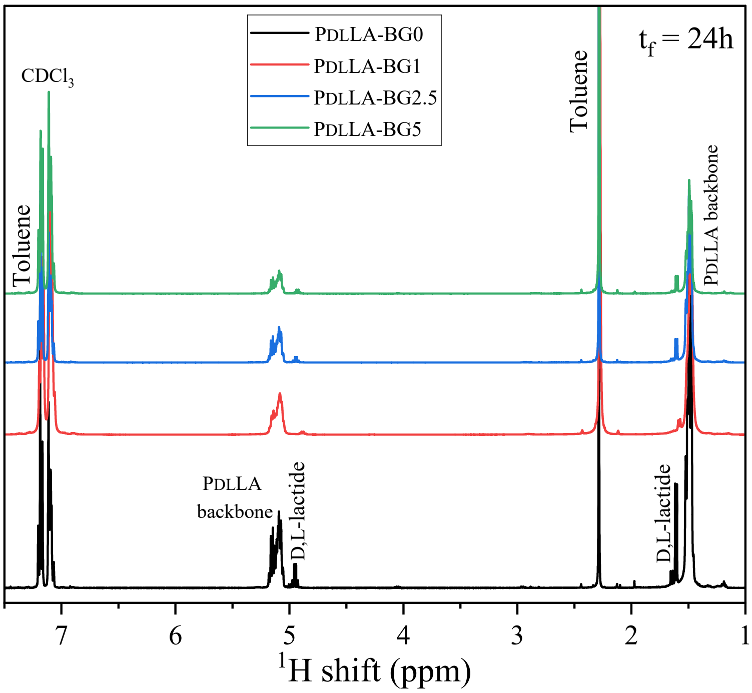

Figure S3. *Stacked ^1H NMR spectra of the four PDLLA-BG samples crude product at the end of the ROP, after 24 hours.*

Ultimately, new theoretical degrees of polymerisation and molecular weights were obtained by multiplying the D,L lactide conversion by the first DP_{th} and $M_{n,th}$ values calculated. Such new values were reported in **Table S2** as well.

Table S1. *Synthesis parameters for the surface-initiated ROP of D,L-lactide from the surface of amino-functionalised spray-dried quaternary bioglass microparticles: mass ratio between microparticles and D,L lactide (f_{MPs}), polymerisation time, monomer concentration, equivalents of each reactant, theoretical degree of polymerisation and molecular weight (DP_{th} and M_n).*

Sample	f_{MPs} (m_{MPs}/m_{DLLA})	**Time (h)**	C_{DLLA} (mol·L^{-1})	**[In]/[Mon]^a/ [TEA]/[Sn] (eq.)**	DP_{th} ^{b,c}	$M_{n,\,th}$ (g·mol^{-1})^d
PDLLA-BG0	0.2	24	4	1/167/0.5/0.2	334	24000
PDLLA-BG1	0.2	24	4	1/177/0.5/0.2	354	25500
PDLLA-BG2.5	0.2	24	4	1/175/0.5/0.2	350	25200
PDLLA-BG5	0.2	24	4	1/215/0.5/0.2	430	31000

[a] *Lactide repeating units.*
[b] *Lactic acid repeating units.*
[c] *Determined from eq. (S.6).*
[d] *Determined from eq. (S.7).*

***Table S2.** Results of the amino-grafting evaluation from TGA analysis: moles of APTES grafted per 100 g of NH_2-BG MPs and APTES grafting density (in $\#NH_2{\cdot}nm^{-2}$ and $mmol{\cdot}g^{-1}$); D,L-lactide conversions, new theoretical degrees of polymerisation and molecular weights.*

Sample	n_{APTES} (mmol)	ρ_{NH2} ($\#NH_2{\cdot}nm^{-2}$)[a]	ρ_{NH2} ($mmol_{NH2}{\cdot}g^{-1}_{MPs}$)[b]	Conv. (%)[c]	DP_{th}[d]	$M'_{n,\,th}$ ($g{\cdot}mol^{-1}$)[d]
NH_2-BG0	16.3	26.8	0.167	-	-	-
NH_2-BG1	15.3	29.8	0.156	-	-	-
NH_2-BG2.5	15.5	27.7	0.158	-	-	-
NH_2-BG	12.7	20.5	0.129	-	-	-
PDLLA-BG0	-	-	-	94	314	22600
PDLLA-BG1	-	-	-	97	344	24700
PDLLA-BG2.5	-	-	-	96	336	24200
PDLLA-BG5	-	-	-	95	408	29400

[a] *Determined from eq. (S.4).*
[b] *Determined from eq. (S.5).*
[c] *Determined from eq. (S.8).*
[d] *Determined by multiplying the DP_{th} and $M_{n,th}$ by the monomer conversion.*

1.3 EVALUATION OF PDLLA GRAFTING DENSITY AND MOLECULAR WEIGHT OF THE GRAFTED CHAINS

The PDLLA grafting density was roughly estimated by adapting the method used by Joubert *et al.* [3], starting from the mass ratio between PDLLA and bare MPs with no organic species grafted. The mass of PDLLA is calculated from the TGA thermograms, and it is equal to the difference between the weight loss of PDLLA-BG and NH_2-BG above 150°C (domains II + III, reported in **Table S5**). Then, the grafting density σ_{PDLLA} is calculated through the following formula:

$$\sigma_{PDLLA}\,[\#chains \cdot nm^{-2}] = \frac{\dfrac{(\Delta m^{II+III}_{PDLLA} - \Delta m^{II+III}_{NH_2})}{100 - (\Delta m^{II+III}_{PDLLA} - \Delta m^{II+III}_{NH_2})} \cdot N_A}{M_{n,\,th} \cdot SSA \cdot 10^{18}} \qquad \text{(S.9)}$$

Where N_A is the Avogadro's number, *SSA* the specific surface area (reported in **Table S4** for all the as-synthesised samples) and $M_{n,th}$ is the theoretical molecular weight of grafted PDLLA considering the monomer conversion (**Table S2**). This last value was used in absence of a real M_n result, since it is impossible to determine it through classical analytical techniques such as SEC. Hence, the grafting density found with the above equation, expressed as the number of grafted PDLLA chains per nm^{-2} of MPs surface, corresponds to the density that the sample would have if the grafted chains had a theoretical molecular weight recalculated by considering the density of primary amines grafted and the monomer conversion. Thus, the σ_{PDLLA} expressed as moles of PDLLA-grafted per g of MPs is obtained by dividing the grafting density found above by N_A/SSA:

$$\sigma_{PDLLA}\,[mol_{PDLLA} \cdot g_{MPs}^{-1}] = \frac{\dfrac{(\Delta m^{II+III}_{PDLLA} - \Delta m^{II+III}_{NH_2})}{100 - (\Delta m^{II+III}_{PDLLA} - \Delta m^{II+III}_{NH_2})}}{M_{n,\,th}} \tag{S.10}$$

Finally, by considering the molar amount of PDLLA-grafted chains contained into 100 g of PDLLA-BG microspheres (equal to $100 \cdot \sigma_{\mathrm{PDLLA}}$), the experimental molecular weight of the grafted chains having that specific grafting density was determined by the formula below:

$$M_{n,\,exp} = \frac{(\Delta m^{II+III}_{PDLLA} - \Delta m^{II+III}_{NH_2})}{100 \cdot \sigma_{PDLLA}\,[mol_{PDLLA} \cdot g_{MPs}^{-1}]} \tag{S.11}$$

All the grafting densities and estimated molecular weights of the grafted chains were introduced in **Table S3** below.

