ANTEC® 2021

2021 PROCEEDINGS

EFFECTS OF STEAM HEAT AND DRY HEAT STERILIZATION PROCESSES ON POLYLACTIC ACID WITH HYDROXYAPATITE COMPOSITE PRINTED BY FFF

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Abstract

3D printing is used for various medical applications, such as the manufacture of guides for surgical operations, custom medical instruments, and low-cost medical applications. In few of these studies that have been performed, the effect of sterilization on these parts has not been considered yet. The fused filament fabrication process (FFF), which is the most widely used today, is used for the making of these guides and instruments. One of the most used materials in the FFF process is polylactic acid (PLA) due to its ease of printing, however, this could be degraded with the sterilization processes by steam heat and dry heat and lose its dimensional accuracy and resistance, something required for medical applications. The purpose of this study is to determine the effects of the steam heat and dry heat sterilization processes on the mixture of PLA and hydroxyapatite (HA) to check whether this mixture can be used in medical applications that are not implantable in the human body. The percentage by weight of hydroxyapatite used is 5%. To study the effect of sterilization processes already mentioned, 3D specimens were printed for flexural, tensile, Shore Hardness and impact mechanical tests. Thermogravimetric analysis (TGA), Differential scanning calorimetry (DSC) and Dynamic mechanical thermal analysis (DMTA) tests were also performed. It is concluded that the blend of PLA and hydroxyapatite increases its resistance to temperature but decreases its mechanical characteristics.

Introduction

PLA Properties

PLA, used in medical applications and in most general applications, is a biodegradable polyester obtained from renewable resources such as corn. It is characterized by being transparent and rigid; and having high mechanical stability and relatively good tensile strength, like polycarbonate (PC). In addition, it has brightness, clarity, and barrier properties. In its manufacturing process, fewer greenhouse gases are generated than in the commodity thermoplastics production process. Its limitations are found in its low impact resistance, hence requiring an elastomer or plasticizer for some practical applications; its low maximum service temperature and low heat deflection temperature. One of the advantages, however, is its high-water permeability and moderate gas barrier properties. PLA is generally used as packaging material, although its price is higher than other packaging and commodity polymers. It is used as a biodegradable packaging film for plastic sleeves, food packaging, bottles, cold drink cups, etc. For its production, PLA starts from a monomer of lactic acid produced from sugar (dextrose) derived from plant starch origin, typically corn, but also sugar beets, wheat, and sugar cane. Its chemical structure is shown in Fig. 1.

PLA is the material mostly used for the FFF printing process [1], [2]. It is a material widely used in 3D printing processes, due to its low glass transition temperature (55°C-60°C), low melting point (175°C) and does not require a high temperature (45°C-60°C) in the printing bed[2]. PLA filament for 3D printing is available in a wide variety of colors, there are trademarks that use ceramic particles and reinforcing woods, additionally compatible with most 3D printers. The working temperature for printing should be set between 195°C and 220°C, with a maximum bed temperature of 60°C, depending on the type of filament.

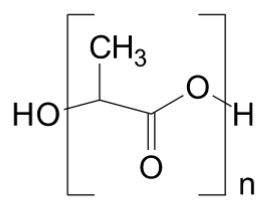

Figure 1. Chemical structural units of PLA.

Hydroxyapatite Properties

Hydroxyapatite (HA) Ca10(PO4)6(OH)2 is a three calcium phosphate used as an implant scaffold material for bone metabolism and cartilage regeneration. Bone metabolism involves resorption of existing bone by osteoclastic. HA powder is used as filler material for subsequent formation of a new bone. Tissue engineering requires a scaffolding capable of supporting the functional properties of osteogenic cells. Calcium phosphate provides the bone with a high resistance to compression, but is relatively brittle; additionally, it is thermodynamically stable to the body's pH, very close to the chemical composition, and is part of the increased bone matrix through osteointegration. Its chemical structure is shown in the Fig. 2.

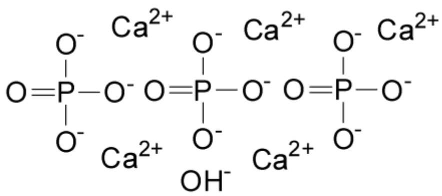

Figure 2. Chemical structural units of hydroxyapatite

PLA composites with HA are used, for example, for bone tissue growth stimulating implants [3], [4] due to its properties of biocompatibility, biodegradability, and bioresorbability. The higher the amount of HA, the PLA-HA blend is considered to increase the dimensional stability of the PLA. PLA and hydroxyapatite blend may be affected by thermal and mechanical loads in such a way that it cannot be used in medical applications.

