Acemannan/alginate/biocompatible ionic liquid-based beads as a sustainable therapeutic approach

Joana M. Gomes[1,2] , Simone S. Silva[1,2] and Rui L. Reis[1,2]

[1]3B's Research Group, I3Bs–Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal;

[2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal

Statement of Purpose: There is a worldwide demand to develop tailored biomaterials using sustainable and efficient green processes. Combining natural polymers and biocompatible ionic liquids (Bio-ILs) would impart matrices with adequate properties to be applied as therapeutic tools for tissue regeneration. We proposed the use of plant- and marine-derived polysaccharides, namely alginate (ALG) and acemannan (ACE), combined with a biocompatible ionic liquid (Bio-IL), cholinium caffeic (Ch[Caffeic]), envisioning the development of bioactive beads. ALG is a polymer with controllable gelation in the presence of di- or polyvalent cations. On the other hand, ACE, the main polysaccharide of aloe vera leaves, possesses Ca^{2+} traces in its composition. As so, earlier proved the development of 3D architectures based on ALG jellification in contact with ACE [1]. Herein, we proposed the formation of a functional system composed of ALG/ACE/Bio-IL. The Ch[Caffeic], a phenolic-based Bio-IL has been selected due to its antioxidant and anti-inflammatory features. Inflammatory diseases as Alzheimer disease, osteoarthritis, and age-related pathologies are characterized by reactive oxygen species (ROS) production[2]. ROS are critical signaling molecules in cell growth and proliferation, which imbalance may induce inflammation. It is expected that the bioactive action of ALG/ACE/Ch[Caffeic] system would contribute to the successful management of these events, which are vital for the success of diseases treatment.

Methods: Ch[Caffeic] was synthesized using an acid-base reaction between cholinium hydroxide and caffeic acid solution, being the purity of the Bio-IL confirmed by ^{1}H NMR. Beads composed of ALG, ACE, and Ch[Caffeic] (AAC) were produced by extrusion dipping a 5% w/v ALG solution in 5% w/v ACE solution containing Ch[Caffeic] (0.5 and 1 % w/v), using a syringe pump. The beads were kept at -4 ºC overnight, washed with PBS, and immersed in a $BaCl_2$ to form stable beads. Then, the architectures were rinsed with PBS, frozen at -80 ºC overnight, and freeze-dried. Morphological features and swelling behaviour were assessed by SEM, and immersion in PBS, respectively. The antioxidant activity of the Ch[Caffeic] and the beads was accessed using the DPPH radical scavenging assay. To evaluate their anti-inflammatory potential, an *in vitro* model was used consisting of a THP-1 cell line stimulated with lipopolysaccharide. The concentration of TNF-α in the medium was evaluated. 6mg/mL Betamethasone solution was used as a control.

Results: Ch[Caffeic] was successfully obtained, and the purity was confirmed by RMN by the presence of the peaks corresponding to the $N(CH_3)_3$ group in the cholinium cation, and the peaks corresponding to the aromatic ring on the caffeic acid structure. AAC beads (± 1mm of diameter) were successfully produced (Figure 1A). The SEM of the different beads (Figure 1 B) containing either 0, 0.5, and 1% (w/v) of Ch[Caffeic] (respectively AAC, AAC0.5 and AAC1) revealed that they present a porous and open structure, with pore size values up to 282.4 ± 17.8 µm, decreasing with the increase of the Ch[Caffeic] content. All conditions have a high swelling ability, up to 2000% after 3h. The antioxidant activity of Ch[Caffeic] and the beads is maintained up to 4h, being higher for AAC1 due to bigger Bio-L concentration. The low TNF-α concentration in the AAC0.5, AAC1, and the control (betamethasone), indicates the therapeutic effect of the architectures. Altogether the findings suggest that the developed AAC beads are promising tools for inflammatory diseases treatment.

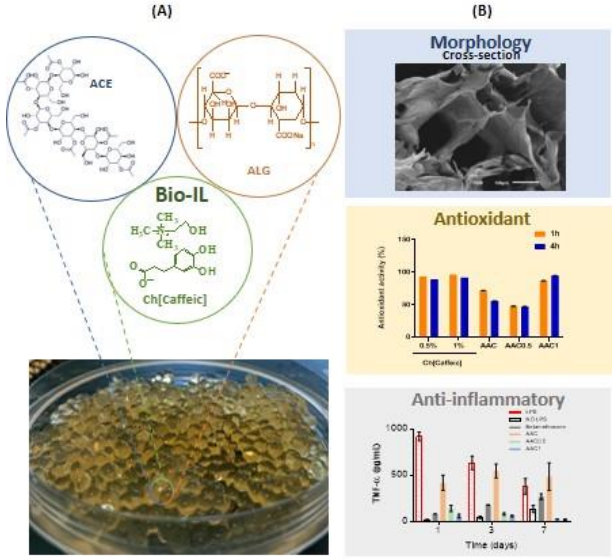

Figure. 1 – (A) Schematic representation of the beads' components and their macroscopical view; (B) their morphological features, antioxidant and anti-inflammatory activities.

References:

(1) Silva, S. S.; SN Appl. Sci. 2019, 1 (7)

(2) Chatterjee, S.; In Oxidative Stress and Biomaterials; Elsevier Inc., 2016 (35-38)

Acknowledgments:

J.M. Gomes acknowledges financial support from Portuguese FCT(PD/BD/135247/2017). This work is also financially supported by the PhD programme in Advanced Therapies for Health (PATH) (PD/00169/2013) and R&D&I Structured Projects with reference NORTE-01-0145-FDER-000021.