CHAPTER 17

BIOMEDICAL APPLICATIONS OF MICROBIAL CELLULOSE IN BURN WOUND RECOVERY

WOJCIECH CZAJA[1]*, ALINA KRYSTYNOWICZ[1], MAREK KAWECKI[2], KRZYSZTOF WYSOTA[2], STANISŁAW SAKIEL[2], PIOTR WRÓBLEWSKI[2], JUSTYNA GLIK[2], MARIUSZ NOWAK[2], AND STANISŁAW BIELECKI[1]

[1]*Institute of Technical Biochemistry, Technical University of Lodz, Stefanowskiego 4/10, Lodz, Poland;*
[2]*Center of Burn Healing, Jana Pawla II 2, Siemianowice Śląskie, Poland*

Abstract

Microbial cellulose (MC) is a very versatile biomaterial. Even though it has already been successfully deployed in such diverse scientific endeavors as electronics, acoustics, and fuel cells, it is particularly well suited for the creation of unique biomedical devices which can significantly improve the healing process. Because of the increased interest in tissue engineered products for the regeneration of damaged or diseased organs, microbial cellulose may become an essential material for a diverse array of medical treatments. Microbial cellulose from *Acetobacter xylinum* recently has been shown to be very beneficial in the treatment of superficial second degree and deep dermal second degree burns. In a clinical study performed on 34 patients, the MC wound dressing materials were directly applied on fresh burns covering up to 9–18% of the body surface. The following analyses were considered during the trials: macroscopic observations of the wound and wound exudates, epidermal growth, microbiological tests, and histopathological studies. The wounds are all very well isolated from the outside environment by application of the MC membranes. 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. In shallow wounds, MC dressing promoted growth of the epidermis and in deep wounds shortened the period of scab demarcation. Factors for this success include but are not limited to the following: (a) a moist environment for tissue regeneration; (b) significant pain reduction; (c) the specific microbial cellulose nano-morphology which appears to promote cell interaction and, tissue re-growth; (d) significant reduction of scar formation; and, (e) easy and safe release of wound care materials from the burn site during treatment. Microbial Cellulose promises to have many new applications in wound

* For correspondence: Tel: (+4842)6313442; Fax (+4842)6366618; e-mail: czajawoj@mail.p.lodz.pl

R.M. Brown, Jr. and I.M. Saxena (eds.), Cellulose: Molecular and Structural Biology, 307–321.

care that extend beyond burn applications including, but not limited to, the following: surgical wounds, bedsores, ulcers, tissue, and organ engineering.

Keywords

Acetobacter xylinum, bacterial cellulose, burns, microbial cellulose, wound dressing, wound healing.

1 INTRODUCTION

Burns of the skin are complicated injuries often resulting in extensive damage to the skin layers. Burns are classified according to depth and identified by three degrees (Latarjet 1995): first-degree burn – usually superficial, affects only the outer layer of skin (epidermis); second-degree burn – either superficial, with damage to the epidermis layer of the skin (second-degree A), or deep when penetrating into the dermis (second-degree B); third-degree burn – a total destruction of all the epidermis and dermis, extending into subcutaneous tissue (skin grafting is recommended). The major goal during treatment of burn patients is to quickly accomplish effective wound closure to increase the rate of healing and significantly reduce pain (Demling and DeSanti 1999; Jones et al. 2002; Prasanna et al. 2004).

The process of healing of burn tissues involves both regeneration of the epidermis and repair of the dermis resulting in formation of scar tissue (Balasubramani et al. 2001). There are several key factors regarding a present standard procedure of burn wound management in order to enhance healing while minimizing or preventing scarring (Latarjet 1995; Gallin and Hepperle 1998): (1) prevention of excessive loss of fluids from wounds (which increases up to 70 times in comparison with normal skin); (2) prevention of wound infection; and, (3) fast and effective closure of the wound, optimally with skin graft or other skin substitutes. The general design of an effective burn procedure relies on its immediate change into the surgical wound which is accomplished by the fast excision of necrotic tissues and closure of the wound with skin substitutes which are either split-thickness autografts (these would be optimal, due to immunological issues), allografts (these are considered as biological wound dressings and cannot replace autogenic skin), or xenografts (Balasubramani et al. 2001).

There is still a need for development of wound care dressing material, which could sufficiently protect wounds against infection or excessive loss of fluid. It is a well-known fact that limitation of these processes significantly reduces metabolic effects associated with burn injury and generally facilitates and accelerates the entire process of the burn treatment. Burns are always associated with serious metabolic and immunological disorders commonly called burn sickness. There are three major phases occurring during burn injury: the shock phase (up to 48 h after the burn event); the catabolic phase (up to several weeks) when a process of wound cleansing from necrotic tissues takes place; and, the anabolic phase, which occurs when the actual process of healing and tissue regeneration takes place. The shorter the period of the catabolic phase, the better are the chances to rescue patients with large area skin burns.

