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Reviews

The Future Prospects of Microbial Cellulose in Biomedical Applications

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Microbial cellulose has proven to be a remarkably versatile biomaterial and can be used in wide variety of applied scientific endeavors, such as paper products, electronics, acoustics, and biomedical devices. In fact, biomedical devices recently have gained a significant amount of attention because of an increased interest in tissue-engineered products for both wound care and the regeneration of damaged or diseased organs. Due to its unique nanostructure and properties, microbial cellulose is a natural candidate for numerous medical and tissue-engineered applications. For example, a microbial cellulose membrane has been successfully used as a wound-healing device for severely damaged skin and as a small-diameter blood vessel replacement. The nonwoven ribbons of microbial cellulose microfibrils closely resemble the structure of native extracellular matrices, suggesting that it could function as a scaffold for the production of many tissue-engineered constructs. In addition, microbial cellulose membranes, having a unique nanostructure, could have many other uses in wound healing and regenerative medicine, such as guided tissue regeneration (GTR), periodontal treatments, or as a replacement for dura mater (a membrane that surrounds brain tissue). In effect, microbial cellulose could function as a scaffold material for the regeneration of a wide variety of tissues, showing that it could eventually become an excellent platform technology for medicine. If microbial cellulose can be successfully mass produced, it will eventually become a vital biomaterial and will be used in the creation of a wide variety of medical devices and consumer products.

Introduction

Rapid progress has been made in recent years in the field of biomedical materials, which utilize both natural and synthetic polymers and which can be used in a variety of applications, including wound closure, drug delivery systems, novel vascular grafts, or scaffolds for in vitro or in vivo tissue engineering. Several microbially derived polysaccharides (i.e., hyaluronic acid, dextran, alginate, scleroglucan) have interesting physical and biological properties and are particularly useful in various biomedical applications. Microbial cellulose (MC), a polysaccharide synthesized in abundance by *Acetobacter xylinum*, has already been used quite successfully in wound-healing applications, proving that it could become a high-value product in the field of biotechnology.[1−3]

Traditional plant-originated cellulose and cellulose-based materials, usually in the form of woven cotton gauze dressings, have been used in medical applications for many years and are mainly utilized to stop bleeding. Even though this conventional dressing is not ideal, its use continues to be widespread. These cotton gauzes, consisting of an oxidized form of regenerated plant cellulose, were developed by Frantz during World War II, and have been successfully used as a hemostatic agent as well as an adhesion barrier.[4−8] Another product, a plant cellulose sponge, has an established clinical application in wound-healing research as a component which stimulates granulation tissue in the wound bed after injury.[9] In addition, several studies described the implantation of regenerated cellulose hydrogels and revealed their biocompatibility with connective tissue formation and long-term stability.[9,10] Other in vitro studies showed that regenerated cellulose hydrogels promote bone cell attachment and proliferation and are very promising materials for orthopedic applications.[10−13]

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Table 1. Properties of Microbial Cellulose Membranes and How They Relate to the Properties of an Ideal Wound Dressing Material[a]

properties of ideal wound care dressing	properties of microbial cellulose
maintain a moist environment at the wound/dressing surface	high water holding capacity (typical membrane can hold up to 200 g of its dry mass in water); 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. Physical processing of the membrane (i.e., squeezing) can remove part of the initial water and allow the membrane to be more absorbable
sterile, easy to use, and inexpensive	membranes are easy to sterilize (by steam or γ-radiation) and package. The estimated cost of production of 1 cm^2 is \$0.02
available in various shapes and sizes	ability to be molded in situ
provide easy and close wound coverage, but allow easy and painless removal	high elasticity and conformability
significantly reduce pain during treatment	the unique MC nanomorphology of never-dried membrane promotes specific interaction with nerve endings
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 [Young's modulus value of several GPa]

[a] Refs 90−97.

Although chemically identical to plant cellulose, the cellulose synthesized by *Acetobacter* is characterized by a unique fibrillar nanostructure which determines its extraordinary physical and mechanical properties, characteristics which are quite promising for modern medicine and biomedical research. In this review, the structural features of microbial cellulose and its properties are discussed in relation to the current and future status of its application in medicine.

The Significant Biomedical Potential of Microbial Cellulose Stems from Its Unique Structure and Properties

Cellulose synthesis by *Acetobacter* is a complex process and involves (A) the polymerization of single glucose residues into linear β-1,4-glucan chains, (B) the extracellular secretion of these linear chains, and (C) the assembly and crystallization of the glucan chains into hierarchically composed ribbons.[14] As a result of these processes, a three-dimensional, gelatinous structure is formed on the surface of a liquid medium. The physical and mechanical properties of microbial cellulose membranes arise from their unique structure, which differs significantly from the structure of plant cellulose. Basically, well-separated nano- and microfibrils of microbial cellulose create an extensive surface area which allows it to hold a large amount of water while maintaining a high degree of conformability. The hydrogen bonds between these fibrillar units stabilize the whole structure and give it a great deal of mechanical strength.[15−17] Even though plant cellulose is composed of microfibrils which are similar to those found within microbial cellulose, the plant cellulose microfibrils are part of a larger aggregation of the cell wall. Thus, microbial cellulose can absorb much higher volumes of liquids than plant-derived cellulose materials. On the basis of its recent clinical performance and according to the results of other research on the properties of this particular biomaterial, MC can be considered an ideal material for high-quality wound dressings. Table 1 summarizes most of the physical and mechanical properties of microbial cellulose which characterize it as an ideal wound dressing material. Interestingly, many *Acetobacter* strains display significant differences in the cellulose production process (i.e., the rate of cellulose ribbon extrusion from a single cell may significantly vary between strains), as well as in the structure of the synthesized polymer. Figure 1 presents SEM images of cellulose structures synthesized by two different strains of *Acetobacter*. The differences in the size of the cellulose ribbons can be clearly seen. From a bioengineering point of view, these structural differences are of great importance since they can be used to create hybrid materials with desired properties consisting

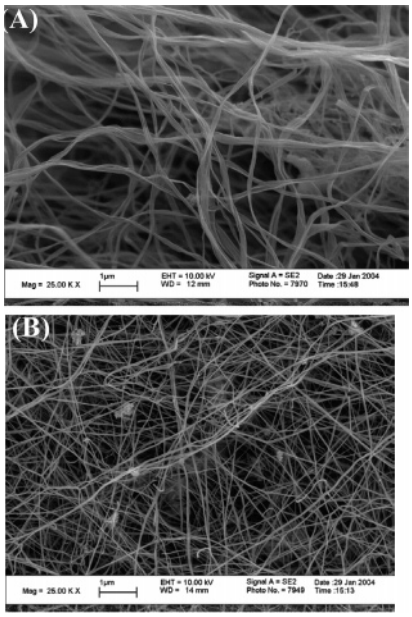

Figure 1. Structure of cellulose produced by two different *Acetobacter* strains clearly indicate differences. (A) NQ5, (B) E_{25}; much larger cellulose ribbons of NQ5 are clearly distinguishable. Whereas the NQ5 strain creates a highly compact and rigid membrane, the E_{25} strain produces a more gelatinous, yet still rigid form of cellulose, which is highly translucent (images captured by Dwight Romanovicz, University of Texas at Austin).

of cellulose products synthesized by different *Acetobacter* strains.

