In Vivo Biocompatibility and Biodegradation Test of Two Barrier Membranes for Guided Tissue Regeneration

Machiavelli Clavesia[1]
Stephani Dwiyanti[2]
Veronika Maria Sidharta[3]
Dyonesia Ary Harjanti[4]
Mora Octavia[2]
Eko Adi Prasetyanto[5]
Tena Djuartina[6]

[1] School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440
[2] Department of Dental Medicine, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440
[3] Department of Histology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440
[4] Department of Anatomical Pathology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440
[5] Department of Pharmacy, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440
[6] Department of Biomedical and Anatomy, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440

Corresponding author: Yohanes Eko Adi Prasetyanto
E-mail: prasetyanto@atmajaya.ac.id

Abstract

This study evaluates biocompatibility and biodegradation properties of PCL/Chitosan/BG-NP/Tetracycline and compares them with commercially available SureDerm membrane in Sprague Dawley Rats.Two different membranes (PCL/Chitosan/BG-NP/Tetracycline membrane and SureDerm membrane) were randomly inserted into subcutaneous pouches in the backs of 54 Sprague Dawley rats. The animals were sacrificed at day 7, 21, and 63. Biocompatibility properties (number and distribution of inflammatory cells, necrosis, neovascularisation, fibrosis, fatty infiltrate, tissue integration, tissue ingrowth) was assessed according to DIN EN ISO 10993-6. Biodegradation property was assessed macroscopically to check for membrane degradation rate. In terms of biocompatibility properties, the test membrane had overall irritancy score of 0 at day 7 and 21, and 0.66 at day 63, and it is considered non-irritant. Biodegradation of test membrane is faster compared to SureDerm at day 7 and 21. However, there is no significant difference between the two membranes at day 63. The GTR membrane PCL/Chitosan/BG-NP/Tetracycline is a good membrane with comparable biocompatibility and biodegradation properties to the SureDerm membrane.

Key words: Biocompatibility, Biodegradation, In Vivo, Polycaprolactone, Guided Tissue Regeneration

Introduction

Periodontitis is the main cause of tooth loss in adults. Research conducted in Jember Regency for the period January-December 2014, several reasons for tooth extraction were found, such as periodontitis (583 cases), caries (302 cases), impaction (58 cases), and persistence (1 case) [1]. Periodontitis was responsible for the largest number of extracted tooth in all sociodemographic groups [1].
Periodontitis affects many people throughout the world. According to the Ministry of Health of the Republic of Indonesia (Depkes RI), periodontitis ranks second in Indonesia [2].

Authors
Machiavelli Clavesia - School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440

Stephani Dwiyanti - Department of Dental Medicine, School of Medicine and Health Sciences,
Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440

Veronika Maria Sidharta - Department of Histology, School of Medicine and Health Sciences,
Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440

Dyonesia Ary Harjanti - Department of Anatomical Pathology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440

Mora Octavia - Department of Dental Medicine, School of Medicine and Health Sciences,
Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440

Eko Adi Prasetyanto - Department of Pharmacy, School of Medicine and Health Sciences,
Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440

Tena Djuartina - Department of Biomedical and Anatomy, School of Medicine and Health Sciences,
Atma Jaya Catholic University of Indonesia, Pluit Raya No. 2, North Jakarta, 14440

How to Cite
C Machiavelli, S Dwiyanti,VM Sidharta, D Ary Harjanti, M Octavia,
E Adi Prasetyanto, T Djuartina.
In Vivo Biocompatibility and Biodegradation Test of Two Barrier Membranes for Guided Tissue Regeneration
Annali Di Stomatologia, 15(1), 9-16.
https://doi.org/10.59987/ads/2024.1.9-16

Periodontitis is a serious infectious disease and if not treated properly it can result in tooth loss [3]. The buildup of plaque bacteria on the surface of the teeth is the main cause of periodontitis [3]. Plaque buildup initially causes gingivitis, which further develop into periodontitis resulting in tissue damage periodontal support in the form of damage to the fibers, periodontal ligament and alveolar bone. It can eventually cause mobility and tooth loss [3].

One of the surgical treatment of periodontitis is carried out using the Guided Tissue Regeneration (GTR) [4]. This GTR method uses a barrier membrane which prevent epithelial tissue invasion and ensure the growth of periodontal ligament cells in the defect area [4]. GTR membrane should have a good biocompatibility, mechanical strength, biodegradability and antibacterial properties. Barrier membranes are also useful for wound healing, isolation of gingival defects and clots stabilisation [5].