Due to recent advances in the field of biomedical materials, scientists have developed a variety of natural and synthetic polymers which can be used for wound

closure, drug delivery systems, novel vascular grafts, or as scaffolds for the creation of tissue engineered constructs. With respect to wound closure and wound care, many different biological and synthetic wound dressings already have been developed and introduced in the market, and many are still in the development phase. The criteria for an ideal wound care dressing material require it to display the structural and functional characteristics of autograft skin (Quinn et al. 1985; Balasubramani et al. 2001). The present status of a modern, successful wound care dressing material indicates several requisite characteristics: absence of toxicity, creation of a moist environment in the wound, high mechanical strength, elasticity, control of fluid loss, significant reduction of pain during treatment, and absorption of secretions from the wound. Additionally, material should be easy to store and ready for immediate usage, provide easy, close wound coverage, allow for painless removal from the wound, should display an optional shape and surface area, and it should enable the introduction or transfer of medicines into the wound (Quinn et al. 1985; Wu et al. 1995; Ruiz-Cardona et al. 1996; Delatte et al. 2001; Walker et al. 2003; Park et al. 2004).

In our experience, we have clinically investigated a novel biomaterial which meets the requirements for an ideal wound dressing system. Microbial cellulose (MC), which is synthesized and secreted by the GRAM negative bacterium, *Acetobacter xylinum*, displays unique physical, chemical, and mechanical properties including a high crystallinity (70–90%), a high water holding capacity (up to 200 times of its dry mass), a well-developed surface area comprised of nanofibers (3–8 nm), elasticity, mechanical strength (a Young's modulus of ~30 GPa), and biocompatibility (Ross et al. 1991; Brown, Jr. 1996; Watanabe et al. 1998; Bielecki et al. 2002; Krystynowicz et al. 2002; Czaja et al. 2004). Due to its unique nanostructure and remarkable properties, MC has been of great interest for many medical applications including artificial blood vessels, scaffolding for tissue engineering of cartilage, and a wound dressing material for chronic wounds (Ring et al. 1986; Fontana et al. 1990; Klemm et al. 2001; Alvarez et al. 2004; Svensson et al. 2005).

In our study, we describe the use of a modern wound dressing material synthesized by *A. xylinum*. This product, MC, is a new system for wound healing. The objective of our research was to clinically compare the wound healing effects of never-dried MC membranes with conventional cotton gauze wound dressings. We wanted to investigate its applicability as a new wound care dressing material in the treatment of large area, partial thickness skin burns (second-degree A/B).

2 EXPERIMENTAL DESIGN

2.1 Never-dried MC membranes preparation

MC produced by the vinegar fermentation bacterium *A. xylinum* is synthesized in the form of twisting ribbons, which in stationary culture forms a thick, gelatinous membrane on the surface of a liquid medium. The membrane formed in such conditions is characterized by the 3D structure made of an ultrafine network of

cellulose nano and microfibrils. This particular structure of the never-dried MC pellicle determines the remarkable and unique physical and mechanical properties of the wound dressing material which can hold a large amount of liquid and displays high mechanical strength (Table 17-1) *A. xylinum* E_{25} from the collection of Institute of Technical Biochemistry, Technical University of Lodz, Poland, was used in this study.

It is a typical GRAM-negative rod that is an obligate aerobe and it produces a thick membrane of pure cellulose at the gas/liquid interface of a static culture (Hestrin and Schramm 1954). Schramm–Hestrin medium (SH medium) (Hestrin and Schramm 1954) enriched with 1% (v/v) ethanol, with a pH adjustment to 5.7 was used in all experiments unless otherwise specified. The cells for the inoculum were grown in liquid SH medium in 250 ml Erlenmeyer flasks either statically or on a rotary shaker, for 2 days at 30°C. The thick gelatinous membrane that formed in the flask after 2 days was squeezed aseptically to remove the cells embedded inside it. The cell suspension was then transferred as the inoculum for the primary culture. The primary cultures were grown statically in plastic stacking trays of different sizes for 7 days at 30°C. The synthesized cellulose was harvested from the medium and washed with 2% sodium hydroxide (overnight) followed by several changes of distilled water in order to remove cells and any residual culture medium embedded in the cellulose material. The final product, in the form of a transparent, wet membrane, was packaged, sealed, and sterilized by γ-radiation. MC wound dressings used in the clinical study had the following sizes: 18 × 25 cm, 40 × 60 cm.

