THERMO-MECHANICAL CHARACTERIZATION OF NANO-HYDROXYAPATITE AND CELLULOSE REINFORCED POLY(LACTIC ACID) COMPOSITES WITH PROSPECTIVE APPLICATIONS FOR BONE SUBSTITUTE MANUFACTURING

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1 INTRODUCTION

Poly(lactic acid) (PLA) has been widely used in biodegradable thermoplastic applications and also as a primary constituent for biomedical drug delivery vehicles and tissue engineering scaffolds [1]. PLA possess however, some properties undesirable for bone tissue engineering applications. Of these, stress shielding, deficiency in biological signaling present in natural extra cellular matrix, autocatalytic hydrolysis, as well as suppression of the mechanical properties under physiological conditions can be stated [2]. Therefore, modification of poly(lactic acid) via polymer blending, copolymerization, and by composite reinforcement have been applied to overcome these shortcomings [3]. Cellulose, which is the most abundant biopolymer in nature, has a well proven characteristics important for biomedical applications, such as biocompatibility, no toxicity and mechanical compatibility with biological soft and hard tissues [4]. Calcium phosphates, and especially hydroxyapatite (HAp), are bioactive ceramics, which can enhance the osteoconductivity of a modified substrate, thus these are frequently used in bone cell tissue engineering. Their activity is based on direct bonding to the human bone tissue and further, to induce pH stabilization of the physiological surrounding environment during in vivo biodegradation of the substrate [5]. HAp also has a high affinity for proteins due to alternating the surface morphology of the substrate and hence, facilitating protein adsorption on the biomaterial surface [6].

Many reviews have covered the chemical, physical and biological characteristics of biomaterials currently of high interest for tissue engineering applications, particularly those relevant to bone reconstruction [7]. For such scaffolds, the shape, porosity as well as surface properties are important factors. The fabrication process is also important, especially regarding the possibility to combine different materials in one fabrication process, and to ease the scaffolds’ structure-property relationship [8]. Furthermore, individual combination of either cellulosic reinforcements or calcium phosphates in a variety of polymers has been evaluated for in vivo and in vitro tissue engineering applications [4, 9].

In a study, calcium phosphate nuclei formation on regenerated cellulose fibers diminished the deficiency of cellulosic materials bone-bonding capability and tissue compatibility as proved by hydroxycarbonate apatite layer generated on the substrate in simulated physiological body fluid [10]. The impacts conferred on mechanical and thermal properties of poly(lactic acid) by variation in the amount and morphology of the biphasic calcium phosphate and hydroxyapatite has been investigated as well [11]. It has also been shown that scaffolds filled with nano-scale HAp demonstrate higher compressive modulus and protein adsorption
capability when compared with micro-HAp added PLLA [12].

Based on the literature [2, 5], it is evident that the combination of PLA, HAp and regenerated cellulose could provide interesting opportunities in the development of novel bioactive composites encompassing all constitutes at a time. The aim of this study is to evaluate the mechanical and viscoelastic characteristics of melt-extruded poly(lactic acid) films modified with nano-size hydroxyapatite and regenerated cellulose, with the purpose to find an optimal composition which could be suitable for tissue engineering applications. In the study we used two types of regenerated cellulose, a particle type and a fibrous type, as will be described later. The purpose of using two morphologically different celluloses is to assess their influence on HAp distribution and mechanical performance of the composites. Moreover, we wanted to investigate the film surface morphology as it is known that a rough surface facilitates the enhancement of protein adsorption and anchoring. The hypothesis was that the added HAp and regenerated cellulose would affect the surface morphology favorable in this respect [2].

All components incorporated in the composites were chosen based on the inherent positive effects on biocompatibility, demonstrated both in vivo and in vitro [13]. To the best of our knowledge, this is the first time the effect of variation in morphology and concentration of regenerated cellulose on the mechanical and thermal properties of HAp/PLA composites produced by melt extrusion is investigated.

2 Experimental

2.1 Materials

PLA 6201D with specific gravity of 1.24 (g/m³) was kindly provided by NatureWorks®. Hydroxyapatite with a particle size <200 nm and a density of 3.86±0.17 g/m³ was purchased from Sigma Aldrich Co. Two morphologically different types of regenerated cellulose were used, a particle cellulose powder (Tencel CP 12) with average diameter of 12 µm and fibrous cellulose powder (Tencel FCP 10/400) with average fiber diameter of 10 µm, and length of 400 µm. Both were supplied by Lenzing AG, Austria. The density of the FCP and CP fillers were measured to be ca. 1.56 and 1.51 g/m³, respectively. High purity analytical reagent grade of 1,4-dioxane was used as the PLA solvent, purchased from Fisher Chemical, UK. Paraffin wax 76243 of analytical grade was purchased from Fluka with experimentally measured density of around 0.908 g/cm³.