Numerous investigations have focused on the development of resorbable materials for GTR membranes, exemplified in the research conducted by Dikici et al [6]. This research used polycaprolactone (PCL) which show a good results because it has better biological properties than other polymers [6]. The PCL membrane also has good mechanical, biocompatibility properties, and slow degradation rate [6]. This supports the use PCL as one of the composition for GTR membranes. However, our research combines two polymers so that the hydrophobic nature of PCL can be overcome [6].

Chitosan is chosen as another polymer because it has good hydrophilic properties [7]. Chitosan also has antibacterial, anti-fungal, and wound healing properties [8]. Another component, Bioactive Glass Nanoparticles (BG-NP), is added to increase membrane stiffness. BG-NP also shows adequate extensibility in wet conditions and is osteoconductive [9].

An alternative way to increase the antibacterial effect of a membrane is with the help of antibiotics [10]. Several researchers have succeeded in combining tetracycline into a polymer solution to develop a membrane barrier [11].

Tetracycline is an effective bacteriostatic agent against many Gram-negative species including periodontopathogens such as *Aggregatibacter actinomycetemcomitans* [12]. Tetracycline acts as a collagenase inhibitor, has anti-inflammatory action, bone resorption inhibitor, and increases the attachment of fibroblasts to the root surface which increases periodontal tissue regeneration [12].

Based on the description above, we fabricate barrier membranes containing PCL/Chitosan/BG-NP/Tetracycline using electrospinning method. The objective of this research is to evaluate biocompatibility and biodegradation properties of PCL/Chitosan/BG-NP/Tetracycline and to compare them with commercially available SureDerm membrane in Sprague Dawley Rats.

Materials and Methods

Research Design

This is an in vivo experimental research using Sprague Dawley rats.

Ethical clearance for this research was granted prior to the commencement of the study by the Research Ethics Committee of the School of Medicine and Health Sciences Atma Jaya Catholic University of Indonesia. All rats were kept under standard conditions in a purpose-designed room for experimental animals. They were treated according to the Animals in Research: Reporting In Vivo Experiments guidelines for animal care, with free access to water and a standard diet.

Study Materials

In this research, there were 2 types of membranes used: test membranes with the composition PCL/Chitosan/BG-NP/Tetracycline and commercially available SureDerm as control membrane (Table 1).

For negative control, the sham group was used. There were 9 groups in this study, namely;

1. Positive Control 1: Rat inserted with SureDerm membrane, terminated at day 7.
2. Positive Control 2: Rat inserted with SureDerm membrane, terminated at day 21.
3. Positive Control 3: Rat inserted with SureDerm membrane, terminated at day 63.
4. Experimental Group 1: Rat inserted with PCL/Chitosan/BG-NP/Tetracycline membrane, terminated at day 7.
5. Experimental Group 2: Rat inserted with PCL/Chitosan/BG-NP/Tetracycline membrane, terminated at day 21.
6. Experimental Group 3: Rat inserted with PCL/Chitosan/BG-NP/Tetracycline membrane, terminated at day 63.
7. Negative Control 1: Sham group, no membrane inserted, but still undergo incision and suture, terminated at day 7.
8. Negative Control 2: Sham group, no membrane inserted, but still undergo incision and suture, terminated at day 21.
9. Negative Control 3: Sham group, no membrane inserted, but still undergo incision and suture, terminated at day 63.

Animals

Fifty-four Sprague Dawley rats (mass, *± 100-300* g) were used in this study. Each group contained six rats allocated to each of 3 observation time points (7, 21, and 63 days).

Table 1. Test and Control Article Descriptions

Function	Name	Description	Implant Sizes (mm x mm)
Test Membrane	PCL/Chitosan/BG-NP/ Tetracycline Membrane	PCL/Chitosan/BG-NP/ Tetracycline Membrane	10.0 x 10.0
Positive Control	SureDerm Membrane	Homologous acellular dermis and penicillin	10.0 x 10.0
Negative Control	Sham Operation	Without biomaterial insertion	10.0 x 10.0

10.59987/ads/2024.1.9-16

Biocompatibility properties were tested according to the DIN EN International Organization for Standardization (ISO) 10993-6 standard for investigating the effects of subcutaneous implantation on local tissues. Assessment of biodegradation was done with the help of 10x10 mm mica plastic, which was further divided into 9 small boxes. If the membrane covered <1/2 of the small box, a degradation value of 11.11% is given. If the membrane covered >1/2 of the small box, a degradation value of 0% was given.

Subcutaneous Implantation

The rat was anesthetized intramuscularly using a syringe containing a combination of ketamine [90 mg/kg] and xylazine [10 mg/kg]. The rat were then shaved and disinfected in the upper back area, and a transverse incision was made on the rat's backs with a scalpel. A 10x10 mm of GTR membrane was inserted into the back subcutaneously [13] (Figure 1). The incision was closed with standard suture material (Prolene 6.0) [13]. Betadine and gauze was applied on the wound. The rat was housed in separate cage until it was in stable condition, after which the rats were moved back into their respective cages [13].

