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1     **Designing a novel nanocomposite for bone tissue engineering using electrospun**  
2             **conductive PBAT/polypyrrole as scaffold to direct nanohydroxyapatite**  
3                     **electrodeposition**

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11

12     **Abstract:** Electrospinning is a well-recognized technique for producing nanostructured  
13     fibers capable to support cell adhesion and further proliferation. Here, we prepared a  
14     novel electrospun blend from poly (butylene adipate-co-terephthalate) (PBAT), a non-  
15     conductive and biodegradable polymer, and a conductive polymer, namely polypyrrole  
16     (PPy). Therefore, the goal was to create electrically conductive nanoscaffolds for tissue  
17     engineering applications. Furthermore, to improve the scaffold biomimetic features for  
18     bone regeneration purposes, we demonstrated the feasibility of electrodepositing  
19     nanohydroxyapatite (nHAp) onto the new hybrid scaffold. Electrochemical  
20     measurements confirmed the electrical conductivity of the novel PBAT/PPy scaffold,  
21     which allowed for the nHAp electrodeposition, further confirmed via ATR-FTIR  
22     analysis and FE-SEM micrographs. The PPy loading did not change the fibers' average  
23     diameter, although the increase in the solution conductivity was probably responsible to  
24     lead to electrospun mats with smaller beads and lower presence of flattened regions

25 compared to PBAT neat. The hybrid scaffold was more hydrophilic than PBAT neat.  
26 The first presented an advanced contact angle (ACA) of 84°, whilst the latter presented  
27 an ACA of 115°. The incorporation of PPy to PBAT maintained the ability of the  
28 generated scaffold to support cell adhesion with no changes in MG-63 cell viability.  
29 However, PBAT/PPy scaffold presented higher values of alkaline phosphatase, an  
30 important indicator of osteoblasts differentiation. In conclusion, we demonstrated a  
31 feasible approach to create electrically conductive nanoscaffolds, which are capable to  
32 undergo to nHAp electrodeposition in order to generate materials that are more  
33 hydrophilic and with improved cell differentiation. These results show the potential of  
34 application of this novel scaffold towards bone regenerative medicine.

35 **Keywords** Poly (butylene adipate-co-terephthalate); polypyrrole; electrospinning;  
36 electrodeposition; nanohydroxyapatite; cytotoxicity; ALP

37

38

### 39 **1. Introduction**

40

41 In the recent decades, many processing techniques have been employed for  
42 producing nanoscaffolds aiming at tissue engineering applications.<sup>1, 2</sup> In the field of  
43 bone tissue regeneration, it is essential to reach bone-extracellular matrix (ECM)-  
44 architectures like, which in turn play a crucial role in controlling cell adhesion and  
45 further differentiation. Among the aforementioned processing techniques,  
46 electrospinning occupies a prominent place due to its recognized ability to produce  
47 tridimensional fibrous structures, which is mandatory for applications in the field of  
48 bone tissue engineering.<sup>3,4</sup>

49 Electrospun nanofibers scaffolds present a wide range of potential applications,  
50 owing to their high porosity, pore interconnectivity and physical-chemical properties.  
51 We can address applications in the fields of wound dressing,<sup>5</sup> membranes,<sup>6</sup> tissue  
52 engineering<sup>3</sup> or even as carriers for drug delivery.<sup>7</sup> Combining all the above mentioned  
53 properties with the ability of electrospun polymeric scaffolds to support cell adhesion  
54 and further differentiation and proliferation, electrospinning has been used as primary  
55 technique to produce fibrous nanoscaffolds for many tissue engineering applications.<sup>3</sup>

56 Polyesters, from synthetic or natural sources, have been widely studied towards  
57 their potential for biomedical applications, more specifically in tissue engineering  
58 applications.<sup>8-10</sup> Recently, poly (butylene adipate-co-terephthalate) (PBAT), a  
59 copolymer, aroused as a promising alternative.<sup>11-17</sup> This polymer is very flexible and  
60 has a wide range of interesting properties, such as high elongation at break and  
61 biodegradability.<sup>18</sup>

62 Ribeiro Neto et al.<sup>19</sup> prepared nanocomposites based on PBAT and  
63 hydroxyapatite (HA) particles via electrospinning and spin coating. In this study,  
64 Ribeiro Neto et al.<sup>19</sup> verified not only that these novel nanocomposites ensured the  
65 attachment, proliferation and differentiation of adipose stem cells, but also that implants  
66 using these materials triggered only a mild inflammatory response. Recently, our group  
67 has demonstrated the preparation of electrospun PBAT/superhydrophilic multi-walled  
68 carbon nanotubes with enhanced mechanical properties and adequate cell viability  
69 levels.<sup>20</sup> Nevertheless, to date materials from the electrospinning of PBAT and their  
70 blends have received little attention related to their preparation and application.<sup>11, 19, 21,</sup>  
71 <sup>22</sup>

72 Conductive polymers have been often applied to produce scaffolds for tissue  
73 engineering applications.<sup>23-28</sup> Furthermore, it has been also hypothesized a mechanism

74 in which the piezoelectric signals can regulate the bone growth.<sup>29</sup> At the cellular level,  
75 the bone cell type that plays an important role in the bone structure development and  
76 appears to be involved in bone mechanotransduction, the osteocytes, was identified.<sup>30</sup>  
77 Consequently, for bone regeneration, these cells communicate with other bone cells,  
78 such as osteoblasts and osteoclasts. Therefore, the influence of electrical stimulation on  
79 bone healing has been studied *in vitro* and *in vivo*.<sup>31-34</sup>

80 Electrodeposited nanohydroxyapatite (nHAp) presents a great similarity to the  
81 mineral component of natural bone, as regards of dimensions and microstructure, whilst  
82 it shows excellent bioactivity, biocompatibility and osteoconductivity.<sup>35-37</sup> Owing to  
83 these outstanding properties, nHAp has been long evaluated for applications in the field  
84 of bone tissue/regeneration.<sup>37-40</sup> Previous study of our group has demonstrated an  
85 effective, fast and low-cost way to electrodeposit nHAp layers onto modified vertically  
86 aligned multi-walled carbon nanotubes (VAMWCNTs).<sup>41</sup> To date, the  
87 electrodeposition of nHAp onto polyesters polymeric fibers, such as electrospun fibers,  
88 has been underexplored since the lack of conductivity of these scaffolds.

