ISSN 1907-0322

EFFECT OF GAMMA IRRADIATION ON MICROBIAL CELLULOSE MEMBRANE FOR APPLICATION IN GUIDED BONE REGENERATION (GBR)

Darmawan Darwis

Center for the Application of Isotopes and Radiation Technology
National Nuclear Energy Agency, Indonesia
Jl. Lebak Bulus Raya No. 49, Kotak Pos 7002 JKSKL, Jakarta 12070
Telp. 021 7690709, Fax. 021 7691607
E-mail : darmawan_p3tir@batan.go.id

Received 29 Mei 2009; accepted 17 Juni 2009

ABSTRACT

EFFECT OF GAMMA IRRADIATION ON MICROBIAL CELLULOSE MEMBRANE (MC MEMBRANE) FOR APPLICATION IN GUIDED BONE REGENERATION (GBR). The synthesis and effect of gamma irradiation on characteristics of microbial cellulose membrane have been evaluated. Microbial cellulose gel (nata de coco) was produce using bacteria *Acetobacter xylin*um incubated in bacterial growth medium containing coconut water as a micro nutrient source. Microbial cellulose membrane was prepared using mould compression at 120°C for 5 minutes. The membranes were irradiated using gamma rays with doses of 25 and 50 kGy respectively at dose rate of 10 kGy/h. Several parameters such as water absorption, surface morphology, thermal and mechanical properties of un- irradiated and irradiated membranes were analyzed. The results showed that optimum production of microbial cellulose by *A. Xylinum* is 10 to 12 days at incubation temperature of 30°C and pH 4. Chemically treatments of MC membrane by NaOH and NaOCl solution were effective to remove the bacteria contaminant, bacterial cells embedded in the polymer net and endotoxin which occurred during cellulose production as well as produced membrane with more white colour. Water absorption properties of MC membranes showed maximum value at immersion temperature of 25°C, 37°C and 50°C were 110, 137 and 140 %, respectively. Water absorption of MC membrane decreases by increasing irradiation dose. Microscopic photograph of MC membrane showed that the membrane was consisted of interconnected nano to micro porous structures with diameter ranging from 0.05 to 0.5 μm. Thermal properties of MC showed that decomposition temperature of un-irradiated and irradiated MC membrane at dose of 25 and 50 kGy were 328°C, 328°C and 295°C, respectively. Tensile strength of un-irradiated MC membrane in dry state was 102 MPa. Irradiation at 25 and 50 kGy reduced tensile strength to become 85 and 51 MPa respectively. The decrease of thermal property and mechanical strength of MC membrane by irradiation is due to decomposition of polymeric cellulose to the lower molecular weight. This degradation hopefully improve biodegradation and resorption of MC membrane in tissue cell.

Key words : microbial cellulose, gamma irradiation, *Acetobacter xylinum*, Guided Bone Regeneration (GBR)

Jurnal Ilmiah Aplikasi Isotop dan Radiasi
A Scientific Journal for The Applications of Isotopes and Radiation
Vol. 5 No. 1 Juni 2009
ISSN 1907-0322

ABSTRAK

EFEK IRADIASI GAMMA PADA MEMBRAN SELULOSA MIKROBA UNTUK PEMAKAIAN DALAM BIDANG GUIDED BONE REGENERATION (GBR). Sintesis dan efek iradiasi gamma pada karakteristik membran selulosa mikroba telah dipelajari. Gel selulosa mikroba (*nata de coco*) dibuat menggunakan bakteri *Acetobacter Xylinum* yang diinkubasikan dalam media pertumbuhan yang mengandung air kelapa sebagai sumber unsur hara mikro. Sedangkan membran selulosa mikroba dibuat menggunakan teknik mold kompres pada suhu 120°C selama 5 menit. Membran kemudian diiradiasi dengan sinar gamma pada dosis 25 dan 50 kGy pada kecepatan dosis 10 kGy/jam. Beberapa parameter seperti absorpsi air, morfologi permukaan membran, sifat-sifat mekanik dan termal membrane baik yang diiradiasi maupun tidak diiradiasi dianalisa. Hasil yang diperoleh menunjukkan bahwa produksi selulosa mikroba optimum terjadi pada hari ke 10 – 12. Setelah inkubasi pada suhu 30°C dan pH 4. Perlakuan secara kimiawi menggunakan larutan NaOH dan NaOCl terhadap membran selulosa mikroba efektif untuk menghilangkan bakteri kontaminan, sel-sel bakteri yang terperangkap dalam jaringan polimer dan juga menghasilkan membran yang berwarna lebih putih. Sifat absorpsi air dari membran yang dilakukan pada suhu 25°C, 37°C dan 50°C menunjukkan nilai maksimum masing-masing adalah 110, 137 and 140 %. Absorpsi air dari membran SM berkurang dengan bertambahnya dosis iradiasi. Hasil foto mikroskopik membran SM menunjukkan bahwa membran tersusun atas struktur berpori dengan ukuran nano sampai mikro dengan diameter 0.05 s/d 0.5 μm dimana pori tersebut saling berinterkoneksi. Sifat termal membran SM menunjukkan bahwa suhu dekomposisi yang tidak diiradiasi maupun yang diiradiasi dengan dosis 25 kGy dan 50 kGy berturut-tururt adalah 328°C, 328°C and 295°C. Kekuatan tarik (TS) membran dalam keadaan kering yang tidak diiradiasi adalah 102 MPa. Iradiasi dengan dosis 25 kGy dan 50 kGy menurunkan TS menjadi bertturut-turut 85 MPa dan 51 MPa. Penurunan terhadap sifat mekanik dan termal membran SM akibat iradiasi disebabkan oleh terjadinya dekomposisi polimer SM menjadi polimer Sm yang lain dengan berat molekul lebih rendah. Degradasi akibat radiasi ini diharapkan dapat meningkatkan sifat biodegradasi dan bioresorpsi SM dalam jaringan sel tubuh.

