

EVALUATION OF 4D PRINTED PBS, PBS/HA USING PAM PROCESS

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Abstract

Polybutylene succinate (PBS) was used in this study to evaluate its capability to ensure properties of an implanting scaffold. PBS was mixed with an inorganic filler; hydroxyapatite (HA) which is a component of natural bone to enhance cell viability. Biocomposites were prepared using melt extrusion process, trying to obtain the highest filler ratio. Compounds were then shaped using Fused Filament Fabrication (FFF) and Pellet Additive Manufacturing (PAM) which allowed to print biocomposites with high filler ratio. 3D specimens showed an increase in elastic properties with radiation dose due to crosslinking reaction in PBS, but a decrease on the elongation at break with the predominance of chain scission at higher radiation dose. Moreover, PAM process permitted to print biocomposites with a filler ratio of HA up to 40% (w/w). 4D printing of biomaterials using electron beam irradiations looks promising for bone tissue engineering.

Keywords: 4D printing, Bone tissue engineering, Electron beam irradiations, Pellet Additive Manufacturing PAM

1. INTRODUCTION

Additive manufacturing possessed many benefits such as allowing printing object with controlled porosity and high precision in a short time. This process could be used for medical applications, especially for bone tissue engineering [1]. Bone tissue engineering aimed for the regeneration or replacement of bone tissue damaged by disease or injuries. Scaffolds could be manufactured by 3D printing for bone regenerative applications. However, these biomaterials must be in agreement with established specifications containing necessary properties such as biocompatibility and biodegradability. One group of biomaterial-based scaffolds for bone tissue engineering is polymers. Polyesters are commonly used in medical applications due to their biocompatibility with controlled degradation rate. Polyesters with addition of inorganic filler such as hydroxyapatite (HA), main inorganic components of bone, form a biocomposite material with improved mechanical properties and bioactivity [2]. Polybutylene succinate (PBS) was used as polymer matrix due to his excellent mechanical properties and thermal properties [3].

In addition, scaffolds of biocomposite could be designed for the regeneration of bone tissue using additive manufacturing. These scaffolds should be porous in order to promote cell adhesion and proliferation [4]. Pore size greatly affected osteogenic properties and the biodegradation rate. However, the biodegradation rate of these scaffolds cannot be spatially controlled. In this case, 4D printing process is a good way to control this aspect by applying structural modifications on materials with an external stimulus [5], such as electron beam radiation, and change the shape of scaffolds in time via chain-scission/crosslinking reaction. Thereby, molecular weight and crystallinity of polymers are greatly affected by the radiation treatment, allowing to control the bioresorption rate.

In the first place, the influence of an external stimulus such as electron beam irradiation was evaluated on thermal and mechanical properties on PBS. Thus, 4D printed PBS was beforehand studied before starting the manufacturing of biocomposites. Then, PBS/HA scaffolds were elaborated and analyzed using Thermogravimetric Analysis (TGA) in order to determine maximum filler ratio possible and control the amount of filler ratio on each compound produced before printing process.

2. MATERIALS AND METHODS

2.1. Materials

PBS pellets were purchased from NaturePlast (France). The reference PBE003 was extrusion grade with these commercial characteristics: density 1.26, melt flow index (190 °C, 2.16 kg) 4-6 g/10 min, melting temperature 115 °C. Spray-dried HA powder was provided from Medicoat (France) at a granulometry between 45-150 µm, purity of 95 % and 15-63 µm with a purity of 98 %.

2.2. Biocomposites manufacturing

PBS pellets and HA powders were dried at least for 12 h at 60 °C before extrusion process. Compounds were prepared using melt extrusion process with BX10 extruder from Axon Plastics Machinery ab (Sweden). Temperature was set from the feeding area to the die at 135/135/130 °C. Extruder screw rotation was maintained at 15 Hz. Biocomposites have been made with HA proportions ranging from 0 % to 70 %wt. For printing tests, four formulations were performed, combining the PBS matrix with 0, 20, 40 or 60 %wt. HA. Biocomposites were then stored in hermetic plastic bags at room temperature.