89 Polypyrrole (PPy), a well-known conductive polymer, has been often applied as a  
90 biomaterial due to the possibility of generating cellular stimulus, adhesion and  
91 proliferation besides of bacteria reduction.<sup>42-45</sup> To date several authors electrospun  
92 polyesters/pyrrole nano/microfibers for tissue engineering.<sup>25, 46-49</sup> However, so far there  
93 is no study published using PBAT/PPy blends towards tissue engineering applications.  
94 Moreover, there is no study addressing the electrodeposition of nHAp on polyesters  
95 surfaces, as previously mentioned, due to the lack of conductivity of these polymers.  
96 Herein, we presented for the first time the preparation of electrospun PBAT/PPy fibers  
97 aiming at tissue engineering applications. In this context, we evaluated the cytotoxicity  
98 and alkaline phosphatase activity (ALP) using human osteoblasts. This novel

99 biomaterial presented promising properties for future *in vivo* applications aiming at  
100 bone tissue engineering.

101

## 102 **2. Experimental**

103

### 104 **2.1 Materials**

105

106 BASF SE kindly provided the pellets of PBAT (commercial Ecoflex® F Blend  
107 C1200). The solvents used in this investigation were dimethylformamide (DMF, Sigma-  
108 Aldrich,  $\geq 99\%$ ) and chloroform (Sigma-Aldrich,  $\geq 99\%$ ). Calcium nitrate tetrahydrate  
109  $[\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}]$  and ammonium phosphate dibasic  $[(\text{NH}_4)_2\text{HPO}_4]$  were also purchased  
110 from Sigma-Aldrich, with high chemical grade. Any mention of other chemicals has the  
111 respective origin indicated along the text.

112

#### 113 **2.1. Electrospinning of PBAT/PPy fibers**

114

115 Electrospinning was carried out from solutions containing PBAT and PPy at 12  
116 wt% and 1 wt%, respectively, using chloroform and DMF as solvent system (60/40). In  
117 a typical preparation, PBAT was dissolved in chloroform during 2 h, under vigorous  
118 stirring, while PPy was dispersed in DMF under sonication (VCX 500 – Sonics) during  
119 60 min. After PPy was fully dispersed, the two solutions were mixed and the resulting  
120 solution was stirred during 20 h until complete homogenization. Electrospinning  
121 optimal conditions were established as follows: 13 kV, 10 cm as needle-collector  
122 distance, solution flow rate of  $1 \text{ mL h}^{-1}$ . The counter electrode was covered with  
123 aluminum foil to collect the electrospun mats, which were easily displaced to proceed

124 with the characterizations and biological assays. During electrospinning, we carefully  
125 controlled the temperature (21-23 °C) and humidity (45-55%).

126

## 127 **2.2. Electrodeposition of nHAp onto PBAT/PPy fibers**

128 First, we evaluated the electrochemical performance of the PBAT/PPy scaffolds  
129 by collecting cyclic voltammograms using a classical electrode cell with a well-known  
130 potassium ferrocyanide (II) (5 mM, Synth-F1008) in 0.1 M KCl<sub>(aq.)</sub> solution. After that,  
131 we electrodeposited nHAp crystals on PBAT/PPy scaffolds using a standard three-  
132 electrode cell controlled by Autolab PGSTAT 128N. The PBAT/PPy scaffolds were  
133 employed as a working electrode by inserting it inside a copper/Teflon electrochemical  
134 cell, which exposed a fixed electrode area ( $\sim 0.27 \text{ cm}^2$ ) to the solution, and also  
135 established electrical contact to a copper rod on the back-side. A platinum mesh was  
136 used as counter electrode, while Ag/AgCl (3 M KCl<sub>(aq.)</sub>) as reference electrode. The  
137 electrolyte solution used was composed of  $0.042 \text{ mol L}^{-1}$  of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  +  $0.025$   
138  $\text{mol L}^{-1}$  of  $(\text{NH}_4)_2\text{HPO}_4$ . The pH was adjusted to 4.7 and automatically measured  
139 throughout the process of electrodeposition using a pX1000 real-time pH meter (no data  
140 shown, Metrohm). Magnetic stirring and a thermostatic bath were used to maintain the  
141 process at constant stirring and temperature ( $\sim 70^\circ\text{C}$ ), respectively. The nHAp crystals  
142 were produced on PBAT/PPy scaffolds by applying a constant potential of  $-3.8 \text{ V}$  for  
143 30 min. This set-up was chosen to promote stoichiometric nHAp with a Ca/P ratio of  
144  $\sim 1.67$ .

145

## 146 **2.3. Characterization of PBAT/PPy/nHAp fibers**

147

148 *PBAT/PPy fibers*

149

150 ATR-FTIR (Attenuated Total Reflectance Fourier Transform Infrared  
151 Spectroscopy) was performed using a Perkin-Elmer Spotlight 400 FTIR Imaging  
152 System. Data were collected in the range of 4000-450  $\text{cm}^{-1}$  in absorbance mode.

153 FE-SEM (Field-Emission Scanning Electronic Microscopy) was carried out  
154 using a Mira3 TESCAN Microscope, operating at 20.0 kV. Prior to analysis, all samples  
155 were coated with a thin layer of gold (~10 nm) using a sputter-coat system, in order to  
156 improve image acquisition.

157 The dynamic contact angle between a deionized water drop and the surface of  
158 the samples was measured. A Krüss contact angle device (Model DSA 100S) equipped  
159 with a recording system was used. Briefly, a single drop of deionized water (2  $\mu\text{L}$ ) was  
160 deposited on the surface of the samples (fixed on Teflon substrates) by an automatized  
161 dispositive (syringe-needle system) to generate a drop with accurate volume. The  
162 measurements of the angle between the interface were taken in different times (0-2400  
163 s). All measurements were carried out in a controlled humidified atmosphere (~ 60%).