The given medical application should dictate the choice of the particular cellulose structure (specific *Acetobacter* strain). For example, implantable cellulose for artificial skin should ideally display high porosity, with interconnected pores of 50−150 μm, in order to facilitate skin cell integration into the cellulose scaffold, whereas temporary wound dressings should have a nanoporous structure and should keep the wound moist during the healing process.[18,19]

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. A material composed of porous plant cellulose has been shown to be biocompatible with bone tissue and hepatocytes.[9,20] Research conducted on an implanted cellulose sponge showed that it can be regarded as a slowly degradable material.[9] As mentioned by the same authors, this material can be considered nondegradable if used as a temporary wound coverage for a short period of time.[9] Unlike plant-originated cellulose, microbial cellulose is free of lignin and hemicelluloses. However, microbial cellulose is treated with strong bases in order to completely remove bacterial cells embedded in the polymer net.[3,21] There are several in vivo biocompatibility studies that used MC on animal models. For example, Kolodziejczyk and Pomorski implanted pieces of microbial cellulose (1 cm in diameter) into subcutaneous pockets on rabbits and periodically examined them after 1 and 3 weeks.[22] The implants did not cause any macroscopic inflammatory responses, and histological observations showed only a small number of giant cells and a thin layer of fibroblasts at the interface between the cellulose and the tissue.[22] Positive results were also obtained by Oster et al. in an in vitro study using mouse fibroblasts cells.[23] A specific in vivo biocompatibility study of microbial cellulose has also been conducted by Klemm et al., who implanted cellulose in the form of a hollow tube as an interposition segment of the carotid arteries of rats.[24] In a recent, very systematic study by Helenius et al., pieces of microbial cellulose were implanted into rats.[25] Those implants evaluated after 1, 4, and 12 weeks showed no macroscopic or histologic signs of inflammation and no presence of giant cells. Also, according to the authors, no chronic inflammatory responses were observed throughout the course of the studies.[25] Instead, they observed the formation of new blood vessels around and inside the implanted cellulose.[25] Interestingly, the authors also noticed that cells, mostly fibroblasts, were able to significantly penetrate the more porous bottom side of a microbial cellulose membrane. The newly formed tissue, integrated with MC, contained fibroblasts and newly synthesized collagen.

Microbial Cellulose as a Wound-Healing System: Temporary Wound Coverage

Microbial Cellulose in the Treatment of Chronic Wounds and Burns. Wound healing is a dynamic process that involves the complex interaction of various cell types, extracellular matrix (ECM) molecules, and soluble compounds.[26] Typically, normal wound healing progresses through a series of processes including homeostasis, inflammation, granulation tissue formation, and remodeling.[26] Chronic wounds, such as ulcers, do not heal because one or more of these processes fail to function properly. Thus, successful wound treatments improve the tissue repair process by counteracting the inherent abnormalities of the chronic wound. Once the barriers to normal tissue repair are removed, the healing process can begin, which involves autolytic debridement, granulation tissue formation, and re-epithelization.[26]

In order to eliminate the hostile environment within the chronic wound and to facilitate proper healing, wound dressings of various types have been developed and administered. For example, ulcers are typically treated with dressings such as hydrogels, hydrocolloids, synthetic and biological membranes, and alginate.[27] In 1962, George Winter discovered that healing, and specifically re-epithialization, was accelerated if the wound was kept moist.[28] Since then, almost all effective wound dressings are designed to maintain a moist environment within the affected region. In fact, proteolytic activity may be elevated in a moist environment, resulting in the stimulation and accumulation of growth factors.[29] Moist dressings are permeable to water, and this property has advantages for wound healing. For example, high water vapor permeable dressings show enhanced healing, probably due to an increased concentration of growth-promoting factors within the exudate and to the creation of a more extensive ECM of fibrin(ogen) and fibronectin.[30]

Burns are very complex injuries, causing extensive damage to skin tissues. The healing process involves the regeneration of the epidermis and the repair of the dermis, both of which result in the formation of scar tissue.[31] One of the major goals of burn therapy is to quickly accomplish effective wound closure so as to increase the rate of healing and to provide immediate pain relief.[32−34] In addition, proper wound management must prohibit the wound from becoming infected and dehydrated.[35,36] Despite the fact that many different biological and synthetic wound dressings have already been developed, the search for an ideal wound dressing is still in progress. According to the modern approaches in the field of wound healing, an ideal wound dressing system must be structurally and functionally similar to autograft skin.[31,37]

Because of its unique properties, microbial cellulose (MC) has been shown to be an highly effective wound dressing material. In fact, the results of various studies indicate that topical applications of MC membranes improve the healing process of burns and chronic wounds. The progress in this field has been discussed in a recent publication.[1] In addition, a recent study conducted in Poland used never-dried MC membranes in order to treat patients with severe second-degree burns.[38] This study showed that the skin of the patients whose burns were covered with never-dried MC membranes healed faster (faster re-epithelialization) than the wounds of patients who received a conventional wound dressing (such as wet gauze and ointments).[38] The Polish study also found that MC membranes actually performed better than conventional wound dressings in (1) conforming to the wound surface (excellent molding to all facial contours and a high degree of adherence even to the contoured parts such as nose, mouth, etc. were observed), (2) maintaining a moist environment within the wound, (3) significantly reducing pain, (4) accelerating re-epithelialization and the formation of granulation tissue, and (5) reducing scar formation.[38,39] These MC membranes can be created in any shape and size, which is beneficial for the treatment of large and difficult to cover areas of the body (Figure 2).