2.3. Printing settings

PBS samples were manufactured as tensile test specimen type 5A to comply with the standard ISO 527-2, using a 3D printer by Fused Filament Fabrication (FFF) process with a Raise3D N2 printer (U.S.A.). Idea Maker software was used to slice different profiles. The printer nozzle diameter was 0.4 mm and stayed the same for all samples to avoid any deviation. Printing temperature was set at 190 °C for the printer nozzle and 80 °C for printer built-platform and maintained throughout the process. Printing parameters such as printing speed has been settled at 50 mm/s with a layer thickness of 0.2 mm and 0.4 mm as line width with a filling rate of 100 %. The slicing profile used one outer shell and an alternating fill pattern of $\pm 45^\circ$ for all layers.

The stiffness of the biocomposites makes it difficult to print from the FFF process so the biocomposites were printed using another process. Biocomposites of PBS/HA were printed using a 3D printer Pellet Additive Manufacturing (PAM) process with a PAM P-series printer from Pollen AM (France). A modified Cura software (by Pollen AM) was used to slice different profiles. The printer nozzle diameter was 0.2 mm. Printing temperature was set between 140-150 °C for the extruder printing temperature and 160-170°C for the printer nozzle. Perforated platform was used to limit warping effect of PBS during cooling. The printing speed was regulated by the speed of the extrusion screw, which was adjusted according to the viscosity of the polymer. The printing speed was set at around 70 mm/s. Other printing parameters were layer thickness of 0.2 mm, 0.2 mm as line width, one outer shell, an alternating fill pattern of $\pm 45^\circ$ for all layers and a filling rate of 100 %.

2.4. Electron beam irradiations

All tensile tests samples were irradiated in passes of 25 kGy at most, between 0 and 100 kGy, with a high energy electron beam Linac type accelerator of 10 MeV with an average dose rate of 15 kGy/s by using pulsed sourced (IONISOS, Chaumesnil, France). Irradiated samples are then directly stored after irradiation in hermetic plastic bags at 2 °C.

2.5. Thermal analysis

The thermogravimetric analysis (TGA) was carried out by using a thermal analyzer (Netzsch TG 209 F3 Tarsus, Germany). TGA allowed to evaluate the proportion in HA and the influence of HA ratio of biocomposites pellets on the thermal degradation temperature, necessary for 3D printing. About 15 mg of sample was put in a TGA pan and the analysis was performed from 30 °C to 600 °C with a heating rate of 10 °C/min. Nitrogen gas was used to keep an inert atmosphere at a flow rate of 20 mL/min.

The differential scanning calorimetry (DSC) permits to evaluate the influence of radiation dose on thermal and structural properties on irradiated tensile tests samples. It was made using a DSC Q20 from TA Instruments (U.S.A.). About 10 mg was weighed in an aluminium pan. To maintain inert atmosphere, nitrogen gas was used with a flow rate of 50 mL/min. Samples were first heated from -80 °C to 200 °C with a heating rate of 10 °C/min to remove thermal history, then cooled down to -80 °C at

a cooling rate of 10 °C/min. Finally, the samples were reheated until 200 °C at 10 °C/min. TA Universal Analysis software allowed to analyze the measurement data.

2.6. Tensile tests

Tensile tests were conducted at room temperature using an Instron 3366 (U.S.A.) with a 10 kN load cell. Five pieces were 3D printed for each irradiation doses: 0, 10, 25, 50, 75 and 100 kGy. The specimens were printed with dimensions according to 5A-type specimens from ISO 527-2 standard. Traction speed was set at 10 mm/min and maintained constant throughout the tensile tests until the sample break. All data were then retreated to determine modulus Young, breaking stress and elongation at break.

2.7. Biocompatibility assay

Cell viability was conducted on 3D printed PBS scaffold (Fig. 1). Scaffolds were 3D printed in a cylinder with a diameter of 14 mm and a pore size of 400 µm and walls of 200 µm. Each layer had a thickness and height of 200 µm. Samples were immersed in cell environnement containing mesenchymal stem cells from dental pulp for 21 days. Cell proliferation was examined using WST-1® assay.

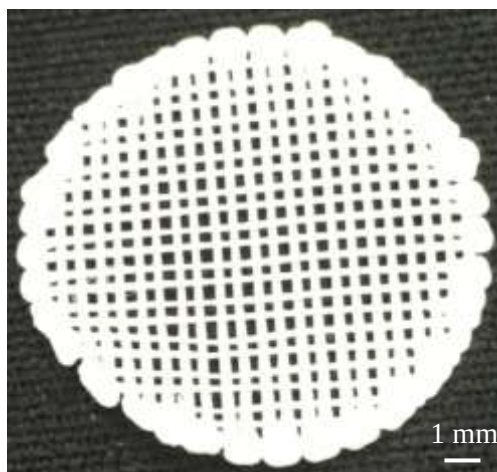


Fig. 1. 3D printed scaffold of PBS with a pore size of 400 µm and walls of 200 µm.

