EPITHELIAL TISSUE ENGINEERING: FROM DEVELOPING SCAFFOLDS TO CLINICAL TESTS

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ABSTRACT – With the increasing growth of populations prone to wound healing complications there is an urgent need for novel strategies to treat this biomedical burden. A therapeutic approach of particular relevance is Tissue Engineering. It is considered a promising biomedical technology, which aids the regeneration of injured tissues. Bacterial Cellulose (BC) synthesized by Gluconacetobacter hansenii is an excellent platform for epithelial tissue engineering and it is reported to function as a scaffold for the regeneration of a variety of tissues. Our group is focused on the development of biologically and physiologically competent BC biomembranes to be applied for tissue repair strategies. Efficient BC composites were successfully developed and pre-clinical studies were performed. The pre-clinical results confirmed the good potential of the developed BC biomembranes as a compatible engineered scaffold and contributed for a better understanding of its promising application in clinical tests.

1. INTRODUCTION

The morbidity and mortality from chronic ulcers of varying etiology present a significant health care problem (Eming *et al.*, 2002). Although advances in multidisciplinary wound care have improved clinical outcomes, the economic and social impact of non-healing wounds continues to grow (Dini *et al.*, 2006). With the increasing growth of populations prone to wound-healing complications there is an urgent and unmet need for novel strategies to both prevent and treat this substantial biomedical burden (Wong and Gurtner, 2012).

One therapeutic approach of particular relevance to wound healing is tissue engineering (TE), a concept described over 20 years ago (Langer and Vacanti, 1993). TE has emerged at the intersection of numerous disciplines to meet a global clinical need for technologies to promote the regeneration of functional living tissues and organs. It employs the principles from the fields of materials science, cell biology, transplantation, and engineering in an effort to treat or replace damaged tissues (Naderi *et al.*, 2011). It is considered a promising biomedical technology, which aids and increases the repair and regeneration of deficient and injured tissues (Zhong *et al.*, 2010). In the field of reconstructive medicine the development of new innovative matrices for skin repair is in urgent need and clinicians have been looking for better skin substitutes for clinical application (Wendt *et al.*, 2011).

Many researchers all over the world have been fascinated by the chance of creating a skin-like substitute without any further harm to the patients (Fohn and Bannasch, 2007). In this perspective, our laboratory is currently focused on the development of biologically and physiologically competent skin substitutes by using bacterial cellulose (BC) biomembranes as scaffold for development of new strategies for tissue repair (Recouvreux *et al.*, 2011). BC is a natural cellulose with nanofibrous structure and it is biosynthesized by certain bacteria. In addition to its common use in medical applications as wound dressings, temporary artificial skin and artificial blood vessels, BC is currently expanding its use as a biomaterial with 3D nano-network for scaffold preparation in tissue engineering field as seen in figure 1 (Fu and Zhang*, et al.*, 2013).

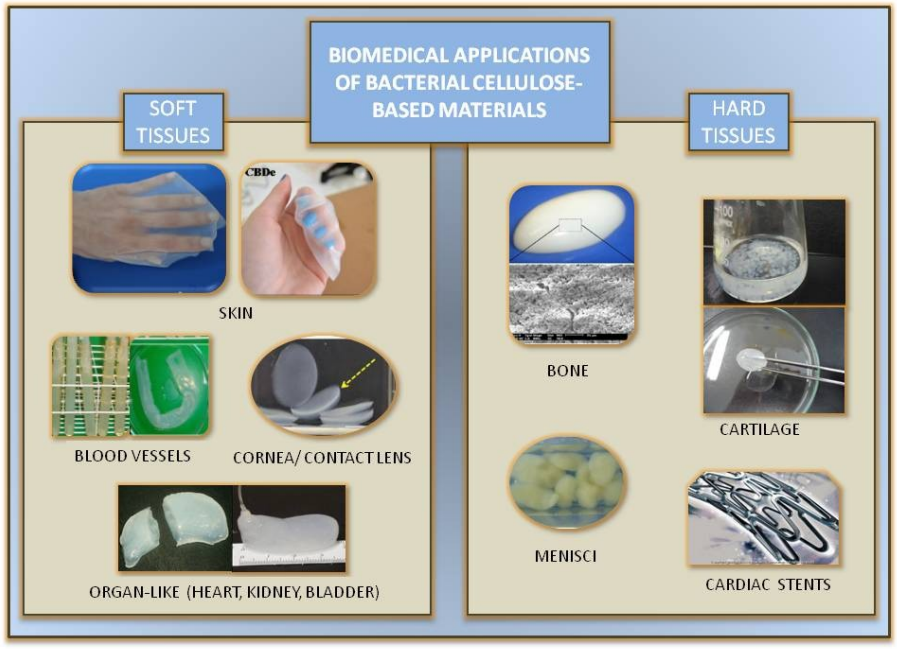

Figure 1: Biomedical applications of BC-based materials (images from InteLab/UFSC).