2.2 Clinical trials

Controlled, randomized clinical trials were conducted on 34 patients. The experimental group of individuals who received the MC consisted of 22 patients in the testing group (5 females with an average age of 49, and 17 males with an average age of 35). The control group consisted of 12 patients. All patients, both from testing and control groups, suffered from severe thermal burns (second-degree A/B) covering 9–18% of the total body surface area (TBSA). The research study protocol was approved by the Bioethical Committee of the Medical University of Silesia (Katowice, Poland) prior to the commencement of the study. Written consent of all patients was taken after presenting them appropriate information about the research project. Patients who were included in the study were between 18 and 70 years of age, had second-degree A/B burns covering 9–18% TBSA, did not have diabetes,

Table 17-1. Physical and mechanical characteristics of the MC wound dressing

Thickness (mm)	Crystallinity degree (%)	Young's modulus (GPa)	Water holding capacity (g water/g cellulose)	Cellulose content in the dressing (%)	Relative total deformation index (%)	Tensile strength (MPa)
3	65	2.7	79	0.6	30.1	92

collagenosis, uraemia, mental disease, cancer, immunosupression, or any blood transfusions for a year prior to the beginning of the clinical study, nor did they have any cytostatic or radiant energy treatments. Patients who did not sign the written consent form, did not understand the objectives and methods of the study, were pregnant, or who incurred serious side effects during treatment were excluded from the study.

After cleansing and disinfecting the wound, MC dressing materials were directly applied on fresh burns (second-degree A/B) closely covering up to 9–18% of the body surface of the experimental patients, and then covered with gauze wraps containing a 3% Braunol (B. Braun Medical) solution. The control group was treated with a standard procedure using gauze wound dressings with a 3% Braunol solution, silver sulfadiazine, povdone-iodine, or flammacerium ointment. The normal wound treatment period for both groups of patients (testing and control) was 10 days. The Parkland formula of fluid replacement with crystalloids was applied to all patients on the first day of hospitalization: body weight (kilograms) × the body surface area burned (percentage) × 4, yielding the intravenous crystaloid volume (in milliliters) to be administered over 24 h. One-half of this volume was given during the first 8 h of the burn treatment, followed by administering the remaining half of the volume over the next 16 h. Starting from the second day of hospitalization, the loss of fluid was determined using the hematocrit value, which is the proportion, by volume, of the blood that consists of red blood cells and is expressed as a percentage by volume.

During clinical evaluations the following diagnoses were considered: (a) macroscopic observations of the wound; (b) epidermal growth; (c) microbiological tests of blood (on the tenth day of the treatment); (d) wound exudates (on the first, fifth, and tenth day of the treatment); (e) urine (on the first and tenth day of the treatment); and, histopathological studies (on the first and tenth day of the treatment). Antibiotics were not used in any cases during the studies. Color photographs of the wounds were taken on the first, fifth, and tenth day of the treatment. Tissue specimens from experimental patients and control patients were obtained for examination by excising the wound during periodic changes of the MC dressing. Tissue specimens were fixed in 10% formalin solution, dehydrated and embedded in paraffin wax. Sections were mounted on plain glass slides and stained with hematoxylin and eosin in a conventional manner. Observations were carried out using bright field light microscopy.

3 CLINICAL OUTCOMES

3.1 High conformability, moisture donation, and faster healing

The medical tests conducted *in vivo* on the rat animal model showed that MC membranes successfully protected the surface of large-area skin burns from an excessive external fluid loss and significantly accelerated the process of healing

(Krystynowicz et al. 2000). These early promising results allowed permission from the Bioethical Committee to perform the clinical trials on humans.

During clinical trials, the MC wound dressing was applied on the fresh wound under sterile conditions. Because of its high conformability, the adherence of MC membrane to the wound surface was excellent (Figure 17-1). In all clinical cases, the dressings adhered very well to the wound sites during treatment, thus avoiding any dead spaces. In order to enhance the patient's mobility, MC dressings were additionally covered with gauze wraps, which helped to keep them in place. The large size of the cellulose membranes employed in this study (e.g., 40 × 60 cm) displayed a full adherence to the variable surfaces throughout the wound. This seemed to be very helpful in accelerating the healing of large area wounds. The applied, never-dried cellulose membrane allowed both: (a) maintenance of a proper moist environment around the wound and (b) due to its highly porous structure, absorbance of the wound's exudates. None of the patients treated during the trial developed any kind of hypersensitive reactions to the applied MC wound dressing.