In studies conducted by Fontana et al. and Mayall et al. a microbial cellulose product called Biofill proved to be a very successful wound covering for skin problems such as burns and chronic ulcers.[2,40] In these studies, Biofill was shown to be more effective than other wound dressing materials in (1) providing pain relief, (2) protecting the wound against infection, (3) accelerating the healing process, and (4) reducing the cost of

Figure 2. A never-dried microbial cellulose membrane shows remarkable conformability to the various body contours, maintains a moist environment, and significantly reduces pain (images courtesy of the Center of Burn Healing, Siemianowice Slaskie, Poland and Professor Stanislaw Bielecki of the Institute of Technical Biochemistry, Technical University of Lodz, Poland).

treatment. Biofill was also shown to be more effective than other skin treatments in studies by Rebello et al. and Wouk et al.[41,42] It is important to note that in all of these studies the Biofill product is actually a partially dried MC membrane.

Another microbial cellulose product called XCell, which is manufactured by Xylos Corporation, was used in a study conducted by Alvarez et al.[3] In this study, the never-dried MC XCell dressing was used to treat patients suffering from chronic venous ulcers. Once again, the MC wound dressing proved to be more effective than conventional wound dressing materials in treating these chronic skin abnormalities. The authors of the Alvarez study concluded that MC was very effective in (1) promoting autolytic debridement, (2) reducing pain, and (3) accelerating granulation, all of which are important for proper wound healing. According to Frankel et al., unlike many other commercially available wound dressing materials, the XCell membrane is the only one that can simultaneously donate and absorb moisture from the wound, which is particularly important for wounds with a large volume of exudates.[43] However, according to Aung, the XCell product requires secondary dressings to maintain the proper moisture balance within the wound.[44]

Augmentation of Microbial Cellulose During and After Synthesis. Normally, *A. xylinum* cellulose synthesized in the form of organized, twisting ribbons, is a highly crystalline I_α-rich cellulose.[45] However, it is known that the cellulose crystallization process can be interrupted by addition of fluorescent brightening agents or cellulose derivatives to the media, which interact with nascent cellulose.[46−49] The structure of cellulose composites formed by the addition of different cell-wall polysaccharides and reagents, like gluco- and galactomannans, xyloglucan, and pectin, were recently investigated using X-ray diffraction, ^{13}C CP/MAS NMR, and electron microscopy techniques.[50−54] Structural interactions between those polysaccharides have been studied, and some interesting properties of such composites were found, such as improved gel strength and stability, the alteration of both ribbon and microfibril structure, lower stiffness, and greater extensibility and strength.[50−54]

Many studies already have shown that a pure microbial cellulose membrane can accelerate the healing process of acute and chronic skin wounds. However, these versatile MC membranes can also be infused with compounds that are known to promote healing. Thus, microbial cellulose when used as a wound dressing or as a scaffold for tissue engineering can be augmented with substances in order to further accelerate the healing process. The cellulose membrane can be augmented with therapeutic compounds either during its synthesis or after it has been created. Microbial cellulose membranes can also be infused with other therapeutic compounds without causing any alteration of its beneficial properties. For example, Legeza et al. created a microbial cellulose wound dressing for the treatment of third-degree burns that was impregnated with superoxide dismutase (an antioxidant) or poviargol (an antibiotic) in order to augment its therapeutic properties.[55] A study by Ciechanska showed that an MC−chitosan composite material could be created during the synthesis of the cellulose membrane by adding chitosan to the culture medium.[56] In other words, the glucosamine and *N*-acetylglucosamine units were incorporated into the synthesized cellulose chains, which was demonstrated in another study by Shirai et al.[57] Ciechanska claims that such a composite material has improved biological and physical properties. For instance, the chitosan-augmented MC membrane is able to retain moisture longer than a pure MC membrane. This may prove to be beneficial because a healing wound needs to be kept moist for as long as possible. In addition, chitosan, when degraded by endogenous enzymes, promotes the healing process by stimulating angiogenesis and tissue regeneration.[56,58] In addition, the mechanical properties of the composite are improved.[56]

Hyaluronic acid, a simple glucosaminoglycan which is found in most mammalian tissues, is especially prevalent in wounds during the healing process, is known to promote the healing of damaged skin, and could be used with microbial cellulose in order to create an even more effective wound dressing material.[59] Indeed, scientists have already begun to investigate the healing potential of augmented microbial cellulose.

Since some tissues require strong extracellular matrices, many bioengineered scaffolds for tissue-engineering purposes must be created with a high level of mechanical strength. Even though pure microbial cellulose is already quite strong, it can be augmented with various compounds in order to make it even stronger. In a study by Yasuda et al. microbial cellulose was immersed in two types of polymer solutions (2-acrylamide-2-methyl-propane sulfonic acid and gelatin) in order to create a hydrogel with enhanced mechanical toughness.[60] The resulting double-network hydrogels (DN), consisting of two independently cross-linked networks of different polymers, can withstand high frictional forces, showing that they are resistant to wear. Thus, these microbial cellulose composites could function as replacement cartilage tissue in damaged joints. Similarly, in a recent study conducted by Wan et al., a microbial cellulose composite material could function as a scaffold for bone tissue regenera-

tion.[61] In this study, Wan and his colleagues were able to create a microbial cellulose membrane that was coated with hydroxyapatite, a compound that is important for bone formation. The resulting composite material retains the mechanical strength and physical properties of microbial cellulose even though it is infused with hydroxyapatite crystals.[61]

Integrated Microbial Cellulose: In Vivo Tissue-Engineering Approach

The physical and mechanical properties of microbial cellulose are attributes that enable MC membranes to function as effective temporary wound dressings. On the other hand, because implantable biomaterials (i.e., scaffolds) are also needed, a new approach has been undertaken to apply cellulose as a material entirely integrated into the body, either as a bone or skin graft.

Skin is a vital organ that provides protection against infection and dehydration. Whenever there is an extensive loss of both the dermal and epidermal layers, surgical grafting of split-thickness autologous skin (skin harvested from the patient) is the standard method of treatment. However, when patients experience widespread full-thickness burns covering 90% of the body, the extensive loss of this vital tissue is usually fatal. Lost skin tissue can be replaced in one of three principle ways: (a) autologous skin grafts, (b) allogenic skin dressings (derived from human cadavers), or (c) synthetic wound dressings. Recent advances in tissue engineering develop skin substitutes by culturing fibroblasts or keratinocytes (or both) on biodegradable matrices. Clinical evaluations of these skin substitutes are reported in several papers; however, the costs involved in their preparation are still very high.[62] Table 2 includes some of the commercially available skin treatments which are used in cases of severe burns or chronic wounds and compares them to microbial cellulose which, besides XCell products, is still in the clinical evaluation process. Most of the commercially available skin substitutes use collagen as a scaffold material.