Sterilization by Steam Heat

Steam sterilization in the form of saturated steam under pressure is one of the most effective methods of sterilization. The vaporization latent heat (e.g. ~540 calories per gram) provides 7 times as much heat on an equimolar basis as dry heat at the same 121ºC temperature, making it at least 12 times faster than dry heat sterilization [5]. Killing power of steam is principally due to the coagulation of proteins in microorganisms, which causes the denaturation of DNA and the break- down of vital enzymes. To avoid degradation of temperature-sensitive materials, sterilization is carried out at 121°C for 20 minutes and at a pressure of 15 psi. High temperatures (134ºC-138ºC) are used if a faster process is required. Drying is the period following exposure and cool-down where condensation can evaporate, and hydration effects are reversed; drying removes residual moisture and polymer hydration [6]. One of its advantages is its simplicity in relation to chemical sterilizations, another is that do not leave toxic residues. The disadvantage of this process is that it corrodes metals and wets and degrades polymers [7], [8], [9], [10], [11], such as PLA [12],[13], [14] and it only works if the steam touches the surface of the material.

Sterilization by Dry Heat

This sterilization process uses heat generated by a hot air furnace, and is used in parts that require heat to reach their whole interior, the required exposure time may vary depending on the volume of the part to be sterilized [15]. Dry heat is easy to control and monitor, being inexpensive, and not generating toxic residues or wastes as EO (Ethylene Oxide) and gamma radiation may have. Dry heat produces very high temperatures (160°C-180°C) which can destroy many microorganisms. Dry heat is used in sterilizing dental instruments to minimize the corrosion of sharp items. One of its disadvantages is the time it takes for the oven to reach the temperature required for sterilization (140ºC), another disadvantage is that the oven temperature is not uniform if a hot air circulation system is not available[15]. Dry heat can distort or melt polymers. The method of annihilating microorganisms is typically depyrogenation [16].

Materials

Sample preparation.

Blend of PLA-HA pellets was kindly supplied by Der Aiju. (Alicante, Spain) It was prepared with 5% by weight and extruded into a Filabot EX2 extrusion machine (Barre, PA, USA) to make the filament with 1.75 mm diameter. It has a density of 1.34 g/cm^3 and melt flow index (MFI) of 6g/10 min (160°C, 2.16 kg). Temperature used to extrude the filament was 185°C.

To compare changes in PLA-HA properties, a commercial gray neat PLA filament obtained from the supplier Prusament was used as a reference. Prusament PLA and Der Aiju PLA-HA were printed on the same 3D printer with similar impression parameters, as shown in Table 1.

The test specimens for mechanical testing were modeled using the Solidworks 2017 software. Then they were saved as a .STL file. Finally, to generate G code, Slic3r Prusa Edition 1.41.i3 MK3 software was used. The Original Prusa i3 MK3s 3D printer was used to manufacture all test specimens.

Main printer parameters were preconfigured in Slic3r, in Table 1 are summarized these parameters.

Table 1. Main 3D printing parameters for PLA-HA and PLA, obtained from the Slic3r Prusa Edition software.

Characteristic	**Slicer Prusa Edition parameters**		
Filament	Parameter	Units	PLA/PLA-HA
	Color	-	Grey/White
	First layer temperature	°C	215
	Other layer temperature	°C	210
	First layer bed temperature	°C	60
	Other layers bed temperature	°C	60
	Filament diameter	mm	1.75
	Supplier	-	Der Aiju/Prusament

Velocity	Perimeter velocity	mm/s	45
	Infill Velocity	mm/s	80
Layers and perimeters	Layer height	mm	0.15
	First layer height	mm	0.2
	Vertical shell number of perimeters	-	2
Infill	Infill type	-	Rectilinear
	Infill density	%	80
	Infill angle	°	45

Material Characterization

Mechanical and thermal characterization were performed to analyze the effects of the sterilization process on the properties of 3D printed PLA-HA blend. The results were also compared with a commercial PLA without any filler to evaluate the amount of change in each property.