In the general procedure used during our clinical trials, the MC dressing remained in contact with the wound for about 24 h or until it had become "almost dry". The dressing was then replaced with a new one. Due to the moisture still present in the never-dried cellulose structure, the release of the dressing from the wound was an entirely painless operation. However, in the beginning phase of our study, a single MC dressing was kept on the wound for more than 24 h, even up to 5 days, with or without its periodic rewetting by 0.9% NaCl. Later on, the completely or partly dried membrane was rewetted in order to achieve a painless removal from the wound (Figure 17-2b). An interesting preliminary observation was made during the extended treatment of burns with dry MC membranes. We observed that under completely dried MC dressings (that

Figure 17-1. Application of a large size MC dressing on a second-degree burn (*See Color Plate of this figure beginning on page 355*)

Figure 17-2. A second-degree A/B burn of both forearms. MC dressing applied on the wound (**a**); MC dressing dried on the wound in the second day of the treatment; left hand has been treated with the control technique (**b**); dry MC dressing removed from the wound after 4 days of treatment revealed a clean area with a fully regenerated epidermis underneath; left forearm treated with control procedure displayed presence of necrotic tissues (**c**); wound after 3 weeks upon burning shows a complete re-epithelialization on the healed right forearm whereas on the left forearm granulation tissues have just been formed (**d**) (*See Color Plate of this figure beginning on page 355*)

were not changed for up to 5 days), the process of healing was accompanied by the entire removal of necrotic tissues and wound exudates (Figure 17-2c). On the other hand, the negative effects of such a treatment included a painful dressing release from the wound and slight damage to the fresh epidermis or even deeper parts of the skin caused by the dry dressing removal. In several cases, the effect of wound squeezing generated by the continuously rewetted MC membrane has been found to be very helpful in the reduction of swelling.

The patient shown in Figure 17-2 suffered from second-degree A/B burn of the both forearms caused by exposure to flames. During clinical studies, the patient's right arm was treated with MC wound dressing and the patient's left arm (the control), with treatment consisting of moist gauze and ointment. In this particular case, the MC membrane was not changed daily, so the process of healing was carried out both in moist (for the first 18 h) and dry (during next 3 days) environments. Despite this fact, an entirely regenerated epidermis was revealed upon removal of the MC dressing from the wound after 4 days of treatment

(Figure 17-2c). At the same time, the left, control forearm still displayed the presence of the necrotic tissue, which finally started to separate from the wound during the next 2 days. Three weeks after burning, the right forearm was completely healed, whereas the left, control forearm had just initiated the formation of red granulation tissues (Figure 17-2d). This particular case shows that even under unfavorable, dry conditions during the longer periods of treatment, the MC provided rapid wound healing without the necessity for frequent dressing changes. More research is needed to elucidate the specific interactions between skin cells and the unique MC nanostructure in order to draw any more conclusions about the treatment with dry MC dressing.

Table 17-2 summarizes the healing process in the testing group of patients, treated with MC dressing and in the control group, treated with the standard technique over the period of 10 days. Analysis of these data clearly shows that the specific moist environment created in the wound upon application of MC membrane promotes its rapid self-cleaning. A significant decrease in the presence of necrotic tissue at the bottom of the wound was observed for patients treated with MC dressing. The data from the table shows that at the fifth day of the treatment, the presence of necrotic tissue at the bottom of the wound was clearly observed only in 3 patients from the testing group (a decrease of 75%) in comparison with 8 patients (a decrease of 33%) continuously displaying necrosis in the control group. Consequently, the formation of pink and red granular tissue in the wound took place much earlier in the group of patients treated with MC dressing than in the control group. Finally, it was proven that by the tenth day of the treatment period, the process of reepithelialization had begun in 7 patients from the testing group (58.3%) in comparison with 4 patients (33.3%) from the control group. These results show that the application of MC burn dressing in the treatment of partial thickness burns promotes the creation of a favorable

Table 17-2. The progress (A–E) of wound healing in the testing (treated with microbial cellulose dressings) and control group over the period of 10 days. The data points are number of patients and percentage of the group that fit in the particular progress category

		A	B	C	D	E
Day of observation	Group of patients	Necrotic tissue at the bottom of the wound	Bottom of the wound covered with pus or wound exudate	Pink granular tissue	Red granular tissue	Epidermis growth
1	Testing	12(100%)	–	–	–	–
	Control	12(100%)	–	–	–	–
5	Testing	3 (25%)	5 (41.7%)	4 (33.3%)	–	–
	Control	8 (66.7%)	–	1 (8.3%)	1 (8.3%)	2 (16.7%)
10	Testing	–	2 (16.7%)	3 (25%)	–	7 (58.3%)
	Control	–	6 (50%)	2 (16.7%)	–	4 (33.3%)

environment for fast wound cleansing, and consequently its rapid healing. No clinical signs of wound infections were noted at any time during treatment. For all patients included in the trials, there were generally no significant differences in the incidence of positive bacterial cultures from the MC and control dressing-treated wounds at days 1, 5, and 10 of treatment. Swabs typically grew organisms including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumani*.