Kata kunci : Selulosa mikroba, iradiasi gamma, *Acetobacter xylinum*, Guided Bone Regeneration (GBR)

INTRODUCTION

The term of guided tissue or bone regeneration has been used in tissue engineering for some years and actually is a specialized sub area of tissue engineering [1, 2]. Guided bone regeneration (GBR) refers to surgical procedure by which utilizing sterile membranes or membranes as a mechanical barrier to create a secluded space around the defects to permit bone regeneration and preventing non desired tissues, like fibrous tissue from growing in and filling the defect [2, 3, 4, 5]. While Guided tissue regeneration refers to surgical procedure in which a barrier is used to keep the

ISSN 1907-0322

epithelium away from root surface [6]. Both of these techniques require a sterile membrane or scaffolds as physical barrier to encourage new bone to grow and prevent the growth of scar tissue in the grafted site as well as to prevent non-desired tissue or epithelium from the defect area. GBR membrane is occasionally utilized with dental implant or bone grafting materials. Bone graft and GBR are needed when a part of our body is missing a portion of bone. This missing portion of bone is called a bony defect. Some examples of application of this techniques are: defect surrounding root of teeth (periodontal defect); defect which occur following tooth extraction; generalized decrease in quantity of jaw bone from trauma or long-term tooth loss; defect surrounding dental implant and defect resulting from cyst or tumor surgery [7,8]. The illustration of these cases is depicted in Figure 1.

Non-degradable as well as biodegradable polymeric materials are the most widely studied scaffolds for GBR. In the market, conventional material of GBR membranes are non-degradable expanded polytetraflouroethylene (e-PTFE: Gore-Tex), and degradable polylactic acid (Guidor) and poly-glatin (Vicryl) [1, 9]. Although e-PTFE membranes have shown excellent clinical results, second surgery procedure is required to remove the membranes after new bone generation. On the other hand, degradable GBR membranes have a benefit to avoid second surgery. However, these are still challenges with respect to (1) barrier of tissue invasion associated with rapid degradation of membranes, (2) osteoconductivity to achieve large area repair and (3) mechanical stability of membrane to sustain surgery treatment [9].

Cellulose, the most abundant component of plant cell, is found in nature almost exclusively in plant cell wall [10, 11, 12]. It is synthesized by variety of organisms ranging from multicellular and unicellular plant to bacteria [13]. Cellulose is a long chain polymer, made up of repeating unit of D-glucose, a simple sugar. Glucose units are in 6-membered rings, called pyranose. They are jointed by single oxygen atoms (acetal linkages) between the C1 of one pyranose ring and C4 of the next ring [13]. The chemical structure of cellulose is shown in Figure 2. There are several genera of bacteria known to synthesize cellulose, but only the Gram-negative *Acetobacter xylin*um species produce sufficient amount of cellulose to warrant commercial interest

ISSN 1907-0322

[10, 11, 13 15]. The cellulose produced by bacteria is known as microbial cellulose or biocellulose.

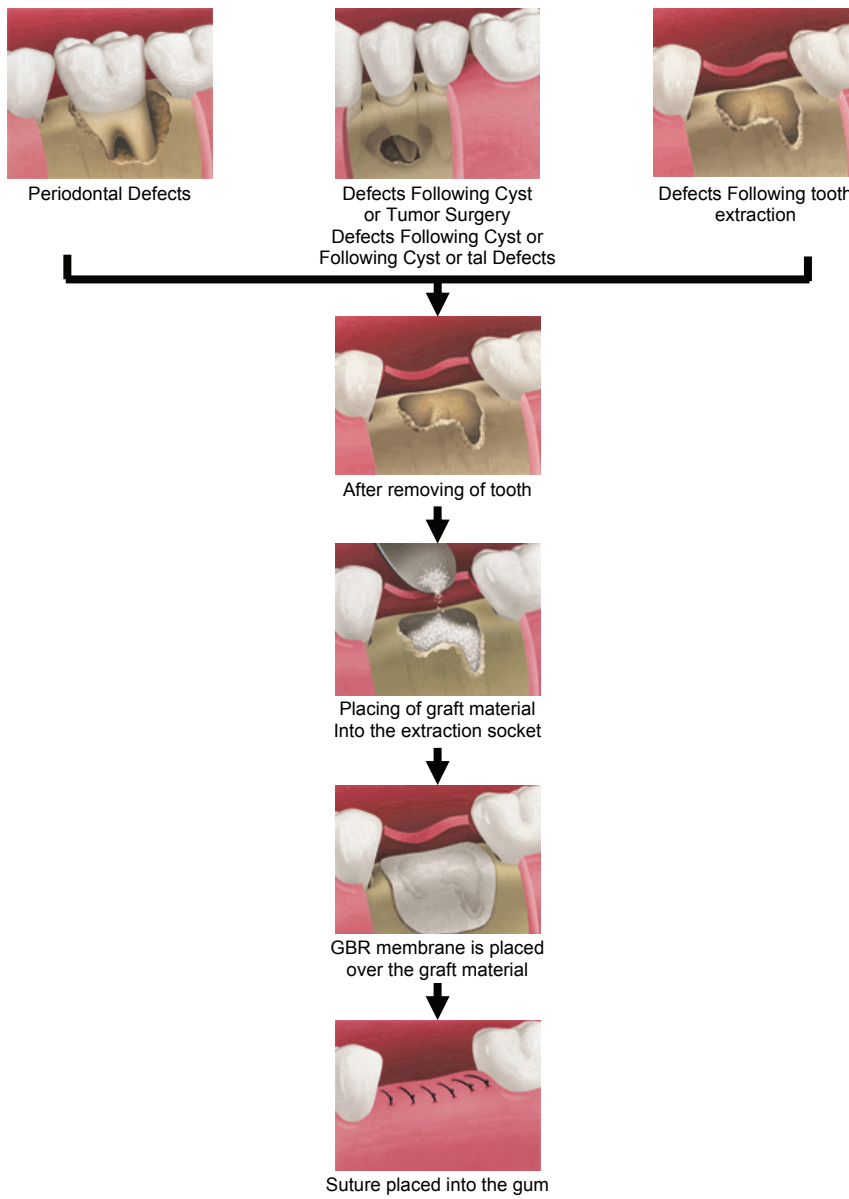

Figure 1. Example of applications of GBR and GBR membrane in the treatment of teeth extraction