Table S3. *Results of the PDLLA grafting evaluation from TGA analysis: PDLLA grafting density (in #chains·nm^{-2} and µmol·g^{-1}) and moles of PDLLA grafted per 100 g of PDLLA-g-BG MPs; experimental molecular weight and degree of polymerisation of PDLLA-grafted chains.*

Sample	**σ_{PDLLA} [a] (#chains·nm^{-2})**	**σ_{PDLLA} [b] (µmol·g$^{-1}_{\mathrm{MPs}}$)**	**n_{PDLLA} [c] (mmol)**	**$M_{\mathrm{n,\,exp}}$ [d] (g·mol^{-1})**	**DP_{exp} [e]**
PDLLA-BG0	0.34	2.09	0.209	21600	300
PDLLA-BG1	0.38	2.01	0.201	23600	328
PDLLA-BG2.5	0.60	3.44	0.344	22300	310
PDLLA-BG5	0.24	1.48	0.148	28200	392

[a] *Determined from eq. (S.9).*
[b] *Determined from eq. (S.10).*
[c] *$n_{\mathrm{PDLLA}} = 100 \cdot \sigma_{\mathrm{PDLLA}}$ (µmol·g^{-1}).*
[d] *Determined from eq. (S.11).*
[e] *Lactic acid repeating units.*

2. RESULTS

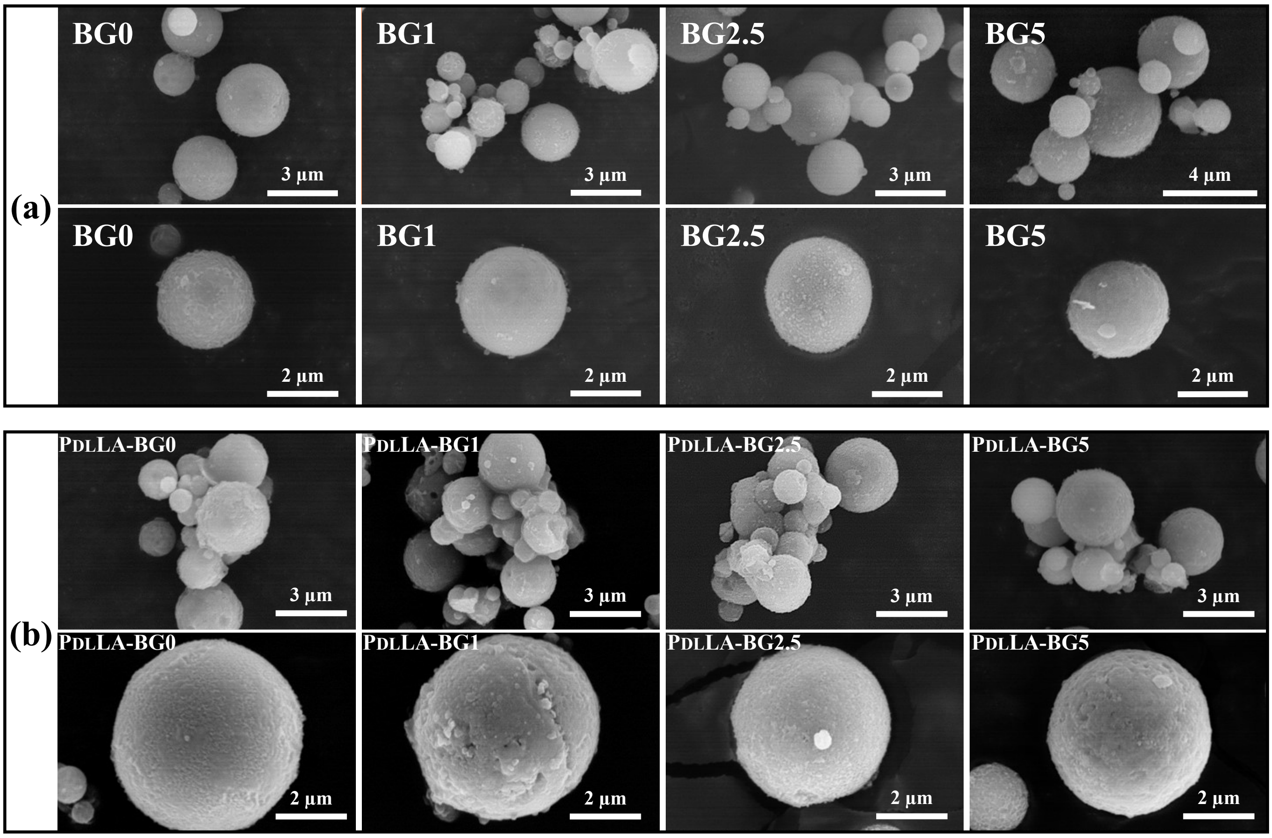

Figure S4. *SEM micrographs of groups of particles (top) and individual particles (bottom) of a) spray-dried BG samples (adapted from G. Vecchio et al. [4]), and b) PDLLA-BG samples, divided by their Cu content (0 to 5 at.% (nominal composition)).*

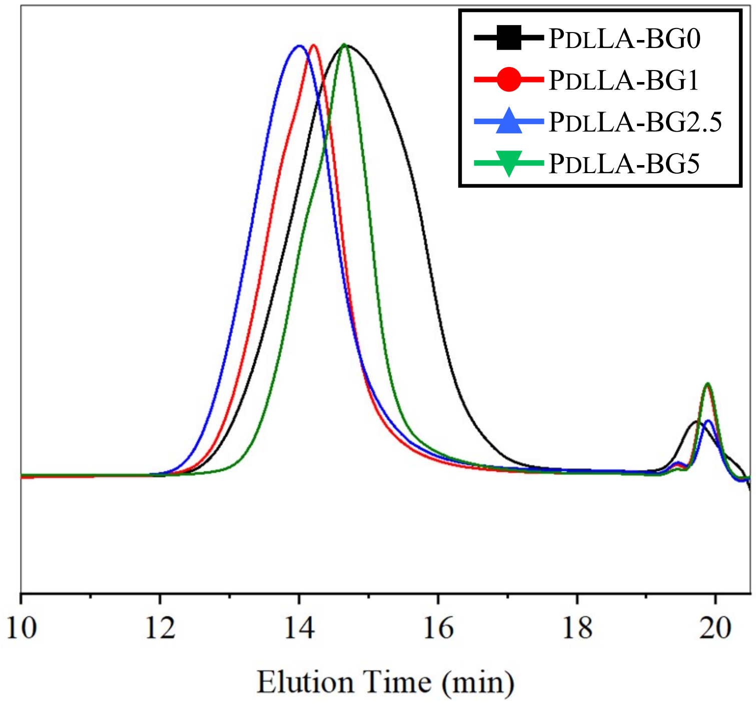

Figure S5. *SEC chromatograms of the free PDLLA chains separated from the PDLLA-BG samples.*

Table S4. *Specific surface area of the as-synthesised BG samples, molecular weight and dispersity of the free PDLLA chains separated from the PDLLA-grafted BG samples.*

Sample	SSA_{BET} **(m^2·g^{-1})** ^{a}	M_n^{SEC} **(g·mol^{-1})**^{b}	$Đ^b$
PDLLA-BG0	3.74	21000	2.18
PDLLA-BG1	3.16	45000	1.75
PDLLA-BG2.5	3.44	54000	1.75
PDLLA-BG5	3.8	32000	1.50

[a] *Specific surface area of the spray-dried BG microspheres.*

[b] *Free PDLLA chains separated from the PDLLA-grafted BG. SEC analysis in THF (PS calibration).*