3. RESULTS

3.1. Thermal properties

Thermal properties of PBS under irradiation were analyzed by DSC. Thermal history of samples has been cleared with a first heating cycle in order to evaluate the effect of irradiations on material. Figure 2 report thermograms of the second heating and Table 1, data obtained from DSC curves from the second heating, including glass-transition temperature (T_g), melting temperature T_m and crystallinity (X_c).

At first sight on thermograms, thermal properties of PBS don't seem to be affected by even at a radiation dose of 100 kGy.

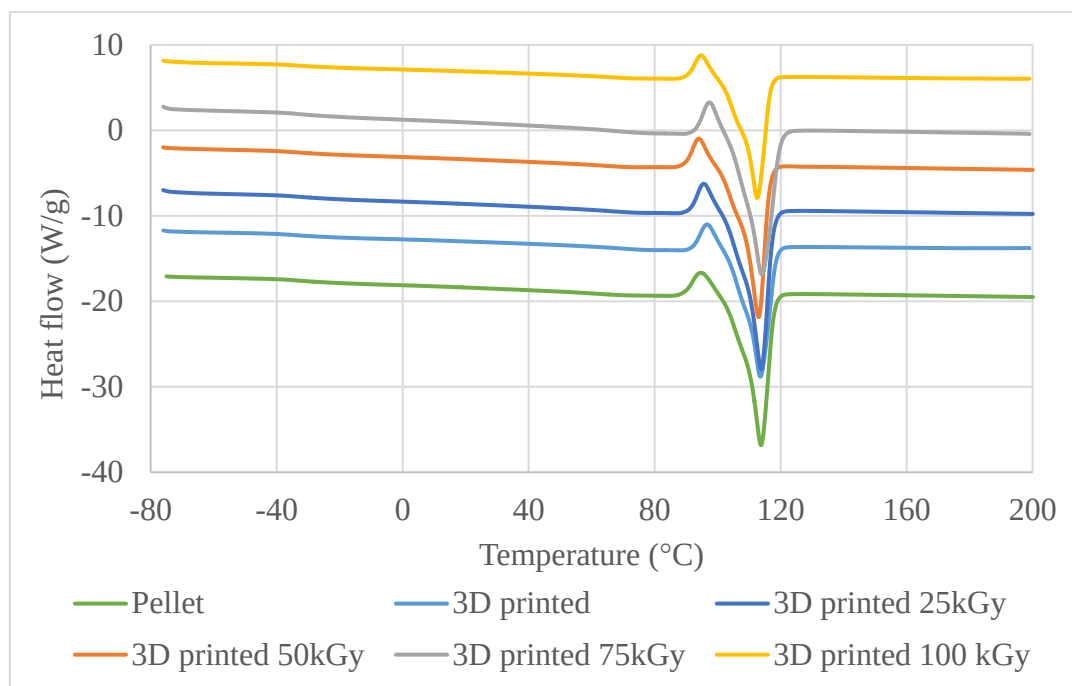


Fig. 2. DSC curves of the second heating of PBS at different radiation dose.

Effects of irradiation on T_g are minimal. T_g retained at -35°C for 3D printed non irradiated PBS to -36°C . However, there is a small decrease of 1°C observed when a radiation dose of 25 kGy was applied on samples, then increased to -35°C at 75 kGy before decreasing once again at -36°C . This can be explained that under radiation, PBS may be the subject of crosslinking and chain scission. At low radiation dose under 75 kGy, cross linking phenomenon could be the predominant reaction whereas chain scission may be dominant at higher radiation dose. This effect was also observed in the work of Byung Kuk K. *et al.* [6] with PBS crosslinked by triallyl isocyanurate (TAIC). Mobility of PBS chains is more restricted at low radiation due to crosslinking and tends to increase T_g .