3.2 MC is particularly useful in the treatment of facial burns

It is a well-known fact that deep, large facial burns are difficult to heal and often require hospital care (Demling and DeSanti 1999). Facial burn injuries very often result in cosmetic disfigurement. Two factors, which play a key role in minimizing disfigurements are: (1) a fast and timely wound closure; and (2) application of pressure to maturing facial scars (Manigandan and Dhanaraj 2004). Due to its unique nanostructure the MC dressing can hold a large amount of liquid, and this makes it quite heavy, but at the same time it is still a highly conformable material. When applied on the face in the form of a mask (Figure 17-3b) the MC dressing initiates a positive pressure on the maturing scars, thus preventing extensive collagen overproduction. In deep burns, pressure therapy to limit collagen formation and reduce scarring normally is recommended as soon as healing allows (Roques 2002). By applying MC dressing on fresh burns, this positive effect of pressure can be achieved from the beginning of the healing process. Figure 17-3, images a–f show a patient with the severe deep second-degree burns of the facial surface caused by exposure to flame. In this particular case, closure of the entire face was achieved with a single sheet of MC membrane in which the holes for eyes, nose, and mouth were made after placement. By comparison, most of the commercially available, standard skin dressings and substitutes usually are too small to cover the entire facial surface, so two or three sheets attached to each other with staples must be normally applied. Shortly after application of the moist dressing on the wounded face, the swelling gradually decreased, and the feeling of pressure on the facial surface had subsided. Seventeen days after the burns, the process of wound healing had significantly progressed (Figure 17-3c). The epithelialization in the regions of wound edges and from the deep epidermal appendages was clearly observed at that time. After 44 days, the wounded face was entirely healed with no need for skin grafting and no significant signs of extensive scarring (Figure 17-3d). The same patient was examined for scar tissue formation after about 20 months post burning. The patient displayed several shallow, nonovergrowing (nonhypertrophic) scar tissues on the facial surface where a third-degree burn occurred (Figure 17-3e). Additionally, the lack of tissue fragments on the right ear was also observed (Figure 17-3f).

One of the positive effects of treatment with MC dressing was a significant reduction of scar tissue formation on the facial surface affected by partial and

Figure 17-3. A deep second-degree facial burn caused by the exposure to flame (**a**); a highly conformable mask of the MC dressing with holes on eyes, nose, and mouth was tightly applied on the wounded face (**b**); the epithelialization in the regions of wound edges and from the deep epidermal appendages has been clearly observed at 17 days upon burning (**c**); the entirely healed face after next 28 days (**d**); examination after about 20 months upon burning showed the shallow, nonhypertrophic scar tissues on the facial surface where third-degree burn occurred (*front*) (**e**); and the lack of tissue fragments on the right ear (**f**) (*See Color Plate of this figure beginning on page 355*)

deep thickness burns. Another positive effect was the character of the remaining scars, which were not overgrown and did not cause any contractions, especially in the region of eyelids. Based on our observations, we can conclude that MC dressing appreciably improved the management and healing rate for deep

second-degree facial burns compared to a standard technique with moist gauze dressing and ointment.

A significant decrease of pain has been observed throughout the treatments with MC material. After daily patient interviews, there were only two cases in which patients terminated treatment with the MC dressing, explaining that they could not withstand the psychological effect of wearing the mask on their wounded faces.

To date, the standard procedure of care for facial burns remains that of an open technique, using topical antibiotics (Hartford 1997; Demling and DeSanti 1999). This is mostly due to the difficulty of using occlusive dressings or skin substitutes on facial burns (Hartford 1997; Demling and DeSanti 1999). Application of the MC dressing, which can be easily formed in the shape of a face mask, could entirely overcome those obstacles. Its physical properties allow excellent molding to all facial contours, displaying a high degree of conformability even to moving facial parts. The histological appearance of the typical wound tissue in Figure 17-4 clearly shows how tightly the MC membrane adhered to the surface of the wound during the overall process of healing.