2.2 Solvent mixing

The proper amount of the cellulose and HAp were mixed with 1,4-dioxane at room temperature for 4 hours. Figures in the abbreviations used for representing composites’ compositions demonstrate weight% of the material with respect to poly(lactic acid) matrix. In ternary composites 10-wt% HAp is set as default and is not shown to prevent redundancy. After adding the PLA granules, the mixture was kept stirring for additional 12 hours at 25°C until the PLA granules were fully dissolved. The mixture was then drop-wise added to a flask containing liquid nitrogen to form solid composite granulates. The granulates were then freeze-dried for 24 hours and finally oven-dried at 50°C for the same period of time to evaporate any residual solvent. This was done to obtain porous and non-toxic granulates, which is essential for further cell culture tests. By doing the mixing in a solvent, it is possible to ensure proper dispersion of the HAp and the cellulose in the PLA matrix, which will facilitate the film extrusion and prevent possible thermal and mechanical degradation of the polymer matrix due to longer mixing time in the extruder.

2.3 Composite Film Production

The films were produced in a lab-scale twin-screw micro-compounder (15cc DSM Xplore® micro-compounder, the Netherlands), equipped with a film extrusion die of 0.4 mm wide. The extrusion was done at constant mixing time, temperature and rotational speed of 5 min, 195°C, respectively 50 rpm. These processing parameters were selected based on extrusion trials to achieve optimal film quality. The die temperature was 180°C, and a force control set to 950 - 1000 N was maintained during the extrusion in order to obtain uniform film thickness. As-extruded films were received by immediate cooling to room temperature after the die by constant air flow.

3 Characterization

3.1 Scanning Electron Microscopy (SEM)
The morphology of the composite films was examined with a table-top scanning electron microscope (TM-1000, Hitachi, Japan). Prior to SEM analysis, the tensile-fractured cross-sections of the samples were mounted on a metal stub and stored in a Denton vacuum under 0.1 mbar vacuum pressure. The test specimens were then coated for ca. 30 s with a Gold powder layer (∼10 nm) using an Agar high-resolution sputter coater (model 208RH), equipped with a gold target/Agar thickness monitor controller. The SEM analysis was performed on low vacuum mode (LV-SEM) to take micrographs at various magnifications.

3.2 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was done in a Q500 TGA from TA Instruments under nitrogen atmosphere and by heating from room temperature to 700 °C with a 10 °C/min heating ramp. Three samples were randomly taken from the starting, middle and the finishing part of the extruded films, in order to determine the HAp and cellulose content distribution in the films along the extrusion direction.

3.3 Tensile Testing

Prior to the mechanical testing, the films were conditioned according to DIN EN ISO 291 in a Memmert HPP108 condition oven. The extension of the films (of length 20 mm and width 3.97 mm in the strip-like section of the dumbbells) were then measured from the movement of the upper bar in the tensile testing machine. The tensile tests were performed with a load cell of 100N, crosshead speed of 5mm min⁻¹, and preload of 0.1N in a Tinnius-Olsen H10KT (PA, USA). At least ten specimens were tested for each composition.

3.4 Dynamic Mechanical Analysis (DMA)

Dynamic mechanical thermal analysis was performed using a Q800 DMA from TA Instruments. Strain amplitude, fixed frequency, temperature ramp, and preload tension of 3 μm, 1 Hz, 2°C min⁻¹, and 0.01N were chosen, respectively. Three strips were cut from each film in the extrusion and the transversal direction to characterize storage modulus ($E'$), loss modulus ($E''$), and damping factor ($\tan\delta$). The distance between the clamps was set to 12 mm. The temperature range was 25 to 130°C.

3.5 Statistical Analysis

Statistical analysis of the experimental results was performed using Minitab® 16 software package. A general linear model (GLM) was applied to perform an analysis of variance (ANOVA) so as to identify the most influential factors on essential property responses. Unless otherwise stated five percent level of significance was considered for further pair-wise comparison of the properties through Tukey post hoc method.

4 Results and Discussion

4.1 Distribution of HAp and cellulose assessed by TGA

The distribution of the HAp and the cellulose in the extruded films was analyzed by TGA analysis of samples taken at different points along the films containing 3, 10 and 20-wt% CP respective FCP. Uniformity of the CP and FCP dispersion in the cross-sections of the films was investigated by comparing the temperatures at which 5 % ($T_5$) respective 80 % ($T_{80}$) weight loss occur. Variation in both of the indices at central and marginal sections were not significant for all the compositions (data are not provided).