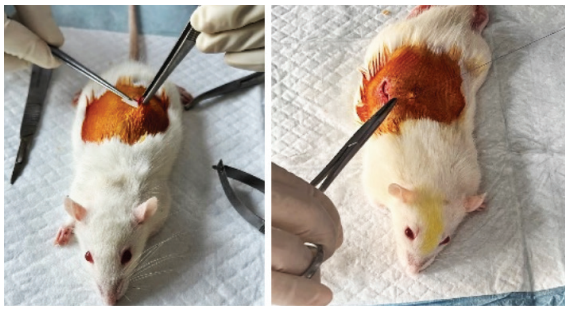

Figure 1. Membrane Implantation into the Subcutaneous Back of Rats

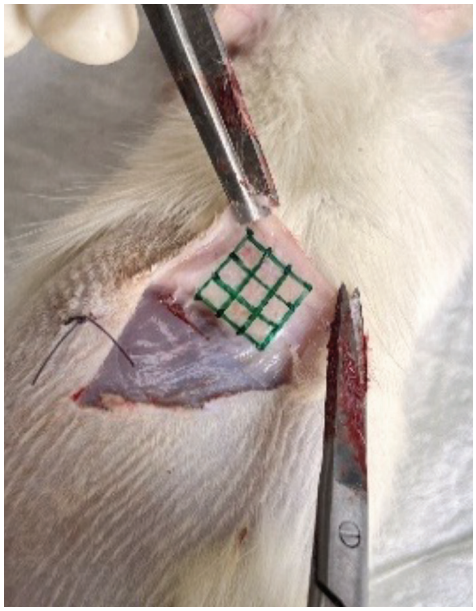

Figure 2. Macroscopic Aspects of Tissue Response to Different Membranes After Implantation

Explantation and Biodegradation Test

After trial periods of 7, 21, and 63 days, each group received an overdose of Ketamine/Xylazine for euthanasia. By adding a safety margin of approximately 5 mm on each side of the implanted membrane, a 20x20 mm sample was removed from the back of the rat using a scalpel, blade, and blunt scissors. Mica plastic was put on top of the biopsied sample to measure the degradation value of the membrane (Figure 2).

Fixation and Histopathological Staining

The sample was placed in a 10% Neutral Buffered Formalin solution for 24 hours for fixation and were then sent for histological processing [13,15]. After fixation, each sample was dehydrated in a series of alcohol solutions of increasing concentration and subsequently embedded in paraffin [15]. The samples were cut into 4 parts using a scalpel, after which they were further cut into 4 μm thick sections and stained with Hematoxylin and Eosin and Masson Trichrome for descriptive and semiquantitative histological evaluation [15].

Semiquantitative Histological Analysis According to DIN EN ISO 10993-6

Semiquantitative histological analysis was performed on each subcutaneous slide scanned according to the area of interest, without overlap, and captured at 400× magnification producing a score value indicating greater dominance among them. The response of tissue-membrane biological parameters is evaluated and assessed, as follows: (Table 2) [14].

1. The number and distribution of inflammatory cells semiquantitatively by looking at changes in neutrophils, lymphocytes, plasma cells, macrophages, and giant cells as a function of distance from the material/tissue [14].
2. Presence and extent of necrosis [14].
3. Inflammatory response parameters (neovascularization, capillaries with supporting fibroblastic structures, degree of fibrous capsule fibrosis stained with Masson Trichrome staining, and fat infiltration) [14].

Measurements were scored according to ISO 10993-6 guidelines: 0, none; 1, slight; 2, moderate; 3, marked; 4, complete/severe [15].Neovascularization was scored according to capillaries present: 0, none; 1, minimal capillary proliferation; 2, groups of 4–7 capillaries ; 3, broad band of capillaries; 4, an extensive band of capillaries [15]. Inflammation is descriptively assessed based on the number of macrophages, polymorphonuclear cells, lymphocytes, plasma cells, and giant cells present [15]. Graded using the following scoring system: 1, Rare, 1-5/phf; 2, 6-10/phf; 3. heavy infiltrate; 4, packed [15]. Necrosis was evaluated, with the following grading system: 1, minimal; 2, mild; 3. moderate; 4, marked [15]. Fibrosis was evaluated, with the following grading system 1, narrow band; 2, moderately thick band; 3, thick band; 4, extensive band [15]. Finally, fatty infiltrate is assessed using the following scoring system 1, minimum amount of fat; 2, several layers of fat; 3, elongated and widespread accumulation of fat cells; 4, extensive fat completely [15]. Irritation score is obtained by adding up scores of PMN cells, lymphocytes, plasma cells, macrophages, giant cells, necrosis which will then multiplied by two, then

adding up with scores of neovascularization, fibrosis, and fatty infiltrate.

