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Leading Opinion

Microbial cellulose—the natural power to heal wounds ☆

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Abstract

Microbial cellulose (MC) synthesized in abundance by *Acetobacter xylinum* shows vast potential as a novel wound healing system. The high mechanical strength and remarkable physical properties result from the unique nanostructure of the never-dried membrane. This article attempts to briefly summarize the recent developments and applications of MC in the emerging field of novel wound dressings and skin substitutes. It considers the properties of the synthesized material, its clinical performance, as well as progress in the commercialization of MC for wound care products. Efficient and inexpensive fermentation techniques, not presently available, will be necessary to produce large quantities of the polymer.

Keywords: Microbial cellulose; *Acetobacter*; Wound healing; Wound dressing

1. Introduction

Recent advances in the field of biomaterials and their medical applications indicate the significance and potential of various microbial polysaccharides in the development of novel classes of medical materials. Several of the microbially-derived polysaccharides possessing novel and interesting physical and biological properties already have been applied in biotechnology products or are presently being widely investigated (i.e. hyaluronic acid, dextran, alginate, scleroglucan). Among them, microbial cellulose (MC), a polymer synthesized in abundance by *Acetobacter xylinum*, belongs to the most promising class of biopolymers, despite the fact that its potential of becoming a high-value product of biotechnology has not yet been fully estimated or discovered [1]. The unique physical and mechanical properties of MC as well as its purity and uniformity determine applications that range from high-quality audio membranes [2] and electronic paper [3] to fuel cells [4] and medical materials [5–7]. This last, emerging area seems to be particularly important since many efforts have been devoted in recent years to explore new skin substitutes and modern wound dressing materials using tissue engineering approaches. Various polymeric materials recently have been investigated for wound dressing application yielding many successful outcomes, but the search for an ideal skin-graft substitute with properties and functionality similar to human skin is still continuing. We believe that MC, while chemically the same as plant cellulose, displays novel physical properties determined by the particular genetics of the organism. In such a case, MC has a distinctive nanofibrillar structure that may become a perfect matrix as an optimal wound healing environment.

☆ *Editor's Note*: Leading Opinions: This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

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2. Biosynthesis, structure and properties of MC

A. xylinum is a simple Gram-negative bacterium which has an ability to synthesize a large quantity of high-quality cellulose organized as twisting ribbons of microfibrillar bundles [8]. During the process of actual biosynthesis, various carbon compounds of the nutrition medium are utilized by the bacteria, then polymerized into single, linear β-1,4-glucan chains and finally secreted outside the cells through a linear row of pores located on their outer membrane. The subsequent assembly of the β-1,4-glucan chains outside of the cell is a precise, hierarchical process. Initially, they form subfibrils (consisting of 10–15 nascent β-1,4-glucan chains), then later microfibrils, and finally bundles of microfibrils consisting of a loosely wound ribbon, which is comprised of about 1000 individual glucan chains [9]. The thick, gelatinous membrane formed in static culture conditions as a result of these processes is characterized by a 3-D structure consisting of an ultrafine network of cellulose nanofibres (3–8 nm) which are highly uniaxially oriented [10]. Such a 3-D structure, not found in vascular plant cellulose, results in high cellulose crystallinity (60–80%) and an enormous mechanical strength. Particularly impressive is the fact that the size of MC fibrils is about 100 times smaller than that of plant cellulose (Fig. 1). This unique nano-morphology results in a large surface area that can hold a large amount of water (up to 200 times of its dry mass) and at the same time displays great elasticity, high wet strength, and conformability. The small size of MC fibrils seems to be a key factor that determines its remarkable performance as a wound healing system. Furthermore, the never-dried cellulose membrane is a highly nano-porous material that allows for the potential transfer of antibiotics or other medicines into the wound, while at the same time serving as an efficient physical barrier against any external infection. The cellulose produced in the form of a gelatinous membrane can be molded into any shape and size during its synthesis, depending on the fermentation technique and conditions used [1]. Unlike celluloses of plant origin, MC is entirely free of lignin and hemicelluloses. A vigorous treatment with strong bases at high temperatures allows the removal of cells embedded in the cellulose net, and it is possible to achieve a non-pyrogenic, non-toxic, and fully biocompatible biomaterial (Fig. 2) [6,7,11].