164

165 *PBAT/PPy/nHAp fibers*

166

167 FE-SEM (Field-Emission Scanning Electronic Microscopy) was carried out  
168 using a Mira3 TESCAN Microscope, operating at 20.0 kV, in order to characterize the  
169 nHAp crystals morphology. Prior to analysis, all samples were coated with a thin layer  
170 of gold (~10 nm) using a sputter-coat system, in order to improve micrograph  
171 acquisition. The microscope was coupled to an OEM easyEDX detector in order to  
172 determine semi-quantitatively the content of calcium (Ca) and phosphorous (P) and also  
173 to perform a mapping of these atoms directly live in the SEM micrograph. EDX and

174 mapping analyses were performed at 10.0 kV. For EDX analysis the samples were not  
175 coated with gold.

176 X-ray diffraction (XRD, X-Pert Philips) with Cu K- $\alpha$  radiation generated at 40  
177 kV and 50 mA was used to characterize the microstructure and phase content of the  
178 nHAp crystals. The results were compared to the standards for HAp powder (JCPDS  
179 01-072-1243).

180

#### 181 **2.4. Cell culture**

182

183 Human osteoblasts from MG-63 cell line (ATCC® CRL-1427™) were cultured  
184 with Dulbecco's Modified Eagle Medium (DMEM, GIBCO) supplemented with 10% of  
185 Fetal Bovine Serum (FBS, GIBCO) at 37°C.

186

#### 187 **2.5. Cellular adhesion analysis**

188

189 SEM (EVO MA10, Zeiss) was used to analyze the adhesion of cells over the  
190 scaffolds. Osteoblasts cultivated for 24 h over the polymeric scaffolds were fixed with  
191 fresh prepared 4% paraphormaldehyde/2.5% glutaraldehyde (Sigma-Aldrich) solution  
192 for 10 min at room temperature. Dehydration was carried out sequentially in the dishes  
193 with acetone (Sigma-Aldrich) at concentrations of 50%, 70%, 90% and 100% for 10  
194 min each, followed by 1:1 vol/vol acetone/HMDS (Sigma-Aldrich) solution incubation  
195 for 30 min and then 100% HMDS for 30 min. The surface of the samples was sputter-  
196 coated with a thin gold layer (~10 nm).

197

#### 198 **2.6. Cellular viability assay**

199

200 The cellular viability of cultured cells was determined with the MTT  
201 colorimetric assay, adapted from the method proposed by Mosmann<sup>50</sup>. During  
202 incubation, the MTT was reduced by dehydrogenase enzyme from mitochondria within  
203 the viable cells, precipitating the insoluble formazan crystals. All the samples pieces (10  
204 x 10 x 1 mm) were sterilized with ethanol (70% v/v) and rinsed with PBD. MG-63  
205 human osteoblast cells were seeded at a concentration of  $2 \times 10^4$  cells/well. The  
206 incubation was performed under a CO<sub>2</sub> (5%) atmosphere, at 37 °C, for 1 and 7 days.  
207 Latex fragments were used as positive control of cell death at the same dimensions of  
208 the substrates. After the incubation period, the samples were removed from their  
209 respective wells. Only adhered cells were incubated with MTT solution (1 mg mL<sup>-1</sup>,  
210 Sigma-Aldrich, Saint Louis, Missouri, USA) for 3 h at 37 °C.

211 After removal of the MTT solution, dimethyl sulfoxide (DMSO) (Sigma-Aldrich  
212 Saint Louis, Missouri, USA) was added to each well and incubated under stirring for 15  
213 min. After complete solubilization of the dark-blue crystal of MTT formazan, the  
214 absorbance of the content of each well was measured at 570 nm with a  
215 spectrophotometer Spectra Count (Packard). The blank reference was taken from wells  
216 with DMSO only, and its value subtracted from samples and control OD. The cell  
217 viability was expressed as percentage related to the control.

218

### 219 **2.7. Alkaline Phosphatase assay (ALP)**

220

221 Osteoblasts differentiation is dependent on the expression of alkaline  
222 phosphatase enzyme. Therefore, osteogenic stimulation by the scaffolds is directly  
223 correlated to the enhancement of ALP activity. To assess the scaffolds ability to

224 stimulate osteoblasts differentiation, MG-63 cells were cultured on the samples in a 24  
225 well plate for 14 and 21 days and the ALP content analyzed. The wells were washed  
226 three times with PBS at 37 °C and incubated with 2 mL of 0.1% sodium lauryl sulfate  
227 (SLS) for 30 min. The SLS/cells solution was mixed with Lowry solution (Sigma-  
228 Aldrich) and incubated for 20 min at room temperature. Folin–Ciocalteu phenol reagent  
229 (Sigma-Aldrich) was added for 30 min at room temperature to allow color development.  
230 Absorbance was measured at 680 nm. The total protein content was calculated based on  
231 albumin standard curve and expressed as  $\mu\text{g mL}^{-1}$ . To determine ALP activity through  
232 the releasing of thymolphthalein monophosphate, we used an Alkaline Phosphatase Kit  
233 (Labtest Diagnóstica, Belo Horizonte, BR) in accordance with the manufacturer's  
234 recommendations. First, 50  $\mu\text{L}$  of thymolphthalein monophosphate were mixed with 0.5  
235 mL of 0.3 M diethanolamine buffer for 2 min at 37 °C. The solution was then added to  
236 50  $\mu\text{L}$  of the lysates obtained from each well. After 10 min, at 37°C, 2 mL of 0.09 M  
237  $\text{Na}_2\text{CO}_3$  and 0.25 M NaOH were added for color development for 30 min. Absorbance  
238 was measured at 590 nm using a UV 1203 spectrophotometer. ALP activity was  
239 correlated with total protein content and expressed as ALP  $\mu\text{mol}$   
240 thymolphthalein/min/mL.