An invention by Oster et al. describes a method of preparing implantable microbial cellulose by dehydrating *Acetobacter*-derived cellulose with several organic solvents such as methanol, ethanol, or acetone.[23] The authors claim that cellulose prepared in this fashion might be useful as a tissue repair material or as a human tissue substitute. Additionally, an invention by Brown et al. describes a process wherein microbial cellulose is implanted into the wound bed.[63]

Permanently implanted MC can be penetrated by skin cells which are able to push away the MC fibrils and migrate deep into the cellulose net (up to 100 μm).[25] This fact may be very important for the treatment of third-degree burns, where new dermis has to be completely replaced and regenerated. With MC, the fibroblasts and keratinocytes would be able to penetrate the microporous net of cellulose, synthesize an extracellular matrix (ECM), and eventually form dermal tissue. Despite the fact that microbial cellulose is not a biodegradable material (at least not in the short term), it could stay in the body forever without causing any toxic or inflammatory reactions. A novel material which can stay in the body for an extended period of time is highly valued for the treatment of burned skin since a good substitute for skin grafting is not currently on the market.

In Situ Moldability of Microbial Cellulose for Artificial Cardiovascular Tissues. One of the greatest advantages of microbial cellulose is its ability to be molded into almost any size and shape during its synthesis without causing any significant alteration of its physical properties. Because of recent developments in implant technologies and microsurgical techniques, small, versatile, microbial cellulose objects may prove to be quite useful in this area of biomedical research. Roberts et al. described and patented one of the first production methods for the creation of shaped (molded) objects.[64] The Roberts et al. method involves inoculating *A. xylinum* into a suitable liquid medium, which is then transferred into a mold consisting of an oxygen-permeable polymer, such as polyvinyl chloride.[64] One of the sides of this fermentation vessel stays in contact with oxygen while the other side remains in contact with the liquid medium, where the cellulose is produced.[64] With this stationary culture technique, various three-dimensional objects of potential biomedical importance can easily be synthesized (Figure 3).

Using a similar molding technique, Yamanaka et al. developed a process for the creation of long, hollow, microbial cellulose tubes with an i.d. of 2−6 mm.[65] These MC tubes could be used as replacement blood vessels or other tubular structures such as the ureter, the trachea, or the digestive tract. The development of functional small-diameter vascular grafts (with an i.d. of less than 6 mm) has always been of great importance since an ideal vascular graft of this size has not yet been developed.[24,66] Although several synthetic vascular grafts have been used successfully in the treatment of large arteries (i.e., poly(tetrafluoro ethylene) (e-PTFE), poly(ethylene terephthalate) (Dacron), polyethylene), thrombosis, the formation of a blood clot, continues to be a problem for small-diameter blood vessel replacements.[66,67] According to Kakisis et al., there are three basic requirements for the construction of an artificial vessel: (a) a sufficient structural scaffold which provides the desired shape and support for cell growth, (b) the proliferation of vascular cells, and (c) a proper nurturing environment.[68] The development of synthetic, small-diameter grafts with mechanical properties similar to those of native arteries, and which are easy to store and handle, is significantly important for certain medical applications.[68] According to Yamanaka et al., the hollow microbial cellulose tubes proved to be biocompatible, especially with blood, and exhibited high durability.[65] Studies on animal models were used to evaluate blood compatibility by substituting the parts of the descending aorta and jugular vein of an adult dog with an artificial blood vessel composed of microbial cellulose. Tests performed a month later revealed that a slight adhesion of thrombi was observed in the sutured portion, but no substantial adhesion of thrombi was observed on the inner surface of the blood vessel, leaving the center portion of the tube unobstructed.[65] The molding technique used by the authors involves culturing *A. xylinum* in a hollow, oxygen-permeable container composed of silicon, cellophane, or other materials.[65] As mentioned before, since *Acetobacter* is an aerobic bacteria, the cells tend to approach the well-aerated zone located near the inner surface of the container where they ultimately produce and deposit cellulose. As a result of this process, a gelatinous membrane having thickness of 0.01−20 mm can be formed on the surface of the container.[65] Another molding technique mentioned by the authors involves culturing *Acetobacter* in the cylindrical space between two different diameter glass tubes which is filled with liquid medium.[65]

According to Kakisis, an artificial blood vessel composed of viable tissue can be considered as an ideal vascular graft.[68] Alternative approaches include the production of fibrocollagenous tubes within the recipient's body or by designing such vessels from acellular native tissues.[68] Most of the existing scaffolds for vessels are based on collagen matrices or other biodegradable polymers which have the proper mechanical properties. According to the authorities in the field, ideal artificial blood vessels should be composed of viable tissue,

Table 2. Skin Treatments for Severe Burns and Chronic Wounds[a]

product	company	product cost	use	type	advantages	disadvantages
Epicel	Genzyme Biosurgery	$18.90/cm^2	burns: deep partial thickness and full thickness	autologous: cultured autologous keratinocytes	not rejected due to autologous cells	1 day shelf life, very fragile, inferior cosmesis, product must be tailor-made for each patient (which takes several weeks), autograft required, risk of infection, success depends on the high input and abilities of skilled practitioners, the resulting skin is fragile for 3 years and usually blisters
Alloderm	LifeCell Inc.	$10.60/cm^2	burns/full-thickness wounds	allogenic: acellular cadaver dermis	not rejected, 2 year shelf life, used on >100000 patients	cadaver skin requires extensive washing, X-ray radiation, and preservation by either freezing or glycerol, limited donor population, time-consuming preparation, very expensive, cannot cover all needs, autograft required
Apligraf	Organogenesis Inc.	$25.60/cm^2	chronic wounds: venous/diabetic ulcers	allogenic: type I bovine collagen, human keratinocytes and fibroblasts	good safety record in >20000 patients	only 5 day shelf life, awkward logistics of ordering and use, needs to be cultured for 3−4 weeks, difficult to apply, product must be tailor-made for each patient
TransCyte	Smith & Nephew Inc.	$14.20/cm^2	burns: full and partial thickness	allogenic: polymer membrane, porcine collagen, silicone, human keratinocytes	1.5 year shelf life (if frozen), transparent, allows fluid and gas exchange	silicone membrane must be removed, autograft required, must be stored frozen (and thawed just prior to use)
Dermagraft	Smith & Nephew Inc.	$12.90/cm^2	chronic wounds: diabetic foot ulcers	allogenic: polyglycolic acid or polyglactin mesh scaffold, human fibroblasts	cryopreserved product, mimics function of dermis (dermal replacement)	difficulties in ordering and application, less effective than Apligraf and Orcel in healing chronic wounds
Biobrane	Mylan Laboratories	$0.90/cm^2	burns: partial thickness, repair donor sites	synthetic: nylon mesh, collagen, silicone	3 year shelf life, good barrier function and water exchange, good adherence, safe, easy to apply and remove, flexible, durable, reduces pain	no antimicrobial properties, must be fastened to patient with staples
Integra	Integra LifeSciences Corp.	$6.00/cm^2	burns: deep partial thickness and full thickness	synthetic: collagen, glycosaminoglycan—composed of chondroitin-6-sulfate, silicone	good barrier function, used in > 10000 patients, moderate shelf life, immediately available	operative removal of silicone layer (requiring the use of skilled practitioners), autograft required, resulting skin is fragile
microbial cellulose (MC)		$0.02/cm^2	burns: all types (first-, second-, and third-degree) chronic wounds: all types (venous, diabetic, and pressure ulcers)	synthetic: cellulose—a natural compound but “synthetic” to human skin	10 year shelf life, many other advantages[b]	may require numerous dressing changes