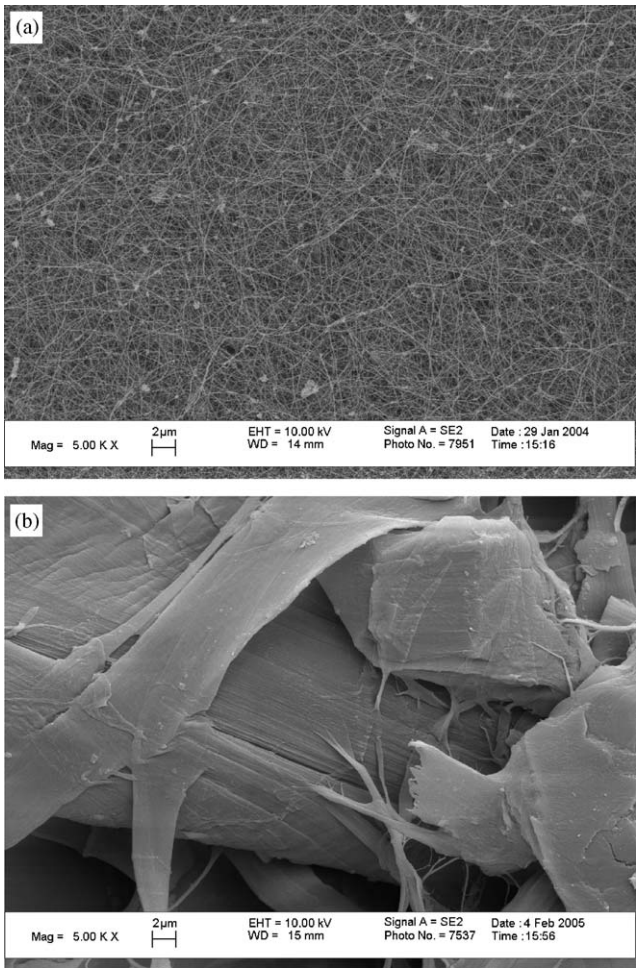

Fig. 1. A comparison of microfibrillar organization between *Acetobacter* cellulose (a) and wood pulp (b) (both at 5000x). Ultrafine net of microbial cellulose left has a very smooth network of microfibrils. Newsprint from wood pulp right has similar microfibrils, but they are part of a larger aggregation of the cell wall remains (*SEM images courtesy of Dwight Romanovicz, University of Texas at Austin*).

Fig. 2. The never-dried microbial cellulose membrane is a non-pyrogenic and fully biocompatible biomaterial with high mechanical strength.

3. Brief overview of the commercialization potential of MC for wound care products

The first efforts to commercialize MC on a large scale were initiated by Johnson & Johnson in the early 1980s. This company pioneered in exploratory investigations on the medical application of MC in the treatment of

different types of wounds [12,13]. However, no data of any clinical trials involving the use of MC as a wound dressing has ever been published, according to our knowledge. The Johnson & Johnson Company did not launch any commercial product out of their inventions, most probably due to the problems with the development of an efficient, large-scale fermentation system. This critical factor of efficiency in the production technology still seems to play a major role for any potential company wanting to commercialize MC for any type of product. A Brazilian company, BioFill Produtos Bioetecnologicos (Curitiba, PR Brazil) independently investigated the unique properties of cellulose biopolymer and created a new wound healing system based on MC produced by *Acetobacter* [5,14,15]. Their line of products includes the following: Biofill® and Bioprocess® (used in the therapy of burns, ulcers as temporary artificial skin), and Gengiflex® (applied in treatment of periodontal diseases) [16]. According to manufacturers, differences in the technology of manufacturing involve: variable initial concentrations of carbon sources, surface/volume (S/V) ratios, and extended times of fermentation. Biofill® is produced within 2 days, whereas Gengiflex®, which is much thicker, requires 8 days of fermentation [16]. US patent No. 4,912,049 protects the company's technology of cellulose film preparation.