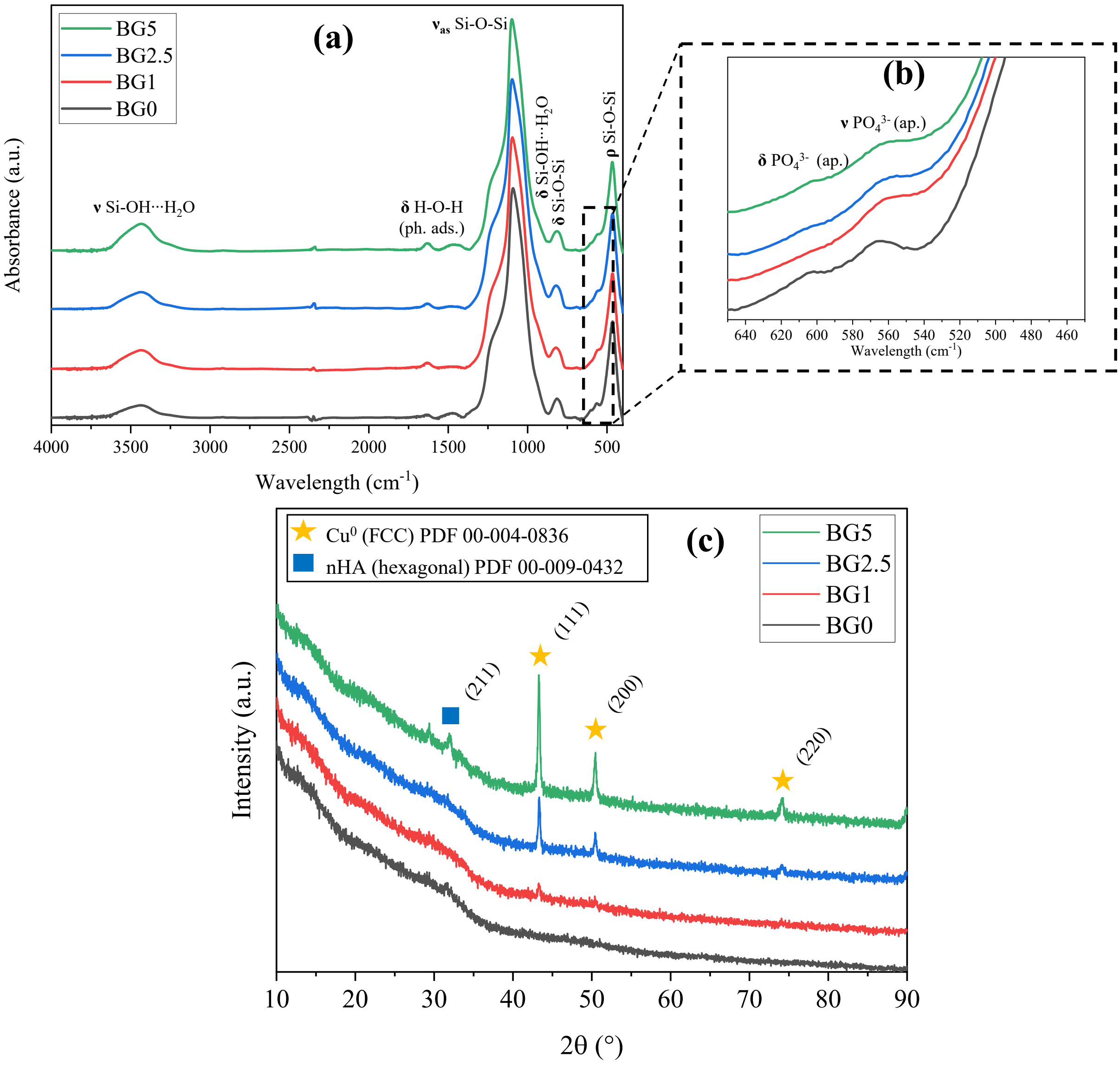

Figure S6. *a) FT-IR spectra of the spray-dried BG samples, with a zoom b) of the orthophosphate region in the range 450-650 cm^{-1}; c) XRD diffractograms of the spray-dried samples (adapted from [4]).*

Table S5. *Percentages of weight loss of bare, amino-functionalised and PDLLA-grafted BG samples in each thermal domain.*

Sample	**Δm (TGA weight loss) (%)**		
	Zone I (25-150°C)	**Zone II (150-350°C)**	**Zone III (350-800°C)**
BG0	0.38	0.35	1.8
NH_2-BG0	0.06	0.29	2.08
PDLLA-BG0	0.13	5.13	1.76
BG1	0.21	0.15	0.68
NH_2-BG1	0.09	0.19	0.9

PDLLA-BG1	0.23	4.79	1.07
BG2.5	0.18	0.15	0.83
NH_2-BG2.5	0	0.23	1.06
PDLLA-BG2.5	0.47	7.7	1.27
BG5	0.18	0.17	1.19
NH_2-BG5	0.07	0.21	1.25
PDLLA-BG5	0.16	4.52	1.12

