Optimization of the Mechanical Performance of Bacterial Cellulose/Poly(L-lactic) Acid Composites

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ABSTRACT Understanding the nature of the interface between nanofibers and polymer resins in composite materials is challenging because of the complexity of interactions that may occur between fibers and between the matrix and the fibers. The ability to select the most efficient amount of reinforcement for stress transfer, making a saving on both cost and weight, is also a key part of composite design. The use of Raman spectroscopy to investigate micromechanical properties of laminated bacterial cellulose (BC)/poly(L-lactic) acid (PLLA) resin composites is reported for the first time as a means for understanding the fundamental stress-transfer processes in these composites, but also as a tool to select appropriate processing and volume fraction of the reinforcing fibers. Two forms of BC networks are investigated, namely, one cultured for 3 days and another for 6 days. The mechanical properties of the latter were found to be higher than the former in terms of Young's modulus, stress at failure, and work of fracture. However, their specific Young's moduli (divided by density) were found to be similar. Young's modulus and stress at failure of transparent predominantly amorphous PLLA films were found to increase by 100 and 315%, respectively, for an 18% volume fraction of BC fibers. BC networks cultured for 3 days were shown to exhibit enhanced interaction with PLLA because of their higher total surface area compared, as measured by nitrogen adsorption, to the material cultured for 6 days. This enhanced interaction is confirmed by using the Raman spectroscopic approach, whereby larger band shift rates, of a peak initially located at 1095 cm^{-1}, with respect to both strain and stress, are observed, which is a quantitative measure of enhanced stress transfer. Thermal analysis (differential scanning calorimetry) and electron microscopy imaging (scanning electron microscopy) of the samples also confirms the enhanced coupling between the resin and the BC networks cultured for 3 days, compared to those cultured for 6 days. These results are shown to have implications for the use of BC networks for composite reinforcement, whereby less material can be used for the same specific mechanical properties. The technique also gives opportunities to study the interfaces in these composite materials in detail.

KEYWORDS: Raman spectroscopy • micromechanics • bacterial cellulose • poly(L-lactic) acid • composite

INTRODUCTION

There is a timely opportunity to create green/environmentally friendly composites for industrial applications having reduced end-of-life impact and cost compared to conventional composites. By recycling, as well as composting green composites, their impact on the environment could also be mitigated. So-called green composites are obtained from renewable resources and are potentially fully biodegradable. One approach to make green composites is to use plant fibers, comprising predominantly cellulose, and a potentially biodegradable resin. There are several sources of cellulose, namely, higher plants, algae, bacteria, and sea animals called tunicates (1, 2). Bacterial cellulose (BC) has been shown to be a very promising material for the mechanical reinforcement of both thermoplastic and thermoset resin materials for composite applications (3–10). BC is produced by a family of bacteria referred to as *Gluconacetobacter xylinum*, and is characterized by a high degree of polymerization and crystallinity (70−80 %), biocompatibility, excellent moldability during culturing and high mechanical properties. Estimated values of 78 ± 17 GPa (11) and 114 GPa (12) for Young's modulus of BC filaments have been reported, indicating their great potential for use in composite materials. However, an important question remains on the nature of stress transfer between BC fibers and polymeric resins, and how this can be best optimized.

BC cultured in static conditions typically comes in the form of a reticulated network of fibers, and can form laminated structures, with weak links between the layers (13). Interaction with polymeric resins and the laminated structure of BC networks has not been thoroughly investigated. It is therefore important to study this interaction in order to determine the optimum loadings required for stress

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transfer, using the least amount of material possible. This could lead to savings on costs if large-scale manufacturing of these composites were to take place.

It is not clear that resins can interpenetrate the small pore and laminated structure that is present in BC fibrous networks. It has been recently shown that theoretical nanofibrous networks have small mean pore sizes (14), which would make it difficult to get impregnation of resin into their structure for effective stress transfer, particularly if the networks are dense. Therefore, the stiffness and strength of a composite material comprising BC relies on both the interaction of the resin material with the fibrous network, and the interactions between fibers within the network.

Raman spectroscopy has been recently reported as a possible analytical tool to study the micromechanical properties of cellulosic-based fibers and composites (15–19). The Raman spectrum of cellulose is characterized by a number of Raman bands, one of which is initially positioned at approximately 1095 cm^{-1}, which correspond to C—O and C—C stretching modes along the cellulose polymer backbone (19, 20). This Raman band, used for stress-transfer quantification, has been reported to shift towards a lower wavenumber position when fibers are subjected to external tensile deformation.

Poly(L-lactic) acid (PLLA) is a "green" polymer, and has been widely investigated as a matrix material for composites (3, 5, 7, 21). There are very few reports on blending PLLA with BC for composite applications. Two examples of recent research report on the transparency of films generated from these materials (5, 12) and the other on enhancing the interface between a conventional cellulose fiber and a resin material (22–24). Transparent BC-based composites have possible applications as packaging materials, display devices, coatings and lenses (3, 5, 8, 13). Another possible application is in the biomedical sector, given that both BC and PLLA have been shown to have excellent biocompatibility (25–28). In these previous publications, BC networks were fully impregnated by resin materials resulting in transparent nanocomposites because of the "nano-size" effect of the BC filaments. The method of production, the viscosity of the resin as well as the porosity and pores sizes of BC networks are crucial for good impregnation, and for obtaining true nanocomposites, where the resin-fiber stress-transfer can be maximized. Depending on how BC networks are produced, their porosity and pore size will vary. In the present study, BC networks were not fully impregnated, resulting in laminated composites. These composites could be relevant for the design of green multilayered packaging. BC networks would be used for their good mechanical and thermal properties, and potentially good printability, and amorphous PLLA for its good sealing properties and transparency. By combining BC networks and PLLA we show that it is possible to follow stress-transfer from the matrix material to the fibrous reinforcing phase using Raman spectroscopy, thus opening opportunities for further in-depth studies of these interesting materials. We also show how lower-density networks can yield the same specific mechanical stiffness of the composite via enhanced stress-transfer from the matrix to the fibers, and how the Raman technique is instrumental in showing this. We also show how the laminated structure of networks cultured for longer times reduces the mechanical efficiency of the resultant composite material. Both these results have implications for the future processing of lightweight yet mechanically efficient BC-based nanocomposites.

EXPERIMENTAL METHODS

Materials. *Gluconacetobacter xylinum* (no. 13693; National Institute of Technology and Evaluation, Tokyo, Japan) and Hestrin-Schramm (HS) medium (29) was used to produce BC networks. The cells for the inoculum were cultured in test tubes statically at 27 °C for 2 weeks. The thick gel produced was then squeezed aseptically to remove the embedded cells. The cell suspension (25 ml) was then transferred as an inoculum for the main culture (500 ml of medium), which was incubated statically at 27 °C for 6 days. A more planar lower density network of fibers was also produced using an incubation time of 3 days, the properties of which were compared to the more laminated networks (6 days). BC networks (35 mm in diameter) were purified by boiling with 2% NaOH for 2 h, and then by washing with distilled water, followed by hot pressing at 2 MPa and 120 °C for 4 min to completely remove the bulk water. The densities of BC pellicles were determined by measuring their dimensions and weights. Values of 0.70 ± 0.10 g cm^{-3} and 1.00 ± 0.02 g cm^{-3} were obtained for BC networks cultured for 3 days and 6 days, respectively. These values were taken into account to calculate volume fractions and specific mechanical properties of BC networks.