ISSN 1907-0322

One of the most important features of microbial cellulose is its chemical purity, which distinguishes this cellulose from plant cellulose, usually associated with hemicelluloses and lignin, which inherently difficult removal [16]. They also have different physical and chemical properties. The diameter of microbial cellulose is about 1/100 of that of plant cellulose and Young's modulus of MC membrane is almost equivalent to that of aluminum [11]. It was reported that microbial cellulose is biocompatible to tissues and it's also biodegradable. Therefore, it is expected that microbial cellulose would be a new biodegradable biopolymer materials.

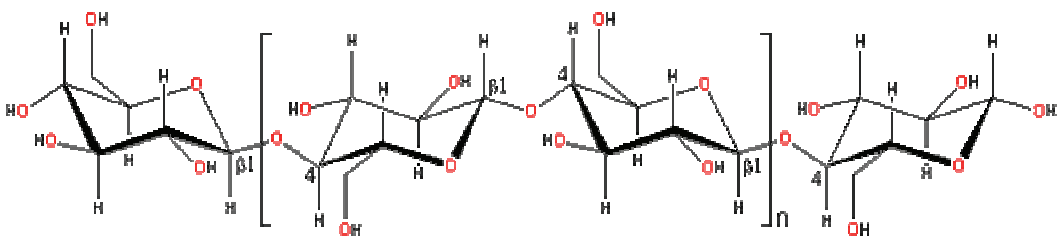

Figure 2. Chemical structure of cellulose

Microbial cellulose is produced by the bacteria *Acetobacter xylinum* either in static, shaken or agitated cultures. In a static culture, the cells produce cellulose as secondary assembles into thick cellulosic mats, otherwise known as pellicle. The pellicle normally covers the entire surface of the medium which is exposed to air. In shaken and agitated cultures polymer fibers of nanometer size are produced. The bacterium grows and produces cellulose from a wide variety of substrates. The bacterium grows and produces cellulose optimally under acid medium of pH 4-5, temperature of 30-32C and enough oxygen and carbon sources. Widely available commodities accepted *Acetobacter* as a carbon source including dextrose (glucose), sucrose, fructose, invert sugar, ethanol and glycerol [17]. It is well known that coconut water has been extensively explored to produce microbial cellulose pellicle known as nata de coco. In production of nata, coconut water is used together with bacterial growth medium as a micro nutrient source. Therefore the nata produced using coconut

ISSN 1907-0322

water is called nata de coco. Nata de coco gel when dried can produce thin membrane which is very potential to be used in tissue engineering applications. Philippine is the biggest world producer of nata de coco [19]. It was reported that Indonesia and Philippine are the two biggest producer of coconut in the Asian and Pacific region [20]. However, in Indonesia, traditionally the coconut water is not optimally used. In traditional markets, after the coconut meat was taken, the coconut water is consider as waste and throw away. Taken this into consideration, it is potential to improve the usage of coconut water to produce valuable products such as microbial membrane especially for application in biomedical fields.

High energy radiation especially gamma rays is well known to be use for sterilizing medical devices. Apart of this, it can be used also for improvement of degradability of cellulose. In this work, gamma irradiation was used both to sterilized and degrade microbial cellulose membrane in order to be used safely in tissue engineering applications.

MATERIAL AND METHODS

Materials

In this experiment, *Acetobacter xylinum* was used as bacteria to synthesize cellulose. Coconut water supplied from traditional market at Bogor, West Java was used as micro nutrient source used for bacterial growth media. Sugar cane (sucrose) and ammonium hydroxide (NH_4OH) were used as carbon and nitrogen sources, respectively. To fix acidity of media to pH about 4, acetic acid glacial was used. Alkaline solution of sodium hydroxide (NaOH) was used to remove viable organisms and pyrogens (endotoxins) produced by bacteria from the pellicle. A typical bleaching solution of sodium hypochlorite (NaOCl) was used to bleach pellicle. All the chemicals used are pro analysis grade (p.a. grade). and all the supporting equipments are sterilized before used.

ISSN 1907-0322

Methods

Preparation of pellicle (nata de coco). Five liters of fresh coconut was filtered using cotton mesh to remove the debris, then left for 5 days**.** The filtered coconut water was put into 10 liters volume of beaker glass and to this solution, 500 grams of granulated sugar (10% of water coconut) and 25 ml of ammonium hydroxide solution (0.5% of coconut water) was added. The solution was then heated at 95°C for 1 hour. After cooling to 45°C and adjusted the pH to 4 by adding acetic acid glacial. This solution was called substrate/growth medium.

About 1000 ml of *A. xylinum* recovered from inoculation flash was added to growth media and stirred using a glass stirrer until homogeneous. Two hundred (200) ml of propagation culture of *A. xylinum* were then poured into polystyrene trays with a size of 10 cm x 10 cm x 5 cm. The Polystyrene trays were sealed and incubated at ambient temperature (30 ± 2°C) until growth of pellicle of microbially-derived cellulose was completed. Normally, it takes about 10 to 12 days. The pellicle was removed from the trays and chemically treated using 1.25 M concentration of sodium hydroxide solution at 90 to 95°C in order to remove bacterial by-products and residual media. The chemically processed cellulose pellicle was then rinse with filtered water and followed by bleaching process using sodium hypochlorite solution of 0.05 % w/v. The white pellicle was then rinse thoroughly with filtered water for 2 hours.

