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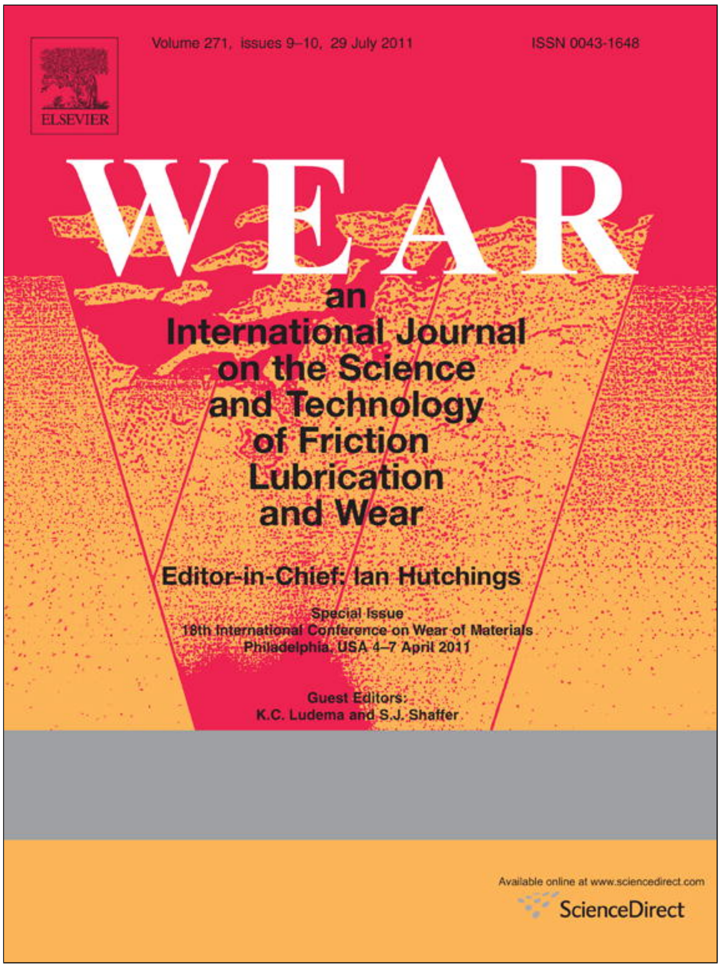

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Friction and wear behaviour of bacterial cellulose against articular cartilage

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ABSTRACT

Bacterial cellulose (BC) is a natural and biocompatible material with unique properties, such as high water holding capacity, ultra-fine fibre network and high strength that makes it an attractive material for the repair of articular cartilage lesions. However, data on the tribological properties of BC is very scarce, particularly if natural articular cartilage is involved in the contact. In this work, unmodified BC pellicles were grown from *Gluconacetobacter xylinus* in order to be used as tribological samples against bovine articular cartilage (BAC) in the presence of phosphate buffered saline (PBS). The tribological assessment of the sliding pairs was accomplished using reciprocating pin-on-flat tests at 37 °C. The reciprocating sliding frequency and stroke length were kept constant at 1 Hz and 8 mm, respectively. Contact pressures ranging from 0.80 to 2.40 MPa were applied. The friction coefficient evolution was continuously monitored during the tests and the release of total carbohydrates into the lubricating solution was followed by means of the phenol–H_2SO_4 method as an attempt to evaluate wear losses. The morphology of worn surfaces was characterized by SEM/EDS and the main wear mechanisms were identified. Low friction coefficient values ($\sim$0.05) combined with the preservation of the mating surfaces (BC and BAC) indicate the potential of BC to be used as artificial cartilage for articular joints.

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1. Introduction

Bacterial cellulose (BC) possesses an array of unique properties, including high water holding capacity, high crystallinity, ultra-fine fibre network, high tensile strength in the wet state and the possibility to be shaped into three-dimensional structures during biosynthesis. These properties allow wide applications, particularly in human and veterinary medicine, pharmaceutical, biotechnological, food and paper industries.

This material is biocompatible, presenting an insignificant foreign body reaction, not eliciting any chronic inflammatory response in vivo. In tissue engineering, novel BC biomaterials have been proposed as scaffolds and in vitro and in vivo studies demonstrated their suitability for implantation of cell-containing or cell-free devices that induce regeneration of tissue function. BC has proved promising for the treatment of chronic wounds and burns, as artificial cardiovascular tissues, and as scaffolds for guided regeneration of bone, nerve and cartilage [1–3]. However, in order to extend the application of BC to load-bearing applications, such as the articular cartilage, the tribological response of this material needs to be investigated under conditions mimicking the in vivo situation.