241

## 242 **2.8. Statistics analysis**

243

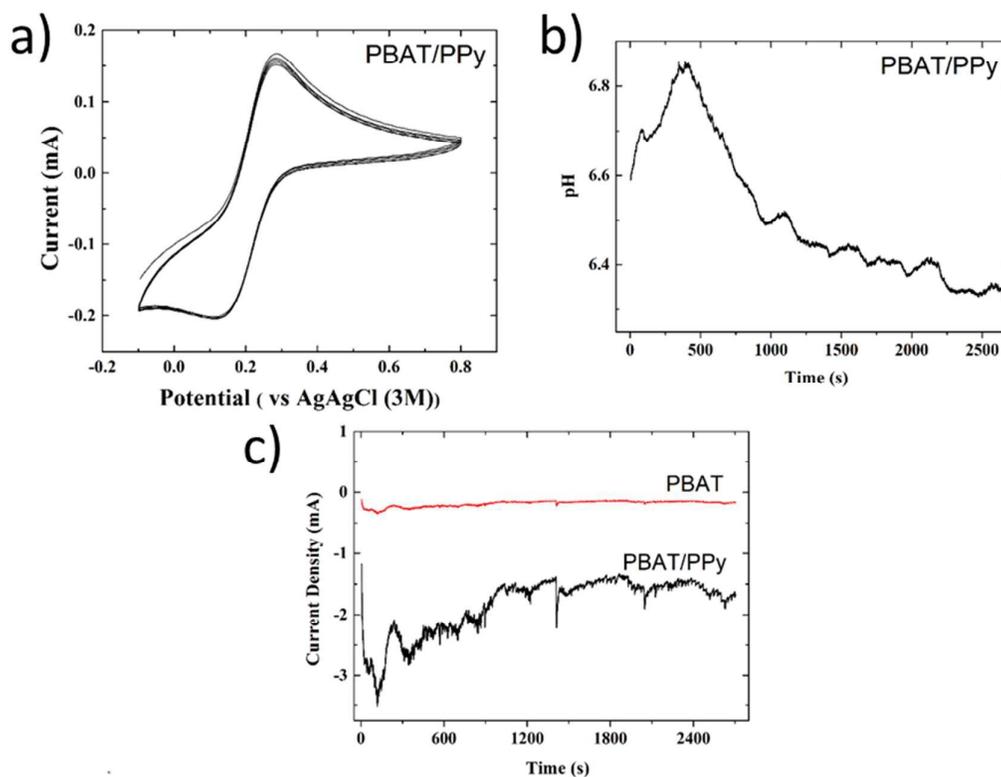
244 Cell culture experiments were conducted in quadruplicate and all values reported  
245 as mean  $\pm$  standard deviation. The difference between groups was analyzed by  
246 ANOVA test followed by Tukey's post-hoc ( $p < 0.05$ ).

247

## 248 **3. Results and Discussion**

249

250 **Fig. 1** shows the electrochemical response (a), pH measurement (b) and current  
 251 density (c) of PBAT and PBAT/PPy scaffolds.



252

253 **Figure 1:** (a) Cyclic voltammograms of PBAT/PPy scaffolds taken at 10, 25, 50, 100  
 254 and 200  $\text{mV s}^{-1}$  in 5 mM  $\text{K}_3\text{Fe}(\text{CN})_6/0.1 \text{ M KCl}_{(\text{aq})}$ . (b) pH and (c) Current density  
 255 transients during electrodeposition of nHAp on PBAT/PPy scaffolds at  $-3.8 \text{ V}$  vs.  
 256 Ag/AgCl and  $T = 70 \text{ }^\circ\text{C}$ .

257

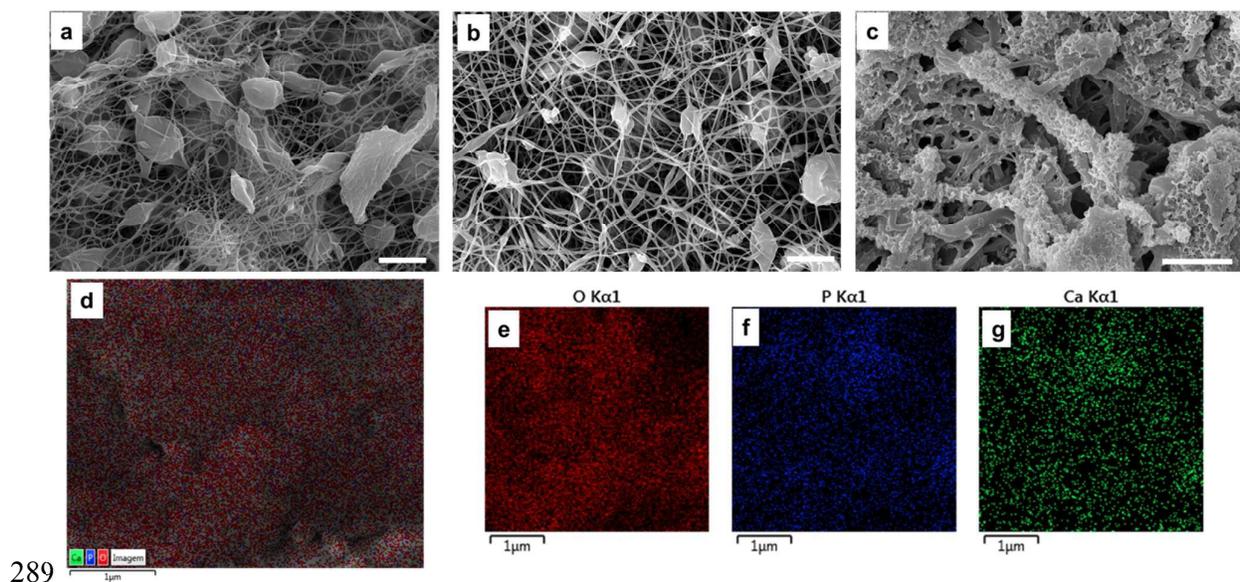
258 **Fig. 1a** shows the voltammograms recorded in 0.1 M KCl aqueous electrolyte  
 259 with a sweep rate of 10-200  $\text{mV s}^{-1}$  of the PBAT/PPy scaffolds. As depicted, the  
 260 current-voltage curve of composite presented capacitive behavior between the potentials  
 261 of 0.8 -0.1 V vs Ag/AgCl (3 M). The oxidation-reduction peaks at 0.28 V and 0.12 V  
 262 vs. Ag/AgCl (3 M) are respectively attributed to reactions of conductive PPy polymer

263 incorporated to PBAT. As a direct result, PBAT/PPy scaffolds have current capacitance  
264 and current density.