Microbial cellulose commercial production was intensively investigated in the 1990s by several, major Japanese companies and national governmental organizations which set up interdisciplinary research programs with major aims to develop efficient mass production techniques. The venture was called Biopolymer Research Co., Ltd and was supported by the Japan Key Technology Center, a joint organization under Ministry of International Trade and Industry (MITI) and the Ministry of Post and Telecommunications, together with six private companies: Ajinomoto, Shimazu Construction, Nikki, Mitsubishi Paper, Nikkiso and Nakamori Vinegar [17]. The well-funded project (approximately $45 million) resulted in several patents and publications, but to our knowledge, it has not yet succeeded commercially, with the exception of audio speakers from MC by Sony [2].

Also in the 1990's, intensive fundamental and applied studies on cellulose biosynthesis were undertaken at the Technical University of Lodz in Poland. The governmental support by the Ministry of Scientific Research and Information Technology endorsed the production of different types of MC wound dressings. In addition, this led to the initiation of clinical trials on humans, and these are still successfully ongoing [7]. Furthermore, this research has produced a particularly efficient strain of *Acetobacter* [18], which grows on an inexpensive, relatively simple nutrient medium. These advances promise fermentation scale up with better possibilities for commercialization. In addition, intellectual property is being secured for uses of MC in wound care [19].

The general failure of a large-scale commercialization effort for MC seems to have been mainly caused by the lack of an efficient fermentation system. Unlike other microbial polysaccharides that can be synthesized economically in large stirred-tank fermenters, MC must be grown in static, non-agitated cultures. Sheer stresses in agitated fermenters always damage the cellulose during synthesis. Another scale-up approach has been based on a combination of stationary and agitated culture and takes place in horizontal fermenters where optimal conditions for media supply and cell attachment to the surface of rotating discs or roller are created [20,21].

In 1996, another company, Xylos, a US-based corporation, negotiated exclusive licensing agreements with Johnson & Johnson to use their patents on cellulose-based wound-care products. Since that time, the company developed its own improved manufacturing technology and was able to successfully obtain FDA approval on its products. The XCell® family of wound-care products offered by the company includes XCell® Cellulose Wound Dressing and XCell® Antimicrobial Wound Dressing that have been marketed in the US since 2003 [22]. In its strategy, Xylos Co. is first concentrating on the rapidly growing field of chronic wound care. According to the manufacturer, XCell® is specifically engineered and characterized by a dual-functionality of hydration and absorption to maintain the ideal moisture balance required for the good healing process [22].

Besides the efforts to commercialize MC for strictly medical purposes, two US-based companies: Cetus Co. (Emeryville, California) and Weyerhaeuser Co. (Tacoma, Washington) used a deep tank fermentation technique and a patented, genetically improved *Acetobacter* strain that was able to synthesize cellulose in agitated culture, to create a product called Cellulon® with application as a food stabilizer and thickener [23]. In the mid-1990's, Kelco, Inc. (US) purchased the MC business from Weyerhaeuser, and launched the product as PrimaCel® aimed to food industry.

To our knowledge there are no other existing companies manufacturing and marketing MC or MC-based wound dressings. However, there are many more R&D units making continuous and rapid progress in the field of MC biosynthesis and application. We feel certain that more players will soon join this multi billion-dollar market.

4. MC as a wound healing system

Healing of skin wounds is a complex process which requires the involvement of many different tissues, cell

Table 1
Characteristics of the modern wound care dressing material

Non-toxic, non-pyrogenic, biocompatible
Able to provide barrier against infection
Able to control fluid loss
Able to reduce pain during treatment
Able to create and maintain a moist environment in the wound
Provide easy and close wound coverage
Enable introduction or transfer of medicines into the wound
Able to absorb exudates during inflammatory phase
Display high mechanical strength, elasticity and conformability
Display an optional shape and surface area
Allow for easy and painless release from the wound

types and matrix components [24,25]. There are three major directions in which wound-healing research is aimed presently [25]: (a) improvement of wound healing by elements which may potentially accelerate healing and reduce scarring, (b) development of novel skin substitutes as equivalents of autograft skin, and (c) identification of signals that trigger the process of healing by regeneration rather than repair (scar formation). The present status of modern wound healing systems generally requires that materials used for the wound cover should create an optimal environment for epidermal regeneration by providing a barrier against wound infection and fluid loss. Many different biological and synthetic wound dressings have been developed in order to treat surgical and non-surgical lesions [25–30]. Some of these have been quite successful in wound closure, however a search for the ideal wound dressing material is still continuing. According to modern approaches in the field of wound healing, an ideal wound dressing system must display similarity to autograft skin, both structurally and functionally [25,31]. Table 1 shows the set of requirements to be fulfilled by a modern, successful wound care dressing material.