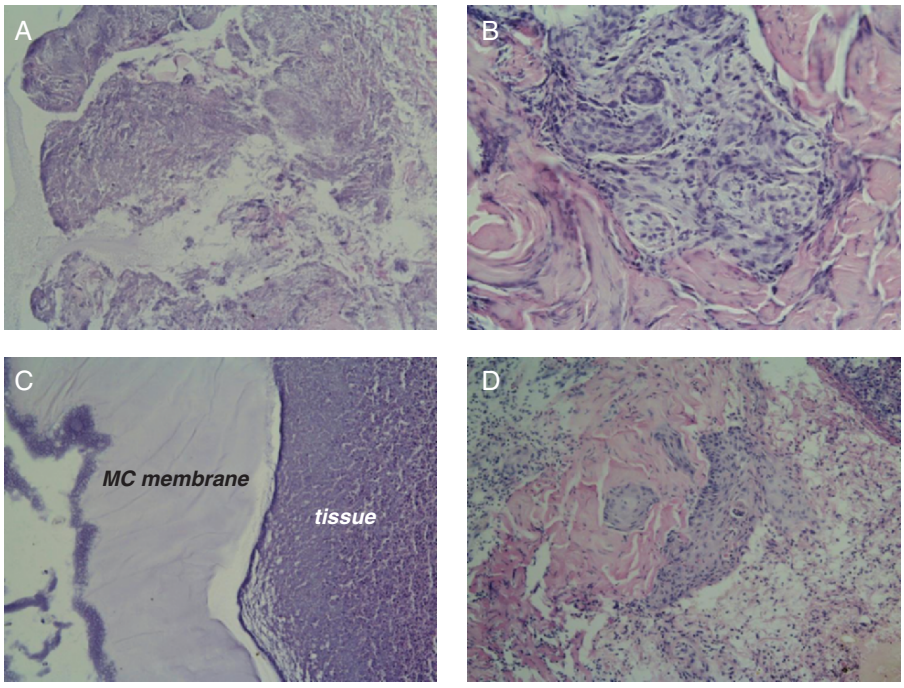

Figure 17-4. Photomicrographs of a biopsy specimen from a wound treated with MC dressing. (**a**) Necrosis of wound tissues; (**b**) growth of granulation tissue and keratinocytes from the appendages of skin (fifth day of treatment with MC dressing); (**c**) fragment of MC dressing tightly adhered to the wound tissue (fifth day of treatment with MC dressing); and (**d**) a fresh epidermis growing in the wound after 10 days of treatment with MC dressing (*See Color Plate of this figure beginning on page 355*)

A similar situation can be observed during application of the MC dressing on the burn wounds of ears. Most ear burns cause a partial loss of some parts of the ear cartilage, and consequently reconstruction is required (Manigandan and Dhanaraj 2004). Generally, the process of ear healing faces many difficulties during the treatment due to the weak blood supply of ears and a shallow position of the cartilage. Another difficulty is the anatomical shape of the ear, which does not allow for easy coverage. Application of MC membrane, which easily and tightly adheres to the ear's contours, creates a specific close contact of a moist layer that entirely isolates the wound from the external environment (data not shown). Another interesting and important advantage of the MC dressing includes its transparency, which allows for continuous clinical observation of the healing progress.

The unique 3D nanostructure of MC seems to be a key factor, which determines its usefulness as a wound dressing material (Figure 17-5). A specific occlusive, moist environment created upon close contact application of the MC membrane significantly facilitated the process of necrotic debris removal (autolytic debridement) in comparison with the control group of patients. In addition, this environment improved the development of granulation tissue and accelerated the entire process of re-epithelialization. It also created an optimal healing environment by maintaining a moisture layer for new cell migration and growth. A significant decrease in daily wound care needs, degree of pain, and the overall time of healing was observed in the treatment with MC dressing in comparison with the control procedures. Many other studies have shown that wound healing of partial-thickness burns can be successfully obtained with appropriate dressing material. Promising results have been obtained using different membranous, occlusive materials like Allevyn, Aquacel, OrCel, Biobrane-L, TransCyte, Integra, and other materials (Demling and DeSanti 1999; Loss et al. 2000; Innes et al. 2001; Vloemans et al. 2001; Still et al. 2003). All of the above dressings provided a moist healing environment, which was associated with a short healing time. Generally, the transparent films and colloids which resulted in a smooth