Mechanical Characterization

Tensile test

Tensile tests were performed in accordance with ISO 527-1, the specimens were 3D printed in XY Plane direction. The tension specimens are small and have the dimensions specified in ISO 527-1.

Flexural Test

Flexural tests were performed in accordance with ISO 178, the specimens were printed in XY plane. A universal testing machine ELIB 50 of S.A.E. Ibertest (Madrid, Spain) was used with a 5 kN load cell. It was set to test the speed at 10 mm / min in both tests. The test specimens for the flexural test have the dimensions recommended in ISO 178, their dimensions are 10x4x80mm^3.

Impact Test

The impact tests were carried out according to ISO 179 with a 1-J Charpy pendulum from Metrotec (San Sebastián, Spain). All tests were performed at room temperature with at least 5 unnotched samples per test, the dimensions of the specimens were 80x4x8mm^3. Charpy impact test specimens were printed horizontally following rectilinear printing patterns with 80% infill.

Hardness Test

A Shore D hardness measurement was also performed according to ISO 868, a 676-D model hardness tester by J. Bot Instruments Vilassar de Dalt JBA (Barcelona, Spain) was used. Hardness was measured in a 7 mm thick square and 50mm of the side printed on the XY plane, using the preconfigured printing profile for each material, according to table 1, but with 100% infill and a layer thickness of 0.2mm. The 3D file was generated in SolidWorks 2017 and saved as .STL; for printing and generating the G code, the printer's own Slic3r software was used. The specimen was adjusted to these dimensions so that the defined in point 5 and 6 of ISO 868 is complied with, to perform 5 penetrations in each piece.

Thermal Characterization (TGA, DSC, DTMA)

A differential scanning calorimeter (DSC) model Mettler-Toledo 821 (Schwerzenbach, Switzerland) was used. The average sample weight ranged from 8 to 10mg and were subjected to a thermal cycle for each material according to the following: PLA-HA, first heating from 25ºC to 250ºC, cooling to 25ºC and heating up to 350ºC. Cooling and heating rates of the DSC were 10ºCmin^{-1}, the circulation flow in the furnace was 100 mL-min^{-1} of N2. Melting temperature was defined from the maxima of the first DSC-curve peak.

Crystallinity index(X_C) was calculated based on the cold crystallization enthalpy(ΔHcc), melting enthalpy(ΔHm) of the first warm-up and melting enthalpy for theoretically 100% crystalline material PLA (ΔH100 = 93.7 J·g−1) [14] according to Ec. (1),[17].

$$X_C(\%) = \frac{(\Delta H_m - \Delta H_{cc})}{\Delta H_{100}} \times 100 \quad (1)$$

Thermogravimetric study (TGA)

Thermal stability of printed materials, filaments and sterilized samples were evaluated with a thermogravimetric study (TGA). A Linseis Model TG-PT1000 thermogravimetric analyzer (Selb/Germany) was used. A warm-up curve was applied from 30ºC to 700ºC, with a sampling interval of 1s, N2 flow of 10mL-min^{-1} and a warm-up speed of 20ºC/min. The weight of all samples always ranged from 16mg to 20mg. The degradation temperature "onset" (TO) was defined as the temperature at which 5% of mass loss occurs. In addition, the maximum degradation temperature (Tmax) was obtained as the corresponding peak of the first derivative (DTG).

Dynamic Mechanical Thermal Analysis (DMTA)

DMTA test was performed on an AR-G2 rheometer from TA Instruments (New Castle, USA) with a special clamp system for solid samples, which worked in shear-torsion mode. Samples of dimensions of 4x10x40mm^3 were subjected to a sweeping program from 30ºC to 140ºC for PLA-HA at a rate of heating of 2°C/min, at a frequency of 1 Hz, and at a maximum shear deformation of 0.1%.

Sterilization process

Sterilization by Steam Heat

A pressure cooker Monix Clásica was used with 400 cm^3 of water placed inside. The specimens were subjected to a 45-minute sterilization cycle consisting of a heating cycle up to 121˚C in 5 minutes, and an exposure of 121˚C for 20 minutes and a cooling to room temperature. Temperature was monitored with a Raytek RS373-8499 thermometer.

Sterilization by Dry Heat

A Selecta oven was used, the specimens were placed in trays and heated up to 140˚C for 70 minutes, sterilized at 140˚C for 3 hours, and cooled from 140˚C inside the furnace to room temperature in approximately 4 hours.