5. Clinical performance of MC wound dressing

There have been several publications and reports on the successful use of MC as a medical product. In 1990, Fontana et al. [5] first reported the application of cellulose pellicles of varying thickness, produced by *Acetobacter*, as temporary skin substitutes. The product, called Biofill®, has been used for several skin injury treatments such as basal cell carcinoma/skin graft, severe body burns, facial peeling, sutures, dermabrasions, skin lesions, chronic ulcers, and both donor and receptor sites in skin grafts [5]. According to Farah, the thickness of the film was adjusted using the following variables: concentration of carbon and nitrogen sources in the culture medium, temperature, and fermentation time. The final product of biosynthesis was dehydrated while stretched [14].

Chronic wounds such as venous leg ulcers, bedsores, and diabetic ulcers are difficult to heal, and they represent a significant clinical challenge both to the patients and to the health care professionals. The treatment of chronic wounds involves the application of various materials (hydrocolloids, hydrogels, biological or synthetic membranes) that provide a moist wound-healing environment that is necessary for optimal healing [32]. Wound dressings play an important role in the entire management of these types of wounds, and recent reports on applications of MC dressings in the treatment of chronic wounds suggest that it displays properties superior to other existing wound-healing materials. Mayall et al. [33] used a Biofill® skin substitute in the treatment of trophic ulcerations of the limbs and showed that this material was very effective by shortening the cicatrisation time, reducing contamination, and the cost of treatment. According to Farah [14], the film applied on the lesion region with a loss of epithelial tissue acts as a new skin, eliminating pain symptoms (by isolating the nerve ending) and enhancing absorption of wound exudates. According to Fontana et al. [5] advantages of using Biofill® as a biological dressing have been confirmed in more than 300 treatments. The authors mentioned the following advantages: immediate pain relief, good and close adhesion to the wound bed, good barrier against infection, easiness of wound inspection, faster healing, improved exudates retention or reduced time of treatment, as well as reduced costs [5]. Rebello et al. [34] described the use of Biofill® in the treatment of skin transplants sides (both donor and receptors) and reported that cicatrisation occurred upon 11 days of treatment. In clinical and histopathological studies by Wouk et al. [35], a comparison of different skin promoters was performed on animal models. Using criteria of healing quality and adhesion to the wound, they found Biofill® dressing to be the most effective among the four other tested.

The osmotic-diffusive properties of Bioprocess®, a MC wound dressing, were analyzed by Slezak et al. [36]. They measured values of the following coefficients: hydraulic permeability, reflection, and diffusive permeability and showed that the cellulose membrane is characterized by a low selectivity and is easy permeable for water and other solutions (aqueous solution of glucose, sucrose, ethanol, NaCl, KCl). The authors stated that the material might be used in the therapy of scalds and ulceration. In the report by Kucharzewski et al. [37], two methods of treating non-healing venous leg ulcers were compared. The experimental group of patients was treated with MC wound dressing (Bioprocess®), whereas the control group was treated with Unna's boot hydrocolloid dressing, which is widely used

in the therapy of these types of wounds. In the clinical procedure, MC dressing with a thickness of 0.05 mm was applied on the clean wound with gauze pads placed on the top of the dressing. The limb was then bandaged, and sodium chloride solution poured over the bandage a few times a day in order to keep it moist all the time [37]. The membrane was changed every 7 days until the wound was completely healed. The results showed that 15 out of 27 patients of the experimental group treated with MC were completely healed of ulcerations after 8 weeks of treatment, whereas only 4 out of 27 patients from the control group showed completely healed wounds after the same treatment times. The remaining 12 patients from the experimental group were healed within the next 6 weeks, whereas the process of healing for patients from the control group was completed after 20 weeks. Based on the results of these clinical studies, the authors concluded that MC wound dressing was more effective in the treatment of the chronic venous leg ulcers than Unna's boot [37].