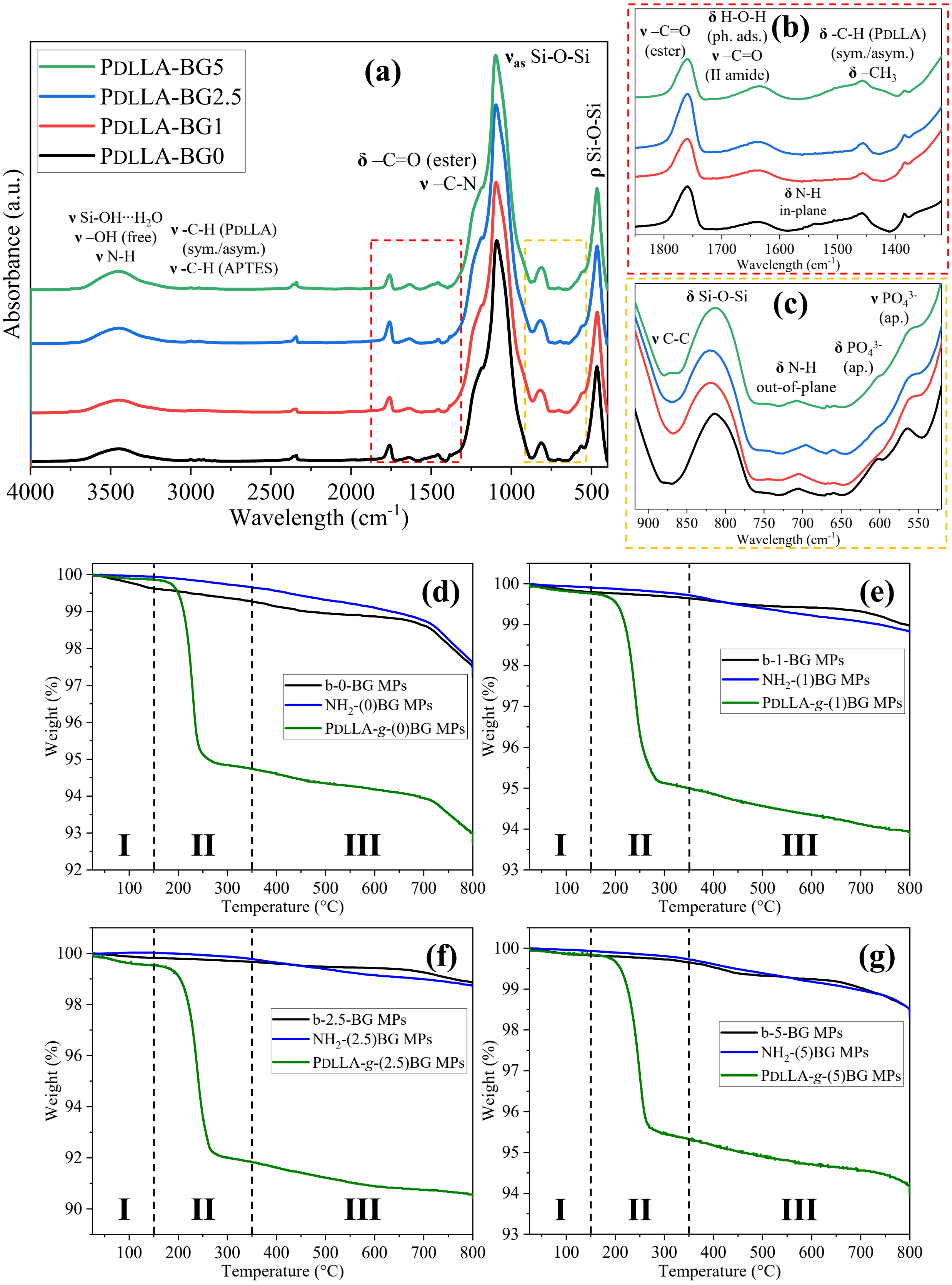

Figure S7. *(a) Stacked FT-IR spectra of PDLLA-BG samples, with zooms onto the (b) 1300-1800 cm^1 and (c) 500-950 cm^{-1} regions. TGA thermograms of (d) BG0, NH_2-BG0 and PDLLA-BG0; (e) BG1, NH_2-BG1 and PDLLA-BG1; (f) BG2.5, NH_2-BG2.5 and PDLLA-BG2.5; (g) BG5, NH_2-BG5 and PDLLA-BG5.*

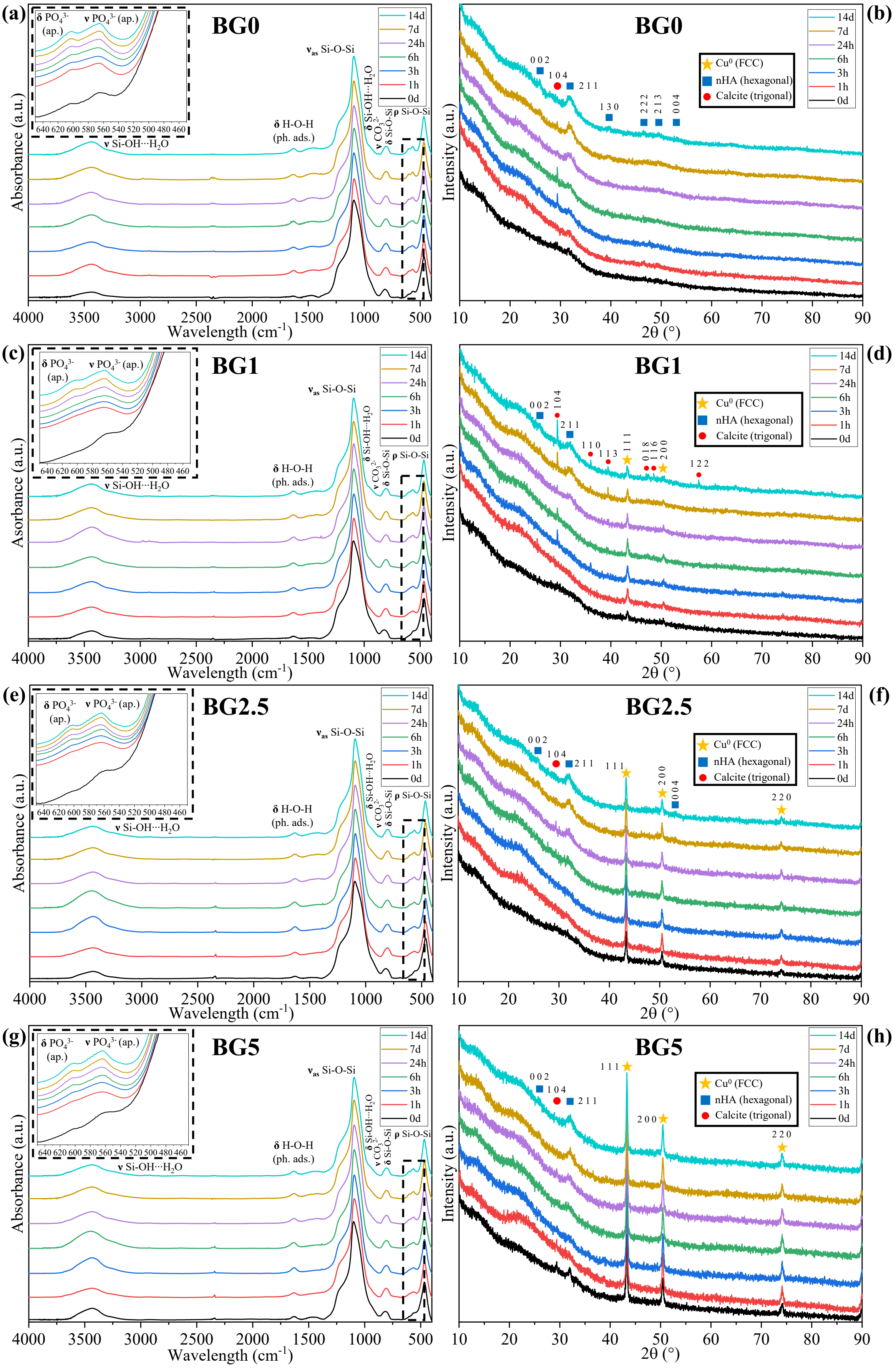

Figure S8. *FTIR spectra with a zoom of the orthophosphate region in the range 450-650 cm^{-1} of (a) BG0, (c) BG1, (e) BG2.5 and (g) BG5 before and after 1 hour to 14 days of immersion in SBF. XRD diffractograms of (b) BG0, (d) BG1, (f) BG2.5 and (h) BG5 before and after 1 hour to 14 days of immersion in SBF (adapted from [4]).*