Preparation of microbial cellulose membrane. Microbial cellulose membrane was prepared using compression mould. The white pellicle was cut to 10 cm x 5 cm size and put it into 0.05 mm stainless steel spacer. It was then compression mold at 120°C for 10 minutes under 150 kgf. The dried membrane produced was removed from the spacer.

Irradiation of microbial cellulose membrane. The microbial cellulose membranes were placed into polystyrene plastic bag and sealed under vacuum. The samples were then irradiated using gamma rays delivered from cobalt-60 source at 25 kGy and 50 kGy, respectively with a dose rate of 10 kGy/h.

ISSN 1907-0322

Characterization of samples

Water absorption measurement

Water absorption of un-irradiated and irradiated MC membranes was measured in distilled water at various time and temperatures (25, 37 and 50°C). The sample membrane with the size of 10 mm x 20 mm was weighed (Wo) and placed into bottle vial, closed the bottle with the cap and distilled water was added until all the membrane submerged. The bottle was then kept at constant temperatures for a certain predetermined times. It was then removed from water and sweep the outer surface of membrane using tissue paper (blotting technique) to remove water from membrane surface and then weighing the membrane (Wti). The membrane was return back into same bottle vial for another consecutive time. Repeat the process up to 1 month.
The percentage of water absorption was calculated using formula:

$$\text{Percent water absorption} = [(Wo - Wti)/Wo] \times 100$$

Surface morphology

The surface morphology image of MC membranes samples was analyzed using Scanning Electron Microscope (SEM) (SEMEDX type N integration system, Hitachi). The dry membrane was mounted in sample holder and coated by gold. The sample was analyzed at 5000 times magnification.

Thermal degradation

Thermogravimetric Analyzer TGA-50 Shimadzu was used to elucidate thermal decomposition of MC membranes. About 2.1 mg of samples were vacuum dried at 35°C for 24 hours before weighing. Put the sample into an aluminum pan and heat the samples to 500°C with heating speed of 20°C/minute under nitrogen gas flow of 50 ml/minutes. For the isothermal measurement, the sample was kept at 150°C and followed by heating up 200°C and hold the sample at this temperature for 20 minutes then finally proceed heating up to 300°C.

ISSN 1907-0322

Analysis of mechanical properties

Mechanical properties such as tensile strength (TS) and elongation at break (Eb) of MC membranes were measured using Shimadzu Compact Table Top Universal Testing Machine, EZ Test Series equipped with computer program. The membrane sample with rectangular shape of 10 mm x 30 mm and thickness of about 0.005 mm was used for measurement. Before measurement, initial length (gage length) of sample was marked at a length of 10 mm, denotes as Lo. The membrane was mounted to the upper and lower clamps to the tension machine and measurement was carried out at a crosshead speed of 2 mm/minute at room temperature until the sample broke. The length between to marks of sample after the break was denoted as Lz. Tensile strength (TS) and Elongation at break (Eb) were calculated by the following equation:

$$\text{Tensile strength} = P / A$$

P is the tensile force over the sample membrane and A is the cross-sectional area of the sample membrane. Units for tensile stress or tensile strength are newtons per square meter (N/m^2, also called pascals, Pa)

$$\text{Elongation at break (\%)} = [(Lz - Lo)/Lo] \times 100$$

RESULTS AND DISCUSSION

Production of microbial cellulose

Synthesis of microbial cellulose is prepared using modified procedure by Tita Puspitasari [21]. The MC pellicles were produced by *A. xylinum* fermented under medium consisting of coconut water, sugar cane, and ammonium hydroxide at proper composition. In this experiment, incubation of the medium was run at 30 ± 2°C in static condition at an initial pH of 4 for 10 to 12 days, and the pellicles produced an average thickness of 15 mm. After chemically treated using NaOH and NaOCl, the pellicles were then compress molded to produce membranes with a thickness of about 0.05 mm. The membrane after chemically treatment is depicted in Figure 3. From this figure, it is shown that the membrane is slightly opaque and no brown spots are

ISSN 1907-0322

observed. In contrary, the non-chemically treated cellulose pellicle shows a brown spot indicating contamination from other bacterias and the membrane is more yellowish as shown in Figure 4. From these results, it can be said that chemically treatment of MC pellicles by NaOH and NaOCl are effective to remove bacteria contamination where the bacterial cells were embedded in the polymer net and endotoxin occurred during cellulose production as well as produced whiten membrane.

Figure 3. Photograph of membrane prepared from chemically treatment of MC pellicle.

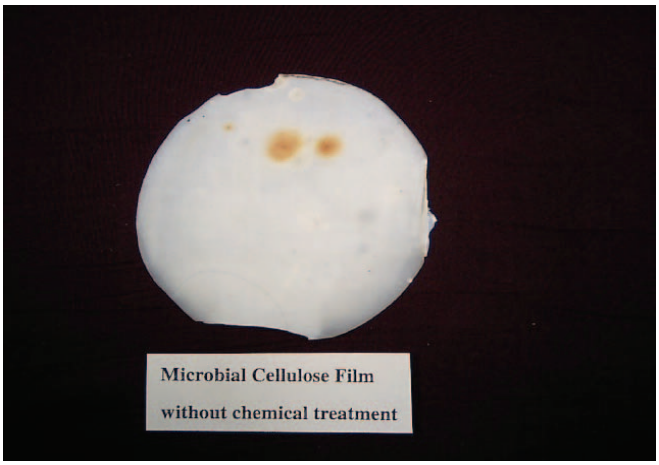

Figure 4. Photograph of membrane prepared without chemically treatment of MC pellicle