Poly(L-lactic) acid (PLLA; grade L9000; molecular weight (M_w) > 150000 g/mol, density 1.3 g cm^{-3}) was purchased from Biomer (Krailing Germany).

Sample Preparation. BC pellicles were cut into strips using a razor blade with the following dimensions, ~25×1×0.007 mm for the networks cultured for 3 days and ~25×1×0.035 mm for the networks cultured for 6 days. PLLA films were prepared by compression molding. PLLA pellets were dried overnight at 40 °C and then melted at 180 °C for 120 s in a thin steel mold and then compressed at 12 MPa for 120 s. The mold was cooled down for 150 s. The cooling rate of the compression molder was measured and found to be ~55 °C min^{-1}. These processing conditions allowed us to obtain transparent PLLA films. These films were then cut into strips with dimensions of ~25 × 1 × 0.160 mm^3. BC/PLLA composites were prepared by further compression molding of BC strips between two PLLA films, under the same processing conditions. A pressure of 1.2 MPa was used to prepare composites with BC networks cultured for 3 days because higher pressures were found to damage them. Their dimensions were ~25 × 1 × 0.120 mm^3. The weight fractions of BC introduced in the composites were determined by weighing BC strips before and after impregnation.

Wide-Angle X-ray diffraction (WAXD). The morphology of processed PLLA films was investigated using a Philips X'Pert powder diffractometer having a 1.79 Å Cobalt X-ray source. 2θ was varied from 10 to 40° in increments of 0.04°.

Differential Scanning Calorimetry (DSC). Thermal properties of processed PLLA films and BC/PLLA composites were investigated using a TA Q100 heat-flux differential scanning calorimeter. Samples of mass 7.5 ± 0.5 mg, enclosed in hermetic aluminum pans, were heated and then cooled and then heated again in the range 25—200 °C at 10 °C min^{-1} under a 50 mL min^{-1} nitrogen purge gas flow. Experiments were repeated at least twice to ensure repeatability and empty pan measurements were performed to ensure reliability of the results.

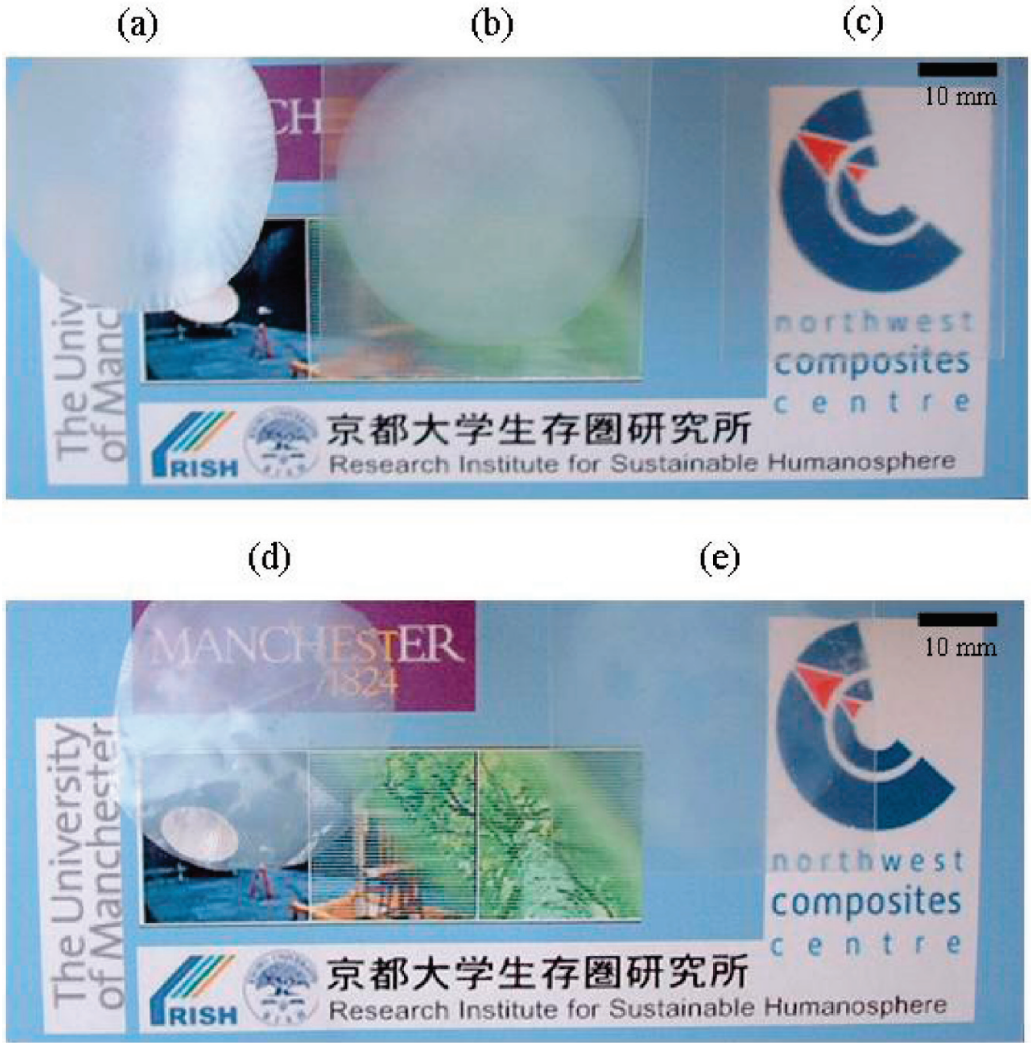

FIGURE 1. Image of the bacterial cellulose networks and composites showing transparency: (a) bacterial cellulose network cultured for 6 days, (b) bacterial cellulose network cultured for 6 days/poly(L-lactic) acid composite, (c) poly(L-lactic) acid, (d) bacterial cellulose network cultured for 3 days, and (e) bacterial cellulose network cultured for 3 days/poly(L-lactic) acid composite.