265 **Fig. 1 b** shows a pH decrease for more acidic levels due to an oxidation reaction  
266 taking place at the anode ( $2 \text{H}_2\text{O}_{(l)} \rightarrow \text{O}_{2(g)} + 4 \text{H}^+_{(aq)} + 4\text{e}^-$ ), which forms  $\text{H}^+$  during  
267 water splitting. The pH was measured between the working electrode and the counter  
268 electrode. The shape of the current transient is different, and the measured current  
269 density is much higher than those reported by Eliaz and Sridhar<sup>51</sup> for electrodeposition  
270 of HAp on CP-Ti at either pH = 4.2 or pH = 6.0. However, the applied potential  
271 reported by Eliaz and Sridhar<sup>51</sup> was  $-1.4 \text{ V vs. SCE}$  (*i.e.*  $-1.356 \text{ V vs. Ag/AgCl}$ ),  
272 which resulted in less hydrogen evolution. The extensive hydrogen evolution in the  
273 present work may have been responsible for the noisy and unsteady current transient  
274 besides electrodeposition on 3D ultrathin fibers. While the kinetics of nucleation is  
275 promoted by the high overpotential, crystal growth is suppressed by the intensive  $\text{H}_2$   
276 evolution. As a consequence, smaller nHAp crystals are formed and the coating is  
277 governed by secondary nucleation processes.

278 **Fig. 1 c** shows a comparison between the average current density measured  
279 during nHAp electrodeposition. Clearly, we noticed that the measured current density  
280 for PBAT/PPy scaffolds was ten times higher than for PBAT. The nHAp  
281 electrodeposition process involved an evolution of hydroxyl ions on the surface  
282 electrode (PBAT/PPy scaffolds). Consequently, the hydroxyl ions, induced acid–base  
283 reaction to form  $\text{HPO}_4^{-2}$  and  $\text{PO}_4^{-3}$ , are responsible for calcium phosphate precipitation  
284 on PBAT/PPy scaffolds.<sup>52</sup> Diffusion process is responsible to control the  
285 electrodeposition process besides of current density and pH changing of the solution.<sup>53</sup>  
286 We presented all these characteristics in **Fig. 1 b and 1 c**.

287 **Fig. 2** shows the micrographs of PBAT, PBAT/PPy and PBAT/PPy after nHAp  
 288 electrodeposition.



290 **Figure 2.** FE-SEM micrographs of (a) PBAT; (b) PBAT/PPy and (c) PBAT/PPy/nHAp  
 291 (bar scales = 2.5  $\mu\text{m}$ ). EDS mapping of PBAT/PPy/nHAp in (d) layers (Ca, P, O  
 292 atoms); (e) O atom; (f) P atom and (g) Ca atom.

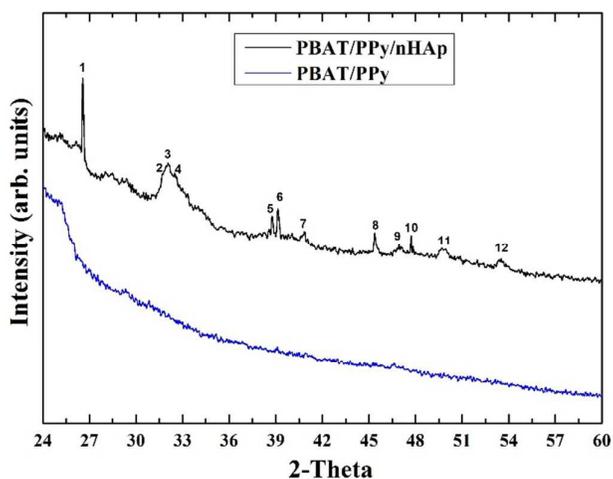
293

294 We observed that the PPy loading did not promote significant changes in the  
 295 average diameter of the fibers. PBAT presented an average diameter of  $111 \pm 26$  nm  
 296 (**Fig 2a**), while PBAT/PPy had a small increase ( $132 \pm 33$  nm, **Fig 2b**). Both samples  
 297 presented a bead-on-a-string morphology, however it can be noted that loading PPy led  
 298 to smaller beads with less flattened regions. This reduction on the beads size and further  
 299 fusiform aspect may be attributed to the increase in the electrical conductivity due to the  
 300 presence of PPy and consequent increase in the neat charge density in the jet.<sup>54</sup>  
 301 Electrical measurements showed that while the PBAT solution presented an electrical  
 302 conductivity of  $0.2 \mu\text{S cm}^{-2}$ , the introduction of PPy increased the electrical  
 303 conductivity to  $36.2 \mu\text{S cm}^{-2}$ . One can note that the electrodeposition onto the

304 PBAT/PPy scaffold surface (**Fig 2 c**) was effective and led to nHAp crystals  
305 homogeneously deposited.

306 **Fig. 2 (d-g)** shows the EDX mapping of PBAT/PPy/nHAp scaffolds. The  
307 mapping distribution of Ca, P and O atoms (**Fig 2d, f and g**) indicated a homogeneous  
308 distribution of electrodeposited nHAp onto the PBAT/PPy scaffolds. We observed a  
309 Ca/P of 1.69, which was quite close to the stoichiometric nHAp (1.67) present in bone  
310 tissue.<sup>36</sup>