The overall irritancy score of the test article at each study time point was calculated as follows: Overall irritancy score = test membrane irritancy score (PCL/Chitosan/BG-NP/Tetracycline membrane) – average irritancy score of control membrane (Surederm membrane) If the result was a negative number, the irritancy score was considered to be 0.0. The irritancy grade was then determined according to Table 3.

Results

Biocompatibility Test

Data of the biocompatibility test in the form of mean histological evaluation for irritation/reactivity-cell type/respone according to ISO 10993-6 for day 7,21, and 63 is presented in Table 4,5, and 6. [15]. Lymphocyte cells, plasma cells, and macrophage cells were found on the SureDerm membrane and PCL/Chitosan/BG-NP/Tetracycline membrane on day 7 and day 21 (Table

Table 2. Histological Evaluation System for Irritation/Reactivity – Cell Type/Response [13]

Respone	Score (phf = Per High Powered (x400) Field)				
	0	1	2	3	4
PMN cells	0	Rare, 1-5/phf	6-10/phf	Heavy infiltrate	Packed
Lymphocytes	0	Rare, 1-5/phf	6-10/phf	Heavy infiltrate	Packed
Plasma cells	0	Rare, 1-5/phf	6-10/phf	Heavy infiltrate	Packed
Macrophages	0	Rare, 1-5/phf	6-10/phf	Heavy infiltrate	Packed
Giant cells	0	Rare, 1-2/phf	3-5/phf	Heavy infiltrate	Packed
Necrosis	0	Minimal	Mild	Moderate	Packed
Neovascularization	0	Minimal capillary proliferation focal, 1-3 buds	Groups of 4-7 capillaries with supporting fibroblastic	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic
Fibrocytes/ fibroconnective tissue, fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the implant site	Extensive fat surrounding the implant

Table 3. Irritancy/Reactivity Grade. Adapted from DIN EN ISO 10993-6 [13]

Overall Irritancy Score	Irritancy/Reactivity Status
0.0 to 2.9	Minimal or no reaction (non irritant)
3.0 to 8.9	Slight reaction (slight irritant)
9.0 to 15.0	Moderate reaction (moderate irritant)
>15.1	Severe reaction (severe irritant)

- Irritation Score: (PMN Cells + Lymphocytes + Plasma Cells + Macrophages + Giant Cells + Necrosis) x 2 + (Neovascularization + Fibrosis + Fatty Infiltrate)
- Overall Irritation Score: PCL/Chitosan/BG-NP/Tetracycline Membrane Irritation Score - SureDerm Membrane Irritation Score

Table 4. Mean Histological Evaluation for Irritation / Reactivity – Cell Type / Response According to ISO 10993-6 Guidelines for Day 7

	PMN Cell	Lympho-cytes	Plasma Cell	Macro-phages	Giant Cells	Ne-crosis	Neovas-cularisa-tion	Fibrosis	Fatty Infiltrate	Tissue Integra-tion	Tissue In-growth
SureDerm Membrane	0	0.5 ± 0.84	0.67 ± 1.21	0	0	0	0	0.5 ± 0.84	0	0	0
Negative Control	0	0	0	0	0	0	0	0.33 ± 0.52	0	0	0
PCL/ Chitosan/ BG-NP/ Tetracycline membrane	0	0.33 ± 0.52	0	0.33 ± 0.52	0	0	0	1.17± 1.33	0	0	0

10.59987/ads/2024.1.9-16

4 & 5). On the other hand, there were no cells found on day 7, day 21, and day 63 for the negative control (Table 4, 5, 6).
The PCL/Chitosan/BG-NP/Tetracycline membrane has a comparable irritation score as SureDerm membrane and irritant status of non irritant on day 7, day 21, and day 63 (Table 7). Exemplary histological images stained with Hematoxylin and Eosin as well as Masson Trichrome of the three groups at day 7, 21, and 63 can be seen in Figure 3 and 4.