ISSN 1907-0322

Water absorption capability

The ability of materials to absorb water is one of the important parameter to be evaluated when the material intended to be used as biomaterials. To which extend the water absorption is needed highly depend on the site of application. For example, wound dressing membrane needs high water absorption capability to absorb the exudates and maintain humidity of membrane. Figure 5. shows water absorption of un-irradiated MC membrane at different immersion time and temperature. Rate of water uptake is fairly rapid in the early stage of immersion especially during the first 30 minutes then gradually increase up to 4 hours until finally reach an equilibrium condition. The water absorption of membrane does not increase anymore even after the membrane kept at room temperature for 2 months. Obviously water absorption of MC membrane is affected by temperature. Water absorption of cellulose showed an increase by increasing the immersion temperature. It can be seen that maximum water absorption of MC at 25°C, 37°C and 50°C were 110, 137 and 140 %, respectively.

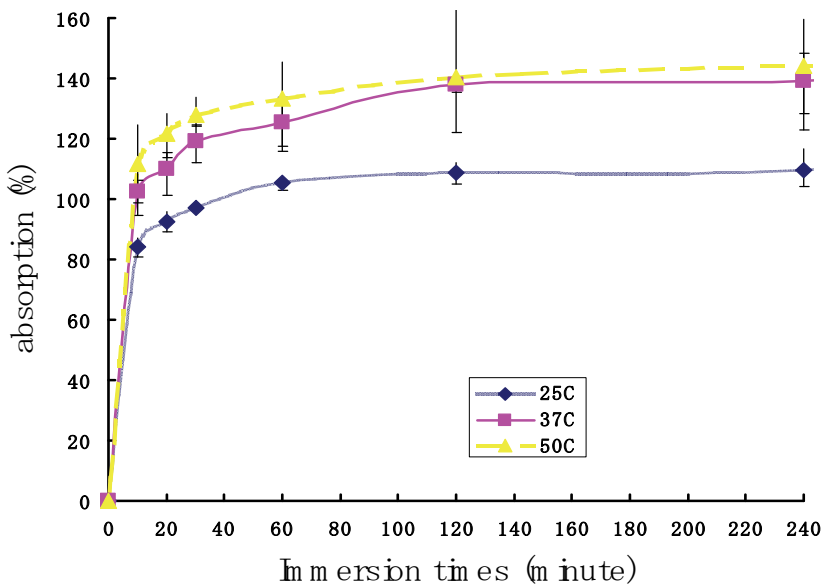

Figure 5. Water absorption profile of MC membranes at different temperatures

ISSN 1907-0322

Water absorption of cellulose membranes after gamma irradiation at 25 and 50 kGy is presented in Figure 6. The same tendency of water absorption profile was observed for irradiated membrane. Irradiation at 25 kGy did not give any significant reduction in water absorption but at 50 kGy, water absorption was reduced significantly as shown in Figure 6. The reduction in water absorption capability of MC is probably due to degradation of polymeric chain of cellulose during irradiation.

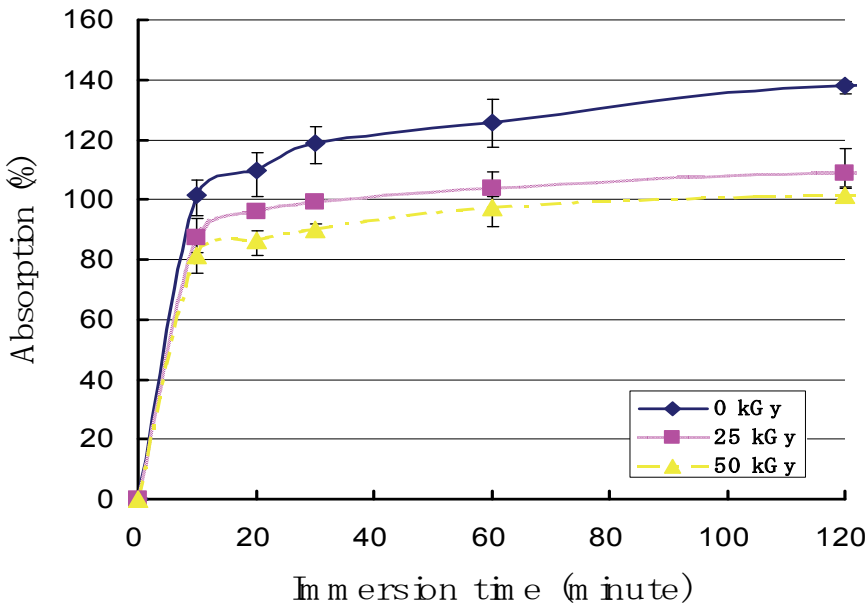

Figure 6. Water absorption of MC membranes at different irradiation doses

Surface morphology of MC membrane

Cellulose is synthesized by *Acetobacter* through a complex process and its 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. As a result of these processes, a three-dimensional, gelatinous structure is formed on the surface of a liquid medium [22]. Figure 7 presents SEM images of cellulose structures synthesized

ISSN 1907-0322

by strains of *Acetobacter xylinum*. This figure shows that MC membrane consists of inter-connected nano to micro porous structures with diameter ranging from 0,05 to 0,5 µm. Cross section image of MC membrane indicate a sandwich structures of cellulose fibres. The size of MC pores is far below the pore size of tissue (2 – 15 µm). From this result, it can be concluded that MC membrane is effective to prevent invasion of soft tissue to the graft site. Many studies 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 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 completed production processes. Microbial cellulose membranes can also be infused with other therapeutic compounds without causing alteration of its beneficial properties [22, 23, 24].