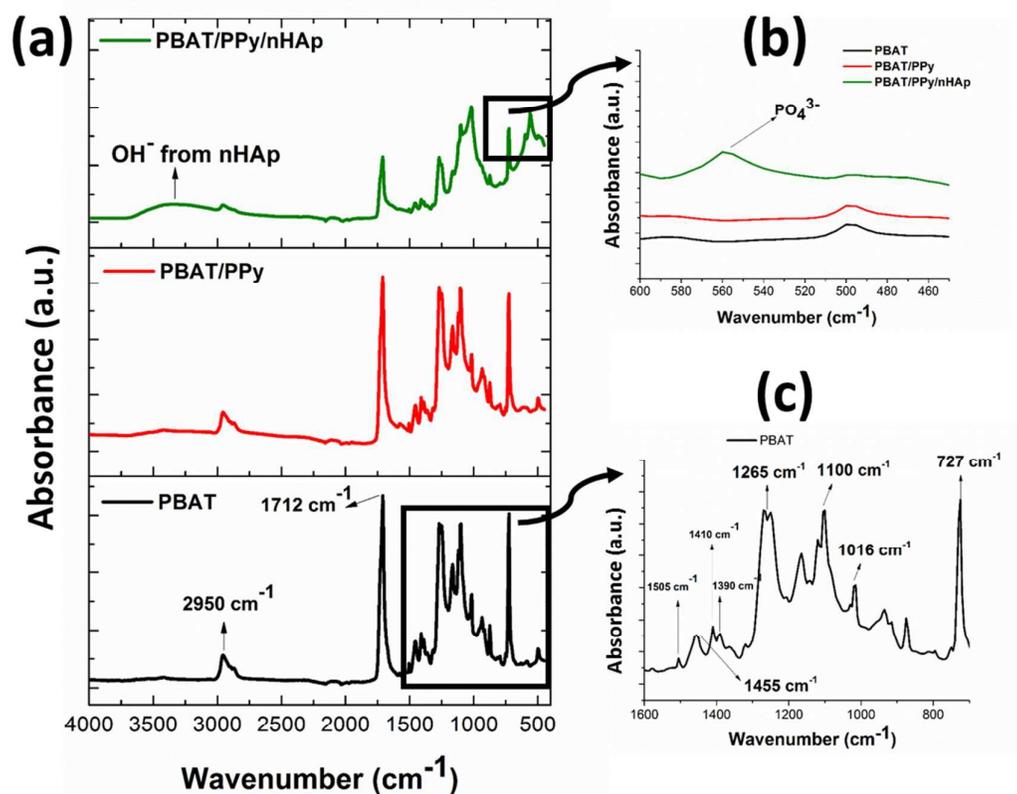
311 **Fig. 3** shows the XRD patterns of PBAT/PPy and PBAT/PPy/nHAp scaffolds.  
312 One can see that the apatite formation is confirmed by the presence of several  
313 characteristic XRD peaks in the diffraction patterns. The principal diffraction peaks of  
314 nHAp appear at 2-Theta values of 25.9° for reflection (002) and at 31.9° (triplet) for  
315 reflections (211), (112) (JCPDS 01-072-1243).<sup>55</sup>



316

317 **Fig. 3** shows the ATR-FTIR spectra of electrospun PBAT, PBAT/PPy and  
318 PBAT/PPy/nHAp.

319



320

321 **Figure 4.** ATR-FTIR spectra of (a) PBAT, PBAT/PPy and PBAT/PPy/nHAp; (b) zoom322 in the 600-450 cm<sup>-1</sup> region, showing the absorbance of the PO<sub>4</sub><sup>3-</sup> group; (c) zoom in the323 1600-700 cm<sup>-1</sup> region for PBAT.

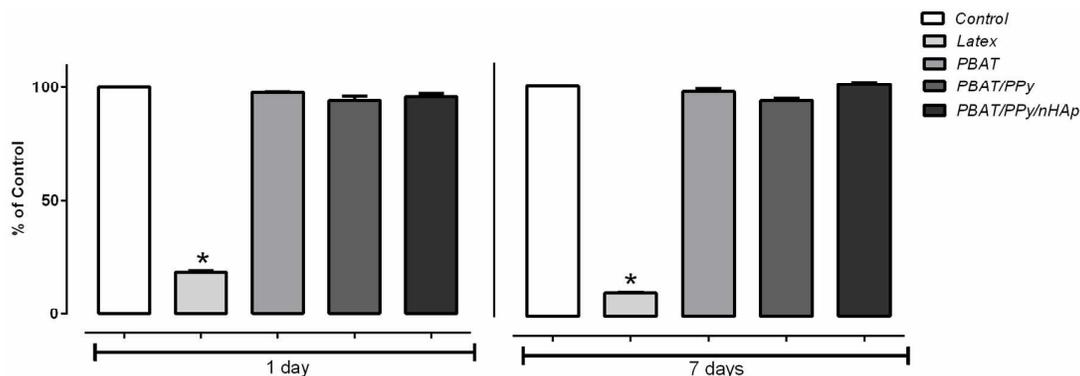
324

325 The ATR-FTIR spectra of the electrospun PBAT, PBAT/PPy and PBAT/PPy

326 after nHAp electrodeposition (PBAT/PPy/nHAp) (**Fig. 4**) showed the characteristics327 peaks of the polyester (PBAT).<sup>20</sup> The asymmetric stretching vibration of CH<sub>2</sub> groups328 can be identified at 2950 cm<sup>-1</sup>; stretching vibration of C=O at 1710 cm<sup>-1</sup>; stretching of329 phenylene group at 1455 and 1505 cm<sup>-1</sup>; trans-CH<sub>2</sub>-plane bending vibration at 1410 and330 1395 cm<sup>-1</sup>; symmetric stretching vibration of C-O at 1265 cm<sup>-1</sup>; C-O left-right331 symmetric stretching vibration absorption at 1100 cm<sup>-1</sup>; bending vibration absorption of332 CH-plane of the phenylene ring at 1016 and 731 cm<sup>-1</sup>.

