

## Development of bacterial cellulose wound dressings with controlled delivery of vitamin D<sub>3</sub>

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Wounds, in particular traumatic (e.g. burns) and chronic ones, are a major cause of morbidity and impaired life quality. They often result in long hospitalization stays, taking up substantial health resources in developed countries. This proposal aims at developing a safe, easy-to-use and non-expensive approach to efficiently address this problem, by attaining faster and proper wound healing. Recent studies showed that an antimicrobial peptide (AMP), LLKKK18, released from conjugates with dextrin embedded in a Carbopol hydrogel significantly improved burn wound healing. In addition to antimicrobial activity, this peptide stimulates vascularization, thus supporting a faster healing and tissue regeneration[1]. As such, one can hypothesize that a hydrogel comprising drugs that stimulate the expression of LL37 will improve wound healing while keeping the wound area infection-free.

This work comprised the approach towards the development of a novel bacterial nanocellulose (BNC) dressing. BNC, already used clinically for the treatment of burn wounds due to the unique properties like high water holding capacity, high crystallinity, ultrafine fiber network, high resistance, high moldability and biocompatibility[2]. In this work BNC will be used as drug carriers for the controlled release of drugs, namely of vitamin D<sub>3</sub>, an inducer of an endogenous expression of AMP LL37, known for accelerating the wound healing process, and as a protective barrier against exogenous agents (dust, microorganism) that can impair wound healing.

Since vitamin D<sub>3</sub> is poorly water soluble, and thus not easily incorporated in the highly hydrophilic environment of the BNC membrane, vitamin D<sub>3</sub> was loaded in to a newly developed hyaluronic acid (HA)-based amphiphilic nanogel and then incorporated in different types of BNC membranes. Such, nanogel was attained by conjugating a hydrophobic molecule to the HA chain. In aqueous environments, it self-assembles in nanosized structures with a hydrophilic shell and a hydrophobic core, able to incorporate hydrophobic molecules.

The new HA nanogel was successfully produced with a degree of substitution of 10.8±0.9% (out of 15 %, the maximum possible). Several vitamin D<sub>3</sub> concentrations were loaded with high stability into the nanogel (VitD<sub>3</sub>-HA), successfully achieving an encapsulation efficiency between 70-91%, with better results at small drug concentrations. Two wet BNC membranes, with different initial thickness were evaluated in terms of swelling and vitamin D<sub>3</sub> *in vitro* release rate. Wet membranes with lower thickness revealed the best swelling results with a maximum water absorption after 8 h. Vitamin D<sub>3</sub> was released from both wet BNC membranes gradually, starting 2 h after the initial contact with the release medium. The thinner BNC membranes had a faster release profile than the thicker membranes. The HA conjugate and vitamin D<sub>3</sub> were tested for cytotoxicity, revealing their safety for *in vivo* applications.

These study revealed a great potential of BNC as natural drug-delivery system of vitamin D<sub>3</sub>, but further research and development is necessary to explore its full potential on wound treatment still has to be confirmed *in vivo*.

[1] Silva JP, Dhall S, Garcia M, et al. Improved burn wound healing by the antimicrobial peptide LLKKK18 released from conjugates with dextrin embedded in a carbopol gel. *Acta Biomater.* 2015; 26:249–262.

[2] Czaja, W.; Krystynowicz, A.; Kawecki, M.; Wysota, K.; Sakiel, S.; Wroblewski, P.; Glik, J.; Nowak, P.; Bielecki S. Biomedical Applications of Microbial Cellulose in Burn Wound Recovery. In: *Cellulose: Molecular and Structural Biology*. Springer, 2007: 307–321.