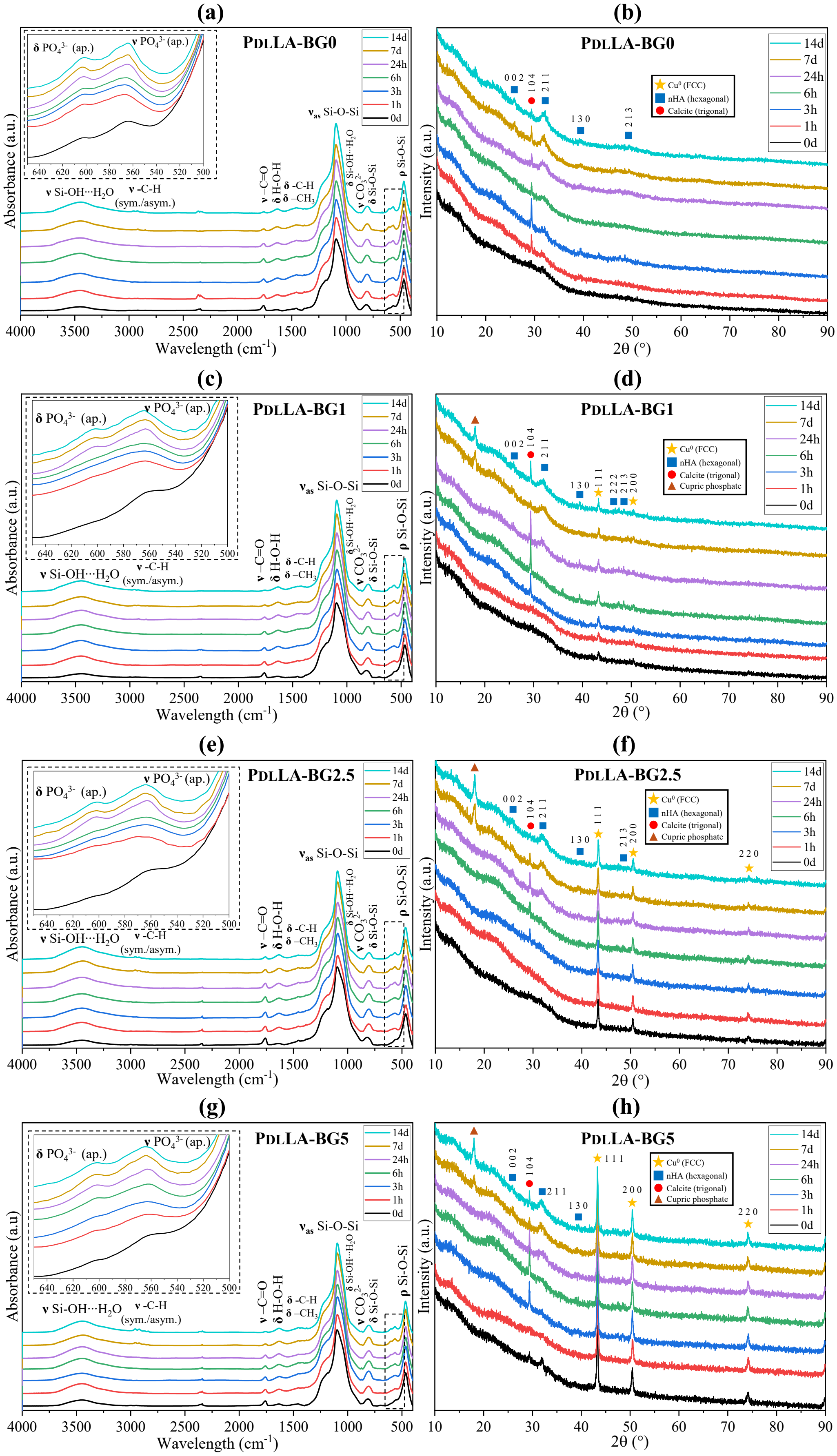

Figure S9. *FTIR spectra with a zoom of the orthophosphate region in the range 450-650 cm^{-1} of (a) PDLLA-BG0, (c) PDLLA-BG1 and (e) PDLLA-BG2.5 before and after 1 hour to 14 days of immersion in SBF. XRD diffractograms of (b) PDLLA-BG0, (d) PDLLA-BG1 and (f) PDLLA-BG2.5 before and after 1 hour to 14 days of immersion in SBF.*

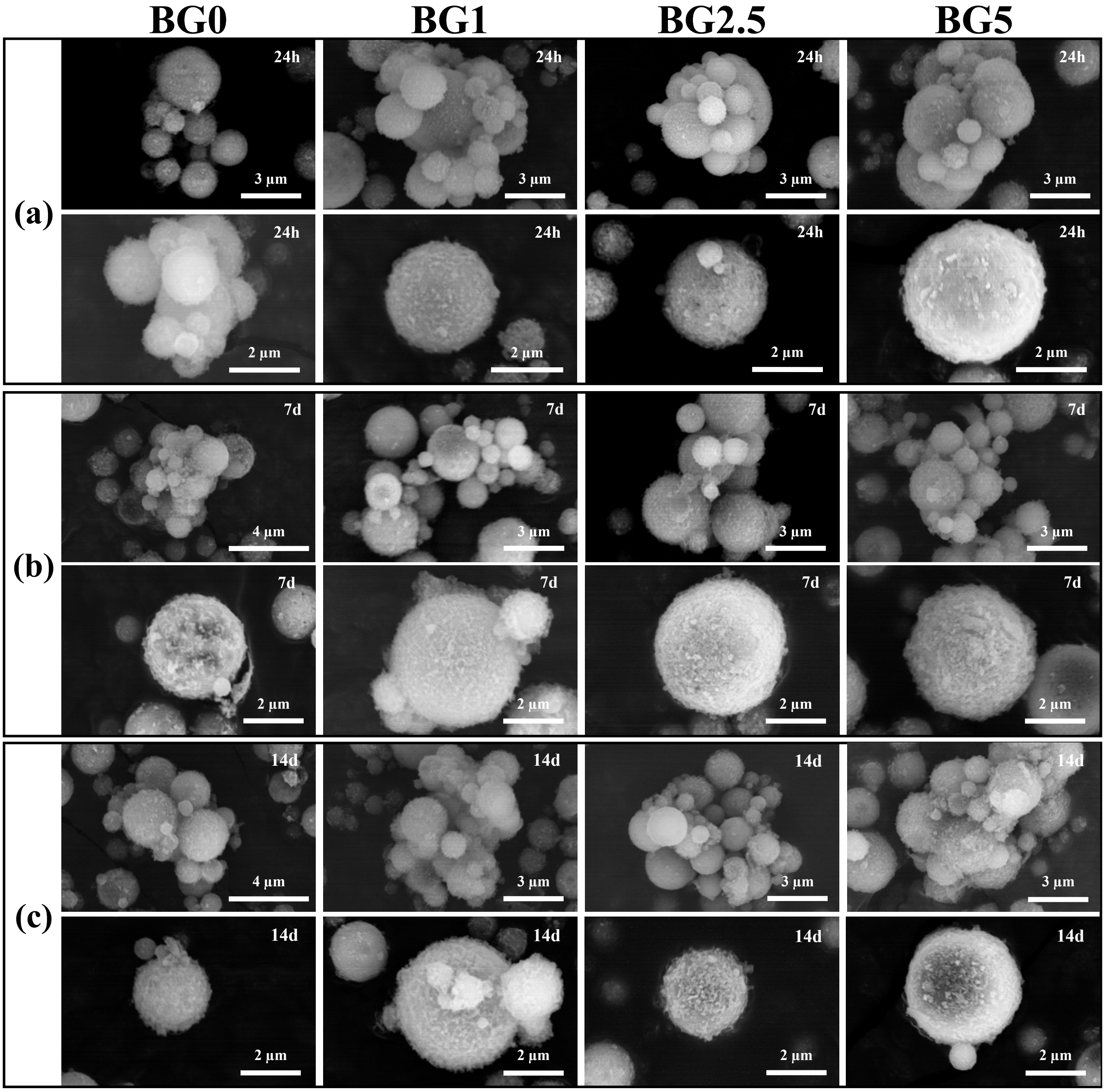

Figure S10. *SEM micrographs of agglomerated (top) and individual (bottom) particles of the as-synthesised Cu-doped BG samples after (a) 24 hours, (b) 7 days and (c) 14 days of immersion in SBF.*

