The potential of bacterial cellulose as hemostatic material

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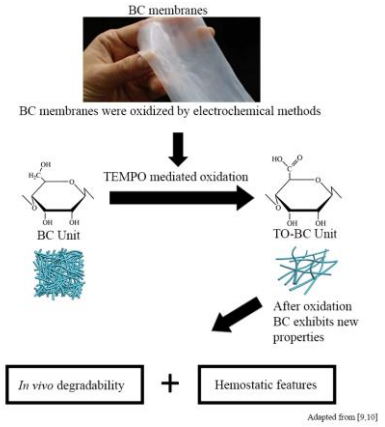

Bacterial cellulose (BC) produced by *Komagataeibacter* exhibits suitable properties for biomedical applications. However, BC is not biodegradable being mandatory its chemical modification by oxidative methods, making it biodegradable when implanted in the human body. Tetramethylpiperidine-1-oxyl (TEMPO) radical has been applied to oxidize cellulose since allows the selective oxidation of the primary hydroxyl group at C6 into carboxyl groups. Thus, we aimed at developing a hemostatic material based on the oxidized BC. The oxidation of BC was achieved through electrochemical methods using TEMPO. The oxidation of the hydroxyl groups was analysed by FT-IR and the selective oxidation of C6 was confirmed by ^{13}C-NMR. SEM was used to assess the surface morphology of BC membranes and the *in vitro* degradation was investigated in ultra-pure water. The hemostatic behavior was evaluated using whole blood coagulation tests.

Plants hold the most common cellulose biosynthesis pathway, although it can be also synthesized by bacteria, algae and fungi. Vegetable cellulose is almost an unlimited organic polymer resource on earth. However, bacterial cellulose (BC) produced by *Komagataeibacter* represents an alternative source with great potential for some specific applications, given its very special technical properties. In particular, the purity and ability to absorb a very high amount of water makes BC a unique source of cellulose [1]. Beside its good mechanical stability and high crystallinity, BC is also a biocompatible, hydrophilic and non-toxic material that has great mechanical properties (Young's modulus around 15-35 GPa and tensile strength of 200-300 MPa), high degree of polymerization and high water content - up to 200 times its dry weight [2]. Overall, BC is an astonishing material with a huge potential to be applied in several fields such as in food and cosmetic industries and also in biomedical field where biodegradation is a desirable requirement for many applications. The modification of BC in order to increase and improve its biodegradability has been attempted. BC may be chemically modified through its hydroxyl groups. Thus, an improvement on biodegradation may be achieved through the oxidation of BC making it reabsorbable by the organism. Besides this, after oxidation BC displays other characteristics such as hemostatic features being a suitable raw material for some applications as medical devices [3]. In this work, the oxidation of BC membranes was achieved using tetramethylpiperidine-1-oxyl (TEMPO) radical through electrochemical oxidation methods. TEMPO has been chosen as the primary method to convert polysaccharides into the corresponding polyuronic acids through the selective oxidation of the primary hydroxyl groups, i.e., only the primary hydroxyl group at C6 is oxidized to carboxyl groups. Besides this, this approach is a suitable alternative to the chemical co-oxidants like NaClO-NaBr, water-acetonitrile-NaClO-$NaClO_2$ and is considered as cleaner since it is possible the anodic regeneration of the oxidizing species instead of the primary oxidant [4].

This project thus aims to develop a hemostatic and resorbable material based on BC. After oxidation, using a total current of 400 and 700C, BC membranes were deeply characterized through different techniques such as: FT-IR, SEM and ^{13}C-NMR. The carboxyl content after oxidation was also determined, and the *in vitro* degradability in ultra-pure water was evaluated after 3, 7, 14 and 63 days. Finally, the hemostatic behavior was investigated through whole blood coagulation tests.

FT-IR spectra of the oxidized cellulose showed an increase of the absorption band around 1628 cm^{-1} attributed to the carboxylic acid vibration, in comparison with that obtained for non-oxidized cellulose [5]. The bands were also more intense on membranes with a higher degree of oxidation. On the other hand, through SEM analysis it was possible to assess the morphology of BC network with and without oxidation. The obtained imagens revealed that the morphology of the membranes was not changed by the oxidation which is in accordance to the literature [6]. It was also performed ^{13}C-RMN analysis to evaluate the specific oxidation of BC membranes on C6. The obtained results showed the usual six signals of BC ascribed to each C atom. The signal around 62 ppm corresponding to C6 primary hydroxyl group on the microfiber surface decreases after the oxidation, showing the selective oxidation of C6 [7]. Titration was performed to quantify carboxyl groups in the oxidized BC membranes. It was possible to conclude that the oxidation degree achieved increased with applied current from 12% to 23%, respectively for 400 and 700C as could be expected [8]. The *in vitro* degradability of oxidized BC membranes was evaluated using Surgicel® as control since this material is composed of oxidized regenerated cellulose and it is as hemostatic agent widely used on medical field. The obtained results revealed that almost no degradation occurred in the non-oxidized BC membranes showing the relevance of the oxidation on the improvement of the *in vitro* BC degradability. Moreover, the membranes with higher degree of oxidation degraded more extensively and faster. However, after 63 days of incubation, even the membranes with higher degree of oxidation did not degraded completely, while Surgicel® degraded almost completely after 14 days. The results related with the hemostatic behavior of BC membranes were obtained through the whole blood clotting times assay. The obtained results demonstrated that the oxidized BC exhibited hemostatic activity, although not as effective as Surgicel ®.

Acknowledgements
This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

References

[1] Y. Huang, C. Zhu , J. Yang, Y. Nie, C. Chen, D. Sun, Cellulose, 21 (2014) 1–30.

[2] I. Reiniati, A.N. Hrymak, A. Margaritis, Crit. Rev. Biotechnol., 37 (2017) 510–24.

[3] C. Lai, Z. Shujiang, L. Sheng, S. Liao, T. Xi, Z. Zhang, Colloid Polym. Sci, (2013);

[4] P. Parpot, K. Servat, A.P. Bettencourt, H. Huser, K.B. Kokoh, Cellulose, 17 (2010) 815–24.

[5] P.D. da Silva, S. Montanari, M.R. Vignon, Biomacromolecules, 4 (2003) 1417–25.

[6] C. Lu, S.Y. Chen, Y. Zheng, W.L. Zheng, C. Xiang, H.P. Wang, Nano-Scale Materials, Materials Processing and Genomic Engineering. Trans Tech Publications, (2014) 90–4.

[7] C. Lai, S. Zhang, X. Chen, L. Sheng, Cellulose, 21 (2014) 2757–72.

[8] Q. Cui, Y. Zheng, Q. Lin, W. Song, K. Qiao, L. Shumin, RSC Adv., 4 (2014) 1630.

[9] Y. Chen, S. Chen, B. Wang, J. Yao, H. Wang, Carbohydrate Polymers, (2017).

[10] H. Luo, G. Xiong, D. Hu, K. Ren, F. Yao, Y. Zhu, et al, Mater Chem Phys., 143 (2013) 373-9.