It is clear from previous studies that materials derived from BC can provide a promising future for biomedical application (Chang and Zhang, 2011). Therefore, based on fundamental concepts of tissue engineering, this review addresses the successful pre-clinical application of BC biomembranes as an engineered scaffold, aiming to contribute for a better understanding of its potential application for epithelial tissue repair in clinical tests.

1.1. Tissue Engineering

The past three decades have seen the emergence of an endeavor called Tissue Engineering (TE) in which scientists, engineers, and physicians apply tools from a variety of fields to construct biological substitutes that can mimic tissues for diagnostic and research purposes and can replace (or help regenerate) diseased and injured tissues (Berthiaume *et al.*, 2011). TE applications can be broadly classified into therapeutic applications, where the tissue is either grown in a patient or grown

outside the patient and transplanted, and diagnostic applications, where the tissue is made *in vitro* and used for testing drug metabolism and uptake, toxicity, pathogenicity, etc.

An important feature of TE includes a scaffold, which provides an architecture where seeded cells can organize and develop into the desired organ or tissue prior to implantation. Scaffolds are porous, degradable structures fabricated from either natural materials or synthetic polymers. They can be sponge-like sheets, gels, or highly complex structures with intricate pores and channels fabricated using new materials-processing technologies (Griffith, 2002).

Scaffold provides an initial biomechanical profile for the replacement tissue until the cells produce an adequate extracellular matrix during the formation, deposition, and organization of the newly generated matrix. Then the scaffold is either degraded or metabolized, eventually leaving a vital organ or tissue that restores, maintains, or improves tissue function. Properties of biocompatible scaffolds can be considered from different aspects including optimal nutrient and waste transport, delivery of bioactive molecules, material degradation rate, cell-recognizable surface chemistries, mechanical unity, and the ability to promote signal transduction pathways (Naderi *et al.*, 2011). The success of tissue organization and development mostly depends on these properties, because they can eventually induce cell adherence, nutrient/waste transport, cell differentiation, cell viability, and matrix organization.

1.2. Bacterial Cellulose biomembranes

Several biopolymers have been investigated for applications such as scaffolds, among which stands out the bacterial cellulose (Svensson *et al.*, 2005). Cellulose is well known as one of the most abundant biodegradable materials in nature and has been the topic of extensive investigations in macromolecular chemistry (Chang and Zhang, 2011). Cellulose is a linear homopolymer of glucose $(C_6H_{10}O_5)_n$ (Figure 2) with n ranging from 500 to 5000, and it is a widespread polymeric material in nature (Müller *et al.*, 2006). It is insoluble in water and it is degraded specific enzymes (Martson *et al.*, 1998).

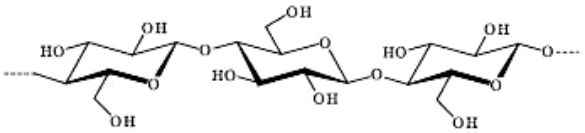

Figure 2: Chemical structure of cellulose.

BC is a promising natural cellulose with nanofibrous structure and it is biosynthesized by certain bacteria, including the bacterium *Gluconacetobacter hansenii*, formerly known as *Acetobacter xylinum* (Kondo *et al.*, 2001). This bacterium constructs a cellulose film, as seen in figure 3, between the culture medium and the gaseous surface, which has a dense layer on one side and a thick gelatinous (and porous) layer on the opposite side (Helenius *et al.*, 2006). In addition to its common use in medical applications as wound dressings, artificial skin and artificial blood vessels, BC is

currently expanding its use as an excellent biomaterial with 3D nano-network for scaffold preparation in tissue engineering field.