Articular joints withstand two to four times the body weight in a normal walking cycle. However, as simple as the movement might be, it may involve rapid changes in load and speed, and to accomplish that cartilage must be healthy. In most cases, it may remain functional for about 70 years, considering an approximation of 1 million cycles per year [4]. These tribological marvels have not yet been matched by any substitute made by man. When damaged, cartilage has a limited capacity of self-regeneration [5,6]. The outcome of such lesions is progressively incapacitating, by generating pain and hence limiting motion. Materials – plain or combined, natural or synthetic – have been sought or modified so as to adequately interact with the surrounding natural tissue, to cope with the problem of the low regeneration capacity of cartilage. Either as scaffolds to promote new tissue growth or as a surrogate material working in harness with natural cartilage, numerous materials and combinations have been proposed, and special attention has been dedicated to hydrogels [7–12], although very few authors have focused on their tribological response [13].

This study describes the tribological properties of BC against bovine articular cartilage in the presence of a saline solution. The effect of contact pressure on the tribological response of the gel was evaluated and the wear mechanisms of BC were characterized.

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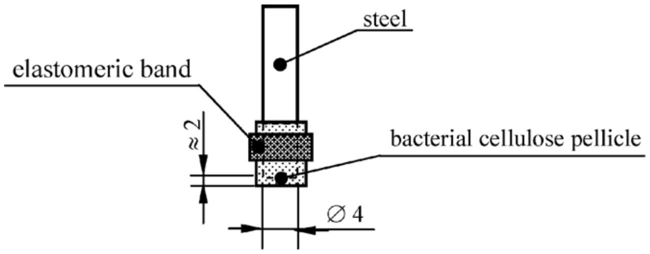

Fig. 1. Schematic representation of the pin sample consisting of a steel rod covered with a BC pellicle.

2. Experimental details

2.1. Bacterial cellulose production

Gluconacetobacter xylinus (ATCC 53582) purchased from the American Type Culture Collection was grown in Hestrin–Schramm medium, pH 5.0. The inoculated medium was added to 24-well polystyrene plates, and incubated statically at 30 °C. BC pellicles were purified by 2% SDS treatment at 70 °C, for 12 h, followed by 4% NaOH at 70 °C, for 90 min and washed extensively with distilled water. Samples were autoclaved and stored in PBS (pH 7.4), at 4 °C, prior to use.

2.2. Experimental set-up

Pins for friction and wear tests were prepared from stainless steel rods. The cylindrical extremity of these pins was fully covered with a BC pellicle, the flat base of which undergoing the tribological interaction, as exemplified in the schematic representation in Fig. 1. The flat contact surface had a slightly rounded edge in order to avoid undesirable loading effects. The hydrated BC pellicle, having ≈2 mm thickness, was fixed to the metallic pins by mechanical compression around the lateral side of the rod by means of an elastomeric band (Fig. 1). With this procedure, BC pellicle perfectly adapted to the cylindrical extremity of the rod without displacement of the pellicle even under the combined action of the normal applied load and tangential friction force, being therefore an effective method of fixation.

Bovine articular cartilage (BAC) flat plates, freshly obtained from a local market, were cut out from fresh bones, sonicated in distilled water for 3 min and immersed in phosphate buffered saline (PBS) at room temperature, prior to testing. The time span between cutting the BAC plates and each test was less than 15 min for all tests performed.

2.3. Tribological tests

The tribological testing of BC against BAC was performed on a pin-on-flat reciprocating sliding tribometer (CETR-UMT2, USA). Tests were conducted at 37 °C lubricated by PBS mimicking to some extent the in vivo environment. Increasing contact pressures ranging from 0.80 to 2.40 MPa were applied. Both the reciprocating sliding frequency and stroke length were kept constant at 1 Hz and 8 mm, respectively. Test duration was typically 30 min, but tests of up to 6 h duration were also performed. Before sliding, the pin was immersed in PBS during 10 min in order to attain the equilibrium water content (EWC). Under these conditions, the highly compliant contact surface of BC pellicle on the pin adapted perfectly to the surface topography of the cartilage plate. The friction coefficient evolution was continuously monitored during the tests and surface damage of the mating surfaces was investigated. Periodically (every 30 min), a sample of the supernatant was collected and assayed for total carbohydrates by the phenol–H_2SO_4 method [14].

