Bacterial cellulose–kaolin nanocomposites for application as biomedical wound healing materials

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Abstract
This short communication provides preliminary experimental details on the structure–property relationships of novel biomedical kaolin–bacterial cellulose nanocomposites. Bacterial cellulose is an effective binding agent for kaolin particles forming reticulated structures at kaolin–cellulose interfaces and entanglements when the cellulose fraction is sufficiently high. The mechanical performance of these materials hence improves with an increased fraction of bacterial cellulose, though this also causes the rate of blood clotting to decrease. These composites have combined potential as both short-term (kaolin) and long-term (bacterial cellulose) wound healing materials.

Keywords: biomedical materials, bacterial nanocellulose, microbial nanocellulose, nano-kaolinite, blood clotting, wound healing

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1. Introduction

Kaolin is well established as a blood clotting agent [1] and is already used in commercial biomedical materials [2]. The negatively charged areas on the surfaces of kaolin accelerate the blood clotting process by binding to the Hageman factor (factor XII). Bacterial celluloses are biocompatible materials used to enhance longer-term wound healing. The nano-fibrillar structure of bacterial cellulose exacerbates the rate of wound healing. These non-toxic bacterial celluloses both form nucleation sites upon which the collagen can bind and provide an important preliminary biocompatible covering [3, 4]. This material furthermore has the effect of retaining moisture [5], which enhances re-epithelialization during fibroblastic proliferation and assists the process of wound healing/tissue growth [6, 7]. This research concerns the combination of short-term and longer-term wound healing mechanisms in a single composite system, using no adhesives to bind the cellulose and kaolin. Reported herein, are the first results, emphasizing the microstructural and material characteristics of these composites in relation to their mechanical properties, clotting potential and mass transfer properties.

2. Methods

2.1. Composites manufacture

Bacterial cellulose (BC) was extracted from nata de coco (PT Kara Santan Pertama, Bogor, Indonesia). The extraction procedure closely followed that reported in [8] and the resulting material was a gel comprising pure bacterial cellulose and water. The solids content of the gel was determined using a Precisa HA 300. Ultrafine kaolin particles (shape factor 40–120, size distribution 50–800 nm) were subsequently added to the gel in specific weight percentages.
in order to produce slurries containing %w/w BC:K ratios of 5:95, 15:85 and 30:70. These slurries were then pressure filtered into sheets and dried at 80 °C. The resulting BC–K composite sheets were very thin (<0.5 mm) and flexible.

2.2. Determination of morphological properties and chemistry

Scanning electron microscopy (SEM) coupled to energy-dispersive x-ray spectroscopy (EDS) was used alongside x-ray photoelectron spectroscopy (XPS) to characterize the morphology and chemical nature of the composites. Mercury porosimetry was also conducted on the materials at a pressure of 400 MPa and at 27 °C in order to determine the pore space characteristics as well as the densities of each composite.

2.3. Mechanical testing

Tensile tests were conducted using an Instron 8872. The composites were cut into dog-bone shaped samples for mechanical testing. At least five specimens were tested from each test group (5:95, 15:85 and 30:70). Each group was tested under three different conditions of humidity. The temperature was kept at 23 °C and the humidity variables were 0, 56 and 85%.

2.4. Blood droplet spreading and clotting tests

Two separate tests were conducted. Both involved extracting blood from mice of the FVB/n strain. The animals were bred and maintained at the Central Animal Laboratory at the University of Turku. The mice were euthanized by CO₂ inhalation and cervical dislocation. Blood was collected by cardiac puncture. All animal experiments were approved by the National Laboratory of Animal Care and Use Committee, Finland. The first set of tests involved releasing 20 μl pure blood droplets onto samples from each test group under conditions of room temperature (23 °C) and relative humidity (RH) of 56%. The blood in these tests was untreated and dropped immediately after extraction from the mice. The second set of tests involved the measurement of contact angle changes of blood droplets on the surfaces of the composites. Since these tests required more time, to prevent premature blood clotting, the blood was put into eppendorph tubes that were pre-coated with 0.5 M ethylenediamine tetraacetic acid (EDTA) pH 8, mixed well by pipette, and placed on ice. Blood droplets, 5 μl in volume, were placed onto the composites and contact angles were recorded using a Cam 200 Contact Angle Meter with the Young–Laplace method of analysis. Clotting was defined as the point at which the contact angle vanishes.

3. Results and discussion

BC within a kaolin particle matrix tends to form secondary interactions with the edges of the kaolin particles (figure 1(a)). BC networks and binds the kaolin particles not only through secondary interactions with the kaolin, but also through entangling BC fibres (30% w/w BC) (figure 1(b)). Entanglements such as these become more frequent as the relative fraction of BC to kaolin is increased. Interestingly, in this figure entanglements of the smaller cellulose nanofibres can be seen to occur in the regions where the larger BC fibres attach to the kaolin. These reticulating patterns of BC attachment, much like the reticulate patterns of veins in insect wings, serve to further reinforce and stabilize the connections between the BC and kaolin particles.