Statistical analysis

Significance in the data differences were statistically analyzed by one-way variance analysis (ANOVA) by using Origin Pro 2017 SR2. Tukey's test with a 95 % confidence level was used to identify which data groups were significantly different from others.

Discussion and Results

Effects of HA on PLA Mechanical Properties

The tensile and flexural properties of the PLA and PLA-HA are reported in Table 2 and Table 3. It was found that neat PLA tensile modulus (TM), flexural modulus (FM), tensile strength (TS) and flexural strength (FS) are greater than the same PLA-HA properties ($p<0.05$), elongation at break (ε_b) is higher for PLA-HA, these results indicate that PLA-HA is less ductile than neat PLA. If the mechanical properties of neat PLA are compared after steam heat sterilization processes, TS, FS, TM and FM are greater than mechanical properties of PLA-HA ($p<0.05$), ε_b is greater for PLA-HA, which indicates that the material decrease its strength and ductility, as shown in fig.3. For PLA-HA, TM, TS, ε_b, FM and FS are minor for the dry heat sterilization process in relation to neat PLA, which indicates that the ductility has been reduced.

Table 2. PLA-HA tensile test results.

Material	Tensile Modulus MPa	Tensile Strength. MPa	Elongation at Break %
PLA	2095,97±25,8[a]	40,62±1,78[a]	4,39±0,18[a]
PLA-SH	1068,68±143,91[b]	29,01±2,04[b]	4,39±0,23[a]
PLA-DH	1952,55±114,15[a]	35,4±4,83[a]	3,6±0,14[b]
PLA-HA	1052,75±24,65[b]	11,41±1,1[c]	5,63±0,49[c]
PLA-HA-SH	1208,69±19,22[b]	7,66±1,04[d]	0,76±0,09[d]
PLA-HA-DH	1634,93±34,84[c]	11,22±0,69[c]	0,75±0,12[d]

SH= Steam heat ; DH=Dry heat. [a–e] Different letters within the same property show statistically significant differences between sterilization process($p < 0.05$), applying Tukey's pair comparison.

Table 3 also shows the report of changes in Charpy impact resistance (IS) and Shore Hardness. It is observed that IS does not change ($p>0.05$) between neat PLA and PLA-HA. Results show that PLA-HA hardness is lower than neat PLA ($p<0.05$), which is consistent with the results of the flexural and tensile tests.

Table 3. PLA-HA flexural, Impact and Shore D Hardness test results.

Material	Flexural Modulus MPa	Flexural Strength MPa	Charpy Impact Strength kJ/m^2	Shore D Hardness Shore (D)*
PLA	2839,48±73,68[a]	63,92±1,6[a]	12,8±0,84[a]	80,33±0,21[a]
PLA-SH	2231,02±159,81[b]	52,92±2,31[b]	19,60±1,21[b]	73,00±0,45[b]
PLA-DH	3229,38±141,55[c]	56,46±4,51[b]	13,47±0,66[a]	82,50±0,34[c]
PLA-HA	1130,35±226,49[d]	18,94±3,92[c]	13,79±1,29[a]	72,17±0,6[b]
PLA-HA-SH	1515,28±295,14[d]	17,02±3,32[c]	16,04±0,57[a]	73,33±0,67[b]
PLA-HA-DH	635,02±42,79[e]	16,34±3,05[c]	15,36±0,50[a]	75,50±0,34[d]

SH= Steam heat ; DH=Dry heat. [a–e] Different letters within the same property show statistically significant differences between sterilization process($p < 0.05$), applying Tukey's pair comparison.

Effects of Steam Heat Sterilization Process on PLA-HA Mechanical Properties

TM and FM of PLA-HA-SH increases in relation to the same PLA-HA properties, see Fig. 3. ε_b is reduced from 5.63% to 0.76%, equivalent to a reduction of 86.50%, this is due to the ceramic increase in the mixture. TS reduces until 7.66MPa, this is may be because the print layers are separated by heat, probably due to the poor interface and adhesion between PLA and the HA, which is essential for the load transfer [19] and it can also be the result that the HA content, having less tensile strength, decreases the strength of the blend. FS, IS and Shore Hardness statistically remain the same ($p>0.05$).