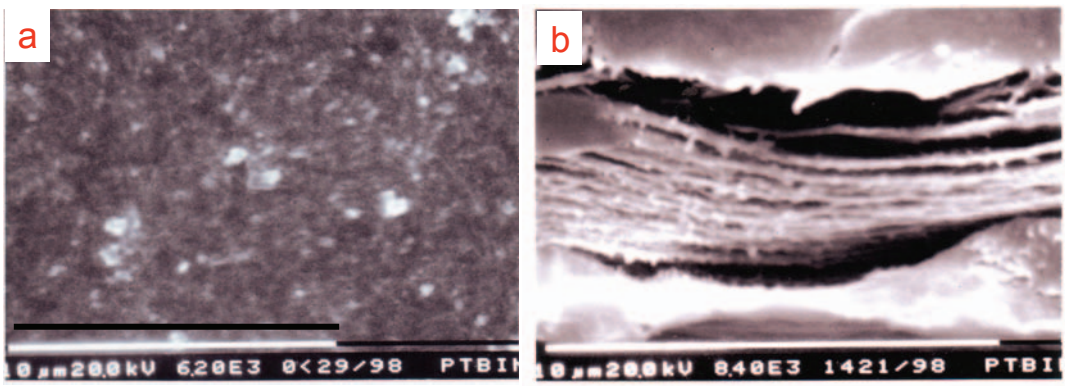

Figure 7. SEM micrograph of microbial cellulose membranes,
A. surface area (magnificataion = 6200),
B. crossection area (magnificataion = 8400)

Thermal stability of MC membrane

Thermogravimetric analysis was used to elucidate thermal properties of MC membrane. Figure 8 shows temperature decomposition profile of MC membrane. While thermal stability of MC membrane is presented in Figure 9. As can be seen in

ISSN 1907-0322

Figure 8, MC membrane (0 kGy) has sufficiently high decomposition temperature at around 328°C. This is due to strong inter-chain hydrogen bonds in the crystalline regions of cellulose, which prevent them from melting and give rise to high temperature decomposition [25,26]. Gamma irradiation with dose of 25 kGy and 50 kGy, respectively reduce the decomposition temperature to 317°C and 295°C, respectively, as shown in Figure 8. The decrease in decomposition temperature of irradiated MC membrane is probably cause by radiation degradation of cellulose networks resulting in reduction of the number of hydrogen bond. Thermal stability of materials at a certain temperature plays an important role when the material subjected to high temperature during processing. In this study, MC membrane was obtained by using hot press technique under 120°C for 10 minutes. Isothermal curve of CM membrane at 150°C and 200°C for 30 and 20 minutes respectively is shown in Figure 9. This result indicated that MC pellicle posses good heat stability over 200°C as indicated by 0% decomposition at 150°C and 200°C for 30 and 20 minutes aging, respectively. It also suggests that MC pellicle could be process at temperature arround 200°C without any decomposition.

Mechanical properties of microbial cellulose

Mechanical properties of microbial cellulose membranes are presented in Tabel 1. In this experiment, the thickness of membranes used was 0.04 ± 0.01 mm. It can be seen that, in dry state, MC had high tensile strength around 100 MPa and elongation at break around 2 %. This result implies that MC membranes is stiff and brittle and make it difficult to handle the samples. However, in wet state, the membrane became soft, pliable and elastics as indicated by increasing of elongation at break to around 10 %; and in the same time still maintains high tensile strength around 100 MPa. Characterization of mechanical properties of material in the wet state (after immersion in water at 37°C for a certain time) is very important for material which would be used as implant (especially GBR and GTR) since the material have to maintain their properties during the course of treatment. Immersion of MC membrane in water up to 2 months at 37°C did not give any significant differences in mechanical properties as

ISSN 1907-0322

shown in Table 1. This result implies that MC membrane will maintain its barrier function when used as membrane of GTR. In order to evaluate the effect of thickness of material on mechanical properties, tensile and elongation at break measurements of the membrane with thickness of around 0.2 mm were also carried out. The results show that the tensile strength of MC membrane in dry state is 50 ± 20 MPa and elongation at break is 10 ± 2%, while in wet state TS is 8 ± 1 MPa and Eb is 42 ± 5%. Gamma irradiation reduces mechanical properties of MC. At 25 kGy, there is no significantly reduction in tensile strength shown. However, the decrease in tensile strength is much higher at 50 kGy irradiation which is around 50 MPa. It has been reported that gamma irradiated cotton cellulose at dose ranging from 0 to 130 kGy under nitrogen or oxygen atmosphere resulted in degradation of cellulose to acetaldehyde, acetone, arabinose, desoxy saccharide, formaldehyde, formic acid, glucoronic and gluconic acid, glucose, malonaldehyde, oxalic acid and xylose. It is also reported that the presence of oxygen increased the yields of all radiolytical products [27]. In another study, Kasprrzykl at. al (28) reported that the degree of crystallinity of wood cellulose slightly decreases during the initial stage of gamma irradiation at the dose range of 20 – 120 kGy, whereas in higher doses (300 – 4500 kGy) a decrease of crystallinity is much greater reaching about 42% for 4500 kGy and it becomes zero at the dose of 9000 kGy. In their study [28] they conclude that the minimum dose for the decomposition of cellulose crystals is considered to be above the dose of 120 kGy.

It is understood that cellulose is very difficult to be degraded by body fluid. Therefore degradation of cellulose by radiation is desired because it will increase the biodegradation of MC membrane when it is used as scaffold. It can be estimated that biodegradable MC membrane can be prepared using irradiation technique. To look in more detail on degradability of irradiated MC membrane, it is adviceable to elucidate more experimental test using synthetic body fluid as a model study.