333 The electrodeposition of nHAp on the surface of the electrospun PBAT/PPy mat  
 334 could be confirmed via ATR-FTIR. The vibrational band in the region of  $3500\text{ cm}^{-1}$   
 335 (PBAT/PPy/nHAp, **Fig. 4 a**) can be attributed to  $\text{OH}^-$  absorption peak whilst the  $\text{PO}_4^{3-}$   
 336 absorption peak could be observed at  $566\text{ cm}^{-1}$  (**Fig. 4. b**).

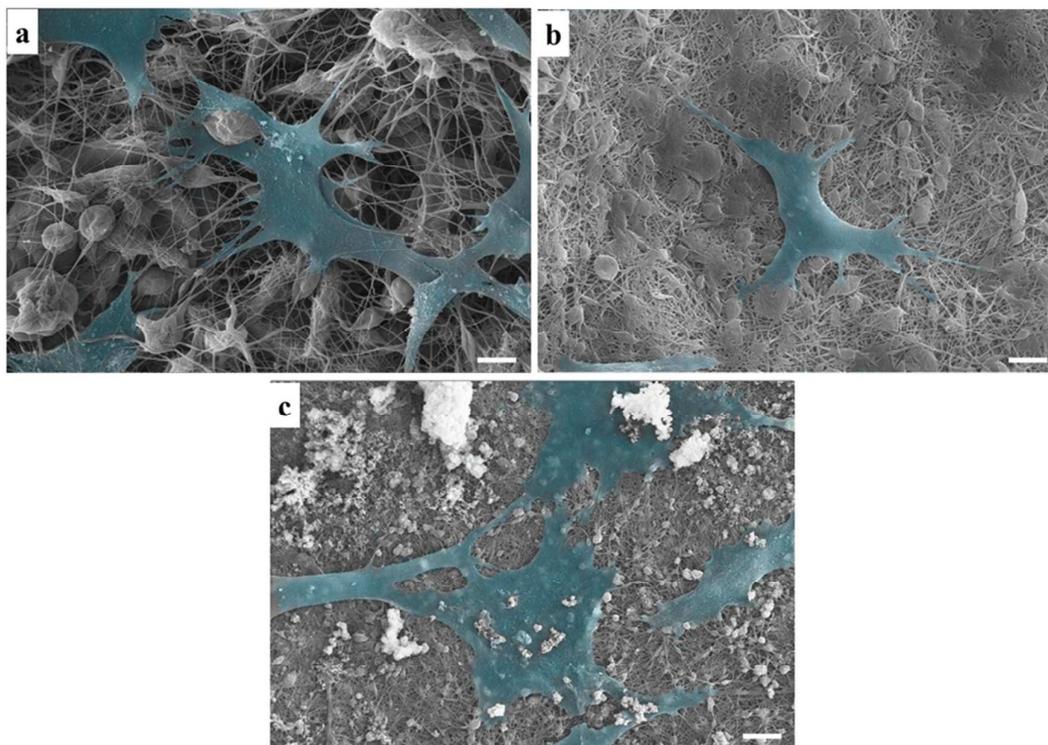
337 PBAT neat or with PPy, and nHAp are known as no cytotoxic materials,<sup>56-59</sup>  
 338 providing a great biologic compatibility. Electrospun PBAT, PBAT/PPy and  
 339 PBAT/PPy/nHAp mats presented no cytotoxic effect after the contact with cells during  
 340 1-7 days. **Fig. 5** shows the osteoblasts viability after cultivation on PBAT, PBAT/PPy  
 341 and PBAT/PPy/nHAp scaffolds for 1 and 7 days.



342  
 343  
 344 **Figure 5.** Cellular viability analysis of MG-63 cultured on polymeric scaffolds after 1  
 345 and 7 days. \* $p < 0.05$  vs control.

346  
 347 Nevertheless, the aim of biomaterial research is not restrictive to the production  
 348 of inert materials, but also to the development of scaffolds capable to improve  
 349 biomaterial integration with organic tissue. The creation of a biomimetic material for  
 350 bone regeneration can be mainly achieved with structure and surface manipulations<sup>60, 61</sup>  
 351 , as for example the addition of nHAp.<sup>62, 63</sup> Nanofiber scaffolds with tridimensional  
 352 structure can enhance cellular adhesion because their arrangement is similar to  
 353 extracellular matrix.<sup>64</sup> As **Fig. 6** shows, polymeric nanofiber mats can provide an ideal

354 scaffold for cells. MG-63 osteoblasts were able to adhere to the scaffolds, maintaining  
355 the classical osteoblast morphology (part of the cells was blue painted).

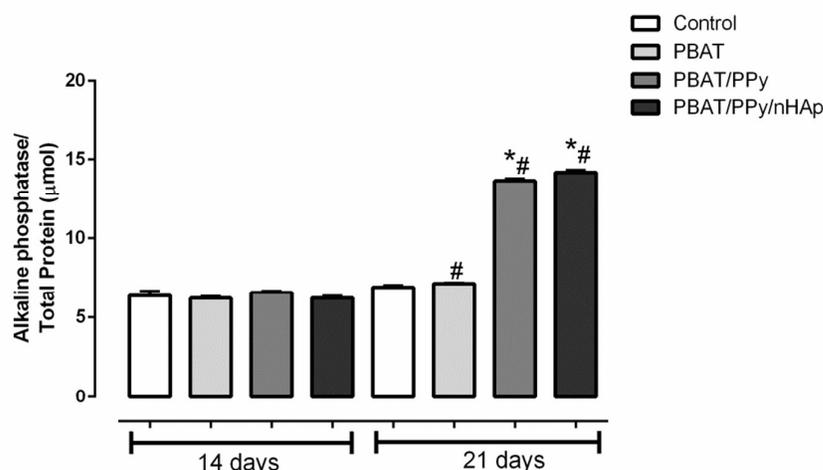


356 **Figure 6.** SEM micrographs of part of MG-63 cells (blue painted) cultured 24 h on (a)  
357 PBAT, (b) PBAT/PPy and (c) PBAT/PPy/nHap scaffolds. Scale bar = 10  $\mu\text{m}$ .