It has been generally shown that a combination of occlusive, moist wound dressings and compression bandages create the proper environment for painless autolytic debridement, improved development of granulation tissue, and accelerated re-epithelization [6]. In one of the most recent articles, Alvarez et al. [6], reported the use of MC in the form of a hydrated membrane (Xcell®, Xylos Co.) in the treatment of chronic venous ulcers. In clinical trials based on 24 patients, MC was more effective than a standard protocol (non-adherent cellulose acetate gauze) in the process of autolytic debridment. According to the authors, MC created a protective, moist environment, very similar to a natural undisturbed wound protected by blister. Unlike MC dressings manufactured by Biofill Co., the Xcell® product is claimed to have an ability to simultaneously donate and absorb moisture from the wound based on the fact that it conforms to wounded and intact skin differently [6]. According to the authors of these studies, the balance of moisture absorbance and delivery can be easily regulated by the secondary dressing, which might either shift the whole system to absorb exudates (i.e. any absorbent material) or to deliver moisture (i.e. polyurethane film dressings). According to recent studies by Aung [38], the dressing was easy to apply and kept on the wound for 7 days without changes. This researcher suggested that such procedures might allow private practice clinicians to offer wound care services within their own practice. According to Aung [38], the use of Xcell® may facilitate an easier dressing change and reduce the amount of material necessary for wound healing as well as reduce the frequency of dressing changes. All of these lead to the highly desired overall cost reduction, however in this particular case of treatment with Xcell® product, there is always a need for a secondary dressing to be used [38].

The most traumatic and complex of all skin injuries are caused by burns, and this results in an extensive damage to the various skin layers [25]. Burns are generally defined according to depth and range from 1st degree (superficial) to 3rd degree (entire destruction of epidermis and dermis). The standard protocol of burn management highlights several factors which accelerate the process of optimal healing [39,40]: (a) control of fluid loss, (b) barrier to wound infection, (c) fast and effective wound closure, optimally with skin grafts or skin substitutes, and (d) significant pain relief.

In the studies by Czaja et al. [7], a new wound healing system based on MC was clinically investigated in Poland on humans for the treatment of large area 2° A/B skin burns. The wound healing effects of never-dried MC membranes and conventional gauze wound dressings as controls were compared in this research. These studies were proceeded by in vivo tests conducted on animal models which showed that MC membranes were fully biocompatible and also successfully protected burn wounds from excessive external fluid loss, thus accelerating the entire process of healing [11].

The great conformability of this cellulose material has been proven during clinical trials on large number of patients [7,41]. The MC dressing adhered to the wound sites very well, and its elastic properties allowed an excellent molding to all facial contours, displaying a high degree of adherence even to the moving parts (such as eyelids, nose, mouth, etc.) (Fig. 3). Due to the problems with use of occlusive dressings on facial burns, the open technique using topical antibiotics is still the standard procedure. In the studies by Czaja et al. [7], a complete closure of the wounded face with a single sheet of MC (original size 40×60 cm) has been achieved. In comparison, most of the commercially available skin

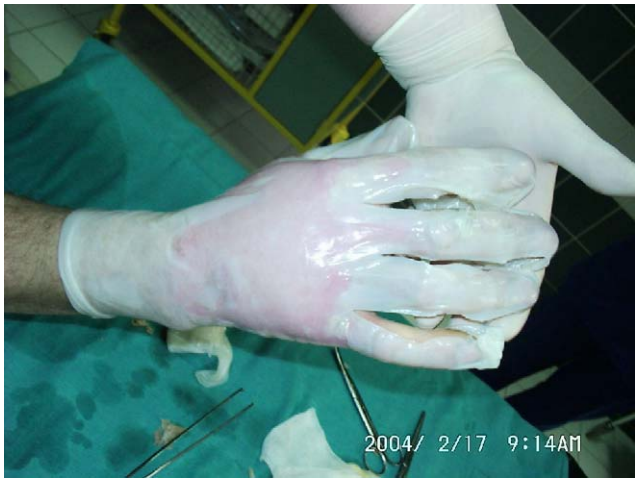

Fig. 3. Microbial cellulose dressing applied on a wounded hand. The unique physical properties of microbial cellulose allow an excellent molding, displaying a high degree of adherence even to the moving parts (*image courtesy of Center of Burn Healing, Siemianowice Slaskie, Poland*).