Table 5. Mean Histological Evaluation for Irritation / Reactivity – Cell Type / Response According to ISO 10993-6 Guidelines for Day 21

	PMN Cell	Lympho-cytes	Plasma Cell	Macro-phages	Giant Cells	Ne-crosis	Neovas-cularisa-tion	Fibrosis	Fatty Infiltrate	Tissue Integra-tion	Tissue In-growth
SureDerm Membrane	0	1± 1.27	0.5 ± 0.84	0.33 ± 0.82	0	0	0	3± 1.10	0	0	0
Negative Control	0	0	0	0	0	0	0	2.67 ± 1.51	0	0	0
PCL/ Chitosan/ BG-NP/ Tetracycline membrane	0	0.5 ± 0.55	0	0.5 ± 0.55	0	0	0	2.33 ± 1.37	0	0	0

Table 6. Mean Histological Evaluation for Irritation / Reactivity – Cell Type / Response According to ISO 10993-6 Guidelines for Day 63

	PMN Cell	Lympho-cytes	Plasma Cell	Macro-phages	Giant Cells	Ne-crosis	Neovas-cularisa-tion	Fibrosis	Fatty Infiltrate	Tissue Integra-tion	Tissue In-growth
SureDerm Membrane	0	0	0	0	0	0	0	1.67 ± 1.86	0	0	0
Negative Control	0	0	0	0	0	0	0	1.67 ± 0.82	0	0	0
PCL/ Chitosan/ BG-NP/ Tetracycline membrane	0	0	0	0	0	0	0	2.33 ± 1.03	0	0	0

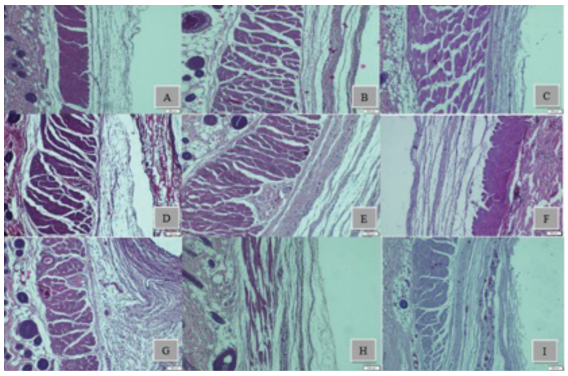

Figure 3. Exemplary Histological Images Stained with Hematoxylin and Eosin of the Three Groups at Day 7, 21, and 63.

A. Negative Control, Day 7; B. Negative Control, Day 21; C. Negative Control, Day 63; D. SureDerm Membrane, Day 7; E. SureDerm Membrane, Day 21; F. SureDerm Membrane, Day 63; G. PCL/Chitosan/BG-NP/Tetracycline Membrane, Day 7, H. PCL/Chitosan/BG-NP/Tetracycline Membrane, Day 21; I. PCL/Chitosan/BG-NP/Tetracycline Membrane, Day 63

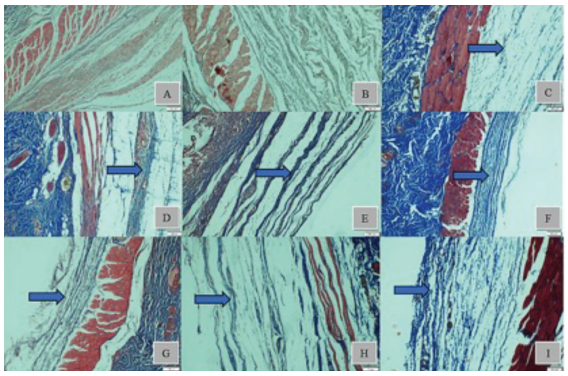

Figure 4. Exemplary Histological Images Stained with Masson Trichrome for Fibrosis Evaluation of the Three Groups at Day 7, 21, and 63

A. Scoring 0, Negative Control, Day 7; B. Scoring 0, Negative Control, Day 21; C. Scoring 1, Negative Control, Day 63; D. Scoring 2, SureDerm Membrane, Day 7; E. Scoring 3, SureDerm Membrane, Day 21; F. Scoring 4, SureDerm Membrane, Day 63; G. Scoring 3, PCL/Chitosan/BG-NP/Tetracycline Membrane, Day 7, H. Scoring 2, PCL/Chitosan/BG-NP/Tetracycline Membrane, Day 21; I. Scoring 3, PCL/Chitosan/BG-NP/Tetracycline Membrane, Day 63

Biodegradation Test

Based on the results of biodegradation tests, it can be seen that the commercial SureDerm membrane was only slightly degraded on day 7 (27.5 ± 6.12%) and day 21 (28.33 ± 14.02%), while the PCL/Chitosan/BG-NP/Tetracycline membrane has greater degradation on day 7 (50 ± 6.32%) and day 21 (50 ± 6.32%) (Table 7).