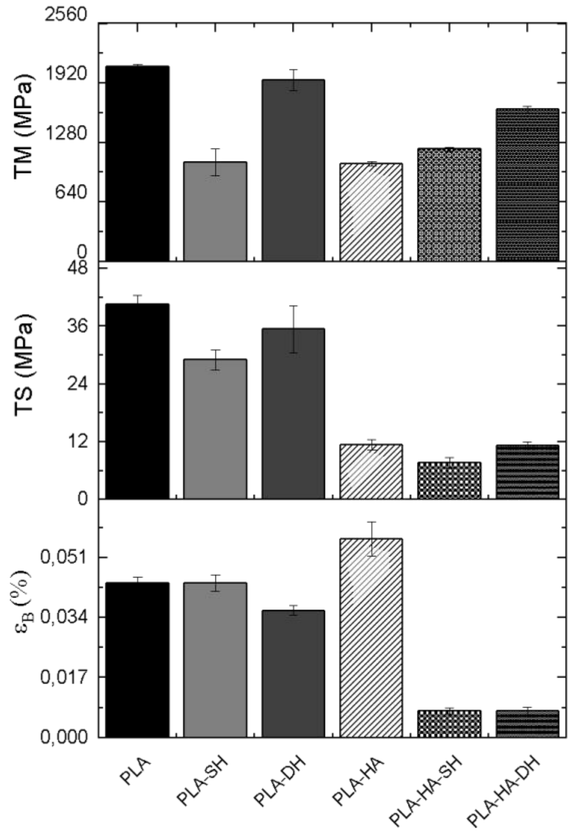

Figure 3. Change of mechanical parameters of FFF PLA and PLA-HA specimens at various sterilization processes as measured in the tensile test of printed PLA-HA. DH=dry heat; SH= steam heat.

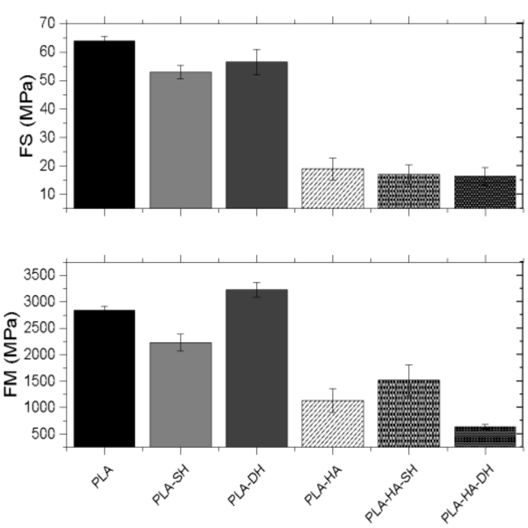

Figure 4. Change of mechanical parameters of FFF PLA and PLA-HA samples at various sterilization processes as measured in the flexural test of printed PLA-HA. DH=dry heat; SH= steam heat.

Effects of Dry Heat Sterilization Process on PLA-HA Mechanical Properties

TM of PLA-HA-DH increases by 55% until 1634.93MPa ($p<0.05$), an increase in Shore Hardness is also shown, this is consistent with the increase in glass transition temperature (Tg) until 63.43°C (DSC results, Table 4), which is also confirmed with the results of DMTA (from 64.8°C to 76.4°C). ε_b is reduced from 5.63% to 0.75%, equivalent to a reduction of 86.60%. FM also suffers a 44% decrease from 1130.35 MPa to 635.02 MPa; on the other hand, FS and IS remain the same ($p<0.05$), see Fig. 4. All these results indicate a decrease in ductility and embrittlement of the material [17].

Thermal Test Results

PLA-HA DSC Characterization

Table 4 shows the thermal parameters of PLA-HA with different sterilization processes. The melting temperature (T_m) of printed PLA-HA is 169.28°C. T_m for PLA-HA-SH decreases up to 162.69°C, and T_m for PLA-HA-DH increases up to 178.85°C.

T_g is maintained in the process for PLA-HA-SH. T_g increases up to 63.43°C for PLA-HA-DH. The 3D printing process does not affect the crystallinity of PLA-HA, crystallinity increases slightly to 29.95%. Steam heat sterilization process increases crystallinity of PLA-HA to 36.33%, this change explains the increase in tensile and flexural strength of the material. PLA-HA-DH decreases the crystallinity to 25.45%, this change explains the decrease in tensile and flexural strength of PLA-HA.