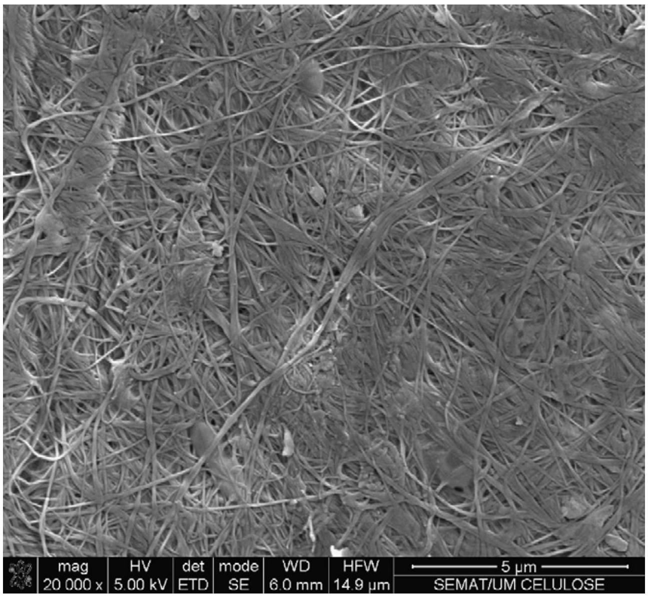

Fig. 2. Surface morphology of bacterial cellulose revealing the characteristic entanglement of nanofibres (⌀ ≈ 70 nm).

The morphology of BC worn surfaces was characterized by scanning electron microscopy – SEM (FEI Nova 200) with EDS X-ray analysis (Pegasus X4M) and the main wear mechanisms were identified. For SEM analysis, samples were dried at room temperature and Au sputter deposition coating was used for high magnification detailed images.

2.4. Wear quantification

Freeze drying as well as evaporation drying are known to reduce significantly the swellability of BC, an effect correlated with a reduction of the absolute number of polymer strands that form the network structure of the membrane [15]. However, because of the high affinity BC has with water, it is not possible to quantify the mass loss caused by wear by using conventional gravimetric methods. An apparently efficient method to utilise would be the phenol–H_2SO_4 method. The acid dissolves any particles or fibres in the supernatant, for BC is a polymer composed by glucose molecules – thus, the premise is that glucose in solution originates from worn BC. Using linear regression analysis it is possible to correlate the referred molecules in solution with mass in solution and estimate the aimed wear quantification.

3. Results and discussion

Table 1 presents typical values for the mechanical properties of BC materials.

The observed exceptional properties justify the interest in using this material as a reinforcement agent for various composites. In combination with a suitable polymer matrix, BC networks show a considerable potential as reinforcement for high quality speciality applications of bio-based composites, because of the small dimensions of its fibrils, which average 70 ± 20 nm diameter, enabling an intimate contact between cellulose and matrix polymers, allowing for a large specific area and thus excellent adhesion [17].

Table 1
Mechanical properties of bacterial cellulose [16].

Elastic modulus (GPa)	Tensile strength (MPa)	Elongation to break (%)
>15	>100	>10

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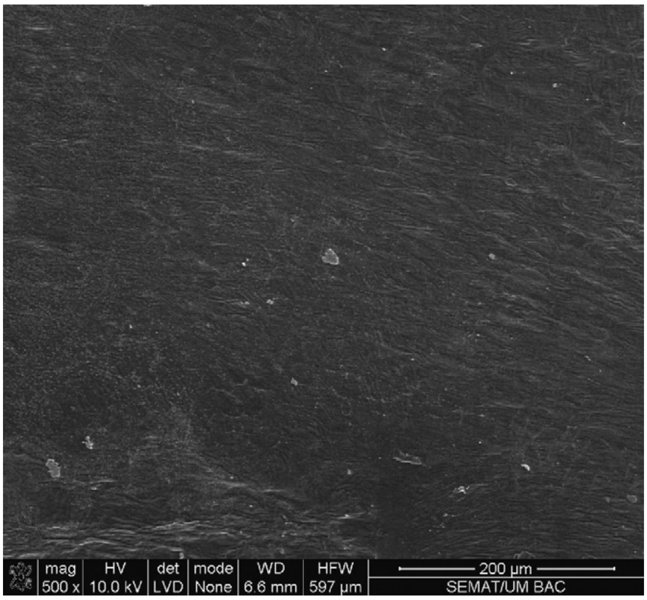

Fig. 3. Surface morphology of bovine articular cartilage in the dry state.