Commercial GTR membrane such as silicon membrane produced by Zao Medsil has mechanical properties such as: TS is not less than 7.0 MPa [29]. From tensile properties, the results of this study showed potential application in tissue engineering.

ISSN 1907-0322

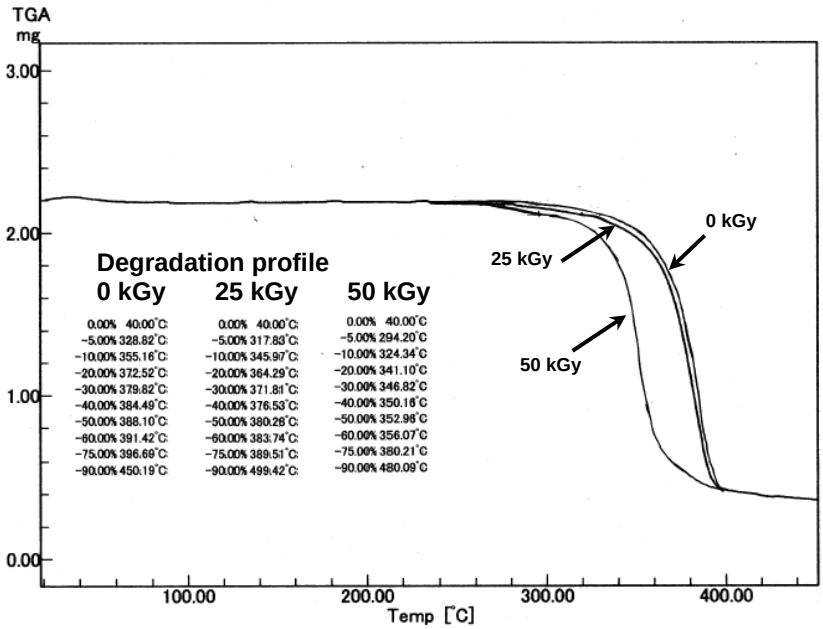

Figure 8. Thermo gravimetric curves of 0 kGy, 25 kGy and 50 kGy γ-irradiation MC membranes

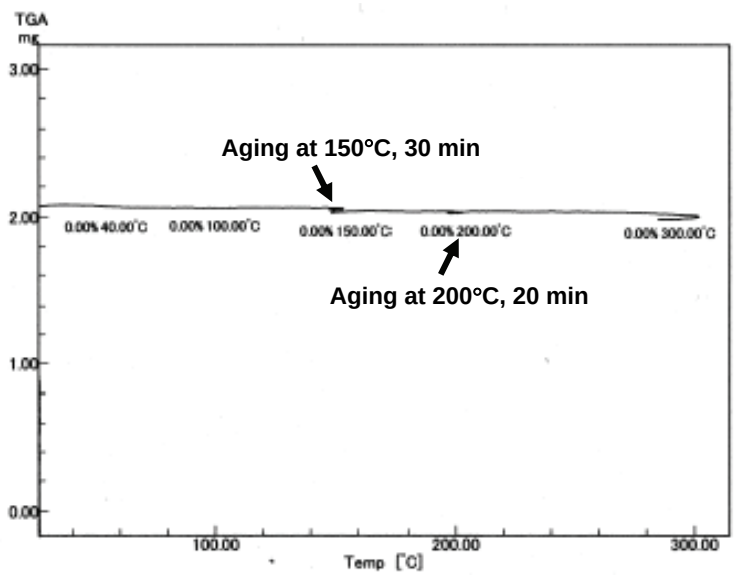

Figure 9. Isothermal curve of MC membrane at 150°C and 200°C for 30 and 20 minutes, respectively

ISSN 1907-0322

Table 1. Mechanical properties of microbial cellulose membranes

Sample condition	Tensile strength (MPa)			Elongation at break (%)		
	0 kGy	25 kGy	50 kGy	0 kGy	25 kGy	50 kGy
Dry state	102 ± 20	85 ± 23	51 ± 7	2.74 ± 0.7	2.0 ± 0.8	1.6 ± 0.5
Wet state: after immersed in water at 37C for						
10 min swelling	95 ± 20	87 ± 25	50 ± 17	11 ± 2.0	7.5 ± 3.0	7.0 ± 2.0
1 days swelling	100 ± 20	90 ± 15	54 ± 30	10.5 ± 5.0	9.8 ± 2.0	5.4 ± 1.7
30 days swelling	96 ± 23	81 ± 25	42 ± 5	11.4 ± 3.0	9.0 ± 2.0	5.1 ± 1.0
60 days swelling	79 ± 21	78 ± 15	46 ± 15	12.4 ± 2.5	10.2 ± 2.0	6.0 ± 2.0

CONCLUSSION

From the experimental results, it is can be concluded that:

1. Utilization of coconut water as micro nutrient source for the growth of bacteria *A. xylinum* at 30°C is effective to produce microbial cellulose pellicle
2. Chemically treatment of MC pellicles by NaOH and NaOCl was effective to remove the bacteria contaminant, bacterial cells embedded in the polymer net and endotoxin which occurred during cellulose production as well as produced whiten membrane
3. MC membrane consists of inter-connected nano to micro porous structures with diameter ranging from 0,05 to 0,5 μm. Cross section image of MC membrane indicate a sandwich structures of cellulose fibers. The size of MC pores is far below the pore size of tissue (2 – 15 μm). Therefore MC membrane is very effective to be used as GBR membrane to prevent invasion of soft tissue to the graft site
4. CM membrane has sufficiently high decomposition temperature at around 328°C. Irradiation up to 50 kGy reduces decomposition temperature of MC to be 295°C.
5. Gamma irradiation degrades polymeric structure of MC. This degradation is very promising because it will increase the biodegradation and bioresorption of MC membrane when it is used as scaffold

ISSN 1907-0322

REFERENCE

1. YOSHIYA U., *et al.*, Usefulness as guided bone regeneration membrane of the alginate membrane, *Biomaterials*, **23** (9), 2027-2033 (2002).