Small p-value for $T_5$ difference, obtained from middle and marginal regions of PLA/HAp10/CP20 implies slightly large difference in CP and/or nano-HAp dispersion at these sites of the composite. Additionally, large discrepancy between total residues left after pyrolysis for this specific composition proves the likelihood of small aggregate formation in its cross-section (Table 1).

Since the interaction between the constituents at molecular level is not clearly known, residues left after TGA analysis can act as a more reliable clue to assess the filler distribution in the composites (Table 1). Accordingly, concomitant presence of particulate cellulosic filler and HAp seem to induce higher dispersion uniformity in the film cross-section. This can be realized by smaller mean difference in the residues at central and marginal sections in the materials composed of 3 and 10-wt% CP than their corresponding films containing FCP. Based on the small p-value - or large differences - observed for $T_5$ at marginal and central parts of the HAp/PLA blend, it is noteworthy to stress the potency of the two regenerated cellulose types in the breakdown of
agglomerated HAp and enhancement of distribution uniformity of the HAp particles in the PLA matrix. It was shown that the porous nature of the cellulose-based materials enable the nano-particle immobilization and its proper dispersion in the composite films which corroborate our results [6].

Overall, the results support proper distribution of the HAp and of both cellulosic fillers in the PLA matrix by using a master batch production method.

4.2 Scanning Electron Microscopy

Figures 1 and 2 illustrate the SEM micrographs of cross-sections of the extruded films at low and high magnification, respectively. The films modified with particulate cellulose (except for 20-wt% CP) have a fairly good distribution of the cellulose particles, as shown in Figure 1 (A–C). In the films modified with the fibrous type cellulose, fiber pull-outs together with poor fiber orientation can be seen in Figure 1 (D–F). This morphology will have an effect on the mechanical properties of the films, as will be discussed later.

In the high-magnification micrographs presented in Figure 2, more detailed interpretations can be done. Firstly, pronounced strain-hardening is realized in the particulate cellulose-filled composites which is absent in the case of fibrous cellulose-incorporated composites. The white regions pointed out by arrows in Figure 2 (A–C), increase with the CP concentration up to 15% (not shown), and disappear at higher loadings, which supports the results acquired for tensile yield and elongation at failure of the corresponding composites. Secondly, it is obvious that the HAp particles are well distributed in both CP- and FCP-filled composites while less particle aggregates are formed in former.

Based on the low-magnification SEM images shown in Figure 1, increase in surface roughness by increased cellulose filler loading is evident which is a desirable feature for cellular interactions [2].

4.3 Differential Scanning Calorimetry

In order to keep the brevity of the report and devote its main share to the mechanical characteristics of the composites, only a brief outcome of the thermal features of the thin films are presented. At all filler concentrations the fibrous cellulose induced higher percentage crystallinity values than particulate cellulose. Nonetheless, analysis of variances performed on the data indicated a greater significance of reinforcement concentration on the degree of crystallinity than for the type of cellulose. Both cellulosic types demonstrate nucleating capacity which resulted in the improvement of the crystallinity of HAp-filled polymer matrix.

Rigid amorphous fractions (RAF) were observed in the DSC thermograms associated with HAp/PLA/cellulose composites which did not exist or were notably small for the HAp/PLA and the neat PLA films. Since only the completely amorphous and free-to-move poly(lactic acid) chains are able to participate in spontaneous nuclei generation during cold-crystallization, it is likely that by increase in the cellulose concentration the portion of RAF develop, leading to low crystallite formation during the cold crystallization, which were lower than that of the HAp/PLA films [14].

4.5 Mechanical Testing

4.5.1 HAp/PLA composites

The 10 wt-% nano-HAp concentration used in this study is presumed to form agglomerates due to poor filler-polymer affinity, slip on each other under the applied forces, act as crack propagating points and eventually lead to reduction in the tensile strength of the HAp/PLA composite compared to neat poly(lactic acid). This has resulted in statistically significant reduction in the tensile strength to 54 MPa from approximately 64 MPa for pure PLA. However, the elongation at break is not considerably decreased compared to the neat PLA matrix (data not presented). Concerning the tensile modulus, the HAp seems unable to induce its stiffening feature as a result of non-uniform filler distribution during the manufacturing process. The same behaviour was addressed previously for HAp (<200 nm) incorporated into PLA (Figure 3) [15]. It can be seen from Table 2 that the work prior to material rupture has remarkably dropped, when compared to neat poly(lactic acid). Although toughness is highly sensitive to the elongation at break, the dramatic reduction in tensile strength of around 16% in the case of binary nanocomposite with regard to neat PLA films weighed down the positive effect of unchanged elongation at failure.
4.5.2 HAp/PLA composites modified with regenerated cellulose