Figs. 2 and 3 present the typical surface appearance of unworn BC pellicles and unworn bovine articular cartilage.

As evidenced in the SEM image of Fig. 2, the surface morphology of BC pellicles is characterized by an entanglement of nano-fibres with an average diameter of ≈70 nm. BC surface is flat and a compact structure is observed.

The cartilage specimen did not undergo any fixation method which resulted in the observed undulated surface with aligned ridges (Fig. 3). These and other surface irregularities are known artefacts caused by dehydration observed in specific imaging studies [18]. However, immediately after excision and while hydrated, before and during sliding experiments, cartilage surface presents an extremely smooth appearance divested of particular features [19,20].

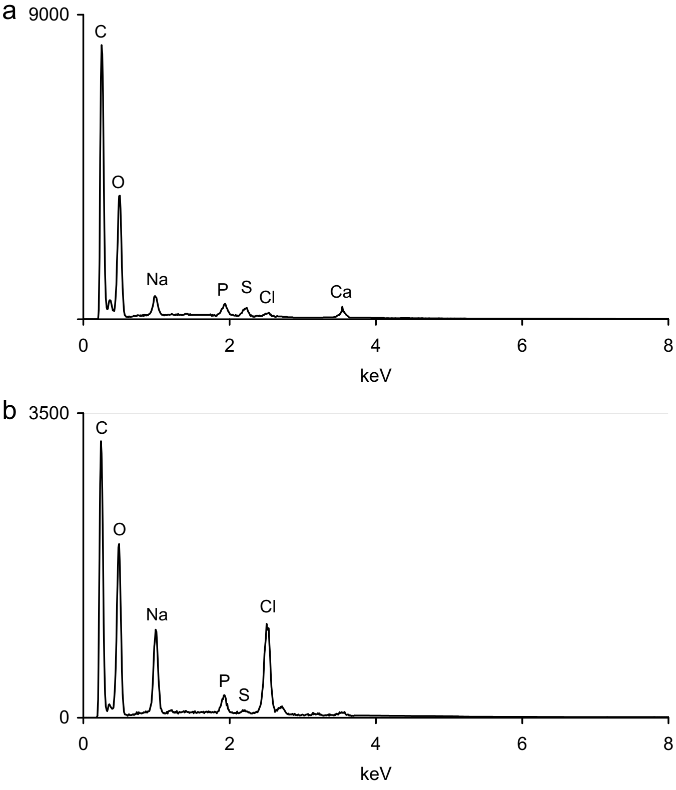

Fig. 4. EDS spectrum after immersion in phosphate buffered saline (PBS) prior to testing: (a) bovine articular cartilage; (b) bacterial cellulose.

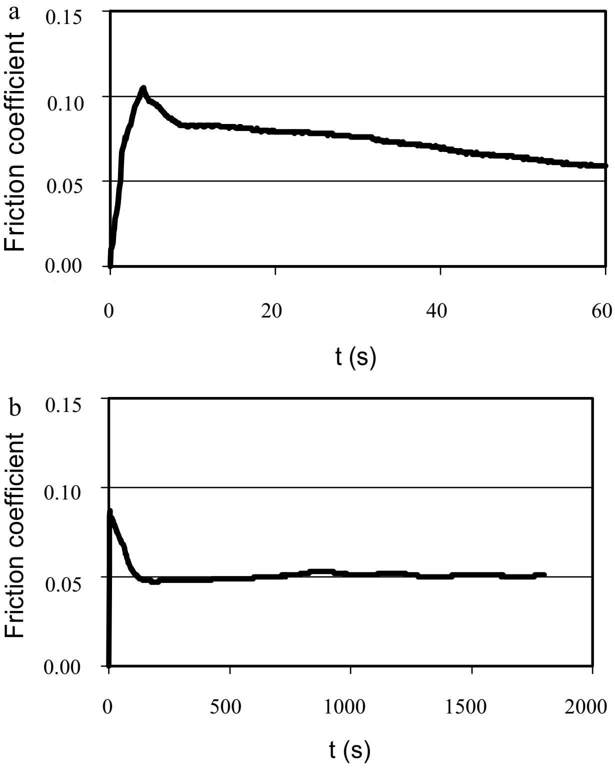

Fig. 5. Friction coefficient evolution during sliding: (a) first cycles of the reciprocating movement; (b) whole test duration ($p = 1.60$ MPa).