Nanofibre attachments at the fibre–kaolin interfaces are also observed in the SEM–EDS image shown in figure 2(a). In figure 2(a), the BC–kaolin interface can be differentiated from the BC fibre by the elemental compositions. The BC fibre will show a greater C content, which decreases at the BC–kaolin interface, while O, Al and Si contents increase. Since kaolin contains more oxygen than cellulose, it is expected that O content will be higher where the kaolin concentration is greater. Similarly, as the BC content is increased, the C-bonding is expected to rise and the O, Al and Si bonds are expected to decrease as the relative kaolin fractions lessen in the composites. These tendencies are confirmed by XPS characterization shown in figure 2(b). The binding potential of BC to kaolin is evidenced in figure 2(c). In this figure, increased humidity raises the elongation to failure of the higher BC content composites (15:85 and 30:70). It is expected that a well bound particulate composite will
Figure 2. (a) Representative SEM micrograph with EDS analysis in the centre of a BC fibre and at a kaolin–BC fibre interface. The reticulate arrays of BC nanofibres joining the ends of larger BC fibres with kaolin particles can also be seen here (b) XPS analysis for 5:95, 15:85 and 30:70 samples and (c) effects of humidity on the elongation to failure for 5:95, 15:85 and 30:70 composites.

Figure 3. (a) Mercury porosimetry analysis showing pore volume peaks, first at about 70–80 nm and again closer to 100 µm. Lower BC content results in a higher nanopore peak and as the fraction of BC is increased the micropore peak rises while the nanopore peak drops. This gives rise to higher rates of imbibition with less BC as is evidenced from the droplet spread sizes in (b) which shows 20 µl blood droplets on samples (left to right) 5:95, 15:85 and 30:70. This is reaffirmed in (c) where the mean spread diameters are plotted against the peak relative pore volumes, (d) toughness plotted against clotting time and (e) strength plotted against clotting time.
undergo a degree of plasticisation under humidity and the elongation will increase. This indicates that both 15:85 and 30:70 composites are sufficiently well networked. In contrast, the 5:95 composite shows the opposite tendency to the 15:85 and 30:70 samples. The 5:95 composites show characteristics typical of weakly connected powder-packing in tension. In such a system, humidity serves to break secondary bonds between the particles and elongation decreases.

Figure 3(a) shows that these composites have two high frequency peaks at about 70–80 nm and at closer to 100 μm. Composites tend to have more of the small pores when the BC content is low and the kaolin content is high. The highest peaks at the higher pore sizes contrarily are coupled to higher BC content and lowered kaolin fraction. In all cases nevertheless, the highest peak pore volume percent is at about 70–80 nm. These pore size distributions affect the spreading-imbibition characteristics of blood droplets as can be seen in figures 3(b) and (c). The mechanical properties can be more severely affected by imbibing fluids. The strength and stiffness of all three groups of composites decrease as a function of increasing moisture content (see table 1). The changes in strength from a 0% RH content are not considerably different in each case, however, there is a distinct loss of strength and stiffness as moisture content rises. If a composite patch is applied to a wound and does not have a sufficiently stable BC network, the material may effectively disintegrate and be unable to hold together a wound opening. That said, provided the BC network is effectively disintegrate and be unable to hold together a wound opening. That said, provided the BC network is sufficiently, the moisture levels will concurrently plasticize the composite more effectively and thus the material would be able to stretch and deform to a greater extremity before failing. Where the lower BC content composites fail in terms of elongation and strength, they make up in respect of blood clotting potential. The greater volume of high capillary pressure pores will allow more of the blood to come into contact with the kaolin particles and hence blood clotting will occur faster. This dichotomy between mechanical properties and clotting potential is illustrated in figures 3(d) and (e). In these figures, the clotting time is determined from the contact angle measurements and the toughness is calculated as the area under the stress-strain curve. The toughness and strength measurements are plotted for samples at room temperature and RH = 56% since these were also the conditions under which contact angle measurements were made. In table 1, the specific values for strength, $E/\rho$, and stiffness, $E/\rho$, are also provided. The densities, $\rho$, calculated for 5:95, 15:85 and 30:70 were 1.1856, 1.1564 and 1.0454 g cm$^{-3}$, respectively. The specific strength values are comparable to concrete for the lowest BC series (5:95) and rubber for the highest BC series (30:70). The specific stiffness values are comparable to polymeric materials in all three series.

To conclude, the preliminary results reported herein confirm that kaolin–BC composites have potential as combined longer and shorter term biomedical wound healing materials. Further research is needed to optimize the mechanical properties with respect to both moisture/fluid ingress and blood clotting potential.

Table 1. Mechanical properties of the different composites under variable conditions of humidity. $E$—elastic modulus, $\sigma_{ult}$—tensile strength, $\rho$—density.

| Humidity | BC (%) | $E$ (GPa) | $\sigma_{ult}$ (MPa) | $\Delta \sigma_{ult}$ (%) from RH = 0% | $E/\rho$ (MN m kg$^{-3}$) | $\sigma_{ult}/\rho$ (kN m kg$^{-3}$) |
|----------|--------|-----------|----------------------|---------------------------------------|-----------------------------|-----------------------------------|
| 0 5      | 0.31   | 4.86      | 0                    | 0.25                                  | 4.10                        |
| 56 15    | 0.29   | 4.07      | −16                  | 0.24                                  | 3.43                        |
| 85 30    | 0.28   | 3.00      | −38                  | 0.23                                  | 2.53                        |
| 0 15 56  | 0.78   | 12.5      | 0                    | 0.67                                  | 10.8                        |
| 56 85 26 | 0.58   | 9.27      | −26                  | 0.50                                  | 8.01                        |
| 85 38 31 | 0.28   | 8.26      | −34                  | 0.24                                  | 7.15                        |
| 0 30 56  | 0.71   | 16.2      | 0                    | 0.67                                  | 15.5                        |
| 56 38 26 | 0.48   | 12.1      | −25                  | 0.46                                  | 11.6                        |
| 85 31 25 | 0.27   | 11.2      | −31                  | 0.25                                  | 10.7                        |

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