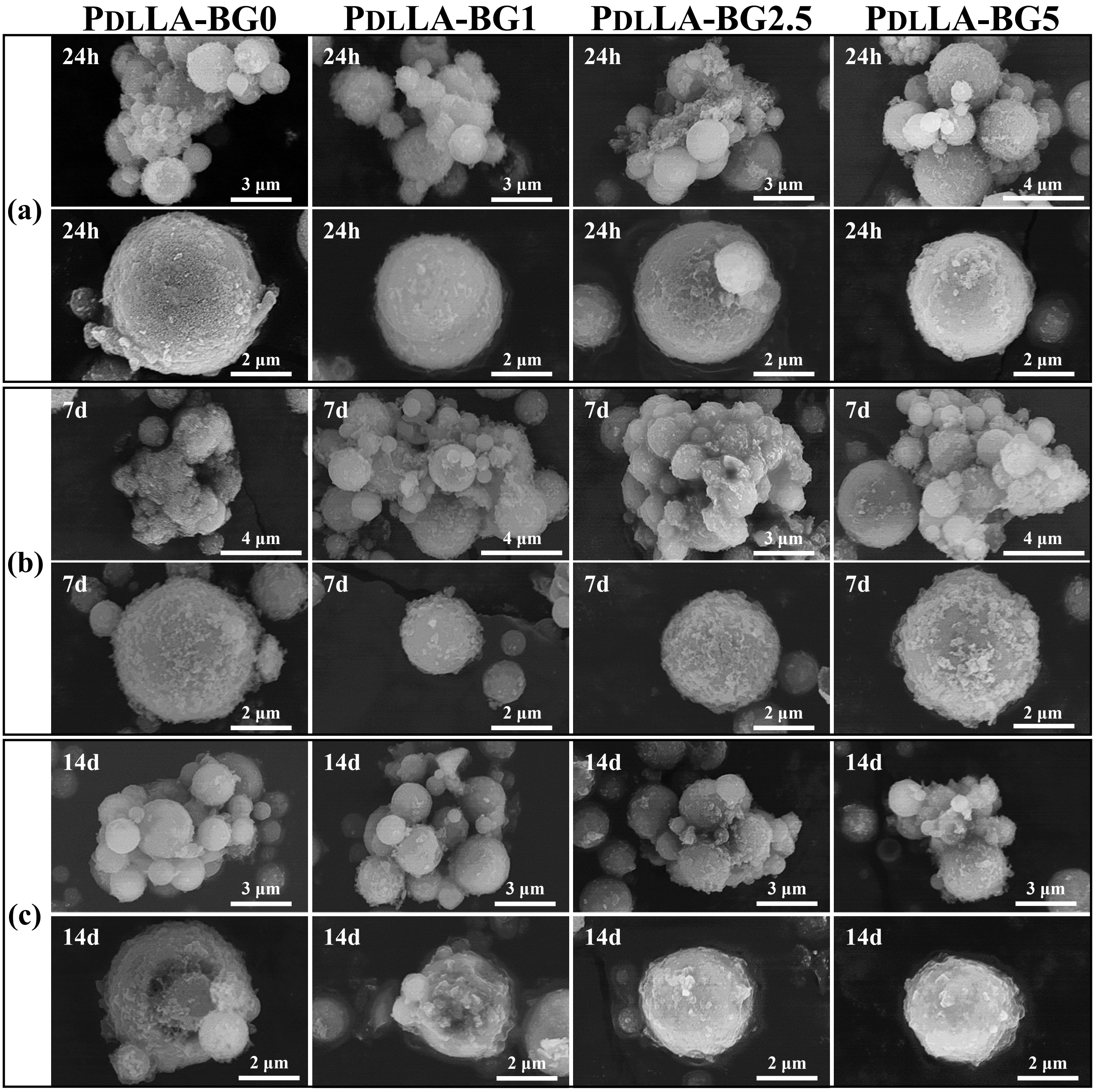

Figure S11. *SEM micrographs of agglomerated (top) and individual (bottom) particles of the synthesised Cu-doped PDLLA-BG samples after (a) 24 hours, (b) 7 days and c) 14 days of immersion in SBF.*

Table S6. *Evolution of the Ca/P ratio of bare and PDLLA-BG samples from 0 to 14 days immersion in SBF.*

Sample	**Ca/P ratios**			**Ca/P evolution (%)**	
	0d[a]	**14d**			
		BG	**PDLLA-BG**	**BG**	**PDLLA-BG**
PDLLA-BG0	5.15	5.83	2.75	+ 13.2	- 47
PDLLA-BG1	4.04	4.30	1.94	+ 6.4	- 52
PDLLA-BG2.5	3.68	3.79	1.71	+ 3.0	- 53
PDLLA-BG5	2.98	3.25	1.41	+ 9.1	- 53

[a] *The Ca/P ratios at 0d are the same for bare and PDLLA-grafted samples.*

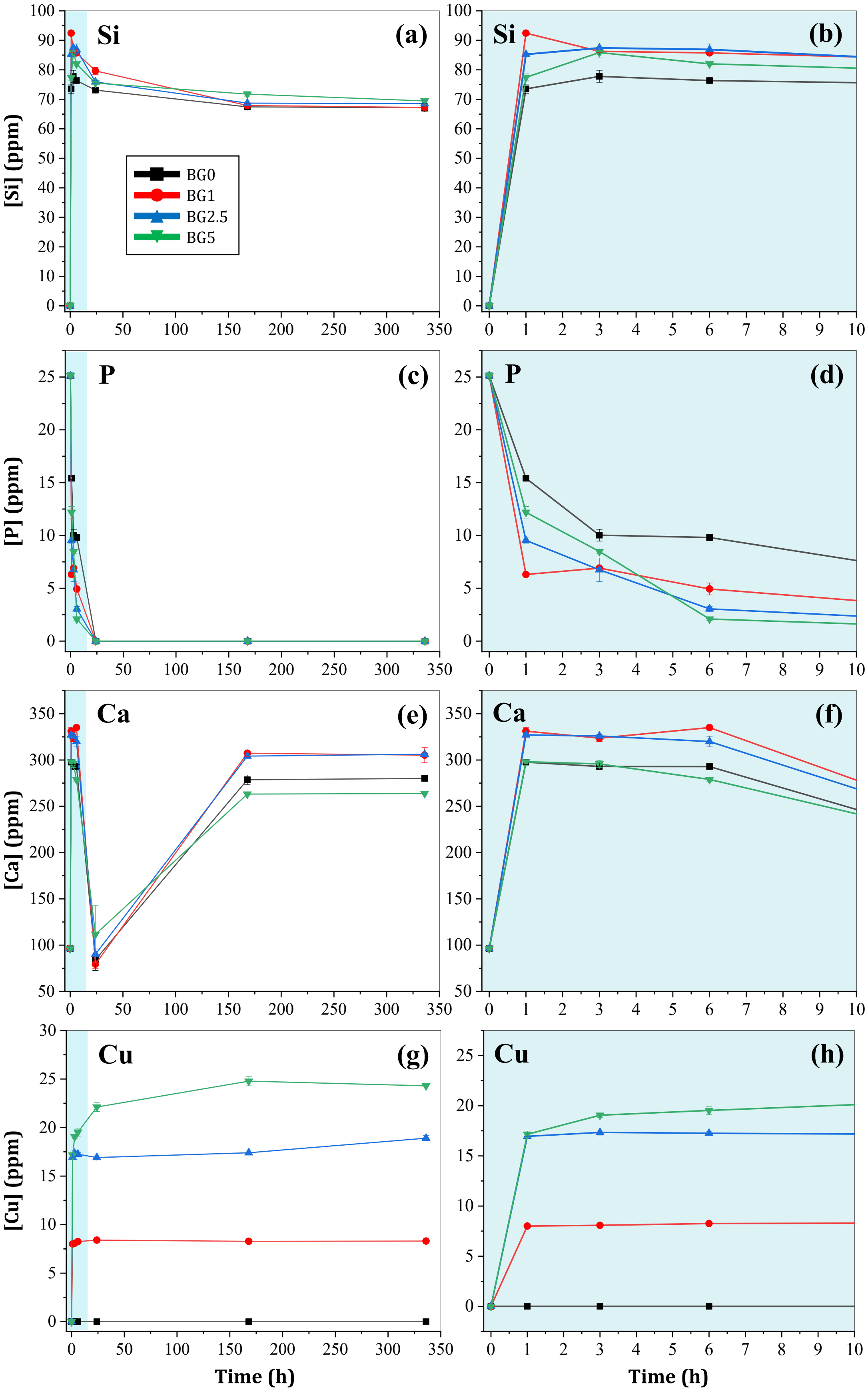

Figure S12. *Ion release profile of the bare spray-dried BG MPs, for each element (a and b for Si, c and d for P, e and f for Ca, g and h for Cu) during in vitro acellular tests in SBF, from 0d to 14d. The graphs on the right (b, d, f, h) represent the zoom of the first time-points (0 d, 1 h, 3 h, 6 h). Every point in the graphs corresponds to the concentration of the element (Si, P, Ca or Cu) detected in SBF solution at each time-point.*