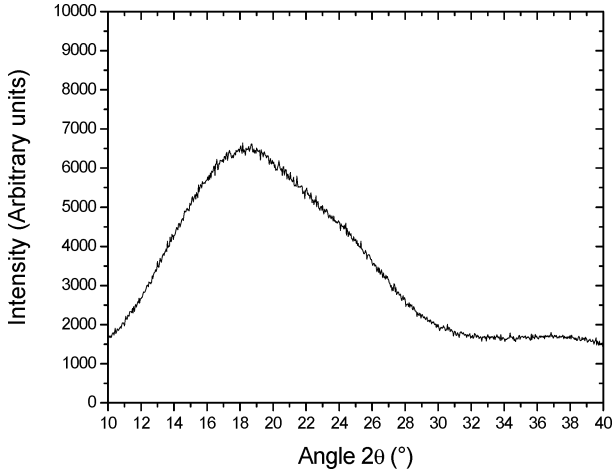

FIGURE 2. X-ray powder diffraction pattern for processed PLLA films in the range of 10 to 40°.

Porosity Measurements. A mercury porosimeter (Pascal 140 and 240 Series, Thermo Electron Corporation) was used to measure the porosity, average pore diameter and pore size distribution of BC networks cultured for 3 and 6 days. Samples were introduced into a dilatometer, followed by degassing and filling using the porosimetry system. Pressurization and depressurization were carried out by the combination of both the Pascal 140 and 240 systems, reaching a maximum pressure of 200 MPa. A blank test on the dilatometer was also carried out to take into account the effect of the compressibility of mercury.

The total surface area of BC networks was measured by nitrogen adsorption in static mode using an ASAP 2010 porosimeter (Software version 5.02, Micrometrics).

Mechanical Properties of BC Networks and BC/PLLA Composites. Pure BC, pure PLLA and BC/PLLA composite strips were mounted onto 20 mm gauge length testing cards and attached using a two-part cold-curing Araldite epoxy resin. Sample widths were determined using an optical microscope, and a micrometer was used to measure the thicknesses of the samples. By dividing the measured force by the cross-sectional area, the engineering stress was determined. The cross-sectional area of all samples was assumed to remain constant during deformation, although in reality Poisson's contraction has to be taken into account. Strain was also assumed to be equal to the cross-head displacement of the tensile testing rigs divided by the original length of the samples, although this may also be different locally in the sample. In this sense, engineering stress and strain is only reported. Mechanical properties of BC networks, BC/PLLA composites and PLLA were performed using an Instron tensile test machine (2511-111) with a 50 N load cell.

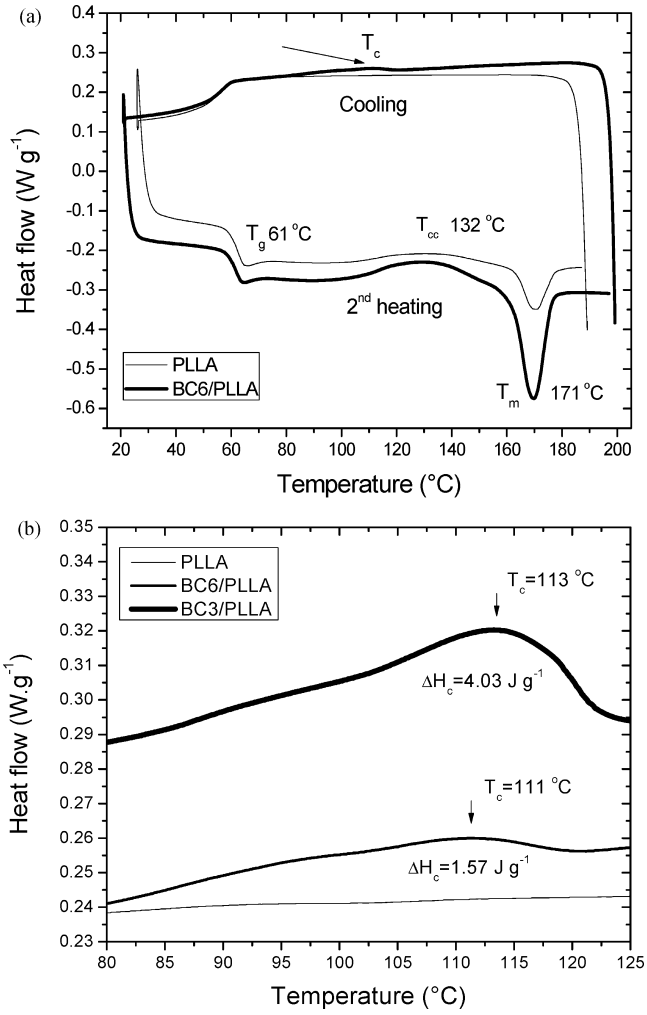

FIGURE 3. Differential scanning calorimetry curves for (a) poly(L-lactic) acid resin (PLLA) and bacterial cellulose networks cultured for 6 days/poly(L-lactic) acid composites (BC6/PLLA) for the second heating cycle and one cooling cycle, where T_g is the glass-transition temperature, T_{cc} is the cold-crystallization temperature, and T_m is the melting temperature, (b) poly(L-lactic) acid resin (PLLA), a bacterial cellulose network cultured for 6 days/poly(L-lactic) acid composite (BC6/PLLA) and a bacterial cellulose network cultured for 3 days/poly(L-lactic) acid composite (BC3/PLLA) between 80 and 125 °C of the cooling curve. T_c is the crystallization temperature. Arrows in both a and b indicate the position of a small crystallization peak.

The compliance of the machine was measured and found to be 4.42×10^{-3} mm N^{-1}. All samples were pre-conditioned 24 h prior to testing at a temperature of 23 ± 1 °C and a humidity of 50 ± 0.5 % and then deformed until failure using a 0.5 mm min^{-1} cross-head speed in the same environmental conditions. At least 6 samples were tested for each material.

The morphologies of the fractured surfaces of BC/PLLA composites were investigated using a Zeiss EVO 60 scanning electron microscope (SEM). Samples were first gold coated and imaged using a spot size of 250 (corresponding to 14.6 nm) and a 5 kV acceleration voltage.

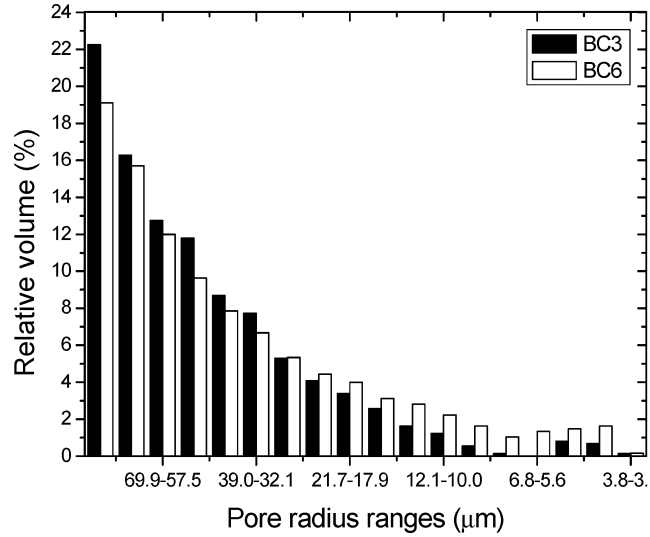

FIGURE 4. Typical pore size distributions of bacterial cellulose networks cultured for 3 days (BC3) and 6 days (BC6) obtained by mercury porosimetry.