359

360 Next, we evaluated the induction of osteoblasts differentiation when cultured  
361 with the polymeric scaffolds. Osteoblast differentiation is a time-dependent  
362 phenomenon that can be modulated by the cell type and stimulus. ALP increase using  
363 MG-63 culture in an osteoinductive media is expected typically after 28 days of  
364 culture.<sup>65</sup> **Fig. 7** shows that PPy induced an increase in ALP activity after 21 days;  
365 meanwhile the presence of nHAp was indifferent. An increase in ALP activity occurs  
366 during osteoblasts differentiation and is commonly related to calcification of bone  
367 matrix.<sup>66</sup> Several authors reported PPy as an enhancer of ALP activity, however the

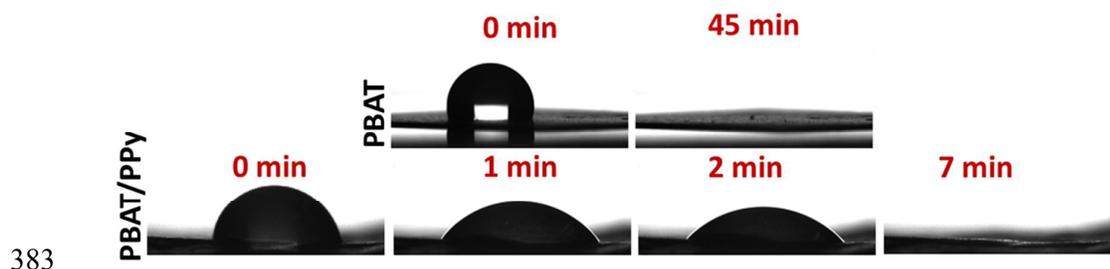
368 experiments involved the use of electrical stimulation<sup>59, 67</sup> or osteoinductive media.<sup>68</sup>  
 369 However, here we proved that only the PPy loading can increase the ALP activity.



370 [ **Figure 7.** ALP activity after 14 and 21 days of osteoblasts culture with PBAT,  
 371 PBAT/PPy and PBAT/PPy/nHAp scaffolds. \* $p < 0.05$  vs control group of the same  
 372 period. # $p < 0.05$  vs control group from 14 days.  
 373

374  
 375 The ability of PPy itself to induce osteoblastic differentiation had not been  
 376 described yet and a possible explanation relies on wettability properties. **Fig. 8** shows  
 377 snapshots taken at different times for PBAT and PBAT/PPy scaffolds. We observed an  
 378 advanced contact angle (ACA, at 0 min) of  $84^\circ$  for PBAT/PPy, while for PBAT the  
 379 ACA was quite higher ( $115^\circ$ ). Furthermore, the water drop was fully absorbed by  
 380 PBAT/PPy scaffold after 7 min, while PBAT took 45 min, which is quite in agreement  
 381 with recent studies.<sup>20</sup>

382



383

384 **Figure 8.** Snapshots taken at different times for PBAT and PBAT/PPy during contact  
385 angle measurements.

386

387 Some authors observed a relationship among surface hydrophobicity and cell  
388 spreading, osteodifferentiation and improvement of metabolic activity.<sup>69-71</sup> Therefore,  
389 changes in the hydrophilicity after PPy incorporation could be the responsible for the  
390 observed osteoblasts behavior and ALP activity.

391

#### 392 4. Conclusions

393

394 Herein we present for the first time the production of conductive and hydrophilic  
395 PBAT/PPy mats using electrospinning technique. We electrodeposited stoichiometric  
396 nHAp crystals onto PBAT/PPy mats and produced a novel nanocomposite with  
397 potential of application in bone tissue engineering. The PBAT/PPy/nHAp  
398 nanocomposites presented biocompatibility, providing a good surface for cellular  
399 adhesion and the induction of osteoblasts differentiation. All these characteristics are  
400 very illustrative and could accelerate bone formation and implant fixation. Further  
401 investigations are required to verify the application of this novel nanobiomaterial and  
402 will be carried out in our lab.

403

404

405

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407

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414 **5. References**

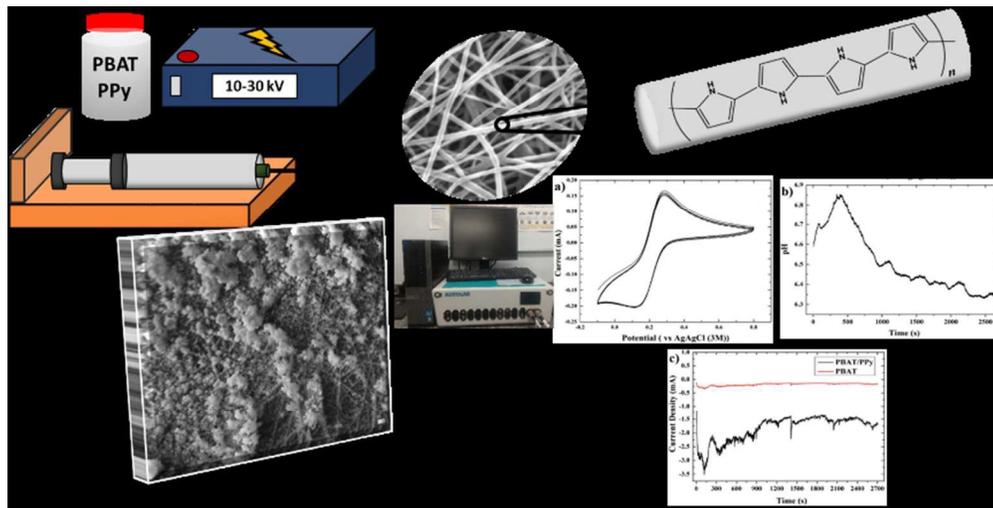
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