Doses (kGy)	T_g ($^\circ\text{C}$)	T_m ($^\circ\text{C}$)	ΔH_m (J/g)	X_c (%)
0	-35.3 ± 0.7	113.8 ± 0.2	74.4 ± 1.2	30.4 ± 0.7
25	-36.1 ± 0.2	113.0 ± 0.1	73.7 ± 2.0	30.3 ± 0.7
50	-35.7 ± 1.1	112.7 ± 0.2	74.4 ± 1.9	30.8 ± 0.7
75	-34.7 ± 1.2	112.6 ± 0.0	76.3 ± 0.6	30.9 ± 0.4
100	-35.9 ± 0.6	112.5 ± 0.1	75.7 ± 3.3	31.1 ± 0.8

Table 1. Thermal properties obtained from DSC curves of PBS samples under electron beam radiation during the second heating

Concerning T_m , a slight decrease was observed from 114°C for 3D printed non irradiated PBS to almost 112°C for 3D printed PBS irradiated at 100 kGy. A first decrease of 1°C was noticed when a radiation dose of 25 kGy was applied on PBS and kept decreasing until 100 kGy. In fact, free radicals that were formed under irradiation tend to recombine or crosslink [7] in the crystalline and amorphous region of PBS. Negrin M. *et al.* [8] highlighted the effects of irradiations by creating defects, leading to the formation of less perfect crystallites due to the recombination of radicals. The impact of generating less perfect crystallite could be seen on the melting temperature which, then, tends to decrease.

By considering a melting enthalpy of 210 J/g for 100 % crystalline PBS [9], crystallinity could be calculated from data collected from DSC curves. Crystallinity value started at 30,5 % for non irradiated PBS and remained globally stable, based on standard deviation, up to a radiation dose of 100 kGy to end at 30,9 %. Due to crosslinking reaction, crystallinity has decreased of 2 % between non irradiated sample and irradiated at 25 kGy. And with the increase of radiation dose, chain scission phenomenon has started to be predominant, permitting to PBS chains to have better mobility and increase crystallinity.

The thermal stability of 3D printed PBS samples were studied by thermogravimetric analysis. The onset thermal degradation temperature (T_o) was taken when 1 % mass loss was seen and the temperature at the inflection point of the degradation curve (T_i) at maximum peak of the derivative form of TGA analysis. Results are reported in Table 2.

Doses (kGy)	T_o (°C)	T_i (°C)
0	307.8 ± 4.1	401.9 ± 1.2
25	300.6 ± 6.7	400.9 ± 0.8
50	285.6 ± 8.1	397.5 ± 2.4
75	282.1 ± 2.7	399.6 ± 1.5
100	290.8 ± 3.9	398.8 ± 0.2

Table 2. Summary of the onset thermal degradation temperature (T_o) and temperature at the inflection point of the degradation curve (T_i) of PBS samples under electron beam radiation

Effect of electron beam irradiation could not be seen on T_i that remained stable at 400 °C. It can be noticed that the beginning of thermal degradation (T_o) moved to lower temperature, going from 308 °C for non irradiated sample to 282 °C at a radiation dose of 100 kGy. A linear decrease of 9 °C was observed up to a radiation dose of 50 kGy, then a larger decrease of 15 °C occurred at 75 kGy. These results could be explained by a slight decrease in PBS molecular weight under low radiation dose, because of few shorter PBS chains, and the drop at 75 kGy conducting to more shorter PBS chains due to many more chain scissions compared to crosslinking [8]. Based on these results, the limit between predominance of crosslinking and chain scission may occurred at 75 kGy. The diminution of molecular weight due to chain scission was also observed on other polyesters such as polylactide (PLA) [10]

3.2. Mechanical properties

Mechanical properties were analyzed by tensile tests. Results of tensile tests such as Young modulus, elongation at break and max stress were presented, respectively, in Fig. 3-4-5 for 3D printed PBS samples in function of radiation doses. The Young modulus of non irradiated 3D printed PBS has showed a value of 303 MPa and this value increased gradually with the increasing of radiation dose. By applying a radiation dose of 75 kGy, the Young modulus increased by 20 MPa compared to non irradiated sample and remained stable at this value at higher radiation dose. By increasing radiation dose the crosslinking density increased gradually [11] and as a result, stiffen PBS under irradiation. However the stability of Young modulus may be explained by the fact that most of PBS chains have crosslinked at 75 kGy. Byung Kuk K. *et al.* [6] observed this phenomenon on PBS and PBS containing TAIC with a stability of the tensile modulus starting at 50 kGy.