The percentage degradation test of SureDerm membrane and PCL/Chitosan/BG-NP/Tetracycline membrane was compared using independent t-test for each timeline. It was found that percentage degradation of the two membranes were statistically significant for day 7 and day 21 in which PCL/Chitosan/BG-NP/Tetracycline membrane was degraded more quickly, but not significant for day 63.

Rate of degradation of the two membranes was faster in the first 21 days, after which the degradation became more slowly (Figure 5).

Discussion

Biocompatibility Test

The GTR membrane has several criteria or requirements to be called an ideal membrane. The first requirement is to have biocompatibility properties [16]. Biocompatibility is the ability of the material to adapt to the environment where the membrane must not harm the body and have non-toxic properties [16]. The membrane must not trigger the host's immune system, sensitization, or chronic inflammatory reactions [17]. Based on the results of the biocompatibility test, it can be seen that the GTR membrane with the composition (g) PCL/Chitosan/BG-NP/Tetracycline = 11/0.5/0.5/0.04 has comparable irritancy score as the SureDerm commercial membrane and the irritancy score overall non-irritant or non-irritating.

This testing is in line with the research of Osathanon et al [18]. Osathanon et al's research discusses the biological basis of GTR membranes in periodontal tissue healing

Table 7. Mean Irritation Score After Membrane Implantation Day 7, Day 21, Day 63

	Study Group	Irritation Score	Overall Irritation Score	Irritation Status
Day 7	SureDerm Membrane	2.84 ± 0.87	0	Non Irritant
	Negative Control	0.33 ± 0.39		
	PCL/Chitosan/BG-NP/Tetracycline membrane	2.49 ± 0.60		
Day 21	SureDerm Membrane	6.66 ± 0.87	0	Non Irritant
	Negative Control	2.67 ± 0.39		
	PCL/Chitosan/BG-NP/Tetracycline membrane	4.33 ± 0.60		
Day 63	SureDerm Membrane	1.67 ± 0.87	0.66	Non Irritant
	Negative Control	1.67 ± 0.39		
	PCL/Chitosan/BG-NP/Tetracycline membrane	2.33 ± 0.60		

Irritation Score: (PMN Cells + Lymphocytes + Plasma Cells + Macrophages + Giant Cells + Necrosis) x 2 + (Neovascularization + Fibrosis + Fatty Infiltrate)

- Overall Irritation Score: PCL/Chitosan/BG-NP/Tetracycline Membrane Irritation Score - SureDerm Membrane Irritation Score

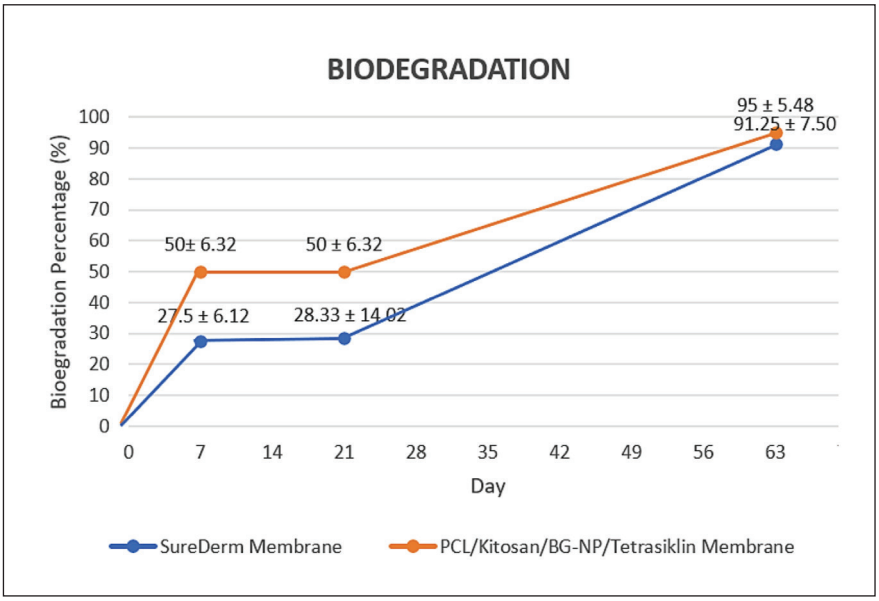

Figure 5. Biodegradation Test Chart

10.59987/ads/2024.1.9-16

Table 8. Mean Biodegradation Test Data Post Membrane Implantation Day 7, Day 21, Day 63

Sample	Biodegradation Percentage (%)			
	Day 0	Day 7	Day 21	Day 63
SureDerm Membrane	0	27.5 ± 6.12	28.33 ± 14.02	91.25 ± 7.50
PCL/Chitosan/BG-NP/Tetracycline membrane	0	50 ± 6.32	50 ± 6.32	95 ± 5.48
P-value	-	<0.01**	<0.05*	>0.05 ns

Note: ns: Non-Significant (p-value >0.05); * : Significant (p-value <0.05); ** : Very Significant (p-value <0.01)

and regeneration [18]. Bioactive GTR membranes have been investigated and developed with the aim of creating membranes that not only act as a physical barrier but also induce biologics to enhance periodontal tissue regeneration [18]. PCL has been introduced as a candidate material for bioactive GTR membranes due to its biocompatibility and simple fabrication procedures [18]. Modification with other agents or biomolecules can be easily made [18]. PCL can be useful for promoting periodontal tissue formation [18].