Table 4. Main thermal DSC parameters of printed PLA-HA with dry heat (DH) and steam heat (SH) sterilization process.

Sample	DSC parameters					
	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_m (J g^{-1})	ΔH_{CC} (J g^{-1})	X_c (%)
PLA-HA-Filament	58.26	92.81	173.13	-51,01	23,93	29,12
PLA-HA 3D	59.75	90.89	169.28	-49,61	21,76	29,95
PLA-HA SH	59.36	-	162.69	-33,79	-	36,33
PLA-HA DH	63.43	-	178.85	-23,67	-	25,45

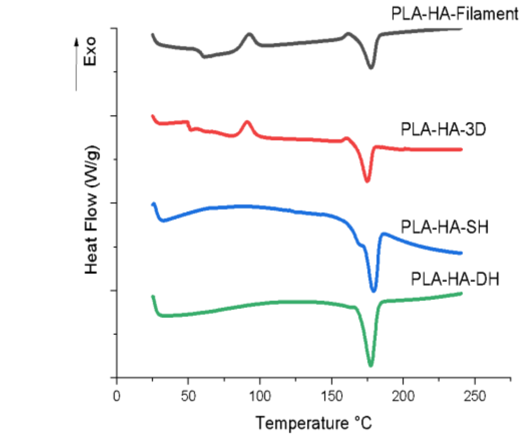

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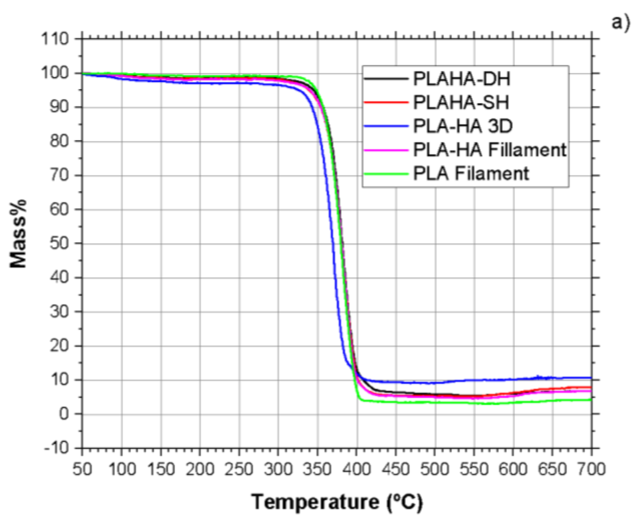
Figure 5. Differential scanning calorimetry (DSC) comparison of PLA-HA sterilized by steam heat (SH) and dry heat (DH).

TGA Results

Thermograms of TGA and differential thermogravimetry (DTG) for 3D printed PLA-HA sterilized by dry heat and steam heat are shown in Fig. 6.



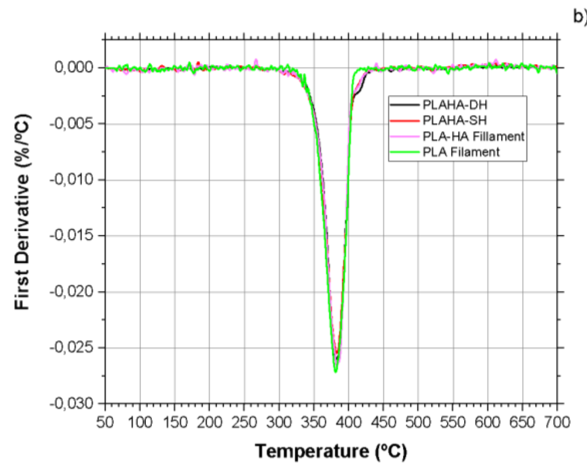


Figure 6. Thermal degradation of FFF printed PLA-HA with dry heat (DH) and steam heat (SH) sterilization process, (a) TG weight loss and (b) first derivative DTG curves.

Comparing the degradation properties of sterilized 3D printed PLA-HA samples, it can be seen that the degradation temperature (T_o) and the degrading temperature (T_d) are high for the sterilized samples by steam heat (383.45ºC). In both sterilization processes, T_o increases in relation to the printed material (325.95 ºC). A summary of TGA test results is shown in Table 5.

Table 5. Summary of Main thermal TGA parameters of printed PLA and PLA-HA with dry heat (DH) and steam heat (SH) sterilization processes.