Therefore brittle sample was also observed as shown results in Fig 4. A diminution of 8 MPa was found between non irradiated sample and irradiated sample at 100 kGy. In fact at higher radiation dose, chain scission was predominant beyond 75 kGy, according to results from thermal properties, leading to a decrease of length of macromolecules and so a decrease of elongation at break. These results were in accordance with the work of Munirad O. *et al.* [12] who studied the influence of electron beam radiation on mechanical properties up to 140 kGy.

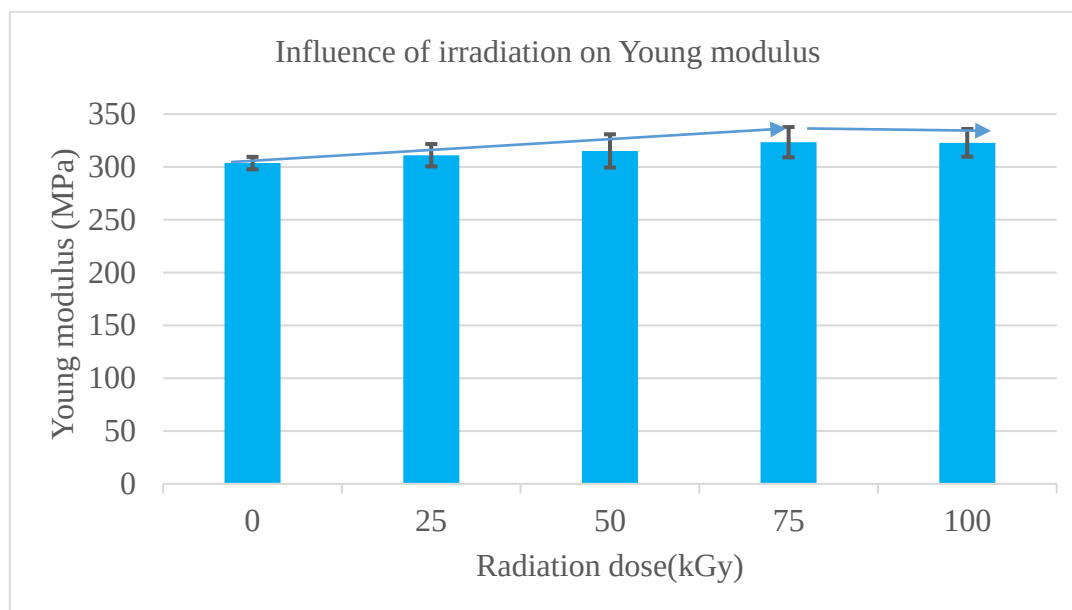


Fig. 3. Impact of irradiation on Young modulus of PBS.

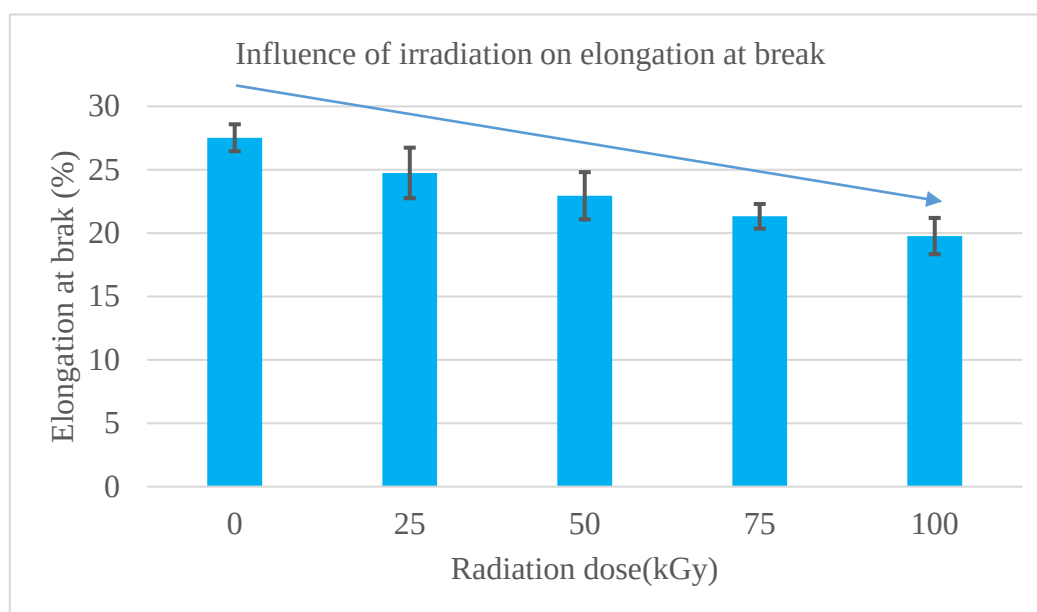


Fig. 4. Impact of irradiation on elongation at break of PBS.