substitutes usually are too small, thus two or three sheets attached to each other with staples must be normally applied. The applied, never-dried cellulose membrane allowed both: (a) maintenance of a proper moist environment around the wound, and (b) due to its highly nano-porous structure, absorbance of the wound's exudates [7,41].

Another interesting and important advantage of the MC dressing includes its transparency, which allows for continuous clinical observation of the healing progress. Generally, the studies showed that MC membranes significantly facilitated the process of necrotic debris removal (autolytic debridement), improved the development of granulation tissue, and accelerated the entire process of re-epithelialization, in comparison with the control group of patients [7]. A significant decrease in daily wound care needs, degree of pain, and the overall time of healing were observed in the treatments with MC dressings in comparison with the control procedures.

The question on why MC works so well has not been fully answered yet. At this moment in vitro research experiments are in progress and we hope they will provide us with an indication of the potential mechanisms of action. We definitely think that due to the unique 3-D nanostructure, MC membranes virtually replicate the wound surface at the nano-scale level and create optimal moist conditions for wound healing and skin regeneration. MC, either as commercial (Xcell®, Biofill®, Bioprocess®) or non-commercial products, used so far in the clinical studies on animals and humans was synthesized by different *Acetobacter* strains, and the final product had the form of either a dehydrated or a never-dried membrane. The results of all of these studies so far suggest that there is a great potential in using this type of cellulose as a wound healing system. However, in our opinion, there remain several variables that influence the overall production process and performance of the final biomaterial. We think that the origin of MC (particular *Acetobacter* strains), its detailed structural characteristic, as well as the fermentation technique used may have a strong impact on the performance of the final product. It is a well-known fact that celluloses produced by different *Acetobacter* strains display some significant structural differences regarding crystallinity index, I_α/I_β mass fractions ratio, or microfibril size [10,42]. The quality of MC strongly depends on the *Acetobacter* strain used in the production process (different conversion ratios of glucose-to-cellulose, rate of cellulose extrusion from the cell, problems with spontaneous mutation), culture techniques and conditions employed. Also post-fermentation operations on the raw materials (the degree of dehydration, physical squeezing, treatment with bases) can strongly influence the final efficacy of the material. In our opinion, the most important factors affecting the overall product performance and production cost-effectiveness are: (a) efficient strains which do not undergo mutations over the time, (b) nutrition media based on inexpensive sources of carbon and nitrogen, and (c) efficient, large-scale fermentation processes. The final price of the biomaterial will be strongly dependent on these variables.

6. Perspectives

Down through the centuries, 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. Knowing what we presently understand about the biosynthetic process, it is possible to envision genetic modification of cellulose producing microbe strains to customize particular products that would greatly benefit from a particular physical form. For instance, the *shape* of MC can be determined by the shape of the fermentation vessel. Thus, if molded non-woven cellulose products are required, they can be synthesized according to the shape of the mold. If the pore structure of never-dried MC is to be custom synthesized, then new strains and fermentation conditions can easily be matched to produce such materials. The degree of polymerization, the crystallization, and the size and shape of the microfibrils and microfibrillar aggregates could all be controlled genetically.

The most significant problem looming on the horizon is the development of large-scale efficient fermentation as discussed above. Because of the inherent novel properties of *Acetobacter* cellulose, it is clear that new fermentation processes based on low shear processing must be developed. This is not an engineering problem akin to the development of the space shuttle! Rather, it is within the grasp of well-educated engineers and scientists who understand the fundamental principles of *Acetobacter* cellulose biosynthesis. If we are to have a vision for the future of novel products of Nature's biosynthetic machines, certainly cellulose is a product that has the capacity to be greatly improved with prolonged benefits to humankind.

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