4.5.2.1 E-Modulus
The Young’s modulus of elasticity is measured at low deformations, until which almost no separation occurs and therefore, it is reasonable that the composite stiffness is actually independent of the filler/matrix interfacial adhesion [16]. In the case of HAp/PLA composites modified with the fibrous type cellulose, the irregular orientation at the composites’ cross-section and the small share of aggregates formed, revealed by SEM and TGA measurements, respectively, concealed the stiffening potential of the fibrous type cellulose. This undesirable effect gets more severe as the fiber content is increased and Young’s modulus decrease from 2 GPa for composite with 3-wt% to 1.8 GPa for the composite with 20 wt.%.

Due to the use of solvent procedure applied in this study, the dispersion remains favorable and severe aggregation is prevented even at high loadings of the particulate type cellulose. The overall outcome is the enhancement of the mean modulus from 1.96 GPa and 1.97 GPa for neat PLA respective PLA/HAp10% composite to 2.42 GPa for 20 wt-% CP-loaded films (see Figure 3).

4.5.2.2 HAp/PLA/cellulose Composites vs. Neat PLA Tensile Strength and Elongation at Break
Proper stress transfer between the particulate respective fibrous fillers and the polymer matrix is a crucial factor in determination of the composites’ tensile strength. Discontinuity in the matrix as a result of weak filler-matrix interfacial adhesion and ineffective stress transfer between the two phases results in decrease in composite strength, which becomes worse by higher filler loading. In Figure 2, large voids are seen in the vicinity of the fibrous and particulate fillers due to de-wetting; these voids can act as stress concentration points. Thus, the tensile strength of the composite attenuates, particularly in the case of composites with fibrous type cellulose, compared to the neat PLA, as shown in Figure 3. Even though considerable improvement in tensile strength is not achieved by the reinforcement addition with respect to neat PLA, it has not dropped considerably up to 15 wt-% in the case of particulate cellulose. Further, statistical analysis (p<0.01) shows that up to 15% cellulose concentration in the PLA/HAp/CP composites gave remarkable improvement in the tensile strength compared to the PLA/HAp. Although it is proved that the existence of FCP modify the composite crystallinity, the fibers have hindered the polymer matrix lamella proper directional arrangement (preferably perpendicular to machining direction), even at low concentrations. Thus, reduction in tensile strength and elongation at failure with regard to neat PLA and PLA/HAp composite with yield strength values of 64.6 and 53.7 MPa, respectively, are not unpredictable [17].

The discussion provided in this section support the reduction in tensile strength and elongation at break of the fibrous cellulose filled composites in comparison with the HAp/PLA composites. This reduction in mechanical strength is significant (p<0.05), especially for the composites with highest content of fibrous cellulose.

4.5.3 Crazing effects in the composites
To measure fracture toughness of materials, tensile testing can be performed on specimens with specific crack geometry. However, in this study, toughness is defined as the amount of energy the material absorbs per unit volume before rupture [18]. These measures are presented in Table 2 and are exploited to perform a comparative study but not to provide exact measure of the fracture toughness. The results for the fibrous cellulose filled composites are ignored, since the toughness values were unsatisfactorily low compared to neat PLA. The craze zone is composed of many highly oriented fibrils which endow high stress-carrying capacity to the material, since the covalent bonds in the fibrils are much stronger than the secondary van der der Waals bonds between the fibrils. Up to 10 wt-% CP concentration, the composite toughness increased, compared to neat PLA films and PLA/HAp10% (Table 2).

The white regions in the SEM images corresponding to 3 and 10 wt-% particulate cellulose composites are indicative of stress-whitening as a result of lamella separation and multiple crazing formation [17]. At low particulate cellulose content, debonding of the partially rigid cellulose particles at polymer interface uphold appearance of multiple crazing, ending up with satisfactory toughness. It is proposed that by appropriate particulate dispersion in the composite cross-section, the post-distribution of stresses on neighboring fibrils right after individual fibril rupture will be stabilized and so, internal flexural momentum appeared due to non-uniform stress distribution is prevented (Note the step-like sharp edges shown in the SEM micrographs of
particulate cellulose filled composites in Figure 1). Thus, superior plastic behavior could be obtained for the particulate cellulose filled PLA compared to neat PLA.