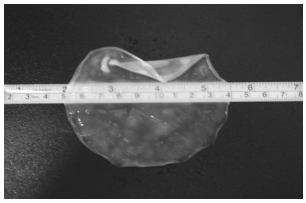

Figure 3: Hydrated BC membrane (image from InteLab/UFSC).

According to previous studies, BC has a great potential to be used as a substrate in tissue engineering because of their properties including high capacity to retain water (hydrophilicity), high crystallinity, high tensile strength, biocompatibility and nanofiber-network conformability, which significantly differ from the structure of plant cellulose (Watanabe and Kondo, 2012). This nanofibrilar structure has proven to be a viable matrix to improve tissue repair and has been also used in a wide range of applications as a scaffold for treatment of second or third degree burn ulcers, for artificial micro vessels and for tissue engineering of cartilage (Helenius *et al.*, 2006). Some of the physical and mechanical properties of BC which characterize it as an ideal material for tissue repair are listed in the table below:

Table 1: Properties of BC membranes and its correlation with an ideal material for tissue repair (adapted from Czaja *et al*., 2006)

Ideal tissue repairing material	BC
maintain a moist environment at the wound/dressing surface	high water holding capacity; high water vapor transmission rate
provide physical barrier against bacterial infections	nanoporous structure does not allow any external bacteria to penetrate into the wound bed
highly absorbable	partially dehydrated membrane is able to absorb fluid up to its original capacity
sterile, easy to use, and inexpensive	membranes are easy to sterilize and package. The estimated cost of production of 1 cm^2 is \$0.02
provide easy and close wound coverage, but allow easy and painless removal	high elasticity and conformability
provide porosity for gaseous and fluid exchange	highly porous material with pore sizes ranging from several nanometers to micrometers
nontoxic, nonpyrogenic, and biocompatible	biocompatible, nonpyrogenic, nontoxic
provide high conformability and elasticity	high elasticity and conformability
provide mechanical stability	high mechanical strength

To improve the positive features of BC for tissue repair, it can also be modified through incorporation of several composites such as collagen, gelatin, alginate, Benzalkonium Chloride, Poly ethylene glycol (PEG), Cotton gauze and *Aloe vera* as well as through incorporation of bioactive molecules, changes in porosity and crystallinity (Saibuatong and Phisalaphong, 2010). Modified CB could function as scaffold for regenerating a variety of tissues, which may possibly make an interesting biomaterial for medical devices and consumer products.

2. FROM DEVELOPING SCAFFOLDS TO CLINICAL TESTS

Many researchers yearn for the chance to create an ideal wound dressing, which does not cause any harm to the patient and promotes the perfect healing (Fohn and Bannasch, 2007). Recently, our research group is focused on the development of such therapy, using hydrogels of BC combined with polysaccharide fractions and *Aloe vera* gel as a biomaterial basis for the development of new strategies for tissue repair (Recouvreux et al ., 2011).

In this perspective our group has been working on the production and characterization of BC based materials incorporated with different fractions extracted from *Aloe vera*: enriched polysaccharide fraction, the total gel from the pulp of the plant containing fibers and the gel free of fibers (Godinho, 2014). These have been produced *in situ* by the addition of *Aloe vera* fractions at determined concentrations. At the end of the production period, these hydrogels were characterized and changes due to *Aloe vera* incorporation were observed. These changes were satisfactory because they resembled the skin structure, confirming the its potential to be applied as a scaffold in studies for epithelial tissue engineering. After the complete characterization of the scaffold, preclinical trials were performed in order to test the safety and efficacy of the BC-Aloe biomembrane *in vitro* and in animal models.

Briefly, for the *in vitro* tests, cell adhesion, citotoxicity and proliferation assays using human dermal fibroblasts were performed. No cytotoxic effect of BC-Aloe composite biomembranes was observed. Interestingly, an increase in cell proliferation was observed in the groups treated with BC incorporated with *Aloe vera* gel. *In vivo* assays were performed using isogenic Balb/c mice using an experimental model approved by the UFSC Ethics Committee on Animal Research, under protocol number PP00620. Full thickness wounds were created at the back of the mices and treated with BC-Aloe biomembranes in different proportions. Results showed that the use of BC-Aloe biomembranes was effective in the treatment of lesions compared to the control (pure BC). It was also possible to observe satisfactory exudate absorption, moisture maintenance, good mechanical protection of the wound site and protection against pathogenic microorganisms. No signs of irritation, edema, desquamation or pruritus were observed, indicating no toxicity of the biomaterial applied.