Articular cartilage is mainly composed by C and O. EDS elemental analysis shows trace amounts of Na, P, S, Cl and Ca, probably resulting from the physiological media (Fig. 4(a)). The same chemical elements were detected for BC, with the exception of Ca that was absent (Fig. 4(b)).

Fig. 5 shows the friction coefficient evolution during sliding for an intermediate contact pressure ($p = 1.60$ MPa), which is representative of the evolution of all contact pressures frictional response considered in this work. Each test is characterized by two distinct

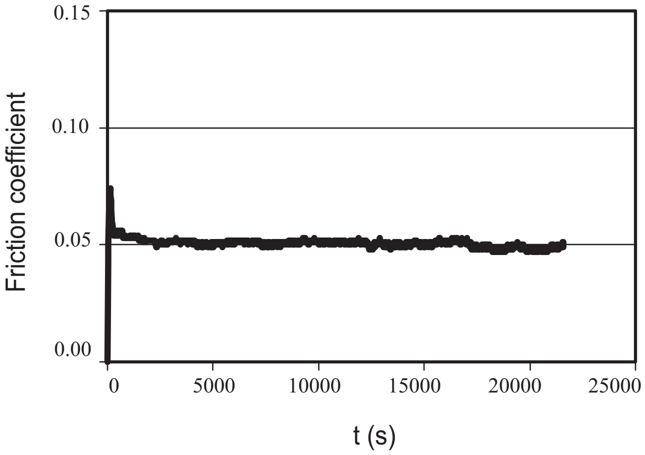

Fig. 6. Friction coefficient evolution during sliding for long test duration ($p = 0.80$ MPa).

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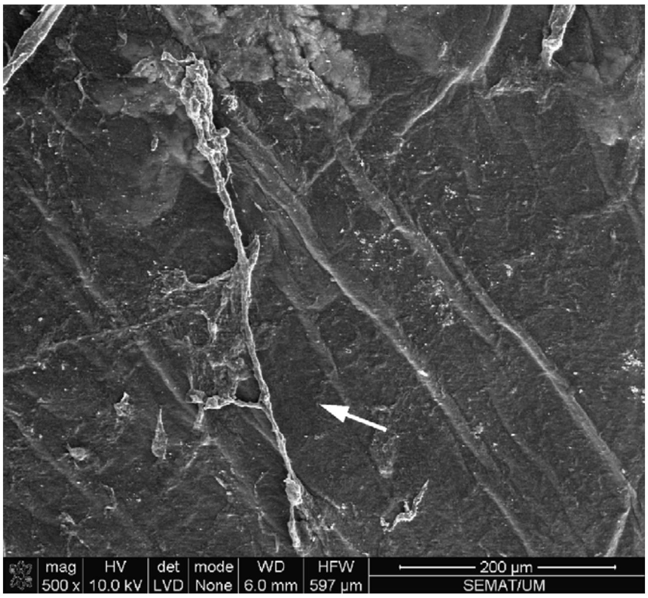

Fig. 7. Worn surface of BC after sliding under p = 2.40 MPa.

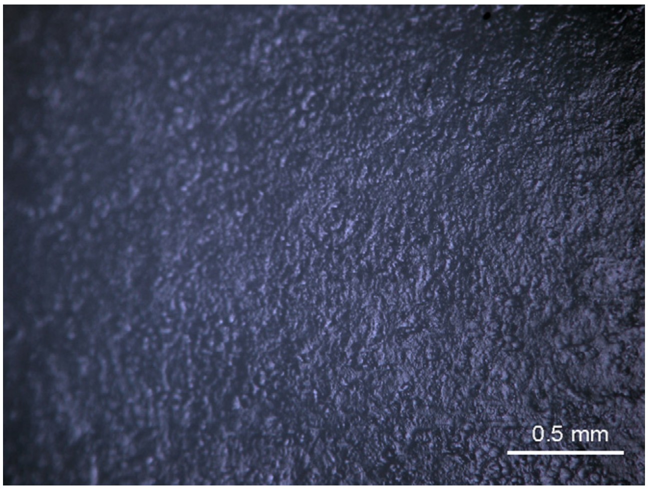

Fig. 8. Optical micrograph of the worn surface of articular cartilage after sliding under p = 2.40 MPa.