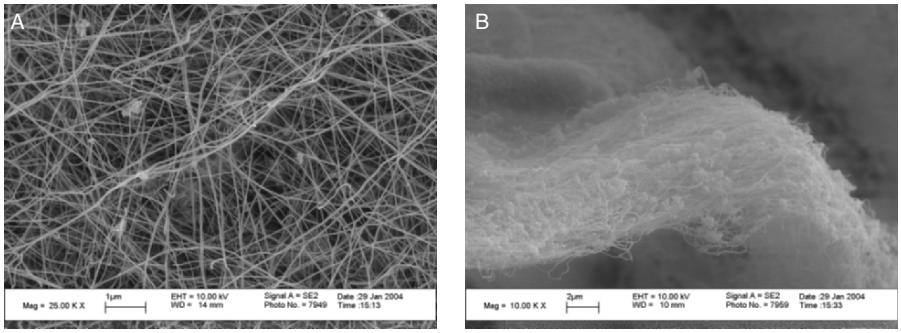

Figure 17-5. Ultrafine net of microbial cellulose (**a**) has a very smooth network of micro- and nanofibrils and (**b**) the cross-section of MC membrane with multilayer architecture

epithelial surface and lowest donor site pain have been shown to be the closest to meet the ideal conditions for fast and stable healing (Innes et al. 2001). The ideal dressing, which possesses all of the qualities of the wound dressing discussed in the introductory chapter of this report, has not yet been developed. However, we believe that the MC dressing, which fulfills all these requirements has the potential to become an ideal wound dressing, particularly for treatment of partial-thickness burns.

4 CONCLUSIONS

In conclusion, our results have shown that wounds are very well isolated from the outside environment by application of the MC membranes. Due to its unique 3D nanostructure, the MC membrane can virtually replicate the wound surface at the nanoscale level and create optimal moist conditions for wound healing and skin regeneration. During the clinical trials, MC dressings were very well tolerated by patients, significantly reducing pain during treatment and allowing for painless removal of the dressing from the wound. In shallow wounds, MC dressing promoted growth of the epidermis and in deep wounds shortened the period of scab demarcation. In our opinion, treatment with MC dressings should be continued until an entirely new epidermis appears, otherwise a second necrosis can take place. The MC dressing needs to be changed every day or rewetted to maintain the desired moist environment. Drying of the dressing on the wound causes the effect of squeezing (pressing). The results to date suggest that MC dressing might be effective in reducing scar formation. In our opinion, cellulose dressings should be applied to fresh wounds immediately after burn injury. Considering this and the capability to produce MC dressing of different sizes and shapes, we think that, in addition to hospitals and ambulatories, MC should be widely used in all emergency responding units such as police, emergency, firemen, army, etc. The unique features of the MC have been demonstrated to be effective in the burn wound healing response.

Acknowledgments

This work has been financially supported by the Grants No. 4 TO9B 056 24 and PBZ-MIN-007/PO4/2003 from the Polish State Committee for Scientific Research. The authors are grateful to Professor R.M. Brown, Jr. and D. Nobles from the Section of Molecular Genetics and Microbiology, University of Texas at Austin, USA for helpful discussions during preparation of this manuscript.

REFERENCES

Alvarez O.M., Patel M., Booker J., and Markowitz L. 2004. Effectiveness of biocellulose wound dressing for the treatment of chronic venous leg ulcers: results of a single center randomized study involving 24 patients. Wounds 16:224–233.

Balasubramani M., Kumar T.R., and Babu M. 2001. Skin substitutes: a review. Burns 27:534–544.

Bielecki S., Krystynowicz A., Turkiewicz M., and Kalinowska H. 2002. Bacterial cellulose. In: Steinbuchel A. (ed.) Biopolymers: Vol. 5. Polysaccharides I. Wiley-VCH Verlag GmbH, Munster, Germany, pp. 37–90.

Brown, Jr. R.M. 1996. The biosynthesis of cellulose. Pure Appl Chem 10:1345–1373.

Czaja W., Romanovicz D., and Brown, Jr. R.M. 2004. Structural investigations of microbial cellulose produced in stationary and agitated culture. Cellulose 11:403–411.

Delatte S.J., Evans J., Hebra A., Adamson W., Othersen H.B., and Tagge E.P. 2001. Effectiveness of beta-glucan collagen for treatment of partial-thickness burns in children. J Pediatr Surg 36:113–118.

Demling R.H. and DeSanti L. 1999. Management of partial thickness facial burns (comparison of topical antibiotics and bio-engineered skin substitutes). Burns 25:256–261.

Fontana J.D., de Sousa A.M., Fontana C.K., Torriani I.L., Moreschi J.C., Gallotti B.J., de Sousa S.J., Narcisco G.P., Bichara J.A., and Farah L.F. 1990. *Acetobacter* cellulose pellicle as a temporary skin substitute. Appl Biochem Biotechnol 24/25:253–264.