4.6 Thermo-mechanical Dynamic Analysis

The results shown in Table 3 provide evidence on non-significant improvement in storage modulus of the 10 wt-% HAp/PLA composite with respect to pure PLA films. Considering the balance between stiffening effect of the rigid HAp particles and the available interfacial surface area between the particles and polymer matrix, this specific filler concentration, as it was implicitly concluded in previous sections, has probably led to the dominance of the particle agglomeration, conferring a non-noticeable $E'$ enhancement at 37°C. Although, the obtained storage modulus for the binary composite at this temperature is inappropriate either for cancellous or for cortical bone tissue applications, it is much higher than and fairly similar to the values reported for PLGA- and PLLA-based nanocomposites, respectively [19, 20]. The percolating behavior of the nano-fillers at temperatures which polymer chains’ mobility play a great role in elastic behavior determination (i.e. above $T_g$), result in higher elastic performance of the filled poly(lactic acid) composites compared to neat polymer films [19]. Mean storage modulus raised from 14.35 to 16.40 MPa for the composite compared with the neat PLA film at 70°C.

Efficient matrix-filler interfacial sliding friction, which seems to be higher in particulate cellulose composites, as a consequence of larger interfacial surface area is a mean to release the absorbed energy [17]. In addition, the physical restriction imposed on the polymer molecular chain mobility by entangled fibrous cellulose at temperatures around the glass transition and their performance as stress carriers is greater than for the particulate cellulose. Thus, the former phenomena dominates in the PLA filled with the particulate cellulose, and results in remarkably higher loss factor (Figure 4), while the latter factors are responsible for higher storage modulus at 70°C of composites aimed to be reinforced with fibrous cellulose (data not shown) [21]. Moreover, ANOVA analysis proved the significant effect of particulate cellulose on $\tan\delta$ height, compared with the fibrous cellulose composites. Compared to the HAp/PLA blends, even low cellulose content of CP and FCP caused $\tan\delta_{\max}$ to reduce from 2.98 for PLA/HAp10 to 2.93 and 2.86, respectively (Figure 4).

As the composite fiber content increases the stress distribution in the composite cross-section become uniform resulting in improvement of storage modulus and/or stiffness at temperatures far lower than $T_g$ [22]. Values attributed to storage modulus of the ternary composites presented in Table 4 prove excelled elastic energy absorption in particulate cellulose filled composites than those with fibrous cellulose. Higher storage modulus achieved by the particulate cellulose filled PLA at 37°C, conforms to the results obtained from tensile tests and is attributed to their well dispersion in the composite cross-section. As previously mentioned, improper distribution and inefficient alignment of the long fibers in the fibrous cellulose-incorporated composites have led to their unsatisfactory stiffness. However, it is presumed that increase in particulate cellulose content up to 20 wt-% in the films results in the stimulation of the intrinsic hydrogen bond formation tendency of the cellulosic particles, which leads to their scattered agglomeration. The storage modulus ascends to its highest value for 15 wt-% particulate cellulose with 3636 and 25 MPa, which is the only composition demonstrated significant enhancement with regards to pure PLA films (p-value= 0.0377<0.05).

The mean value of the storage modulus at 37 and 70°C for the particulate cellulose composites demonstrate improvements compared to the HAp/PLA blends. These superior properties are evident for PLA/HAp10/CP15 with 3636 and 25 MPa as compared with 3298 and 16.4 MPa, respectively.

The results of storage modulus measurements obtained from the transversal dynamic mechanical analysis of representative compositions of the biomaterials are presented in Table 5. Evidently, the transversal storage modulus (TSM) achieved for all of the compositions are significantly lower than directional storage modulus (DSM) reported earlier in this paper. This variation is lower for the particulate cellulose filled PLA-composites due to their symmetrical morphology. Furthermore, the difference between TSM and DSM increase as the reinforcement content increase. The DSM and TSM dissimilarity reaches approximately 500 MPa and 1400 MPa for PLA/HAp10/FCP3 and PLA/HAp10/FCP20, respectively. TSM results for
all the tested compositions are lower than storage modulus of neat PLA films at physiological temperature. However, this argument fairly contradicts the SEM outcomes regarding fibrous cellulose alignment in the composites, discussed earlier. Therefore, it is expected that by further controlling the orientation of the fibrous cellulose and preventing their aggregation at higher contents superior DSM would be achieved for the FCP-filled ternary composites. Although the used cellulose is claimed to have particulate-shape, the viscoelastic characteristics of their composites show anisotropic performance, due to their longitudinal deformation in the extrusion direction during melt-mixing process. It is worth noting that this anisotropic mode is more favorable for bone substitute biomaterials due to the anisotropic complex nature of the natural bone itself [9].