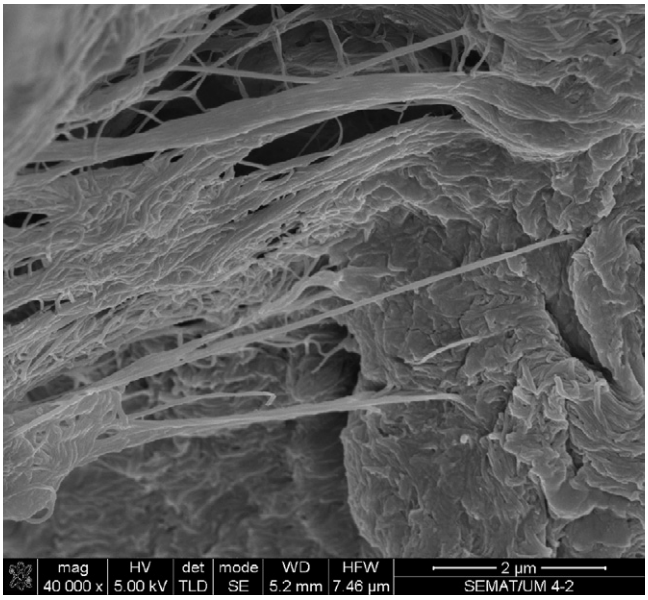

Fig. 9. Detail of BC worn surface, showing high plastic deformation and a nano-fibre network under tension (p = 0.80 MPa).

friction values. At the beginning of sliding, the friction increased suddenly to give a start-up-peak where the friction coefficient could attain values around 0.10 (Fig. 5(a)). As sliding proceeded, friction continuously decreased to a much lower steady-state value (≈0.05).

Table 2 presents the average friction coefficient values in the steady-state regimen obtained for the different contact stresses between the mating surfaces. Very low friction values, in the range 0.046–0.058, were measured evidencing a decreasing friction coefficient with the increasing contact pressure. Such frictional response can be attributed to the amount of liquid lubricant that the compliant gel can expel depending on the contact pressure, thus contributing to the increase of the lubricating effect during reciprocating sliding [21].

Fig. 6 confirms the steady-state friction regimen for long test durations, which indicates that both mating surfaces remain relatively preserved even after a period of 6 h under reciprocating loading.

Friction values of 0.14 [10] and 0.045 [22] were reported for articular cartilage repair biomaterials. Therefore, regarding the present work and comparing the obtained friction results with the values around 0.03 indicated in the literature for self-mated cartilage tribocouples in simulated physiological media [23], it can be concluded that, from the frictional point of view, BC is a gel with potential to be used as artificial cartilage for articular joints.

The morphological wear features of BC after sliding under p = 2.40 MPa, for 30 min, are shown in the SEM micrograph of Fig. 7,

Table 2
Steady-state friction coefficient values for bacterial cellulose/bovine articular cartilage contacts in the presence of PBS (test duration = 30 min).

Contact pressure, p (MPa)	Friction coefficient
0.80	0.058 ± 0.004
1.60	0.049 ± 0.002
2.40	0.046 ± 0.002

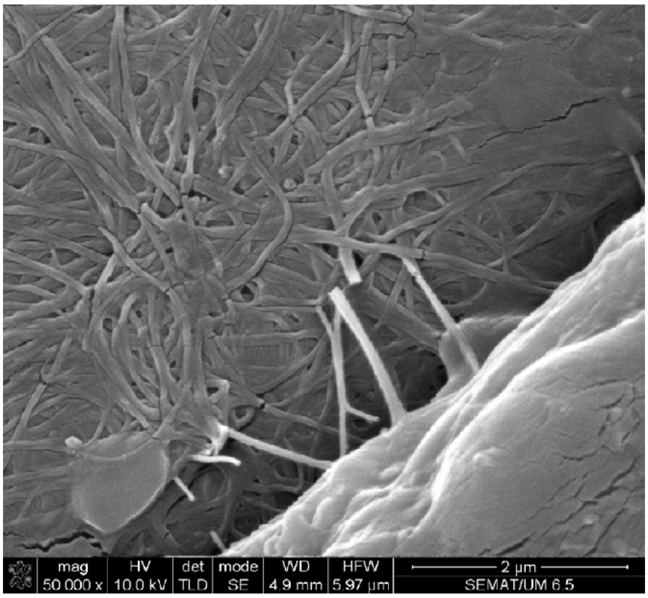

Fig. 10. Detail of the worn BC surface, showing rupture of nano-fibres (p = 1.60 MPa; test duration = 4 h).