Gallin W.J. and Hepperle B. 1998. Burn healing in organ cultures of embryonic chicken skin: a model system. Burns 24:613–620.

Hartford C.F. 1997. Care of outpatient burns. In: Herndon D. (ed.) Total Burn Care. Saunders, Philadelphia, p. 71.

Hestrin S. and Schramm M. 1954. Synthesis of cellulose by *Acetobacter xylinum*: II. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. Biochem J 58:345–352.

Innes M.E., Umraw N., Fish J.S., Gomez M., and Cartotto R.C. 2001. The use of silver coated dressings on donor site wounds: a prospective, controlled matched pair study. Burns 27:621–627.

Jones I., Currie L., and Martin R. 2002. A guide to biological skin substitutes. Br J Plast Surg 55:185–193.

Klemm D., Schumann D., Udhardt U., and Marsch S. 2001. Bacterial synthesized cellulose – artificial blood vessels for microsurgery. Progr Polym Sci 26:1561–1603.

Krystynowicz A., Czaja W., Pomorski L., Kołodziejczyk M., and Bielecki S. 2000. The evaluation of usefulness of microbial cellulose as wound dressing material. 14th Forum for Applied Biotechnology, Proceedings Part I, Meded Fac Landbouwwet-Rijksuniv Gent, Gent, Belgium, pp. 213–220.

Krystynowicz A., Czaja W., Wiktorowska-Jezierska A., Gonçalves-Mi kiewicz M., Turkiewicz M., and Bielecki S. 2002. Factors affecting the yield and properties of bacterial cellulose. J Ind Microbiol Biotechnol 29:189–195.

Latarjet J. 1995. A simple guide to burn treatment. Burns 21:221–225.

Loss M., Wedler V., Künzi W., Meuli-Simmen C., and Meyer V.E. 2000. Artificial skin, split-thickness autograft and cultured autologous keratinocytes combined to treat a severe burn injury of 93% of TBSA. Burns 26:644–652.

Manigandan C. and Dhanaraj P. 2004. An innovative, cost-effective, pressure-relieving device for burned ears. Burns 30:269–271.

Park S.N., Kim J.K., and Suh H. 2004. Evaluation of antibiotic-loaded collagen-hyaluronic acid matrix as a skin substitute. Biomaterials 25:3689–3698.

Prasanna M., Mishra P., and Thomas C. 2004. Delayed primary closure of the burn wounds. Burns 30:169–175.

Quinn K.J., Courtney J.M., Evans J.H., Gaylor J.D.S., and Reid W.H. 1985. Principles of burn dressings. Biomaterials 6:369–377.

Ring D., Nashed W., and Dow T. 1986. Liquid loaded pad for medical applications. US Patent No. 4588400.

Roques C. 2002. Pressure therapy to treat burn scars. Wound Repair Regen 10:122–125.

Ross P., Mayer R., and Benziman M. 1991. Cellulose biosynthesis and function in bacteria. Microbiol Rev 55:35–58.

Ruiz-Cardona L., Sanzgiri Y.D., Benedetti L.M., Stella V.J., and Topp E.M. 1996. Application of benzyl hyaluronate membranes as potential wound dressings: evaluation of water vapour and gas permeabilities. Biomaterials 17:1639–1643.

Still J., Glat P., Silverstein P., Griswold J., and Mozingo D. 2003. The use of a collagen sponge/living cell composite material to treat donor sites in burn patients. Burns 29:837–841.

Svensson A., Nicklasson E., Harrah T., Panilaitis B., Kaplan D.L., Brittberg M., and Gatenholm P. 2005. Bacterial cellulose as a potential scaffold for tissue engineering of cartilage. Biomaterials 26:419–431.

Vloemans A.F.P.M., Soesman A.M., Kreis R.W., and Middelkoop E. 2001. A newly developed hydrofibre dressing, in the treatment of partial-thickness burns. Burns 27:167–173.

Walker M., Hobot J.A., Newman G.R., and Bowler P.G. 2003. Scanning electron microscopic examination of bacterial immobilization in a carboxymethyl cellulose (AQUACEL) and alginate dressings. Biomaterials 24:883–890.

Watanabe K., Tabuchi M., Morinaga Y., and Yoshinaga F. 1998. Structural features and properties of bacterial cellulose produced in agitated culture. Cellulose 5:187–200.

Wu P., Fisher A.C., Foo P.P., Queen D., and Gaylor J.D.S. 1995. *In vitro* assessment of water vapour transmission of synthetic wound dressings. Biomaterials 16:171–175.