Sarasam et al used a PCL/chitosan membrane which improves mechanical properties and cell viability compared to pure chitosan [19]. Chitosan is a polysaccharide that is much sought after in biomedical applications and has been mixed with various macromolecules to reduce undesirable properties [19]. Dissolved chitosan and PCL homogeneously in various mass ratios in a mixture of 77% acetic acid in water and processed into uniform membranes [19]. Dynamic mechanical and thermal analysis shows that the crystallinity of PCL is suppressed and its storage modulus is increased by the addition of chitosan [19].

Another component of the membrane, bioactive glass is a promising material for tissue regeneration due to its controlled degradability and ability to stimulate the formation of new tissue [20]. Bioactive glass has demonstrated excellent bioactivity and biocompatibility when implanted in bone defects [9]. Bioactive glass degradation promotes osteogenesis by stimulating ions. stimulates osteoconductivity [20]. PCL (polycaprolactone) which is hydrophobic in nature was mixed with chitosan which has hydrophilic properties [20]. PCL, known for its robust mechanical strength, can contribute to enhancing the mechanical properties of chitosan, which tends to be inherently brittle [20]. A mixture of the two materials shows good biocompatibility with each other because PCL's low melting point makes it easier to mix the two polymers [19].

Biodegradation Test

Based on the biodegradation test results listed in Table 7, it can be seen that all samples were not completely degraded on days 7 and 21 therefore they act as good barrier and allow sufficient rate of periodontal tissue formation of 4-6 weeks [22]. The addition of BG-NP increase the stiffness of the membrane so that it does not decompose or degrade easily. BG-NPs caused a significant increase in membrane thickness and surface area [21]. Samples on day 63 was completely degraded and invisible to the naked eye. This shows that the test membrane has a degradation capability that meets the ideal criteria of a GTR membrane, but further observations need to be made to determine the time needed for all samples to be completely degraded.

Compared to SureDerm membrane, the test membrane is thinner. The SureDerm commercial membrane has a thickness of 0.26 mm [23]. The test membrane has a thickness of 0.13 ± 0.03 mm [23]. A thicker membrane will have stronger mechanical strength than a thin one. Therefore SureDerm membrane degrade less rapidly due to its thicker thickness compared to the PCL/Chitosan/BG-NP/Tetracycline coated GTR membrane.

Research Limitations

The research has limitations. This research model, using a subcutaneous pouch of rats does not fully reflect the conditions of the oral cavity and the oral microbiome, so there could be variation in biocompatibility and biodegradation properties.

The subcutaneous model is also less representative of the actual condition of GTR, in which the membrane should be placed on a bone defect that has been filled with bone graft, it is recommended that futher research can be carried out on more representative model, such as in bone defect on larger animals such as monkeys.

Conclusion

The GTR membrane PCL/Chitosan/BG-NP/Tetracycline composition (g) 11/0.5/0.5/0.04 is ideal as a GTR membrane because it has a good biocompatibility properties. The PCL/Chitosan/BG-NP/Tetracycline membrane has a comparable irritation score as SureDerm membrane and irritant status of non irritant. The PCL/Chitosan/BG-NP/Tetracycline membrane also has good biodegradation properties which allows sufficient time for periodontal tissue formation. However, the degradation rate is faster compared to SureDerm membrane.

References

1. Fithri Z, Rochim A, Cholid Z. Distribusi pencabutan gigi berdasarkan karakteristik sosiodemografi pada pasien RSGM universitas jember periode januari-desember 2014 (Distribution of tooth extraction based on sociodemographic characteristic of dental hospital of university of jember Pat. Pustaka Kesehatan. 2017 Aug 11;5(1):177–84.
2. Badan Penelitian dan Pengembangan Kesehatan -. Laporan Nasional Riskesdas 2018 [Internet]. Jakarta: Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan; 2020. Available from: https://repository.badankebijakan.kemkes.go.id/id/eprint/3514/
3. American Academy of Periodontology. The American Academy of Periodontology statement regarding gingival curettage. J Periodontol. 2002 Oct;73(10):1229–30.

