Materials Science and Engineering C 82 (2018) 372–383

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Review

In situ and *ex situ* modifications of bacterial cellulose for applications in tissue engineering

Taisa Regina Stumpf [a], Xiuying Yang [b], Jingchang Zhang [b,*], Xudong Cao [a,*]

[a] Department of Chemical and Biological Engineering, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada
[b] Hainan Institute of Science and Technology, 571126 Haikou, China

ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 4 October 2016
Accepted 27 November 2016
Available online 30 November 2016

Keywords:
Bacterial cellulose
In situ modification
Ex situ modification
Tissue engineering

ABSTRACT

Bacterial cellulose (BC) is secreted by a few strains of bacteria and consists of a cellulose nanofiber network with unique characteristics. Because of its excellent mechanical properties, outstanding biocompatibilities, and abilities to form porous structures, BC has been studied for a variety of applications in different fields, including the use as a biomaterial for scaffolds in tissue engineering. To extend its applications in tissue engineering, native BC is normally modified to enhance its properties. Generally, BC modifications can be made by either *in situ* modification during cell culture or *ex situ* modification of existing BC microfibers. In this review we will first provide a brief introduction of BC and its attributes; this will set the stage for in-depth and up-to-date discussions on modified BC. Finally, the review will focus on *in situ* and *ex situ* modifications of BC and its applications in tissue engineering, particularly in bone regeneration and wound dressing.

Contents

1. Introduction

Tissue engineering devices, principally those used as implantable scaffolds, are generally made from biomaterials that offer different structures and properties. To this end, many biomaterials – both synthetic and naturally occurring – have been used for tissue engineering (TE) applications, where additional modifications to the scaffold material such as anchoring of biologically active entities are usually required. Recently, naturally occurring materials such as cellulose, chitosan, hyaluronic acid, and collagen have attracted significant amount of interests as potential materials for applications in TE [1]. Among these materials, cellulose is the most widespread naturally occurring material on earth, with a biomass production estimated to be about 1.5×10^{12} tons annually [2]. It is an important structural component found in cell walls of green plants and some species of bacteria also produce cellulose (*i.e.* bacterial cellulose, BC) in the form of biofilm [2]. While it is necessary to employ different chemical treatments to obtain pure cellulose from plants [3], BC is readily produced in pure form and does not contain any other compound present in the plant pulp or from animal origin [4]. Bacterial cellulose was originally discovered in the 1880s by A. J. Brown [5,6]. The first documented BC applications in biomedical were reported by a Brazilian company in 1986, 1989, and 1990 in a series of patents (see patents WO 08602095, WO 08908148 and US 4912049) that discussed the applications of Biofill® in different

* Corresponding authors.
E-mail addresses: zhangjc1@mail.buct.edu.cn (J. Zhang), xcao@eng.uottawa.ca (X. Cao).

http://dx.doi.org/10.1016/j.msec.2016.11.121
0928-4931/

TE applications, such as skin substitutes for burns and ulcers [7]. In parallel Johnson & Johnson explored the use of BC as a liquid loaded pad for wound care in 1986 and 1987 (see patents US 4588400 and US 4655758). Subsequent studies investigated biocompatibilities of BC using L929 cells (mouse fibroblasts cells) [8] and in rats [9]. Recently more extensive studies on BC have been conducted, and its full potentials in TE applications have started to be gradually realized [10–12].

Unmodified BC has unique physical and mechanical properties not displayed by other biomaterials, such as high purity, ultrafine fibrous network structure with a variable pore geometry [13,14], high water holding capacity, absorbing over 100 times of its own weight in water [15–17], high crystallinity (*i.e.* 84–89%) [18], broad chemical and physical modifying ability [19,20], and the ability to mold into different structures [21]. Moreover, BC sheets and fibers have Young's modulus of 15–18 GPa and 78 GPa, respectively [22,23]. However, its potential as a biomaterial in TE applications has not yet been fully explored. As shown in Fig. 1, there is a significant difference between the number of annual publications when "bacterial cellulose" was used as a key word and those when "bacterial cellulose" + "tissue engineering" were used (data obtained from Web of Science in September 2016). A closer look at the publications of BC with TE applications reveals that the majority of these papers mainly deal with investigations to modify BC scaffold porosities, introducing functional groups and/or antimicrobial molecules, increasing BC degradation rates, and enhancing BC biocompatibilities. Therefore, this review will first discuss general properties of BC and will subsequently focus on recent progress in *in situ* and *ex situ* modifications of BC in TE applications.

2. Bacterial cellulose

Cellulose $(C_6H_{10}O_5)_n$ is a naturally occurring homopolysaccharide formed by a linear chain of monosaccharide β-D-glucose linked by $\beta(1 \rightarrow 4)$ bond. The repeating unit is cellobiose formed by the union of two glucose molecules (Fig. 2(A)). Bacterial cellulose is produced by a few strains of bacteria, such as *Agrobacterium*, *Alcaligenes*, *Pseudomonas*, *Rhizobium*, *Sarcina*, and *Gluconacetobacter* [4,24], and secreted outside of the cells (Fig. 2(B)). These cellulose chains subsequently form elementary sub-fibers, which are then linked together by hydrogen bonding to form microfibrils. Ultimately microfibrils are organized together to form ribbons [25]. Bacterial cellulose biosynthesis has been extensively documented by Ross et al., who presented an account on BC synthesis "machinery", genetics and its regulatory mechanism in a model organism *Acetobacter xylinum*, a non-pathogenic bacteria [26,27]. Recently, many other publications provided overviews of genetic modifications of cellulose producing bacteria, focusing mainly on BC production yields [25,28–30]; interested readers are referred to consult other excellent studies for examples of genetic engineering approaches to produce BC [31–36].