However, value of max stress of 3D printed samples remained stable at 38 MPa based on standard deviation at 50 and 100 kGy. This could be explained by the competition between crosslinking and chain scission that leads to elaborate lower chain length and three dimensionnal network of polymer chains due to crosslinking. Iuliano A. *et al.* [13] have not observed significant variations on tensile strength up to a radiation dose of 26 kGy on PBS/Thermoplastic starch (TPS) but irradiations have had greater impact on Young modulus and elongation at break.

Therefore, electron beam radiation permitted to enhance elastic properties with the crosslinking but on the other hand, elongation at break of irradiated samples were greatly impacted by chain scission process that occurred at higher radiation dose and forming shorter chains, resulting in a diminution of plastic properties.

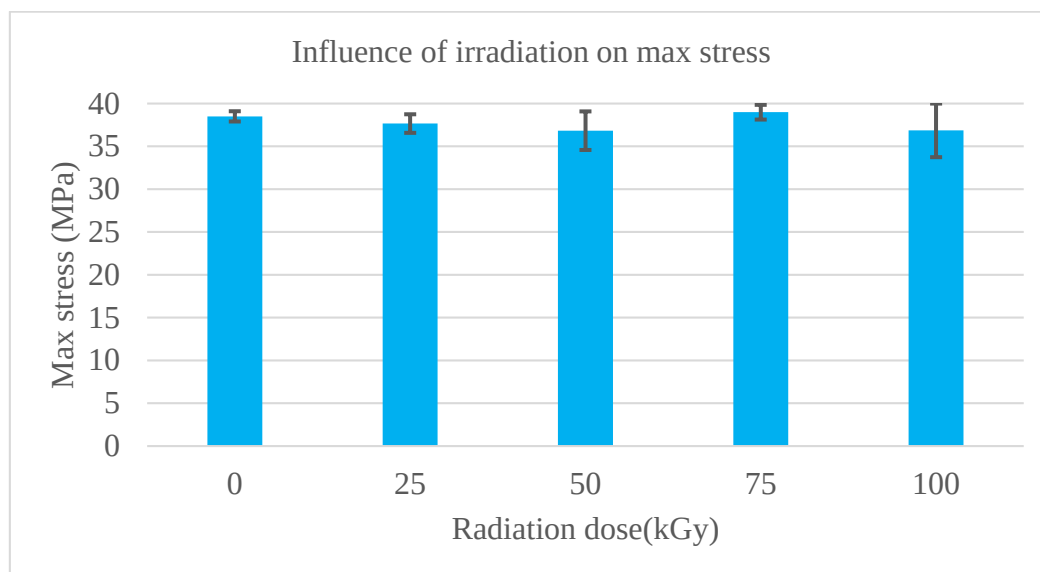


Fig. 5. Impact of irradiation on max stress of PBS.

3.3. Thermogravimetric analysis of biocomposites

Pellets of PBS/HA were performed by melting-extrusion process. Biocomposites were elaborated with a filler ratio up to 70 % (w/w) respectively. In order to control the amount of fillers, TGA were done on three pellets of each batch. Residual mass determined the experimental filler ratio of HA. Results, reported in Fig. 6, showed that manufacturing of PBS/HA was possible with a high amount of HA up to 65 %wt.