A simple method to investigate the presence of various damping mechanisms in a composite is to validate the following relationship. If the damping mechanism in the composite is similar to the pure polymer matrix, which is mainly due to intermolecular friction of polymer chains, the following hypothetical relation should be held [11]:

\[
V_p = \frac{\tan \delta_c}{\tan \delta_p}
\]

(1)

\(V_p\), \(\tan \delta_c\) and \(\tan \delta_p\) are the volume fraction of the polymer matrix in the composite, damping factor of the composite and the pure poly(lactic acid) film at a particular temperature, respectively. The difference in \(T_g\)s reported for the entire tested specimens are not remarkable. Therefore, it seems reasonable to evaluate the damping characteristics of the composites at representative temperatures below (i.e. 37°C) and above (i.e. 72°C) the neat PLA glass transition temperature of approx. 62°C. Corresponding values of the theoretical formulation and the experimental data achieved via DMA measurements are depicted in Table 6 for representative compositions. For the purpose of brevity results associated with above \(T_g\) temperatures are not presented.

At low filler contents though the polymer volume fraction is high, the restrictive effect of the cellulosic reinforcements dominates. This is pronounced with FCP than CP as the corresponding relative ratio to the right hand side of equation (1) is far below the theoretical values, probably due to interlocking of the fibers. As the reinforcement loading increased, the defined damping ratio inclined as well and leveled out the theoretically calculated PLA volume fraction at 20 wt-% FCP and 15 wt-% CP content. This illustrates the mount in severity and intensity of filler-filler and free volume energy dissipation modes by the reinforcement content which is more emphasized for PLA based CP- composites.

The relative damping factor for the binary composite was far higher than that of either type of ternary bio-nanocomposites at 72°C. This illustrates the effectiveness of the cellulosic reinforcements in the reduction of released energy proportion from the total absorbed energy.

At temperatures lower than \(T_g\) namely, 37°C, results presented in Table 6 prove the ascending trend by fibrous cellulose loading. In composites containing particulate cellulose, the damping capacity has not changed significantly by particle loading. In the latter case, the ratio is larger than the volume fraction of the PLA matrix composites at almost all the compositions, which is an evidence for the existence of rigorous damping mechanisms. Implementing GLM with the cellulose type and content as the factors considerable effect of CP on enhancement of the relative damping ratio (p<0.01) was realized. Ternary composites reinforced with CP also did not show significant reduction of the relative damping ratio in comparison with binary composites at 37°C. Consequently, shock absorption efficiency of the particulate cellulose-reinforced composites was enhanced with regard to the biomaterials reinforced with fibrous cellulose and remained constant with regard to poly(lactic acid) simply filled with HAp.

### 4.6.1 Ternary Composites’ Viscoelastic Properties with Respect to Natural Bone

The viscous-elastic behavior of the ternary bio-nanocomposites produced in this study falls below the real cortical bone values (ca. 8 GPa) [23] and it is far higher than values ascribed to cancellous bone (50-500 MPa) [20]. However, 30-90% porosity of the cancellous bone mimicking substitutes should not be neglected [7]. It is worth mentioning that the damping capacity of the CP-reinforced PLA nanocomposite is higher than rigid FCP-reinforced nanocomposites at all of the compositions. It is expected that by addition of higher portion of HAp to the PLA/HAp10/CP15, the HAp may demonstrate synergistic effect on elastic behavior enhancement of the composites with this appropriate cellulose
content and also withstand impact loading due to increased \( \tan \delta \) and loss modulus by the mineral content [23].

5 Possible Application of the Bio-nanocomposites for Bone Tissue Engineering *in vivo*

At *in vivo* conditions, where the composites are in direct contact with body fluid, the storage modulus and tensile testing results obtained for the composites studied in this report will reduce and probably approach those of cancellous natural bone, hence, preventing stress shielding at the scaffold and natural bone tissue interface. The likely enhancement in hydrophilicity by incorporation of cellulosic fillers is believed to upgrade cell spreading on the composite surface [2]. Moreover, well dispersion of the bioactive HAp in the manufactured composite materials are assumed to show improved osteoblast cell adhesion, proliferation and differentiation [24]. Mesenchymal Stem Cells (MSC) are able to sense the surface stiffness, roughness and topography. Although the effect of micro-roughness in comparison with nano-roughness concerning osteoconductivity and cell proliferation is still controversial, evident by some reported studies, it is presumed that textured surfaces similar to those attained in this research facilitate protein adsorption and increase the initial attachment of the cells [25]. Nevertheless, in order to draw firm conclusions, surface measurements and cell assay results should be analyzed and presented in the later publication.