2. MINNA K., HENNA N., KATJA P., NUREDDIN A., TIMO W., PERTTI T., Bioabsorbable scaffolds for guided bone regeneration and generation, *Biomaterials*, **21**, 2495-2505 (2000).

3. HWEY-CHIN Y. and KUANG-WEY H., Guided bone regeneration for fenestration defects in dental implant, *Medical Journal,* **26** (9), 684-688 (2003).

4. MUZAFFER A., GOKSEL S., ERTUNC D., Guided bone regeneration (GBR) on healing bone defects: a Histological study in rabbits, *The Journal of Contemporary Dental Practice*, **5** (2), 1-7 (2004).

5. EDWARDO R.L., www.dentistry.com, diakses tanggal 5 April 2007.

6. GRANGER J.M., *et al.*, Microscopic analysis of rat subcutaneous implant of membrane barrier derived from demineralized cortical bovine bone, http://iadr.confex.com, diakses tanggal 4 Mei 2007.

7. DAVID B. R., Practice Limited to Periodontics & Dental Implants, http://www.periodont.com/bone.htm, diakses tanggal 4 Mei 2007.

8. Bone Grafting & Guided Bone Regeneration http://www.osteohealth.com/Patient Site GBR.html, diakses tanggal 8 Mei 2007.

9. FUJIHARA K., KOTAKI M., and RAMAKRISHNA S., Guide bone regeneration membrane made of polylactone/calcium carbonate composite nano-fibers, *Biomaterials*, **26**, 4139-4147 (2005).

10. LEE R.L., PAUL J.W., WILLIEM H., and ISAK S.P., Microbial cellulose utilization: Fundamentals and biotechnology, *Microbiology and Molecular Biology Reviews*, **66** (3), 506-577 (2002).

11. http://www.rest.titech.ac.jp/junkan/english/cellulose/index.html: Production of biocellulose (bacterial cellulose), diakses tanggal 8 Mei 2007.

12. THI THI NGE, Development of novel biostable/biodegradable biomimetic composites based on bacterial cellulose, Research Institute for Sustainable Humanosphere.

13. http://www.rpii.edu/weberd/althesis.doc, diakses tanggal 4 mei 2007.

ISSN 1907-0322

14. Water structure and behavior of Cellulose, www://http.isbu.ac.uk/water/hycel.htm, diakses tanggal 4 Mei 2007.

15. ALINA K., *et al.*, Molecular basis of cellulose biosynthesis disappearance in submerged culture of *Acetobacter xylinum*, *Acta Biochimica Polanica,* **52** (3), 691-698 (2005).

16. STANISLAW B., ALINA K., and MARIANA T., Bacterial cellulose, www.wiley-vch.de/books/biopolym, diakses tanggal 20 Mei 2007.

17. WHITE, D.G. and BROWN, R.M.JR., Prospects for the Commercialization of Biosynthesis of Microbial Cellulose, in Cellulose and Wood-Chemistry and Technology, Schuerech, C., Ed., John Wiley & Sons, New York, 573 (1989).

18. http://www.engga.uwo.ca/people/wwan/projects.htm WANKEI WAN, biomaterials, diakses tanggal 4 Mei 2007.

19. http://www.american.edu/projects/mandala/TED/coconut.htm, diakses tanggal 4 Mei 2007.

20. http://www.fao.org/inpho/content/compend/text/ch15.htm, diakses tanggal 4 mei 2007.

21. TITA PUSPITASARI, Studi Kopolimerisasi Cangkok Selulosa Microbial dengan Asam Akrilat dan Aplikasinya sebagai Membrane pada Proses Mikrofiltrasi, Master Thesis, Institute Teknologi Bandung (2006).

22. WOJCIECH K.C., http://pubs.acs.org/cgi-bin/asap.cgi/bomaf6/asap/html/-bm060620dAF2, DAVID J.Y., MAREK K. and R. MALCOLM B., JR., *Biomacromolecules*, **06** (9), 1529 (2006).

23. SUSAN O., MARY B., PETER T., and DEBBIE Y., Limb Salvage Using a Bioshintesized Cellulose Wound Dressing: A Case Study, Clinical Symposium on Advances Skin & Wound Care, Phoenix Civil Plaza, Phoenix, AZ (2004).

24. LEGEZA, V.I., GALENKO-YAROSHEVSKII, V.P., ZINOV'EV, E.V., PARAMONOV, B.A., KREICHMAN, G.S., TURKOVSKII, I.I., GUMENYUK, E.S., KARNOVICH, A.G., KHRIPUNOV, A.K., *Bull. Exp. Biol. Med.*, **138** (3), 311-315 (2004).

25. http://www.fiberssource.com, Cellulose, diakses tanggal 8 Mei 2007.

26. JAY, H.H., Why can we melt materials like wax but not materials like wood?, http://www.cellulose.com, diakses tanggal 4 Mei 2007.

ISSN 1907-0322

27. BLUDOVSKY, R., PROCHAZKA, M., and KOPOLDOVA, J., The influence of oxygen on the radiolytical products of Cellulose, *J. Radioanal. Nucl. Chem.*, Letter **87** (2), 69-80 (1984).

28. HENRY, K., KINGA, W., SLAWOMIR, B., The effect of gamma radiation on the supramolecular structure of pine wood cellulose in situ revealed by x-ray diffraction, *Electronic Journal of Polish Agricultural University*, **7** (1), (2004).

29. Silicone membrane for guided tissue regeneration, www.medsil.ru.html, diakses tanggal 4 Juni 2007.