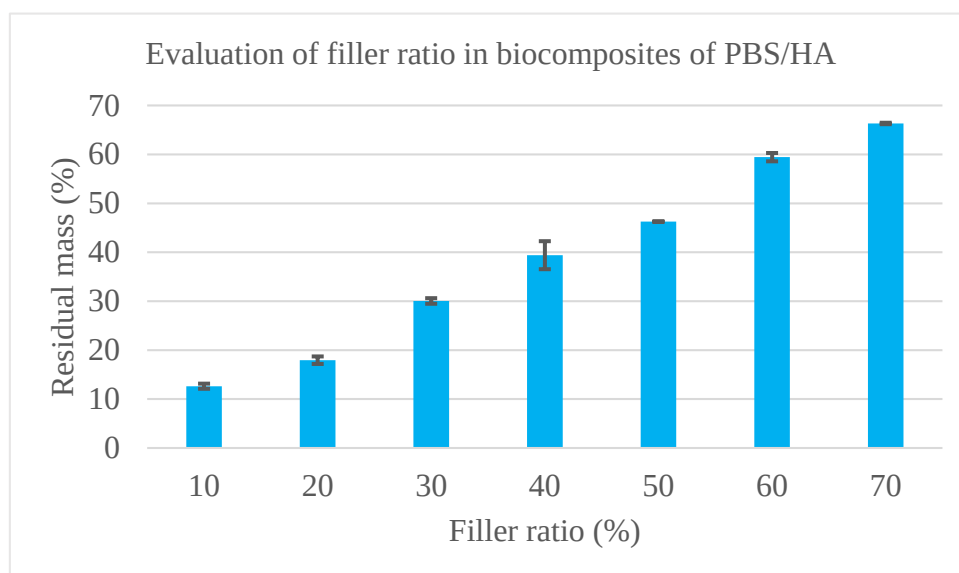


Fig. 6. Evaluation of mass residual in biocomposite of PBS with HA.

In addition, the onset thermal degradation temperature (T_o) was taken when 1 %wt. mass loss was seen and the temperature at the inflection point of the degradation curve (T_i) at maximum peak of the derivative form of TGA analysis. Results in Table 3. showed that the beginning of thermal degradation T_o remained stable at 305 °C up to 40 %wt. of filler ratio before increasing to 335 °C at 70 %wt. of theoretical filler ratio. Firstly, these biocomposites could be 3D printed without thermal degradation. Then

manufacturing biocomposites of PBS/HA with high filler ratio of HA permitted to enhance thermal stability due to the main inorganic part in the biocomposite. There has been a different effect with nano HA [14] where it was found initial thermal degradation decreased with the amount of nano filler up to 30 %.

Filler ratio (%)	T_o (°C)	T_i (°C)
0	307.8 ± 4.1	401.9 ± 1.2
10	304.1 ± 4.8	399.7 ± 0.4
20	303.7 ± 10.1	397.6 ± 0.6
30	308.8 ± 7.7	397.9 ± 3.2
40	306.0 ± 8.0	400.5 ± 1.1
50	312.2 ± 5.2	401.7 ± 0.8
60	325.1 ± 5.0	402.3 ± 1.6
70	334.9 ± 2.4	403.5 ± 1.0

Table 3. Influence of the amount of filler in biocomposites of PBS on the onset thermal degradation temperature (T_o) and temperature at the inflection point of the degradation curve (T_i)

Biocomposites of PBS/HA could be manufactured with a particle size of HA of 15-63 μm at 20, 40 and 60 %wt. in order to be 3D printed using PAM process. Working with lower particle size of HA permitted to limit problem of blockage of the printer head and to get better dispersion of filler in 3D printed architectures. Results in Fig. 7 showed benefits of printing process by homogenizing better the biocomposites. However, printing of PBS/HA (40/60) had not been possible because of too much obstruction of printer nozzle. Increasing the charge rate was not favorable for 3D printing because the output nozzle was quite thin. It would be possible to print these highly filled biocomposites with a larger diameter nozzle, but for the intended application (tissue engineering), it was necessary to make accurate and small prints.