Raman Spectroscopy. A Renishaw system-1000 Raman spectrometer coupled to a 785 nm NIR (near infrared) laser was used to follow the molecular deformation of the samples. These samples were prepared in the same way as described for the determination of the mechanical properties. The laser was focused at a fixed position to a 1−2 μm spot using a 50× long working distance lens. The laser power at the sample surface was ∼1 mW. Samples were deformed in tension using a customized deformation rig (Deben MICROTEST) incorporating a 2 kN load cell. The compliance of this rig was measured and found to be 3.14×10^{-4} mm N^{-1}. Strain was incremented in 0.1 % steps, and the elongation rate was set at 0.033 mm min^{-1} between these increments. At each increment, a Raman spectrum was recorded using an exposure time of 120 s. The peak positions of the Raman band initially located at 1095 cm^{-1} was determined by fitting using a mixed Gaussian/Lorentzian function and an algorithm based on the work of Marquardt (30). The resolution of the spectrometer is <0.1 cm^{-1}, and so shifts greater than this value are observable. Errors associated with band shift rates were found to not exceed ±0.24 cm^{-1} %$^{-1}$, showing that differences between data sets above this threshold level are observable.

RESULTS AND DISCUSSION

Images of the BC networks and the BC/PLLA composite specimens compared to pure PLLA are reported in Figure 1, showing their relative optical transparency. The BC sample produced using a culturing time of 6 days is opaque (Figure 1a), as is the BC/PLLA composite produced using this same material (Figure 1b). As expected, the PLLA sample shows complete transparency (Figure 1c). X-ray diffraction experiments have shown the predominantly amorphous state of processed PLLA films, which makes them transparent. A typical X-ray diffraction pattern from pure PLLA is

Table 1. Summary of the Nitrogen Adsorption and Mercury Porosimetry Measurements for Bacterial Cellulose Networks Cultured for 3 days (BC3) and 6 days (BC6)

technique	property measured	BC3	BC6
nitrogen adsorption	BET total surface area (m^2g^{-1})	94.6 ± 15.0	6.6 ± 2.1
mercury porosimetry	total porosity (%)	11.1± 6.3	16.0 ± 8.5
	average pore diameter (μm)	101.2 ± 2.9	103.2 ± 0.7

Table 2. Summary of the Mechanical Properties for Bacterial Cellulose Networks Cultured for 3 Days (BC3) and Bacterial Cellulose Networks Cultured for 6 Days (BC6)

	Young's modulus (GPa)	stress at failure (MPa)	strain at failure (%)	work of fracture (MJ m^{-3})
BC3	9.4 ± 1.0	109.0 ± 41.8	1.6 ± 0.9	0.8 ± 0.6
BC6	13.0 ± 1.8	218.3 ± 39.5	2.4 ± 0.3	2.8 ± 0.8

reported in Figure 2. A characteristic broad peak, typical of an amorphous polymer, is observed. The BC sample produced using a culturing time of 3 days exhibits some transparency (Figure 1d). The BC/PLLA composite sample with an embedded BC network cultured for 3 days also shows almost complete transparency (Figure 1e), similar to pure PLLA. This must be mainly due to the intrinsic transparency of the BC network cultured for this shorter culturing time, because it is thinner than the material cultured for 6 days. The higher transparency of BC/PLLA composites compared to pure BC networks, comprising this thinner material (3 days), must however also be due to a better wetting and consequently a better interface between the resin and the fibers, than for the thicker BC networks (6 days). These BC composites could be used in applications where transparency is required, whereas when opacity is required, for instance, to protect a product from sunlight, a thicker BC sheet could be used.

The thermal behavior of processed PLLA films and BC/PLLA composites was investigated using DSC, the data from which are reported in Figure 3. As expected, for predominantly amorphous PLLA films, a cold-crystallization at ~132 °C as well as a melting peak at ~171 °C is observed during heating (cf. Figure 3a). During cooling, no crystallization peak is observed which also confirms the amorphous state of the

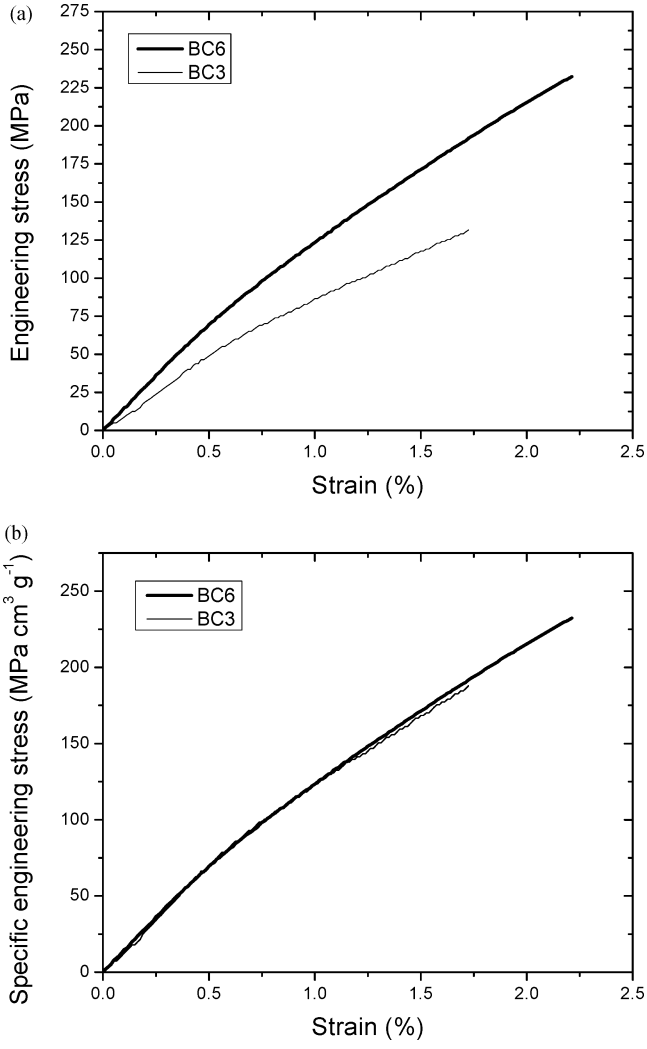

FIGURE 5. Typical stress–strain curves for (a) bacterial cellulose networks cultured for 3 days (BC3) and bacterial cellulose networks cultured for 6 days (BC6) and (b) bacterial cellulose networks cultured for 3 days (BC3) and bacterial cellulose networks cultured for 6 days (BC6) with respect to their density.