[a] Refs 62, 98−100. [b] Unlike all other products currently on the market, MC has all of the following ideal wound dressing properties: much lower cost than other current wound dressing materials, able to donate moisture while simultaneously absorbing exudates, prevents infection by providing a physical barrier to microbes, biocompatible, flexible, durable, sterile, elastic, conforms to almost any body surface, easy to apply and remove (does not involve complex processes such as the production of cell cultures), easy to obtain, does not require the extensive involvement of skilled practitioners, and reduces pain.

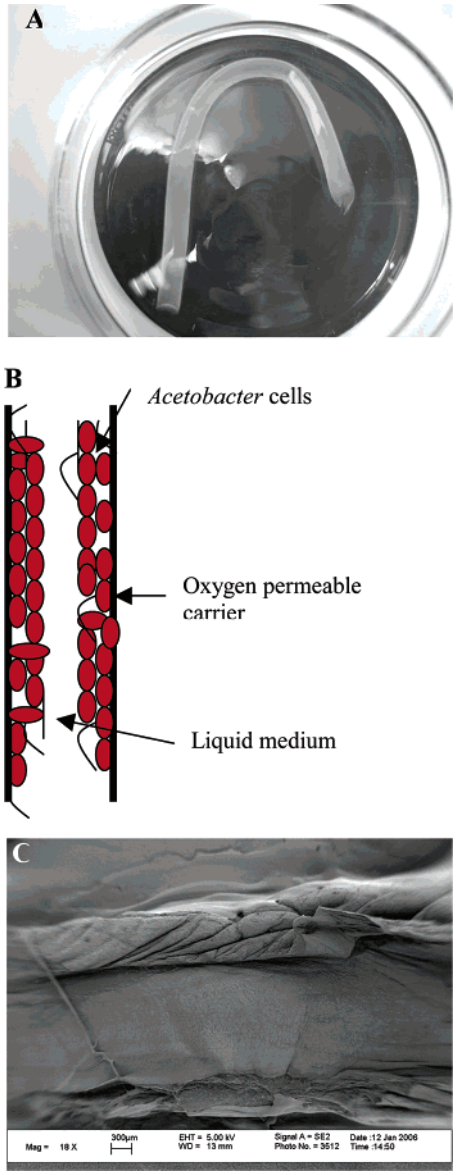

Figure 3. Hollow tube made from microbial cellulose according to the technique described by Roberts et al. (ref 64), using a silicon tube as a mold (A). *Acetobacter* cells which are highly aerobic organisms tend to gather in the oxygen-rich zones near the inner wall of the silicon tube where they produce and deposit cellulose (B). The inner surface (lumen) of the cellulose tube can be made very smooth and highly homogeneous (C).

contract in response to hydrodynamic forces, secrete normal blood vessel products, heal without any immunologic reactions, display a lack of thrombogenicity, and show a resistance to infections.[68] So far, several biodegradable polymers have been used as potential scaffolds for the design of artificial vessels. Among them, polyglycolic acid (PGA), which is highly porous, easy to handle, and can be made into different shapes, is commonly used.[68] However, since PGA matrices tend to be rapidly bioabsorbed and are not able to withstand systematic pressure, several novel copolymers based on PGA have been fabricated in order to remedy these deficiencies.

Using the fact that microbial cellulose can be molded in situ during synthesis, Klemm and co-workers were able to produce tube-shaped cellulose and assessed its potential as a substitute for blood vessels.[69,70] Klemm and co-workers designed an improved patented matrix technology in order to produce a microbial cellulose tube with an i.d. of 1 mm, a length of about 5 mm, and a wall thickness of 0.7 mm.[24,69] The technique used in the Klemm et al. studies includes aspects of stationary culture, where cellulose is grown on the oxygen-rich surface of the liquid medium.[69] During the actual process of fermentation, a glass matrix is immersed in the larger volume of the medium, and microbial cellulose is produced in the portion of the medium in between the outer and inner wall of the matrix.[24] The whole system is externally supplied with oxygen. According to the authors, such a cultivation technique offers some advantages over the other existing methods aimed at obtaining tube-shaped cellulose, which lack the ability to control the texture of thetube's inner surface. Thus, the authors were able to create MC tubes with a significantly smoother inner surface.

The authors also mentioned that a product called BASYC has qualities that are sufficient for experimental microsurgery.[24] Tubes formed using this technique have a smooth inner surface, which resembles normal blood vessels and which is particularly important for artificial microvessels so that blood clots will not form within the inserted artery. Mechanical tests performed on BASYC revealed that its average values of maximal tensile strength (around 800 mN) were comparable to those of normal blood vessels.[24] The tension tests also showed that the BASYC tubes were able to withstand the blood pressure of a rat (0.02 MPa).[24] In fact, Klemm et al. used BASYC to replace part of the carotid artery (4−6 mm) of a rat. Observations performed after four weeks revealed that the microbial cellulose/carotid artery complex was covered with connective tissue and was infused with small vessels.[24] Complete incorporation of the microbial cellulose vessel has been achieved in this experiment, showing that microbial cellulose can be used as a replacement blood vessel. Histological observations showed that 4 weeks after the implantation of BASYC, the inner surface of the microbial cellulose tube was completely covered with properly oriented endothelial cells.[24] The proper orientation of seeded endothelial cells enhances their stability under the shear stresses encountered as blood flows through the vessel.[68] The endothelial cells actively participate in the inhibition of thrombosis and serve as an anticoagulant surface.[71] Thus, the development of entirely endothelialized artificial grafts is one of the most important aspects of artificial vessel implantation.[72] One of the recent strategies in the construction of artificial vessels involves using tubular molds as scaffolds on which autologous or allogenic fibroblasts and endothelial cells are seeded and cultured. The mold is removed prior to implantation of the graft.[68] SEM observations of the implanted BASYC product showed that both the suture line and the suture material were not visible under the cell layer. In comparison, 4 weeks after the completion of end-to-end anastomosis (the union of nerve fibers), the same areas of the control rat (without BASYC) were not completely covered with endothelial cells and the suture material was still visible.[24]

Charpentier et al. used yet another interesting approach in the creation of artificial blood vessels.[73] Specifically, the authors used polyester because it is easy to handle and has good healing capability. However, the polymer surface of these synthetic materials frequently causes thrombosis. In order to reduce this coagulation effect, Charpentier et al. modified their polyester vascular graft by coating it with microbial cellulose.[73] The authors think that this new hybrid material could be ideal for use in the creation of vascular grafts because it (1) is hydrophilic, (2) can prevent thrombin formation, and (3) can be augmented with bioactive agents such as anticoagulation compounds.