The absence of toxicity, combined with the proven healing activity puts the BC-Aloe biomembrane as a promising device for the treatment of ulcers. Therefore, to complete the pharmacological characterization, it is necessary to carry out clinical studies. Phase I clinical trials are the first step of evaluation of a chemical/biological product in humans and are generally preceded by an experimental evidence in animal models; It should be conducted in the same country of the drug discovery and involve a limited number of healthy adult volunteers (OPAS, 1997). In order to

accomplish this goal, the Phase I clinical trial is currently being performed by our research group. Future investigations include phase 2 and phase 3 clinical trials within the next 10 years. The pipeline of de development of BC-Aloe biomembrane, since the scaffold development to its clinical use is summarized in figure 4.

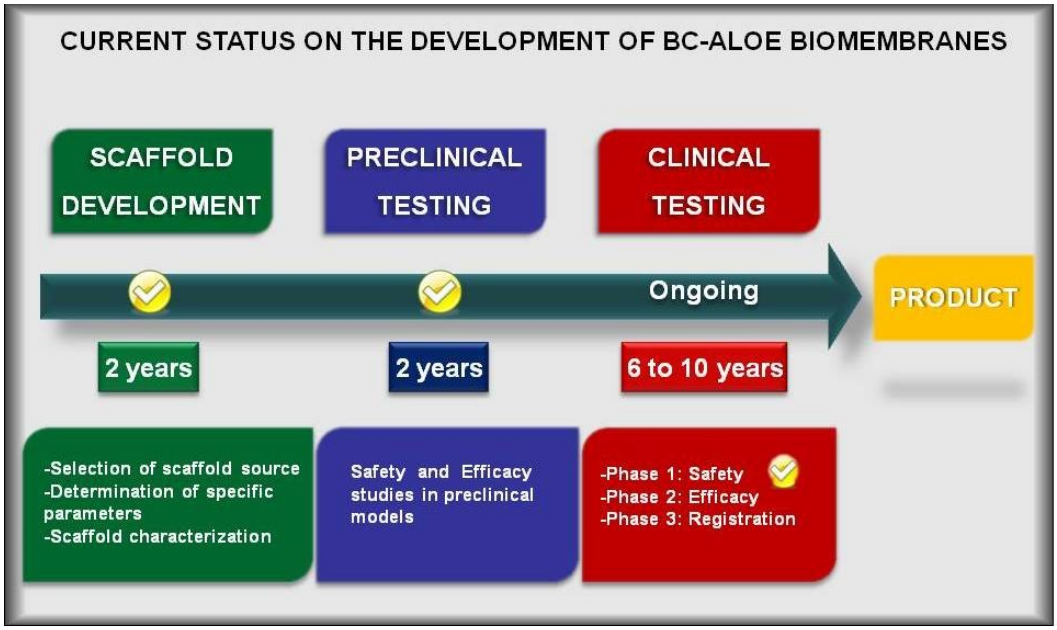

Figure 4: From development of scaffolds to clinical tests. Pipeline of the development of BC-*Aloe* scaffolds at InteLab/UFSC.

3. CONCLUSION

Tissue-engineering techniques bring a new element of rational design and development to therapeutic medicine. Understanding the biology of tissue repair is essential for the improvement of tissue-engineered medical products. One of the main requirements of any biomedical material is that it must be biocompatible, which is the ability to remain in contact with living tissue without causing any toxic or allergic side effects. Some time ago humans have used one form of cellulose or another in medical applications and wound care products. Now, through the serendipity of better understanding a novel form of cellulose assembled by bacteria, scientists are positioned to make good use of the unique properties of such materials. Because of its unique properties, BC has been shown to be a highly effective wound repairing material, by improving healing process of burns and chronic wounds. Efficient BC composites were successfully developed and pre-clinical studies were performed by our research group. The pre-clinical results confirmed the good potential of the developed BC biomembranes as a compatible engineered scaffold and contributed for a better understanding of its promising application in clinical tests. The clinical application of BC-Aloe as an engineered scaffold is still under consideration, opening the field for further investigations taken into account its promising application for wound repair in the epithelial tissue engineering field.

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