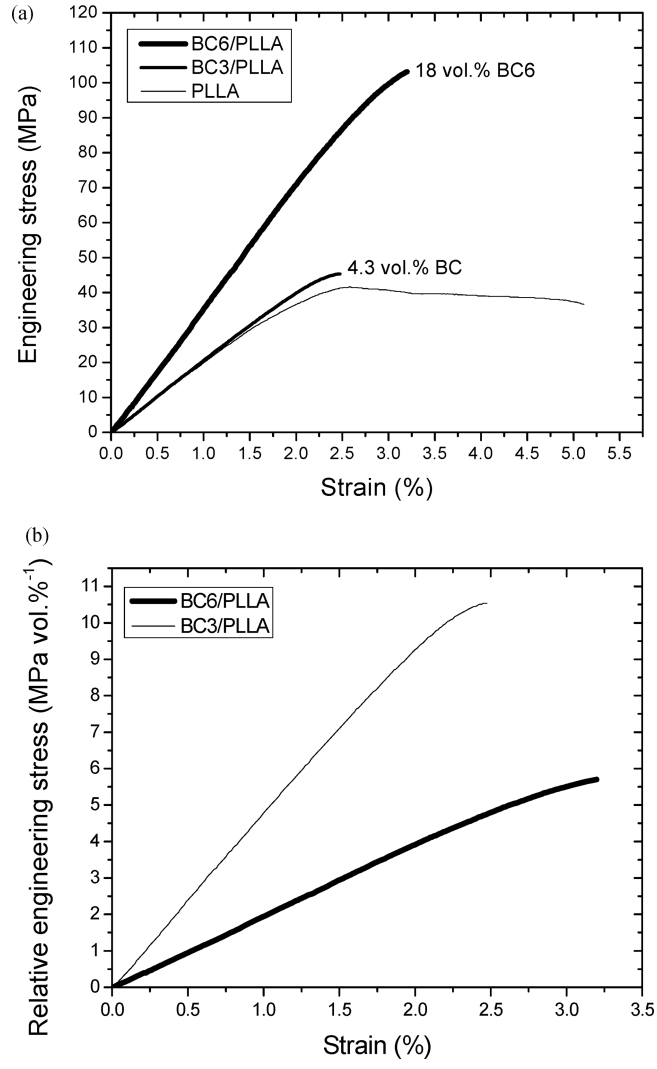

FIGURE 6. Typical stress–strain curves of (a) bacterial cellulose networks cultured for 6 days/poly(L-lactic) acid (BC6/PLLA) and bacterial cellulose networks cultured for 3 days/poly(L-lactic) acid (BC3/PPLA) composites and poly(L-lactic) acid (PLLA) and (b) bacterial cellulose networks cultured for 6 days/poly(L-lactic) acid (BC6/PLLA) and bacterial cellulose networks cultured for 3 days/poly(L-lactic) acid (BC3/PLLA) composites for a 1 vol % bacterial cellulose fraction.

Table 3. Summary of the specific Mechanical Properties for Bacterial Cellulose Networks Cultured for 3 Days (BC3) and Bacterial Cellulose Networks Cultured for 6 Days (BC6)

	specific Young's modulus (GPa cm^3 g^{-1})	specific stress at failure (MPa cm^3 g^{-1})
BC3	13.4 ± 2.4	155.7 ± 63.7
BC6	13.0 ± 0.0	218.3 ± 39.7

Table 4. Summary of the Mechanical Properties of Bacterial Cellulose Networks Cultured for 6 Days/ Poly(L-lactic) Acid Composites (BC6/PLLA) and Bacterial Cellulose Networks Cultured for 3 Days/ Poly(L-lactic) Acid Composites (BC3/PLLA) and PLLA

	Young's modulus (GPa)	stress at failure (MPa)	strain at failure (%)
BC3/PLLA	2.1 ± 0.2	46.9 ± 2.7	2.5 ± 0.1
BC6/PLLA	4.0 ± 0.4	115.2 ± 9.8	3.4 ± 0.3
PLLA	2.0 ± 0.2	27.7 ± 2.5	23.5 ± 17

Table 5. Summary of the Weight and Volume Fractions of Bacterial Cellulose (BC) Introduced in Bacterial Cellulose Networks Cultured for 6 Days/ poly(L-lactic) Acid Composites (BC6/PLLA) and Bacterial Cellulose Networks Cultured for 3 Days/ Poly(L-lactic) Acid Composites (BC3/PLLA)

	weight fraction BC (%)	volume fraction BC (%)
BC3/PLLA	3.0 ± 0.3	4.3 ± 0.7
BC6/PLLA	18.1 ± 2.6	18.1 ± 2.6

Table 6. Summary of the Relative Mechanical Properties for Bacterial Cellulose Networks Cultured for 6 Days/Poly(L-lactic) Acid Composites (BC6/PLLA) and Bacterial Cellulose Networks Cultured for 3 Days/Poly(L-lactic) Acid Composites (BC3/PLLA)

	relative Young's modulus (GPa vol $\%^{-1}$)	relative stress at failure (MPa vol $\%^{-1}$)
BC3/PLLA	0.5 ± 0.1	10.9 ± 1.9
BC6/PLLA	0.2 ± 0.0	6.4 ± 1.1

PLLA films. PLLA resin, like polyethylene terephthalate (PET), is a slow crystallization polymer. If one wants to induce crystallization, cooling rates typically lower than 5 °C min^{-1} must be applied (31). For BC/PLLA laminated composites produced using BC cultivated for 6 days, a small crystallization peak is observed at ~111 °C (cf. Figure 3b). The presence of BC nanofibrils, acting as nucleating agents at the interface, could be responsible for the development of a transcrystalline phase, leading to this small peak. Transcrystalline morphologies have been observed in polypropylene reinforced with cellulose nanocrystals (32). PLLA reinforced with wood floor and microcrystalline cellulose have also been shown to exhibit transcrystallinity (33). The contribution of such a transcrystalline phase to the mechanical reinforcement of composites and its effect on the stress-transfer process are not known. Our DSC experiments were conducted at cooling rates of 10 °C min^{-1}. During processing of our composites, the cooling rate was measured and found