Some investigators are using microbial cellulose in the treatment of other tissues. For instance, Mello et al. described

an interesting application of microbial cellulose in the field of modern neurosurgery.[74] In the Mello et al. study, the authors experimented with animals and replaced a portion of their dura mater, the brain's fibrous outer membrane, with microbial cellulose. In their research, the performance of microbial cellulose, when implemented as a dural substitute on both intact and damaged brains, was carefully evaluated over a 30−270 day period. Duraplasty was performed using relatively thin (50 μm) microbial cellulose membranes (Biofill). The macroscopic and microscopic observations showed that MC did not adhere to either intact or injured cortex.[74] Histology revealed that two newly formed layers of connective tissue enveloped the implanted microbial cellulose. In some tissue samples, the inner membrane consisted of a layer of fibroblasts, which are the most important cells of the dura mater, whereas the external membrane consisted of collagen.[74] Collagen invaded the cellulose membrane, disrupting its structure. Interestingly, the authors noticed a partial disappearance of cellulose, which was in their opinion, caused by the dilution in organic alkalis.[74] In one group of animals, after completion of duraplasty, the authors applied an additional 50-μm thick microbial cellulose film in the extradural space in order to evaluate the antifibrotic effect of microbial cellulose. At sites where microbial cellulose was applied extradurally there was good wound healing and a decrease of epidural scarring in comparison with the control group of animals, those that did not receive the epidural protection. According to the authors, the unique physical properties of microbial cellulose, and its high biocompatibility, demonstrate its suitability for use as a dura mater substitute.[74]

Loures recently invented another interesting microbial cellulose application using a molding technique to form a cylindrical and expandable endoprosthesis which is covered with a microbial cellulose membrane.[75] The resulting device is a microbial cellulose covered wire mesh structure and is intended to be used in the treatment of arterial stenosis, the abnormal narrowing of a blood vessel. Coronary stent implantation is currently performed in more than 80% of percutaneous coronary interventions.[76] Stenting, which involves implanting a metallic mesh to increase blood flow, is rapidly becoming the preferred technique for the percutaneous treatment of coronary artery disease, since it has significant advantages over angioplasty, the mechanical alteration of narrowed or totally obstructed blood vessels.[77] Stents prevent vessel closure and early vessel recoil and improve the long-term patency of arteries.[77] The main drawback of stents is that they can sometimes create in-stent restenosis (ISR), which results from the activation, migration, and proliferation of smooth muscle cells of the inner arterial wall. Another problem associated with the application of conventional stents is that they cannot prevent the release of endothelium fragments into the blood stream, which can occur as the stent pushes against the artery wall.[75]

Various methods have been used to reduce the incidents of ISR including drugs, radiation, and coating the stents with a variety of compounds.[77] According to the invention described by Loures, the gas-permeable mold consisting of a cylindrical stainless steel stent is filled with *Acetobacter* and appropriate media. Once the fermentation is finished, the product, in the shape of a tube, is removed and submitted to chemical treatment in order to remove the cells and any remaining media. The resulting stent, which is covered with microbial cellulose, goes through a drying process so that the final product is tightly wrapped with a dry cellulose membrane.[75] Such a device would form a physical barrier, preventing the smooth muscle cells from migrating toward the vessel lumen. Another advantage of this product is that the cellulose membrane can function as a drug delivery system, capable of delivering restenosis inhibiting drugs.[75] In addition, the release of endothelial fragments, resulting from the compression of the stent against the vessel wall, may be eliminated because the cellulose membrane will keep these fragments in place.[75]

Integrated Microbial Cellulose for Guided Tissue Regeneration (GTR). Guided tissue regeneration is a surgical procedure that utilizes a barrier membrane to enhance the healing process. Various scientists are currently investigating this technique. For example, Dahlin et al. advanced the concept of guided (bone) tissue regeneration when he demonstrated that new (bone) tissue can be formed whenever a physical barrier is used to prevent soft fibrous tissues from infiltrating the healing wound.[78−81] Microbial cellulose was also used as a physical barrier in the regeneration of periodontal tissue.[79,82,83] In other words, MC was used to isolate incised oral epithelial cells and gingival connective tissue from the treated root canal.[79] This separation allows periodontal ligament cells and bone cells to proliferate within the wounded area resulting in bone regeneration. The presence of the physical barrier in this process is important because it prevents fibroblast cell ingrowth and provides enough space to allow osseous cells to grow and function properly.[81]

There are several published clinical studies which used microbial cellulose or microbial cellulose-based membranes as physical barriers for tissue regeneration. The Gengiflex membrane, which was used in most of these studies, is manufactured by the BioFill company (BioFill Produtos Bioetecnologicos, Curitiba, PR Brazil) and is composed of two layers: (a) an internal layer consisting of pure, crystalline microbial cellulose, and (b) an external alkali−cellulose layer consisting of chemically modified microbial cellulose.[79,82] Due to the natural physical and mechanical properties of microbial cellulose, the Gengiflex product is rigid, elastic, strong, and biocompatible.[81,82] The Novaes et al. study found that by implanting a Gengiflex membrane along with hydroxyapatite, which functions as a porous scaffold, bone cells were able to migrate and successfully restore an osseous defect.[79]