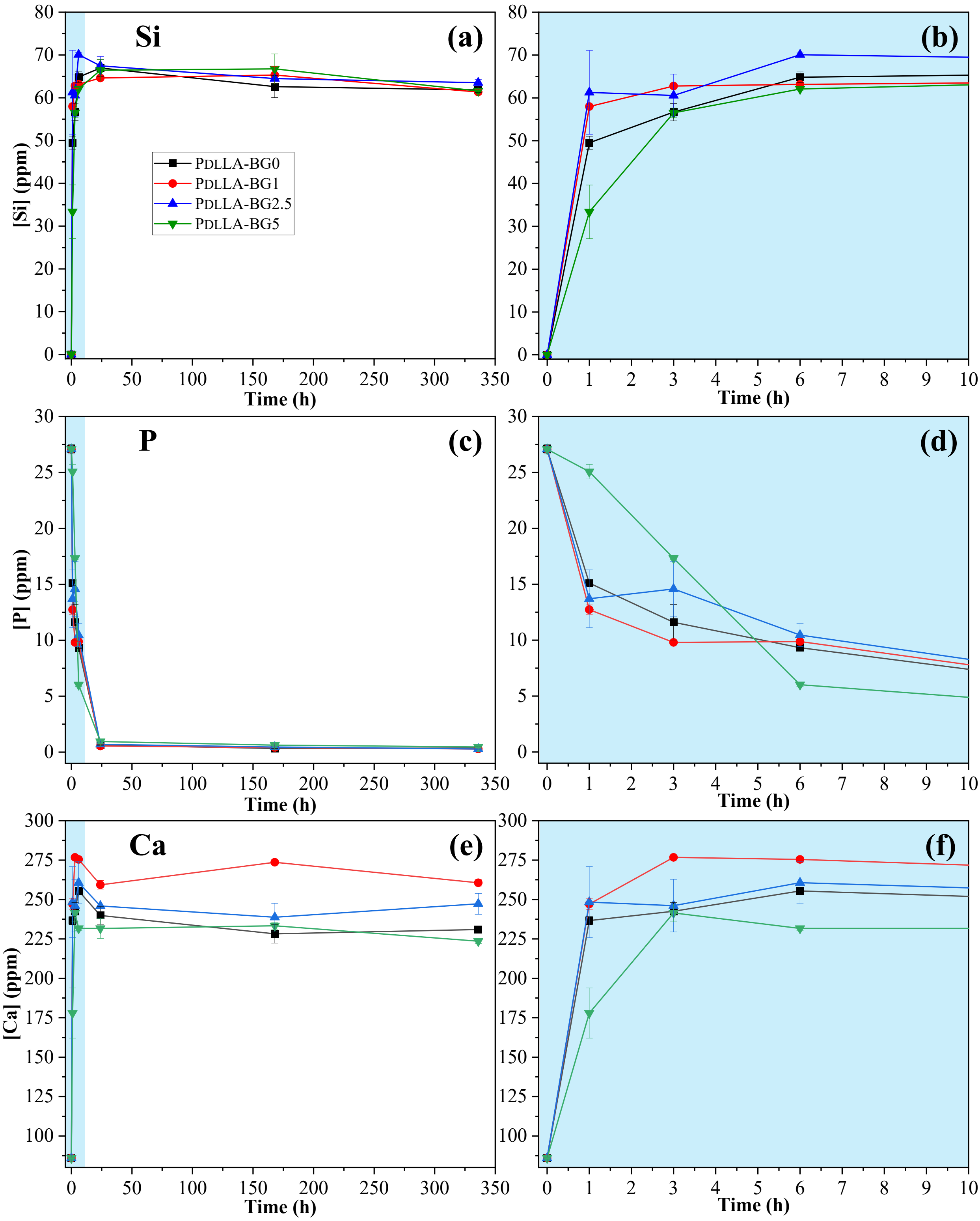

Figure S13. *Ion release profile of the PDLLA-BG samples, for each element (a and b for Si, c and d for P, e and f for Ca) during in vitro acellular tests in SBF, from 0d to 14d. The graphs on the right (b, d, f) represent the zoom of the first time-points (0 d, 1 h, 3 h, 6 h). Every point in the graphs corresponds to the concentration of the element (Si, P or Ca) detected in SBF solution at each time-point.*

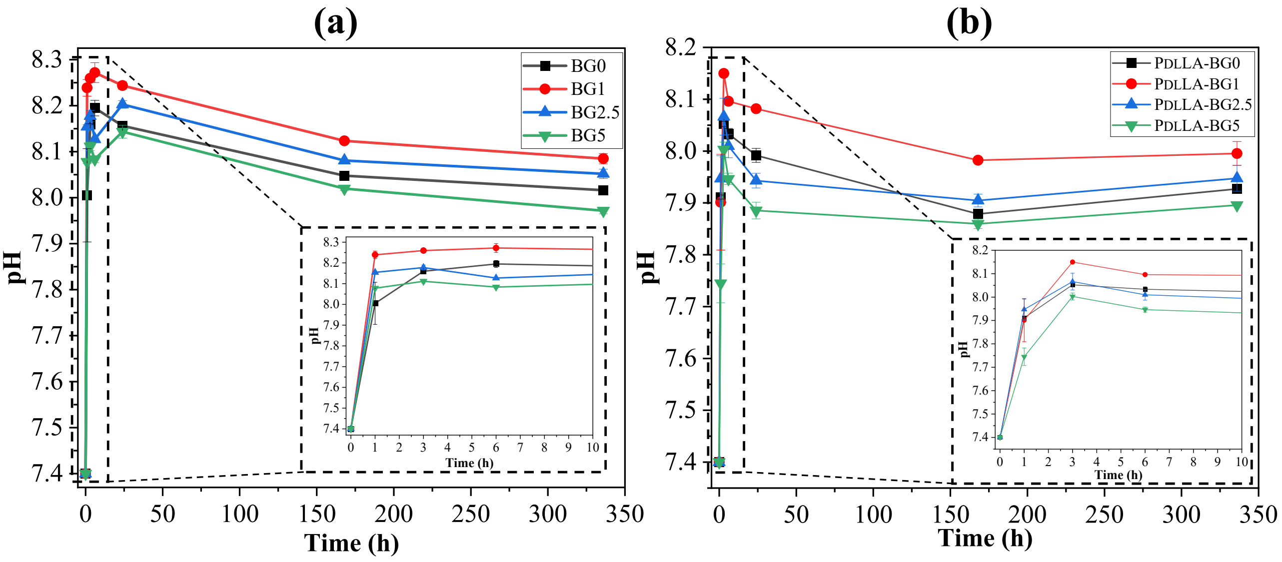

Figure S14. *pH evolution of SBF solutions from (a) bare BG and (b) PDLLA-BG samples degradation at each time-point during the test.*