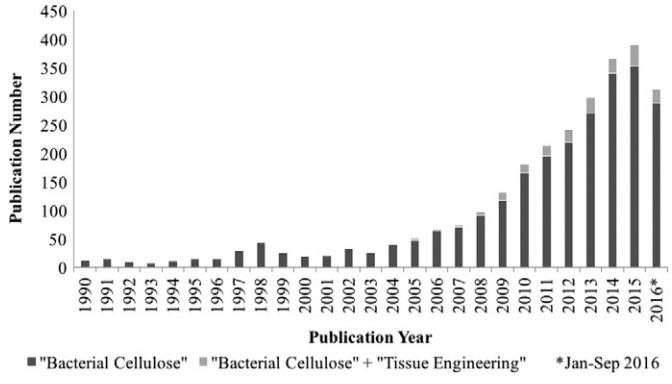

Fig. 1. Annual publications on BC and BC with TE applications since 1990 to September 2016. Search engine used Web of Science™, search term "bacterial cellulose" and "bacterial cellulose" with refined term "tissue engineering".

After BC synthesis, the membrane is further processed to remove bacterial cells, organic acids, salts as well as residual sugars and other components of the culture medium, which could be integrated within the cellulose network. This purification process can be accomplished by various methods, including washing, centrifugation, filtration, and chemical extraction. Among these methods, washing BC with hot and diluted sodium hydroxide solution, rinsing with distilled water, and finally sterilizing the BC by autoclaving is commonly used [9,37–39]. Fig. 2(C) shows a BC membrane obtained by washing with 0.1 M NaOH at 50 °C followed by extensive washing with distilled water. It should be noted that there are no reports in the literature concerning the presence of pathogen-associated molecular patterns in the purified BC; in fact, it will become clear later in this review that BC has been shown to be biocompatible. It is believed that bacteria produce cellulose biofilm in order to protect the organisms from ultraviolet radiation and other chemical or mechanical insults to the bacteria [26], as well as to improve nutrient transport [23]. Bacterial cellulose membrane is formed in a structure with asymmetrical layers at the air/liquid interface, resulting in a denser surface where it is in contact with air and a more gelatinous network on the other side where it is in contact with the liquid [9,40–43].

Bacterial cellulose can be produced in different shapes and molded into 3D structures during *in vitro* cultures, depending on the production method chosen (*i.e.* static culture, agitated culture or airlift reactor) [21,41,44,45]. In addition, the resulting BC properties, such as mechanical properties as well as micro- and macro-structures are also influenced by bacterial culture conditions [25,46–49]. An ideal biomaterial for TE applications must be capable to promote correct tissue regeneration; it must also provide a variety of shapes and sizes, promote cell–biomaterial interactions, and have microstructures suitable for new tissue formation [50]. Due to its structural similarities to extracellular matrix (ECM) components, BC has been reported to show good biocompatibilities. For example, using MTT assay and confocal imaging, Recouvreux et al. showed that human vein endothelial cells proliferated and migrated vertically into a BC hydrogel [21]. In addition, Zang et al. studied differentiation of human adipose-derived mesenchymal stem cells (HASCs) cultured on BC, and showed that the HASCs were successfully differentiated into osteoblasts and formed a consistent layer of osteoblasts on the BC [51]. Moreover, BC scaffolds seeded with the HASCs were implanted into ulna defects of rabbits, and significant mineralization was observed in the defects after 8 weeks when compared with the control group; the researchers also noted that there were no signs of any inflammation responses [51].

Furthermore, BC has been used in many other *in vivo* studies. In a meticulously designed study, Helenius et al. subcutaneously implanted BC membranes on the back of Wistar rats, and subsequently evaluated host responses to the implanted BC material in terms of foreign body reactions, chronic inflammation, angiogenesis, and cell growth for a period of 12 weeks. The study showed that BC was fully integrated with the host tissue and did not induce any inflammation or rejection during the course of the study [52]. In another study, BC tubes were shown to exhibit good biocompatibility when they were cultured with primary Schwann cells and were subsequently implanted in a sciatic nerve injury model in Sprague Dawley rats for 6 weeks [53]. Moreover, to evaluate their potentials as substitutes for small-diameter blood vessels, the BC tubes were used to replace carotid arteries in Texel sheep for a period of 12 weeks. It was observed that a confluent endothelial cell layer without any signs of inflammation was formed along the BC tubes and that vascular smooth muscle cells migrated into the BC tube matrix [54]. Similar results were also observed in a study by Kowalska-Ludwicka et al. who used BC tubes for peripheral nerve regenerations for a period of 6 months [55]. Table 1 summarizes findings described above. Indeed, many studies have demonstrated that BC can be well integrated with host tissues for many different TE applications, as can be seen in Table 2.