In another report, the same authors successfully applied a microbial cellulose membrane for GTR in the treatment of dogs suffering from class II furcation lesions, a type of periodontal disease.[82] After 4 weeks, the dogs in the control group showed no progress. However the dogs treated with microbial cellulose exhibited an increase in osteoblast cells and newly formed collagen fibers. After another 8 weeks of treatment, there was still no improvement in the control group; however, complete bone regeneration was observed in the experimental group. On the basis of their report, the authors concluded that the Gengiflex membrane facilitates the healing of class II furcation lesions in dogs with naturally occurring periodontal disease.[82] In a report by dos Anjos et al. there was no significant difference between microbial cellulose and e-PTFE (polytetrafluoroethylene) membranes in the treatment of class II furcation in mandibular molars.[83] However, two other recently published reports did not favor the application of a microbial cellulose membrane over the more extensively used e-PTFE membrane in the guided bone tissue regeneration process. In the studies performed by Salata et al., the biocompatibility of both types of membranes were compared in vitro and in vivo.[80] The in vitro studies found that both membranes supported osteoblast-like cell attachment, proliferation, and maturation as well as the synthesis of an ECM.[80] However, in clinical applications, bone regeneration associated with a microbial cellulose membrane (Gengiflex) was

predominantly endochondral type (cartilage formation), whereas direct bone formation, without an intermediate cartilaginous stage, was observed when an e-PTFE membrane was applied. In addition, the Gengiflex membrane appeared to disintegrate in vivo which induced a significant inflammatory response and which may have eventually resulted in impaired bone regeneration.[80] It should be mentioned, however, that in the in vivo study, the microbial cellulose membrane was not sutured to the underlying bone. Thus, the membrane may have either moved during the healing process or collapsed into the wound. In similar studies performed on rabbits, Macedo et al. also found that the e-PTFE membrane was more effective than the Gengiflex membrane in the bone regeneration process.[81] For example, incomplete bone formation and inflammation was detected in rabbits treated with microbial cellulose membranes, whereas a nonporous e-PTFE barrier induced proper bone deposition.[81]

Besides artificial blood vessels, molded microbial cellulose was also found to be very useful for nerve surgery, functioning as a protective cover of anastomosis.[24,70] Klemm and co-workers clinically tested a microbial cellulose tube on animals and noticed that it prevented connective tissue from growing into the nerve gap and facilitated the early regeneration of the nerve.[24,70] No inflammatory reaction was observed during these studies. The authors of the study used a BASYC tube and placed it directly over the anastomosis area, holding it in place by two sutures. Due to the transparency of microbial cellulose, the anastomosis areas were clearly visible throughout the whole treatment. Postoperative observations performed during the study revealed that connective tissues, along with their associated vasculature, eventually covered the implanted microbial cellulose tube.[24,70] Faster and improved regeneration of nerve functions was achieved in animals treated with BASYC. In another experiment, a microbial cellulose tube was used to deliver a neuroregenerative compound.[24] Observations performed 8−10 weeks after treatment revealed that the tested drug accelerated the innervations (increased muscle weight). In addition, the animals treated with a drug-infused BASYC tube exhibited improved walking ability.[24] These findings were recently reported by other investigators who determined that the structure of microbial cellulose is effective for drug delivery. For instance, Sokolnicki et al. concluded that the open fiber network of microbial cellulose is ideal for immobilizing harmful compounds while simultaneously allowing nutrients and beneficial compounds to pass from the membrane into the wounded or diseased area.[84]

Microbial Cellulose as a Scaffold For in Vitro Tissue Engineering

The difficulties encountered in repairing or replacing severely damaged skin may be resolved through a process called tissue engineering. This very promising technique involves the in vitro construction of a scaffold material, which successfully mimics the extracellular matrix of normal tissues. Cells of the desired tissue are seeded onto the scaffold which coaxes them to develop into the proper three-dimensional structure. This in vitro tissue construct can then be implanted into the affected area of the body, either as a replacement tissue or even as a replacement organ. Thus, tissue engineering could be very effective in replacing severely burned skin or in repairing chronic, nonhealing wounds, such as ulcers.

One of the key aspects of tissue engineering involves the creation of the scaffold, the three-dimensional matrix which enables the cells to develop into a fully functional tissue construct. Some scientists have proposed that the scaffold material must be biodegradable so that as the seeded cells proliferate, they will secrete their own extracellular molecules, thereby replacing the implanted material.[68] However, this requirement is problematic due to the fact that the temporary scaffold can often degrade faster than the cells can replace it.[85,86] Therefore, the solution to this problem may entail the need for a permanent scaffold material which is biocompatible, porous, and which contains the mechanical properties required for normal tissue function. Preliminary studies indicate that microbial cellulose could actually function as this ideal scaffold material for tissue engineering.[25,87−89]

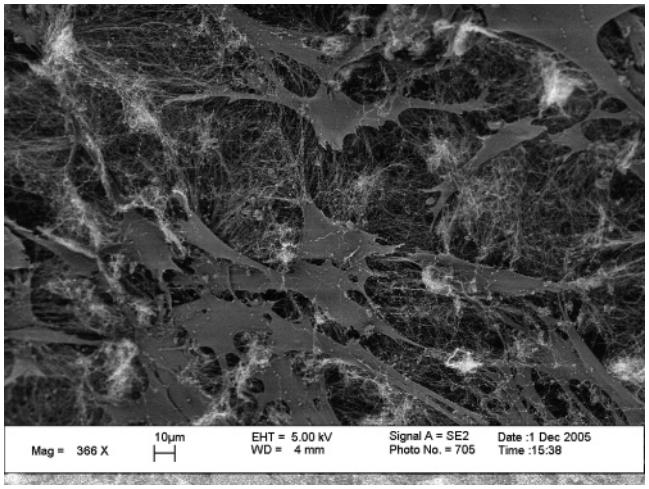

Figure 4. Fibroblasts cells seeded onto a serum-soaked microbial cellulose membrane (image courtesy of Kathryn Bivens and Dwight Romanovicz, University of Texas at Austin).

If cellulose is to be used for tissue engineering, it must be biocompatible. Fortunately, studies have shown that cellulose is not harmful when it is used either as an implanted material or as a substrate for cell cultures. For instance, a study by Watanabe et al. investigated the biocompatibility of microbial cellulose by using it to produce cell cultures.[87] They found that an unaltered MC membrane was not an effective substrate for cell culture or tissue engineering because the cells did not adhere to the MC surface and therefore did not proliferate. However, when the membranes were soaked in serum and electrolytic solutions such as sodium hydroxide, the cells were able to adhere and proliferate, indicating that MC membranes can function as a cell culture substrate and can be used in tissue engineering when infused with the proper substances. The authors also showed that proteins which function as adhesion factors for cells were successfully adsorbed by the MC membranes and that the high permeability of the membranes helped to diffuse the necessary nutrients, growth factors, and other products to the growing cell mass. These results are promising because they indicate that a skin tissue-engineered construct can be created with a cellulose membrane that is seeded with fibroblasts and/or keratinocytes. This construct can be created as a monolayer of cells which can then be placed directly on to the wound bed in order to provide immediate cover for the wound and to initiate the regeneration of skin tissue. Currently, in vitro and in vivo studies are in progress in order to test the efficacy of such a construct (Figure 4).