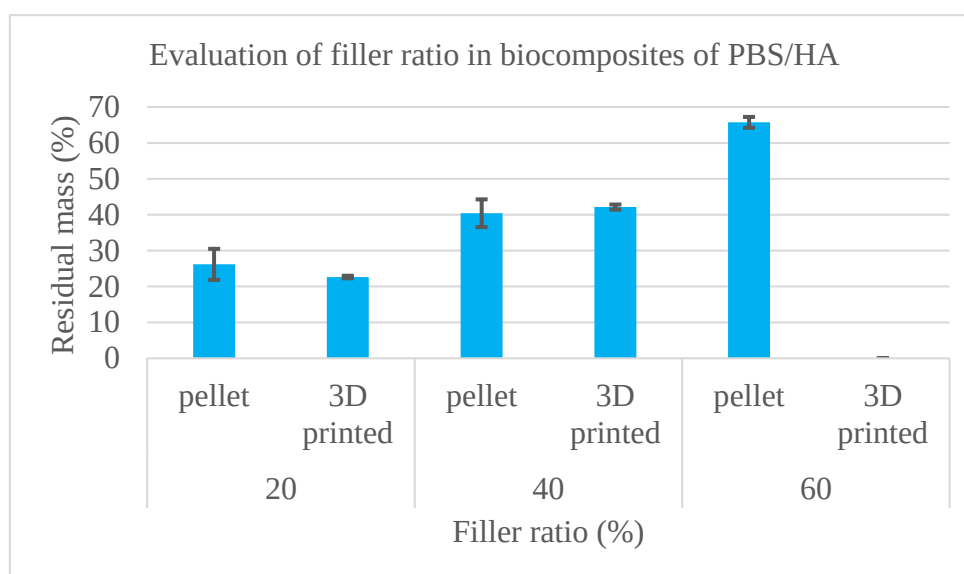


Fig. 7. Evaluation of mass residual in biocomposite of PBS with HA.

3.4. Cell viability

A preliminary study of cell viability on 3D printed PBS scaffold and PBS/HA (20) scaffold was conducted. The results in Fig. 8 showed that the cells adhered more on PBS/HA (20) scaffold than on PBS scaffold, as we observed a better cell viability after 7 days of culture on PBS/HA (20) scaffold. However, an increase in cell viability was observed for both scaffolds after 21 days of culture. These results were in agreement with the work from Grigoriadou I. et al. [15] on PBS and nanocomposites of PBS/SiO₂ nanotubes. However, further analysis have to be done to confirm the cell proliferation on scaffolds and the influence of HA on cell adhesion.

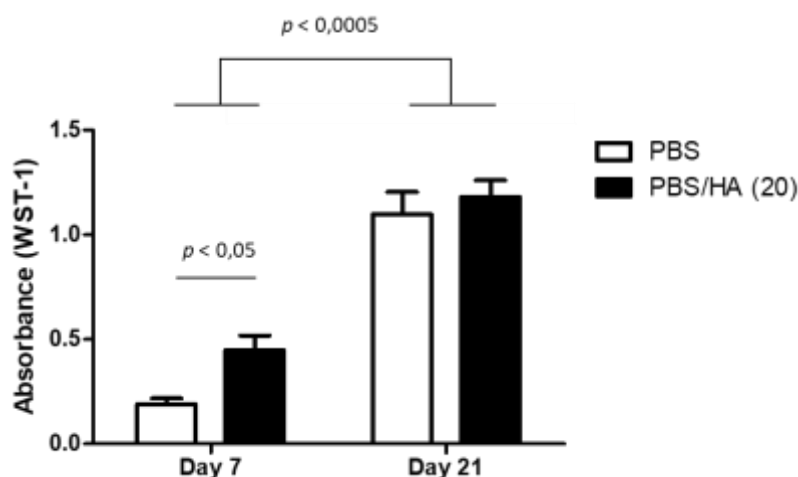


Fig. 8. Evaluation of cell viability on PBS and PBS/HA (20) scaffolds, after 7 and 21 days of culture. Mann & Whitney test

4. CONCLUSIONS

Electron beam irradiations induced different process such as chain scission and crosslinking in polymers. Depending on the radiation dose, one process was predominant compared to the other one. At a radiation dose lower than 75 kGy, crosslinking is predominant in PBS whereas at higher radiation dose chain scission is predominant. Effects could be seen on the thermal degradation temperature that occurred at lower temperature with the increase of radiation dose, because of lower length of PBS chains. Plus, crosslinking reaction due to irradiations had an impact on mechanical properties by increasing elastic properties, such as Young modulus to 20 MPa. However that induced a diminution of the elongation at break of 8 % at the highest radiation dose.

Concerning manufacturing of biocomposites, it was made possible to produce biocomposites with a filler ratio of HA up to 65 %wt. which could be 3D printed up to 40 % afterward for bone tissue engineering applications. Furthermore, 3D printed PBS allowed the cells adhesion.

This was a preliminary study of 4D printed PBS under the influence of electron beam irradiation. Further thermal and mechanical analysis need to be done on manufactured biocomposites. In addition, to conform structure modifications, molar mass analyzes will be performed.

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