4. Ziccardi VB, Buchbinder D. Guided tissue regeneration in dentistry. N Y State Dent J. 1996 Dec;62(10):48–51.
5. Bottino MC, Thomas V, Schmidt G, Vohra YK, Chu TMG, Kowolik MJ, et al. Recent advances in the development of GTR/GBR membranes for periodontal regeneration—A materials perspective. Dental Materials. 2012 Jul;28(7):703 21.
6. Aldemir Dikici B, Dikici S, Reilly GC, MacNeil S, Claeyssens F. A novel bilayer polycaprolactone membrane for guided bone regeneration: combining electrospinning and emulsion templating. Materials. 2019 Aug 20;12(16):2643.
7. Zhang L, Dong Y, Zhang N, Shi J, Zhang X, Qi C, et al. Potentials of sandwich-like chitosan/polycaprolactone/gelatin scaffolds for guided tissue regeneration membrane. Materials Science and Engineering: C. 2020 Apr;109:110618.
8. Xu C, Lei C, Meng L, Wang C, Song Y. Chitosan as a barrier membrane material in periodontal tissue regeneration. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2012 Jul;100(5):1435–43.
9. Mota J, Yu N, Caridade SG, Luz GM, Gomes ME, Reis RL, et al. Chitosan/bioactive glass nanoparticle composite membranes for periodontal regeneration. Acta Biomaterialia. 2012 Nov;8(11):4173–80.
10. Yilmaz Atay H. Antibacterial activity of chitosan-based systems. In: Jana S, Jana S, editors. Functional Chitosan. Singapore: Springer Singapore; 2019;457–89.
11. Mad Jin R, Sultana N, Baba S, Hamdan S, Ismail AF. Porous PCL/chitosan and nHA/PCL/chitosan scaffolds for tissue engineering applications: fabrication and evaluation. Journal of Nanomaterials. 2015;2015:1–8.
12. Seymour RA, Heasman PA. Tetracyclines in the management of periodontal diseases. Journal of Clinical Periodontology. 1995;22(1):22–35.
13. Lindner C, Alkildani S, Stojanovic S, Najman S, Jung O, Barbeck M. In vivo biocompatibility analysis of a novel barrier membrane based on bovine dermis-derived collagen for guided bone regeneration (GBR). Membranes. 2022 Mar;12(4):378.
14. Neto AM, Sartoretto SC, Duarte IM, Resende RF, Alves AT, Mourao CF, et al. In vivo comparative evaluation of biocompatibility and biodegradation of bovine and porcine collagen membranes. Membranes. 2020 Dec;10(12):423.
15. Naenni N, Lim HC, Strauss FJ, Jung RE, Hammerle CH, Thoma DS. Local tissue effects of various barrier membranes in a rat subcutaneous model. Journal of Periodontal; Implant Science. 2020 Oct;50(5):327.
16. Matyjaszewski K, Möller M, editors. Polymer science: a comprehensive reference. Amsterdam: Elsevier; 2012.
17. Sam G, BRM P. Evolution of barrier membranes in periodontal regeneration-"Are the third generation membranes really here?". Journal of Clinical and Diagnostic Research. 2014 Dec 5;8:12.
18. Osathanon T, Chanjavanakul P, Kongdecha P, Clayhan P, Huynh NC-N. Polycaprolactone-based biomaterials for guided tissue regeneration membrane. Periodontitis - A Useful Reference. 2017
19. Sarasam AR, Krishnaswamy RK, Madihally SV. Blending chitosan with polycaprolactone: Effects on physicochemical and antibacterial properties. Biomacromolecules. 2006 Apr;7(4):1131–8.
20. Pajares-Chamorro N, Chatzistavrou X. Bioactive glass nanoparticles for tissue regeneration. ACS Omega. 2020 Jun 9;5(22):12716–26.
21. Lin S, Ionescu C, Pike KJ, Smith ME, Jones JR. Nanostructure evolution and calcium distribution in sol–gel derived bioactive glass. J Mater Chem. 2009;19(9):1276.
22. Fraser D, Caton J, Benoit DSW. Periodontal wound healing and regeneration: insights for engineering new therapeutic approaches. Frontiers in Dental Medicine. 2022 Mar 2;3:815810.
23. Kandelousi PS, Rabiee SM, Jahanshahi M, Nasiri F. The effect of bioactive glass nanoparticles on polycaprolactone/chitosan scaffold: Melting enthalpy and cell viability. Journal of Bioactive and Compatible Polymers. 2019 Jan;34(1):97–111.

10.59987/ads/2024.1.9-16