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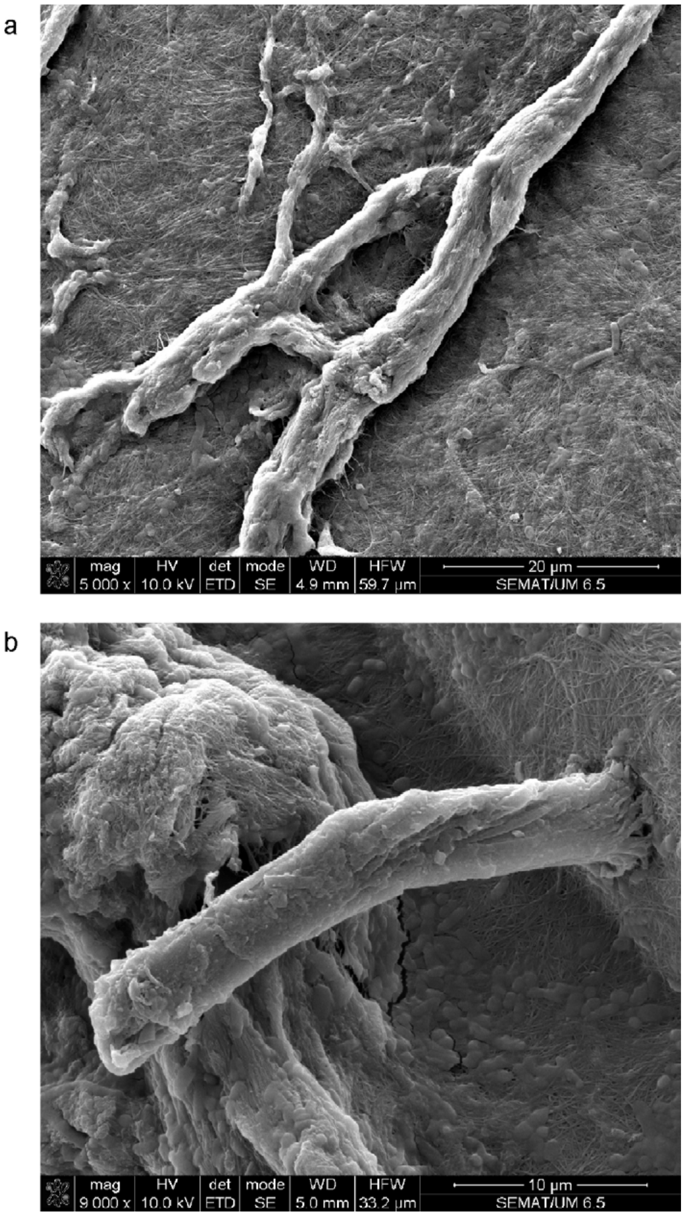

Fig. 11. Tribological rolls of BC: (a) growth of tribological rolls in progress; (b) tribological roll completely formed.

which is also representative of the worn surface appearance resulting from the reciprocating sliding under lower contact pressures ($p = 0.80$ MPa and $p = 1.60$ MPa). For all SEM images considered in this work, the sliding direction is horizontal.

From Fig. 7, BC worn surface is mainly characterised by high plastic deformation, where BC layers tend to be displaced by the combined effect of normal applied load and tangential friction force resulting from the reciprocating motion. These layers and intertwined nano-fibres of displaced material, appear to be strongly seized to the original pellicle. In some places these layers tend to assume the shape of a tribological roll (arrow in Fig. 7). However, as the tribological action proceeds, fibres are further pulled and stretched, with some actually breaking – both the mesh like distribution and anchored fibres, however, prevents the majority of the fibres of being released into the solution. Signs of conventional wear mechanisms such as adhesion and abrasion were not evidenced by BC worn surfaces. In fact, the compliant gel sliding against the smooth articular cartilage surface in the presence of PBS resulted in a particular tribosystem characterized by very low steady-state friction values, under 0.06 (Table 2). This fact, together with the absence of hard wear debris particles at the contact interface prevents the activation of significant adhesive or abrasive wear phenomena. Accordingly to these observations, no signs of wear damage were evidenced at the articular cartilage counterface, as evidenced in the optical micrograph presented in Fig. 8.