In another biocompatibility study by Märtson et al., the authors implanted a porous cellulose sponge into rats in order to assess the resulting cellular interactions.[12] The authors of this study showed that a cellulose sponge, with optimal pore size, exhibited sufficient stability, demonstrating that cellulose can

function as an in vivo matrix for tissue regeneration and can be used to stimulate the formation of granulation tissue.

In addition to being biocompatible, microbial cellulose has unique mechanical properties which makes it well suited for various tissue-engineered constructs. For example, some researchers, such as Bäckdahl et al., are, in addition to those already mentioned, investigating whether MC can be used to replace damaged blood vessels.[88] The authors of the Bäckdahl et al. study showed that smooth muscle cells were able to successfully adhere and proliferate on an MC matrix. In fact, some of the smooth muscle cells were able to migrate into the pores of the microbial cellulose by essentially pushing aside the fibrils. In their studies, Bäckdahl et al. mentioned a well-known fact that a microbial cellulose membrane actually has two distinct sides. As discussed previously, a microbial cellulose membrane is synthesized by *Acetobacter* on the surface of a static liquid medium. The actual formation of the layers of the MC membrane always takes place on the upper-most air-exposed portion of the developing matrix. Thus, the most active layer of cellulose-producing bacteria is always in contact with the air. During the process of fermentation the older layers of cellulose are pushed down by the newly formed cellulose fibrils. As the developing membrane becomes thicker, the liquid medium become a limiting factor for the upper-most cells. As a result, the top side of the membrane develops a rough, less porous, texture. As shown in Figure 5, the air interface side (top side) of a microbial cellulose membrane is more dense, and has a smoother surface, than the side that remains in contact with the liquid medium (bottom side). Thus, the authors of the Bäckdahl study suggest that the air interface side could function as the lumen of an MC replacement blood vessel because endothelial cells more readily attach to a smooth surface.[88]

These preliminary results further substantiate the notion that microbial cellulose could function as a temporary blood vessel matrix material. The microbial cellulose fibers actually enable smooth muscle, endothelial, and fibroblast cells to eventually create a viable blood vessel. The mechanical properties of MC may prove to be ideal for the generation of blood vessels, providing the required tensile strength and the flexibility to withstand the forces generated by the circulatory system.[24] Microbial cellulose may also be useful in the regeneration of other tissues such as bone and cartilage. For example, in a study by Svensson et al., the authors showed that MC could be used as a scaffold for the regeneration of cartilage because (1) it supports chondrocyte proliferation at levels similar to that of native tissue substrates (such as collagen type II), (2) it maintains chondrocytes in their differentiated form (i.e., they do not become fibroblast cells), and (3) it possesses the mechanical properties that are required for the development of proper cartilage tissue.[89]

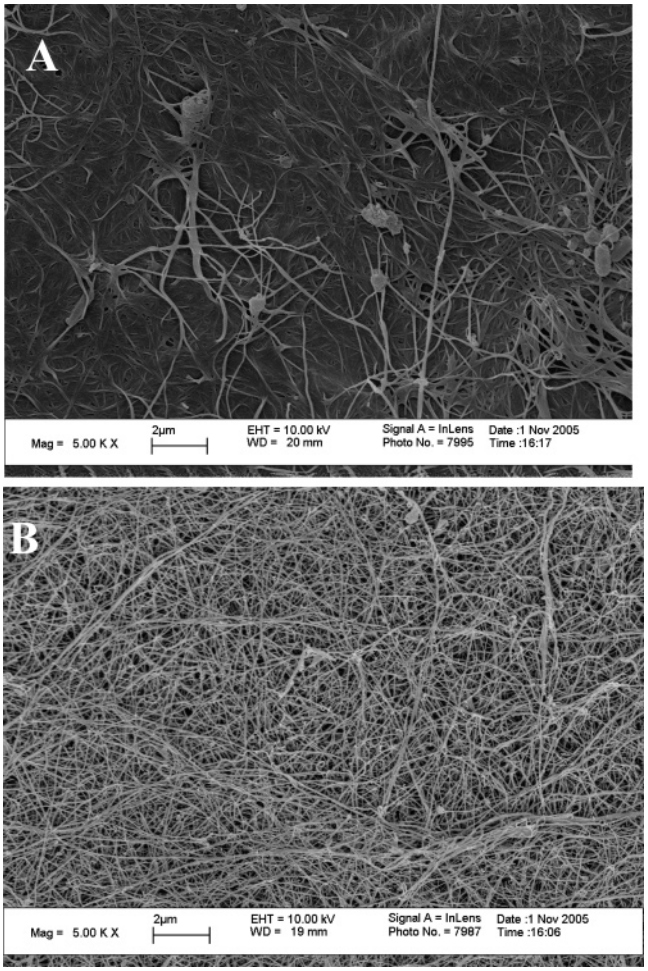

Figure 5. Two distinct sides of a microbial cellulose membrane. The texture of the top side (air interface side) of the membrane (A) results from the limitation of the liquid medium and from its continuous exposure to the surrounding air. The bottom side (the liquid medium side) of the membrane (B) is much more porous than (A) because it has remained in contact with the liquid medium during the entire fermentation process and also it represents the very first layer of cellulose that was deposited by the cells (images captured by Dwight Romanovicz, University of Texas at Austin).

Perspectives

Microbial cellulose is proving to be a very versatile material. It can be used in a wide variety of biomedical applications, from topical wound dressings to the durable scaffolds required for tissue engineering. Many scientists are already trying to develop novel biomaterials from synthetic polymers. These new materials could be used in many biomedical and biotechnological applications, such as tissue engineering, drug delivery, wound dressings, and medical implants. However, many of these synthetic polymers have their drawbacks. For instance, they often do not possess the correct mechanical properties and are usually not biocompatible.[18,85] Initial studies indicate that microbial cellulose is a better candidate for tissue engineering since it is both durable and biocompatible. In fact, microbial cellulose is a particularly interesting material for the development of many different biomedical devices. In some case, such as wound healing and organ replacement, a number of clinical studies have been performed showing its effectiveness in these areas. However, much interdisciplinary research is needed in order to bring microbial cellulose products to successful commercialization. For example, a wide variety of mammalian cells need to be seeded onto MC membranes in order to assess their viability and proliferation. A number of clinical studies will be necessary to prove its usefulness and functionality. If microbial cellulose proves to be effective in wound repair and tissue engineering, then it will have to be produced on an industrial scale. Due to its simple fermentation process, large-scale microbial cellulose production appears to be quite feasible; however, specific engineering details need to elaborated. Also, more biochemical and genetic investigations need to be conducted in order to fully understand and improve the cellulose production process within *Acetobacter*.

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