It was obvious that the damping capacity of the particulate cellulose filled composites were higher than for the HAp/PLA blends and the composites filled with fibrous cellulose at 37°C but dry conditions. This property in addition to higher storage modulus of the former composite compositions makes them favorable for both elderly who need bone substitutes with higher damping capacity features and also where load bearing is the principle demand.

6 Conclusions

Novel composite films constituted of poly(lactic acid), hydroxyapatite and regenerated cellulose were produced by extrusion in a twin-screw micro-compounder. We demonstrated that by introducing 10 wt-% of HAp with a mean diameter of around 150 nm into PLA, dynamic thermo-mechanical and tensile properties of the composite degrades. However, combining the HAp/PLA composite with two different types of regenerated cellulose could improve the properties, and this effect was pronounced for particulate cellulose filled HAp/PLA composites.

SEM and TGA revealed that high cellulosic filler content could prevent the aggregation of HAp in the PLA matrix. Results indicate higher dispersion uniformity of both particulate cellulose and HAp in the composites’ transversal cross-section in comparison with fibrous cellulose reinforced composites, up to 15 wt-% of particulate cellulose content.

Superior tensile properties and dynamical energy storage properties were obtained for the HAp-PLA modified with the particulate regenerated cellulose, while fibrous regenerated cellulose had not as good properties. The highest storage modulus enhancement achieved for the HAp/PLA/cellulose composites were 14 and 10.3 % for 15 wt-% CP compared to pure PLA and HAp/PLA blends. By addition of up to 15 wt-% particulate cellulose, the yield strength was significantly increased, whereas 23% augmentation in ultimate tensile strength was realized by 3 wt-% particulate cellulose powder with added to nano-HAp-filled PLA.

Composites reinforced with fibrous cellulose performed better above \( T_g \), ending up in higher storage modulus due to fiber entanglement by concentration and reduction in the fiber-matrix interfacial and bulk polymer strain occurrence. Although higher matrix crystallinities were obtained for the PLA/HAp/FCP composites compared to the particulate cellulose filled and binary composites, the former composites did not show enhancement in mechanical properties namely, modulus and tensile strength, at dry physiological temperature.

Further research is needed in order to evaluate the capability of cellulosic fibers in HAp/PLA for biomaterials aimed at bone tissue engineering applications as proved by other researchers. Cellulosic micro-particles could improve mechanical properties, energy storage and damping capacity, alter the surface topography, improve HAp dispersion and perhaps improve their hydrophilicity, all demanded characteristics of a prospective candidate for bone tissue engineering applications.
References


Table 1. Weight percent remained at the central (RC) and marginal (RM) sections of the composites after heating to 700°C in an oxygen-free atmosphere

<table>
<thead>
<tr>
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<th>RC</th>
<th>RM</th>
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<tbody>
<tr>
<td>PLA/HAp10</td>
<td>9.24±0.21</td>
<td>9.31±0.18</td>
</tr>
<tr>
<td>CP3-wt%</td>
<td>9.49±0.08</td>
<td>9.55±0.13</td>
</tr>
<tr>
<td>FCP3-wt%</td>
<td>9.49±0.02</td>
<td>9.16±0.45</td>
</tr>
<tr>
<td>CP20-wt%</td>
<td>9.55±0.15</td>
<td>9.07±1.05</td>
</tr>
<tr>
<td>FCP20-wt%</td>
<td>10.22±0.11</td>
<td>10.22±0.07</td>
</tr>
</tbody>
</table>

Fig.1. Low-magnification SEM of ternary films containing 3, 10 and 20 wt-% CP (A-C) and FCP cellulose (D-F)
Fig. 2. High-resolution SEM of 3, 10 and 20 wt-% CP (A-C) and FCP cellulose (D-F) containing films.

Fig. 3. Modulus of elasticity (left) and tensile strength (right) for pure PLA, binary and ternary composites.

Table 2. Total energy demand prior to rupture of neat PLA, binary and CP-reinforced ternary composite thin films. P-values with respect to neat PLA and HAp/PLA composites are included.