Sample	TGA parameters	
	T_0 (^{0}C)[a]	T_d (^{0}C)
PLA-Filament	348.55	380.55
PLA-HA-Filament	344.95	382.80
PLA-HA 3D	325.95	370.95
PLA-HA SH	339.45	383.45
PLA-HA DH	344.95	382.95

Characterization by DMTA.

It can be visualized, in Fig.7a, that the storage modulus (G`) decreases with the increase in temperature, due to the increased mobility of the polymers chain, this happens for all materials studied. The glass transition temperature T_g was obtained through the damping factor analysis (tan δ). Dry heat sterilization process produces the largest increase in G` in all cases. Steam heat process also increases G` value a little less than the effect of G`.

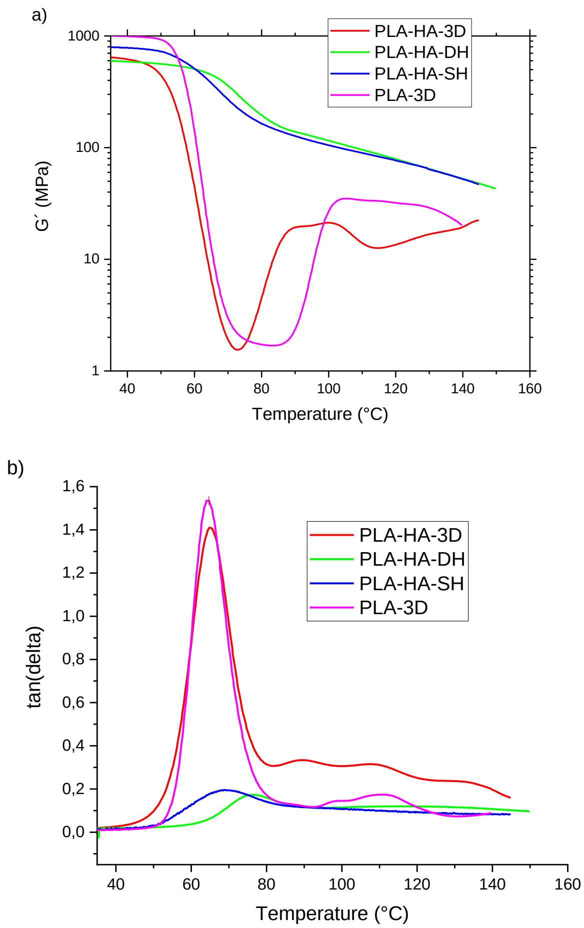


Figure 7. Dynamic mechanical thermal analysis (DMTA) curves a) storage modulus, G' and b) damping factor (tan δ) of PLA and PLA-HA printed, DH (Dry Heat) SH (Steam Heat).

PLA-HA samples sterilized by dry heat present an increase in T_g, (Fig.7b). When sterilized by steam heat, T_g also increases in relation to the printed material (Fig.7b), which is consistent with the decrease in TM and FM. Table 6 presents a summary of DMTA tests for sterilized and unsterilized specimens. The G' values obtained for all 3D printed parts are lower than the values obtained for other studies of the PLA-HA blend[18], this due to the lower adhesion of the 3D printed layers. The difference in T_g between DSC and DMTA methods can be attributed to the different heating rates and different sample dimensions used in the two methods [21].

Table 6. Results of DMTA test for PLA-HA sterilized by steam heat (SH) and dry heat (DH).

Sample Type	**G´at 40 °C (MPa)**	**G´at 110 °C (MPa)**	**Tg(°C)**
PLA-3D	992	33,80	64,4
PLA-HA-3D	617	13,9	64,8
PLA-HA-SH	785	89,5	68,7
PLA-HA-DH	589	95.5	76.4

Conclusions

PLA with 5% of HA by weight and printed by FFF is not suitable for the sterilization processes of dry heat and steam heat, due to the weakening and embrittlement of the material.

Tensile and flexural modulus and strength are increased when subjected to dry heat.

Tensile and flexural modulus and Shore Hardness are increased when subjected to dry heat, while the elongation at break decreases, indicating embrittlement of the material.

From the DMTA results, the sterilization by steam heat increases T_g due to an increase in the crystallinity of the material.

From the DMTA and TGA results, the sterilization by dry heat increases T_g and degradation temperatures.

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