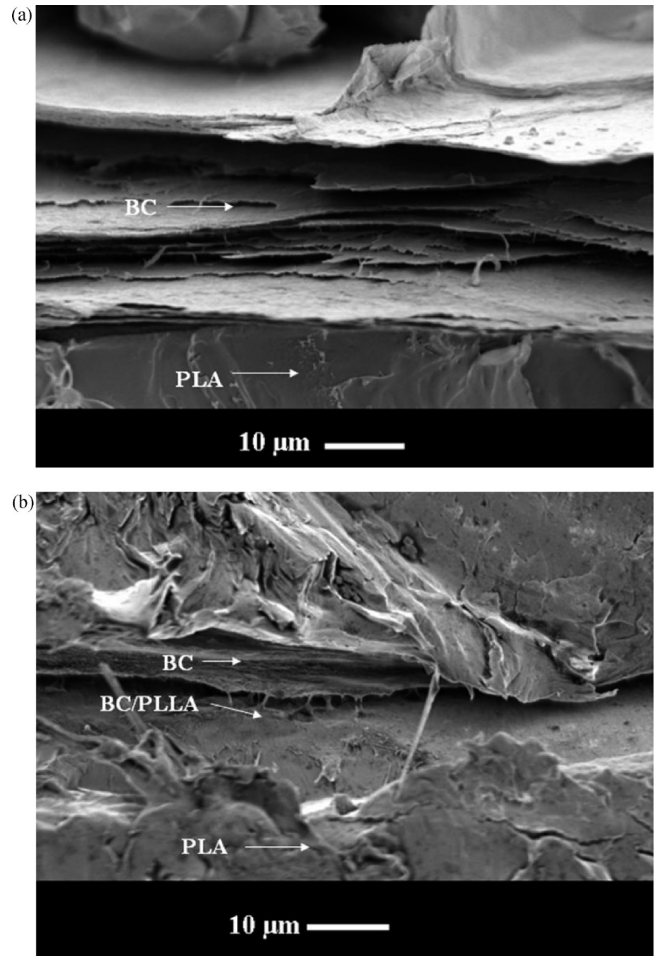

FIGURE 7. Typical scanning electron microscope (SEM) images of fractured surfaces of (a) a bacterial cellulose network cultured for 6 days/poly(L-lactic) acid composite and (b) a bacterial cellulose network cultured for a 3 days/poly(L-lactic) acid composite.

to be ~55 °C min^{-1} (cf. sections on sample preparation and experimental methods). Consequently, at this particular cooling rate, it is unlikely that significant crystallization of the sample will take place. Figure 3b reports a comparison between the crystallization peaks for BC/PLLA composites made with BC networks cultured for 3 and 6 days. One can clearly see a higher enthalpy of crystallization (ΔH_c) for the BC/PLLA cultured for 3 days (4.03 J g^{-1}) compared to the sample with BC material cultured for 6 days (1.57 J g^{-1}). This difference is an indication of a better interaction between the PLLA resin and BC cultured for 3 days, but this will be confirmed later by Raman spectroscopy.

It has been shown that the presence of porosity has a significant impact on the mechanical properties of polymeric materials (34). For instance, for high toughness cellulose nanopaper, the higher the porosity, the lower the Young's modulus for a constant degree of polymerization (35). The stress-transfer process between matrix and fiber must also be influenced by the presence of porosity. Porosity measurements were performed on BC networks cultured for 3 and 6 days (cf. Table 1 for results). Total porosities of 11.1 ± 6.3 and 16.0 ± 8.5%, and average pore sizes of 101.2 ± 2.9 and 103.2 ± 0.7 μm, were obtained for networks cultured for 3 and 6 days, respectively. Figure 4 reports the

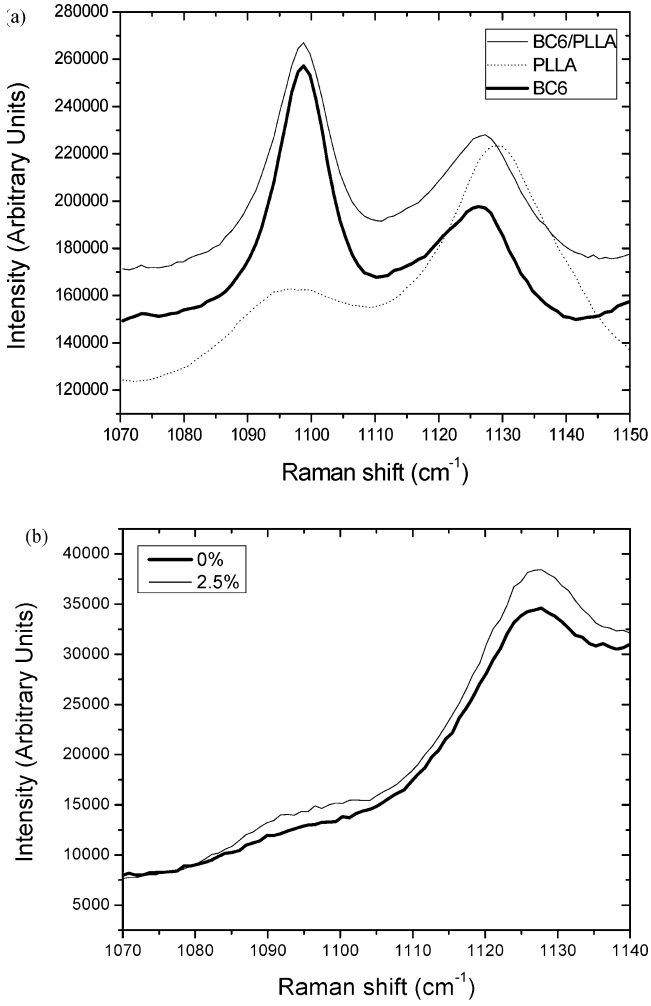

FIGURE 8. Typical Raman spectra for (a) a bacterial cellulose network cultured for 6 days (BC6), a bacterial cellulose network cultured for 6 days/poly(LL-lactic) acid composite (BC 6/PLLA), and poly(L-lactic) acid (PLLA) showing the position of the Raman band initially located at 1095 cm^{-1} and (b) poly(L-lactic) acid in the same region as the BC and BC/PLLA composites before and after 2.5 % strain.

pore size distributions for BC networks cultured for 3 days and 6 days. No significant differences in porosity, average pore sizes, and pore size distributions were obtained for both types of BC networks. The total surface area of BC networks was determined by nitrogen adsorption. Values of 94.6 ± 15.0 and 6.6 ± 2.1 $m^2\ g^{-1}$ were obtained for BC networks cultured for 3 and 6 days, respectively. As a consequence, a better interaction between the PLLA resin and the surface of the BC networks cultured for 3 days is expected, because of a higher available surface area. Mercury porosimetry and nitrogen adsorption measurements allow us to conclude that the predominant difference between BC networks cultured for 3 and 6 days is the total surface area.

A summary of the mechanical properties of BC networks are reported in Table 2. Higher mechanical properties (Young's modulus and strength) are obtained for BC networks cultured for 6 days. This result is also visually clear from the typical stress–strain curves of the BC networks reported in Figure 5a. It is possible that the BC networks cultured for 6 days have a higher degree of polymerization than those cultured for 3 days, because of their longer culturing times, thus leading to a higher Young's modulus and strength. We, however, have no evidence for this. The mean work of fracture of both networks has been determined, from the area under the stress–strain curves. Higher values were obtained for networks cultured for 6 days (2.8 ± 0.8 MJ m^{-3}) compared to those cultured for 3 days (0.8 ± 0.6 MJ m^{-3}) (cf. Table 2). This difference might be related to the laminated structure of the networks cultured for 6 days. More energy is envisaged to be dissipated during the delamination process when these BC networks are mechanically deformed. This delamination of the BC network is also thought to reduce stress-transfer within the network of fibers, although this will be confirmed later by Raman spectroscopy.