<table>
<thead>
<tr>
<th></th>
<th>Toughness (MJ/m³)</th>
<th>P-value (PLA)</th>
<th>P-value (HAp/PLA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PLA</td>
<td>2.05±0.32</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>PLA/HAp10%</td>
<td>1.75±0.26</td>
<td>0.0115</td>
<td>***</td>
</tr>
<tr>
<td>CP1-wt%</td>
<td>2.26±0.71</td>
<td>0.7115</td>
<td>0.0018</td>
</tr>
<tr>
<td>CP5-wt%</td>
<td>1.47±0.15</td>
<td>0.0016</td>
<td>0.4736</td>
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<td>CP7-wt%</td>
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<td>0.0598</td>
<td>0.9879</td>
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<tr>
<td>CP15-wt%</td>
<td>1.37±0.11</td>
<td>0.0003</td>
<td>0.157</td>
</tr>
<tr>
<td>CP20-wt%</td>
<td>1.10±0.07</td>
<td>0.0000</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Table 3. Storage modulus at 37 and 70°C, loss modulus at peak temperature ($E''$) and $\tan\delta$

<table>
<thead>
<tr>
<th></th>
<th>$E'$ at 37°C</th>
<th>$E'$ at 70°C</th>
<th>$E''$</th>
<th>$\tan\delta$ Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>3195.5±119.09</td>
<td>14.35±1.05</td>
<td>62.08±0.59</td>
<td>3.022±0.03</td>
</tr>
<tr>
<td>PLA/HAp10%</td>
<td>3298.6±159.06</td>
<td>16.40±0.21</td>
<td>62.45±0.57</td>
<td>2.98±0.02</td>
</tr>
</tbody>
</table>

Fig. 4. Best fitted regression curves for $\tan\delta_{max}$ heights relevant to particulate cellulose-filled composites. Pure PLA corresponds to 0% cellulose content.

Table 4. Storage modulus at 37°C associated with ternary composites.

<table>
<thead>
<tr>
<th>Particle-type cellulose</th>
<th>Storage Modulus at 37°C</th>
<th>Fibrous cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP3-wt%</td>
<td>3372.7±207.7</td>
<td>FCP3-wt%</td>
</tr>
<tr>
<td>CP5-wt%</td>
<td>3277±299.4</td>
<td>FCP5-wt%</td>
</tr>
<tr>
<td>CP10-wt%</td>
<td>3555.7±60.0</td>
<td>FCP10-wt%</td>
</tr>
<tr>
<td>CP15-wt%</td>
<td>3636±178.3</td>
<td>FCP15-wt%</td>
</tr>
<tr>
<td>CP20-wt%</td>
<td>3403.3±130.4</td>
<td>FCP20-wt%</td>
</tr>
</tbody>
</table>

Table 5. Transversal storage modulus for CP- and FCP-reinforced ternary composites at 37°C.

<table>
<thead>
<tr>
<th></th>
<th>TSM (MPa)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP3-wt%</td>
<td>2922±7.84</td>
<td>13.36</td>
</tr>
<tr>
<td>CP10-wt%</td>
<td>2710±28.29</td>
<td>23.79</td>
</tr>
<tr>
<td>CP20-wt%</td>
<td>2474±1.00</td>
<td>27.30</td>
</tr>
<tr>
<td>FCP3-wt%</td>
<td>2710±24.73</td>
<td>15.81</td>
</tr>
<tr>
<td>FCP10-wt%</td>
<td>2365±32.79</td>
<td>28.53</td>
</tr>
<tr>
<td>FCP20-wt%</td>
<td>1959±65.39</td>
<td>42.35</td>
</tr>
</tbody>
</table>

aThe difference is calculated with respect to corresponding DSM presented in Table 4.

Table 6. PLA volume fraction and relative damping ratio (RDR) of binary, CP- and FCP-added ternary thin film composites at 37°C

<table>
<thead>
<tr>
<th></th>
<th>PLA v/v</th>
<th>RDR at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA/HAp10</td>
<td>0.97</td>
<td>1.27±0.18</td>
</tr>
<tr>
<td>CP1-wt%</td>
<td>0.96</td>
<td>0.95±0.13</td>
</tr>
<tr>
<td>CP5-wt%</td>
<td>0.93</td>
<td>0.99±0.09</td>
</tr>
<tr>
<td>CP10-wt%</td>
<td>0.89</td>
<td>1.07±0.10</td>
</tr>
<tr>
<td>CP15-wt%</td>
<td>0.86</td>
<td>1.20±0.12</td>
</tr>
<tr>
<td>CP20-wt%</td>
<td>0.83</td>
<td>1.09±0.03</td>
</tr>
<tr>
<td>FCP1-wt%</td>
<td>0.96</td>
<td>0.85±0.09</td>
</tr>
<tr>
<td>FCP5-wt%</td>
<td>0.93</td>
<td>0.94±0.08</td>
</tr>
<tr>
<td>FCP10-wt%</td>
<td>0.89</td>
<td>0.98±0.03</td>
</tr>
<tr>
<td>FCP15-wt%</td>
<td>0.86</td>
<td>1.09±0.01</td>
</tr>
<tr>
<td>FCP20-wt%</td>
<td>0.83</td>
<td>1.16±0.06</td>
</tr>
</tbody>
</table>