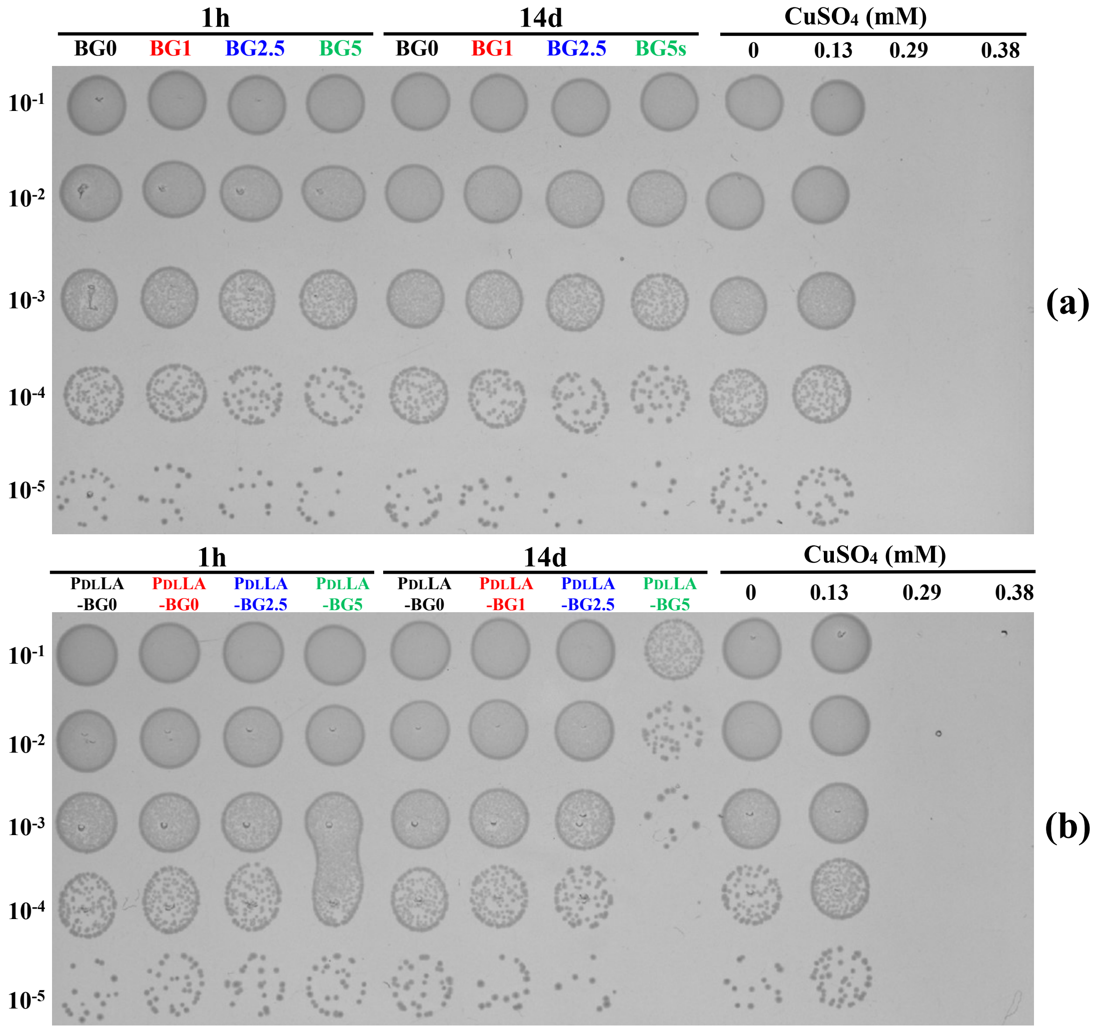

Figure S15. *Survival tests of E. coli/SBF solutions. Serial dilutions (indicated by the exponential factor on the left) of the cultures (ref fig. 6) after the 24 hours incubation were spotted on M9 agar plates. Photos of the plates with (a) bare BG, (b) PDLLA-BG, positive and negative controls on the right of each photo, the latter consisting into $CuSO_4$ solutions with a concentration equal to the nominal Cu amount present in Cu-doped BG particles that would virtually be released if all the particles were completely degraded.*

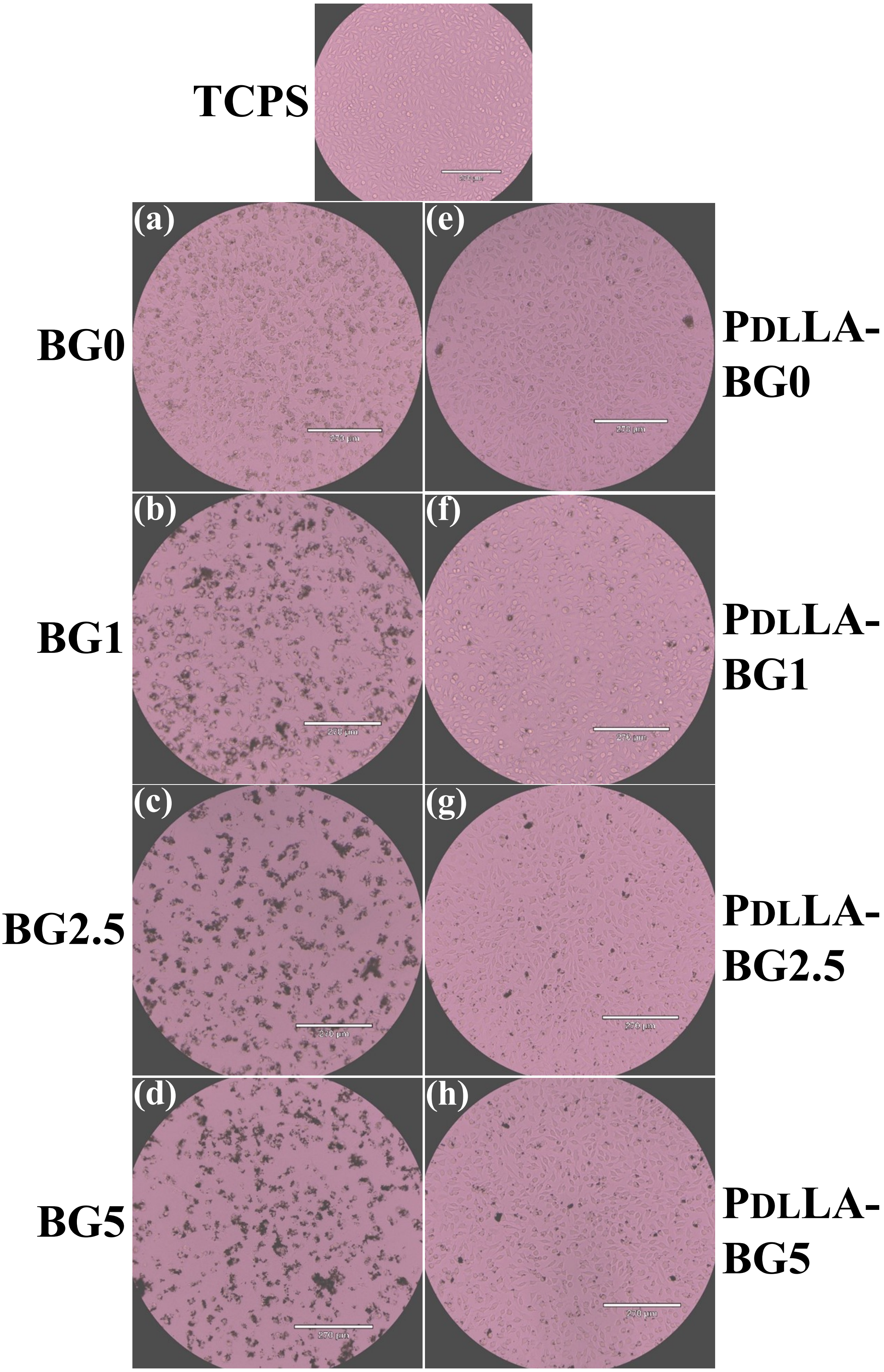

Figure S16. *Brightfield microscope images of L929 after 24 hours treatment with (a, b, c, d) bare BG and (e, f, g, h) PDLLA-grafted BG samples, with the positive control (TCPS) showed on the top (scale bar = 270 µm). The pink colour of the background is due to the culture medium.*

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