The specific mechanical properties of BC networks were determined by dividing Young's modulus and stress at failure by their respective densities. Figure 5b reports typical specific stress–strain curves. It is clear that specific Young's moduli for both BC networks are similar (the values are also reported in Table 3). This result has a direct implication for the design of lightweight composite materials made from BC networks, in that the same specific mechanical properties can be achieved with less material.

Typical stress–strain curves for BC/PLLA composites and pure PLLA are reported in Figure 6a. It is clear that Young's modulus and the stress at failure of PLLA are improved by the presence of BC cultured for 6 days; by 100 and 315%, respectively, for a 18% volume fraction of BC fibers. These values are rather high considering that the resin has probably only slightly impregnated the BC networks. The mechanical properties of BC/PLLA composites and PLLA are reported in Table 4. Their respective weight and volume fractions are reported in Table 5. Young's modulus of PLLA was not significantly improved with a 4.3% volume fraction of BC cultured for 3 days, whereas the stress at failure was significantly improved by 70% (cf. Table 4). If the densities of PLLA and BC cultured for 3 days are taken into account, the specific Young's modulus of PLLA and composites made with BC cultured for 3 days are 1.60 GPa $cm^3\ g^{-1}$ and 1.75 GPa $cm^3\ g^{-1}$, respectively. This means that even if Young's modulus is not significantly improved, the composite material has higher stress at failure and is lighter than pure PLLA.

To compare the reinforcement efficiency of networks cultured for 3 and 6 days, the mechanical properties were divided by the volume fraction introduced in the composites. We refer to these properties as relative Young's modulus, expressed in GPa vol $\%^{-1}$ (cf. Table 6). Figure 6b reports typical relative stress–strain curves for the BC/PLLA composites. One can clearly see that for the same volume fraction, the reinforcement is greater when PLLA is reinforced with BC networks cultured for 3 days than for material cultured for 6 days, leading to a higher stiffness of the composite. It is thought that the higher total surface area of this BC network is the main reason for this enhanced relative

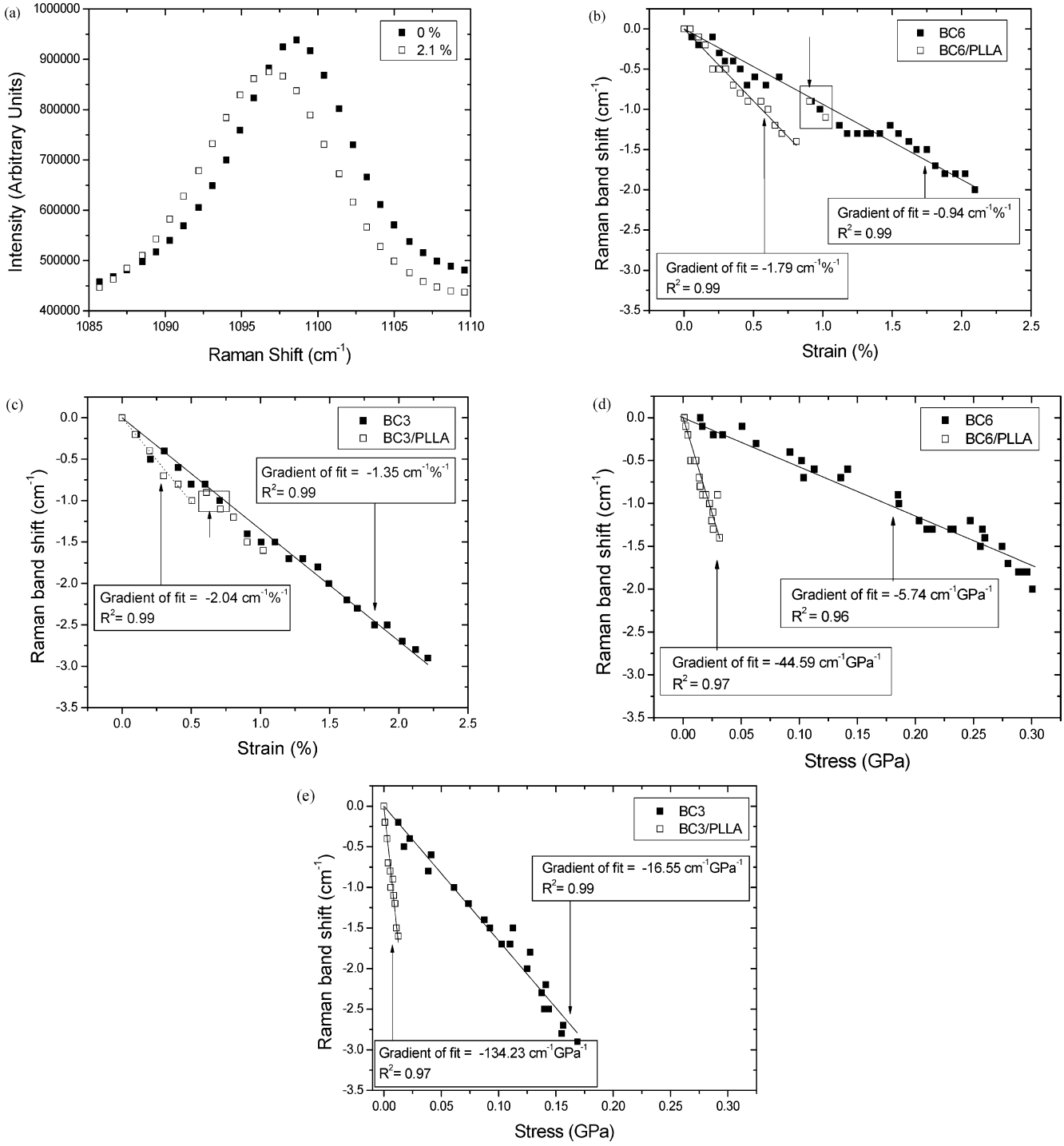

FIGURE 9. Typical (a) shift towards a lower wavenumber of the Raman band initially located at 1095 cm^{-1} upon tensile deformation (2.1% strain) for bacterial cellulose networks cultured for 6 days (BC6), (b) shifts in the peak position of the Raman band initially located at 1095 cm^{-1} for a bacterial cellulose network cultured for 6 days (BC6) and a bacterial cellulose network cultured for 6 days/poly(L-lactic) acid composite (BC6/PLLA) with respect to strain, (c) shifts in the peak position of the Raman band initially located at 1095 cm^{-1} for a bacterial cellulose network cultured for 3 days (BC3) and a bacterial cellulose network cultured for 3 days/poly(L-lactic) acid composite (BC3/PLLA) with respect to strain, (d) shifts in the peak position of the Raman band initially located at 1095 cm^{-1} for a bacterial cellulose network cultured for 6 days (BC6) with respect to stress, and a bacterial cellulose network cultured for 6 days/poly(L-lactic) acid composite (BC6/PLLA) comprising this network with respect to stress, and (e) shifts in the peak position of the Raman band initially located at 1095 cm^{-1} for a bacterial cellulose network cultured for 3 days (BC3) and a bacterial cellulose network cultured for 3 days/poly(L-lactic) acid composite (BC3/PLLA) with respect to stress.

stiffness. Knowing that less culturing time is sufficient for the production of effective BC/PLLA composites is extremely important. At an industrial scale, this could help to reduce the time and therefore cost of BC production.