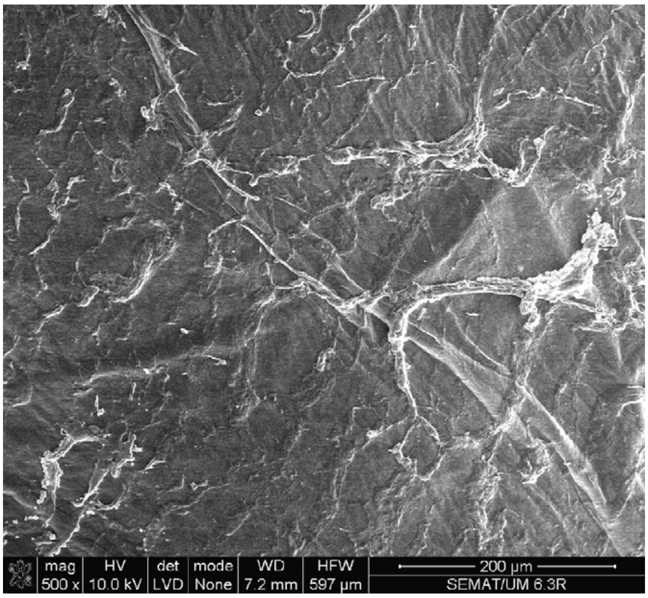

Fig. 12. Worn surface of BC after sliding for long test duration ($p = 0.80$ MPa).

Fig. 9 shows a detailed view of the BC worn surface, confirming the high level of plastic deformation that the gel can accommodate without breaking. A network of fibres under tension can also be identified. The mechanically high-resistant nano-fibres, randomly dispersed and tangled throughout the BC pellicle (Fig. 2), are responsible for the outstanding mechanical properties of the gel (Table 1) and, consequently, have a determinant effect on the preservation of the BC surface in terms of loss of mass under tribological loading.

The detailed view presented in Fig. 10 evidences a particular zone of BC contact surface where large displacement of a layer of material has resulted in fibre rupture and that a partial separation of the layer from the compact structure of BC is in course.

As previously mentioned, during the displacement of BC layers, rolls of compliant material tend to be formed, as evidenced in Fig. 7 and further enhanced in Fig. 11.

Due to the sliding action, the rolls can grow, incorporating more layers of material in displacement by a process similar to a “snow ball” effect (Fig. 11(a)). A high degree of compaction in the rolls takes place, assisted by the contact pressure at the sliding interface. As a consequence, tribological rolls of highly compacted BC material with the characteristic cylindrical shape shown in Fig. 11(b) were formed. These rolls can act as “miniature roller bearings” [24], which is a beneficial effect from the tribological point of view, particularly if they remain entrapped in the contact interface for a long period of time.

Fig. 12 presents the worn surface appearance of BC after a 6 h-long test under $p = 0.80$ MPa. The morphological features are similar to those of the worn surface resulting from a short duration test (Fig. 7). However, a higher amount of displaced layers is evident, resulting in a reduction of the BC pellicle thickness. Therefore, in a long duration test, the contacting BC surface gradually loses part of its initial smoothness, but not necessarily its mass.

Determination of total glucose in solution by the release of particles or fibres to the lubricating medium, showed no correlation

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between the calculated mass dissolved and test duration or contact pressure. This fact indicates that the surface damage of BC by wear occurred essentially by displacement and deformation of material layers with negligible mass loss attributable to wear, defined as such.

Notwithstanding the fact that some wear damage was observed on the BC sliding surfaces, no signs of surface damage were evidenced on the opposing articular cartilage contact surfaces (Fig. 8). This result highlights BC/natural cartilage as a potential tribosystem for in vivo applications.

4. Conclusions

This study evaluated the tribological properties of bacterial cellulose (BC) against bovine articular cartilage (BAC) in the presence of phosphate buffered saline (PBS) and at 37 °C, using reciprocating pin-on-flat wear testing. As main conclusions, it was found that:

A steady-state friction regimen, characterized by friction values around 0.05, was obtained after a relatively short start-up-peak of ≈0.10.

BC evidenced a notable wear resistance assisted by the compact structure of highly mechanically resistant nano-fibres, revealing non-measurable wear from the release of soluble substances in the lubricating medium by the total glucose determination method.

The main wear mechanism of BC consisted on large displacement of thin layers of material due to high plastic deformation combined with formation of tribological rolls at the contact interface.

The low friction coefficient values (≈0.05) combined with the preservation of the mating surfaces (BC and BAC) indicated the potential of BC to be used as artificial cartilage for articular joints.

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