Scanning electron microscopy images of composite samples fractured during tensile testing (cf. Figures 7a and b) reveal a good interaction between the top layers of BC networks and PLLA. Furthermore these images show that delamination is occurring preferably between weakly linked BC layers (13) and not at the interface between BC and PLLA. This observation is even more pronounced for the composites produced with BC networks cultured for 6 days (Figure

7a). This is thought to have a direct influence on the stress-transfer process.

Raman spectroscopy was employed in order to understand the stress-transfer process that leads to the reinforcement effects seen from tensile testing. Figure 8a reports Raman spectra for BC (cultured 6 days) and a BC/PLLA composite comprising the same material. It is clear that the Raman band initially located at 1095 cm^{-1} is still visible when BC networks are incorporated into PLLA, with little interference from Raman bands from the resin. The presence of a small peak from PLLA films, due to the resin scattering was found to not shift after 2.5 % deformation (cf. Figure 8b), whereas the main peak from the cellulose did shift in position. This means this small peak does not contribute to the shift of the Raman band from the cellulose. This result allowed us to study the deformation of BC nanofibrils embedded in PLLA resin, and therefore the stress-transfer mechanism in these two materials can be elucidated.

Figure 9a reports a typical shift in the position of the Raman band initially located at 1095 cm^{-1} for a BC network cultured for 6 days. Greater shifts in the position of this band were observed from the BC when they were combined with PLLA resin, and subsequently deformed in tension (*i.e.* in a BC/PLLA composite). Figure 9b reports the influence of tensile deformation on the position of the Raman band initially located at 1095 cm^{-1} for both the pure BC network material cultured for 6 days, and a BC/PLLA composite of the same material. The position of the peak clearly shifts when both BC materials are deformed in tension, which is indicative of direct molecular deformation of the cellulose backbone structure. The shift in the position the Raman band with respect to strain for the networks cultured for 6 days is found to be lower (-0.94 cm^{-1} $\%^{-1}$) than for values obtained when these networks are embedded in the PLLA resin (-1.78 cm^{-1} $\%^{-1}$). A sudden change in the position of the band was observed for the composite material (cf. arrow and box in Figure 9b). This is thought to be related to fiber/matrix debonding. A value of -1.77 cm^{-1} $\%^{-1}$ has been previously reported for the shift rate of this Raman band with respect to strain for a thin BC network (12). Monitoring of the position of the Raman band initially located at 1095 cm^{-1} from a BC network cultured for 3 days, and a BC/PLLA composite of the same material, was also carried out. These data are reported in Figure 9c. The band shift rate of the BC/PLLA composite (2.04 cm^{-1} $\%^{-1}$) using this material was found to be higher than for the composite system that incoporated the BC network cultured for 6 days (-1.78 cm^{-1} $\%^{-1}$). Again, a small sudden change in the data is observed, which might relate to a debonding of the BC from the PLLA, or delamination of the BC layers (cf. Figure 9c).

The effect of stress-transfer is better understood by plotting the position of the Raman band located at 1095 cm^{-1} against stress. Again, similar to the shifts rates with respect to strain, a much greater band shift rate with respect to stress is obtained for the BC/PLLA composite materials (cf. -44.59 cm^{-1} GPa^{-1} in Figure 9d) than for a pure network of BC (cf. -5.74 cm^{-1} GPa^{-1} in Figure 9d). When the fibers are pressed between layers of PLLA resin, the stress is transferred from the resin to the BC fibers, and hence there is a much greater band shift rate per unit applied stress in the reinforcing phase. The shift rate of the Raman band initially located at 1095 cm^{-1} with respect to strain and stress is higher for the pure BC networks cultured for 3 days compared to networks cultured for 6 days. This is thought to be a result of the laminated structure of the latter networks, leading to inefficient stress-transfer.

When the data for the BC/PLLA composites are compared, it is clear that much greater stress-transfer efficiency occurs for the sample cultured for 3 days than for 6 days (cf. -134 cm^{-1} GPa^{-1} in Figure 9e and 44.59 cm^{-1} GPa^{-1} in Figure 9d). This is direct evidence that composites manufactured with BC material cultured for 3 days have a much greater stress-transfer efficiency between the resin and fibers than those cultured for 6 days. This result explains why the relative mechanical properties are also greater for these samples (cf. Figure 6b). In addition, if one normalizes the band shift rate with respect to volume fractions of BC, the difference between composites made with BC cultured for 3 days and 6 days is even more pronounced. A relative band shift rate of 2.5 cm^{-1} GPa^{-1} vol $\%^{-1}$ is obtained for the samples cultured for 6 days, whereas a value of 31.2 cm^{-1} GPa^{-1} vol $\%^{-1}$ is obtained for samples cultured for 3 days.

CONCLUSIONS

This study has shown that Raman spectroscopy is a powerful tool for following the stress-transfer mechanism in bacterial cellulose networks and bacterial cellulose/poly(L-lactic) acid laminated composite materials. This has been achieved by hot-pressing a predominantly amorphous and transparent film of PLLA resin around sheets of bacterial cellulose. The Raman band initially located at 1095 cm^{-1} from the cellulose has been shown to be spectroscopically distinct from the resin material, making it possible to follow the stress-transfer properties of the composite material. Tensile testing of the samples has revealed that stress-transfer must occur between the resin and fibers, leading to enhanced properties of the composites above that of the pure resin material. Better stress-transfer is obtained for bacterial cellulose networks when pressed within the resin material, and this is revealed by an enhanced Raman band shift rate compared to the pure bacterial cellulose. The bacterial cellulose networks have been shown to have a layered structure for samples cultured for a longer time. Little impregnation of resin is likely to occur into the networks because of a low porosity, and therefore stress must be transferred only with the top layer of the networks. Enhanced stress-transfer is obtained for thinner, less laminated BC networks, and this could have implications for the use of bacterial cellulose for reinforcement of composite materials. For instance, it may be possible to use less material in order to achieve the same reinforcement capability. This is thought to be because stress-transfer efficiency in these materials is a combination of the interface between the resin and the network, and the stress-transfer within the network

structure itself. Characterization from Raman spectroscopic measurements, tensile testing, electron microscopy, and differential scanning calorimetry are in agreement, suggesting a better interaction between the resin and bacterial cellulose networks cultured for less time. The usefulness of the Raman spectroscopic technique for following stress-transfer mechanisms in this form of composite is clear, and further work will develop its use for following the effect of the modification of both the interfaces between fibers within the network and between the resin and the network of fibers.

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