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Comparison of the biodegradability in the membranes for the guided bone regeneration: preliminary study

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Abstract

In this study, 4 different types of GBR membrane were undergone for bio-degradability test; Silk mat from silkworm cocoon (TDI), silk mat from flatwise-spun (FS), collagen membrane (OssGuide), and dPTFE membrane (Cytoplast). Each material was segmented in 10.00 mm length and 0.3 mm thickness. The samples were put into the normal saline at 37°C for 2 weeks. After that, yield strength and tensile strain were measured and compared them with those of before treatment. The morphology of the membranes was observed by SEM. Tensile strain of FS was significantly increased at 2 weeks' normal saline treatment (P=0.018). When compared to OssGuide, TDI and FS showed significantly higher tensile strain at 2 weeks' normal saline treatment (P<0.05). In the SEM images, there were no significant changes in Cytoplast, TDI, and FS after 2 weeks' treatment. However, OssGuide showed damaged surface after 2 weeks' treatment. In conclusion, both TDI and FS did not have any evidence of biodegradability at 2 weeks' observation in normal saline treatment. However, OssGuide showed more than 20 % decrease in yield strength and tensile strain.

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Introduction

The main function of the membrane for the guided bone regeneration (GBR) is a barrier between the bony defect and the soft tissue (Yoo *et al.*, 2016). It maintains the space for bony regeneration. Accordingly, it should not be degraded before bone regeneration. However, the membrane for GBR has been classified as bio-degradable and non-bio-degradable (Kim *et al.*, 2016). Considering the requirement for GBR, even bio-degradable membrane should not be degraded rapidly. As bony regeneration is started in 3 to 4 weeks after operation, bio-degradable membrane should not be degraded within 4 weeks postoperatively (Kim *et al*., 2019a).

In case of non-bio-degradable membrane, the required velocity of bio-degradation has not been announced. As 6 weeks post-operatively is usually recommended for its removal (Karapataki *et al*., 2000), it should not be degraded within 6 weeks post-operatively. PTFE is representative material for non-bio-degradable GBR membrane (Ha *et al.*, 2014). PTFE is not degraded in the body. Silk suture is classified as non-bio-degradable in many countries (Cao and Wang, 2009). Silk suture is mainly composed of silk fibroin protein (Cao and

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Wang, 2009). If GBR membrane is made of silk fibroin only, it should be considered as the different application of the same material. Thus, silk fibroin based membrane should be classified as non-bio-degradable membrane. Silk mat is defined as the mat from natural silk without degumming process (Kweon *et al.*, 2017). When silk mat is produced from silkworm cocoon, the composition of the silk fibroin is approximately 80% (Mondal *et al.*, 2007). Thus, silk mat can be considered as non-bio-degradable. However, silk sericin is approximately 20% in silk mat and it is hydrophilic and considered as bio-degradable (Jo *et al.*, 2017). Accordingly, the classification for silk mat is confusing.

Food and Drug Administration (FDA) of United States provides definite protocol for the assessing bio-degradable materials (FDA, 1996). The tested materials should be dipped into the normal saline or phosphate buffered saline at 37°C for 2 weeks. And then, tensile strength is measured and compared to untreated control. If the materials lose their strength more than 20 % of its untreated control at the specifically required time, these materials can be considered as bio-degradable for the specified purpose. Compared to the implantation experiments in the animal model, this test is simple and more reliable (FDA, 1996).

In this study, 4 different types of GBR membrane were undergone for bio-degradability test. Silk mat from silkworm cocoon (TDI, Spencer biomedical technology, Seoul, Korea), silk mat from flatwise-spun (FS), collagen membrane (OssGuide, SK bioland, Cheongju, Korea) and dPTFE membrane (Cytoplast, Osteogenics biomedical, Lubbock, US). Collagen membrane has been classified as bio-degradable and dPTFE as non-biodegradable. Both commercialized membranes could be used as a positive control in each group. The sericin content is different to the layers of silkworm cocoon (Zhang *et al.*, 2015). It is increased from the innermost to the outermost. In this study, TDI membrane was used for the silk mat from the middle layer of the silkworm cocoon (Kim *et al.*, 2019b). FS has higher content of sericin than that of silkworm cocoon (data not shown). Thus, the effect of different sericin content on the biodegradability of silk mat could be demonstrated in this study.

Materials and methods

In vitro degradation test

Cytoplast, FS, TDI and OssGuide were tested materials. Cytoplast is an undegradable material as negative control and OssGuide is a degradable material as positive control. Each group had 5 samples before treatment and 5 for after treatment. The samples were put into the normal saline at 37°C for 2 weeks.

Each material was segmented in 10.00mm length and 0.3mm thickness. And yield strength and tensile strain were measured by using Instron 2716 testing frame (Instron, Norwood, MA) in Korea Institute of Industrial Technology (KITECH) under 23.5 °C and 50% humidity.

Scanning electron microscopic (SEM)

The morphology of the separated layers was observed by SEM. The images were captured by the SEM (Hitachi, SU-70, Japan) at 5 keV. Images in 100 and 200 magnifications, before hydration and after hydration states were captured.

Statistical analysis

SPSS 21.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Mean and standard deviation for each material yield strength and tensile strain before and after treatment were calculated. The comparison of the same materials between before and after treatment was done by the independent samples' t-test. One-way analysis of variance was done to compare materials' yield strength and tensile strain each other. As post hoc test, Bonferroni's test was used. The significance level was set as $P<0.05$.

Results

The summary of strength measurements was shown in Table 1. Yield strength and tensile strain for Cytoplast were 8.76 ± 0.77 N and 476.64 ± 14.77 % before treatment. They were not shown significant change after treatment. Yield strength and tensile strain were 9.95 ± 0.66 N and 440.91 ± 107.66 % after treatment. Yield strength of FS was similar between before and after treatment. They were 16.46 ± 2.91 N and 17.61 ± 5.24 N at before and after treatment, respectively ($P>0.05$). However, tensile strain was significantly increased after treatment. They were 12.30 ± 4.30 % and 19.52 ± 3.37 % at before and after treatment, respectively ($P=0.018$). Similar trends were shown in

Table 1. Mean and standard deviation for tensile strength and tensile strain for cystoplast, flatwise-spun (FS), TDI, OssGuide before and after intervention.

	Yield strength (N)		Tensile strain (%)	
	Before	After	Before	After
Cytoplast	8.76 ± 0.77	9.95 ± 0.66	475.64 ± 14.77	440.91 ± 107.66
FS	16.46 ± 2.91	17.61 ± 5.24	12.30 ±4.30	19.52 ± 3.37
TDI	29.59 ± 15.96	30.10 ± 18.83	10.64 ± 5.68	15.20 ± 6.98
OssGuide	24.62 ± 7.39	18.56 ± 8.97	26.45 ± 12.37	13.88 ± 8.00

TDI. Yield strength was 29.59 ± 15.96 N and 30.10 ± 18.83 N at before and after treatment, respectively (P>0.05). However, tensile strain was 10.64 ± 5.83 % and 15.20 ± 6.98 % at before and after treatment, respectively (P>0.05). Interestingly, OssGuide showed decreased yield strength after treatment. They were 24.62 ± 7.39 N and 18.56 ± 8.97 N at before and after treatment, respectively. However, the difference was not statistically significant (P>0.05). Unlike both types of silk mats, OssGuide showed decreased tensile strain after treatment. They were 26.45 ± 12.37 % and 13.88 ± 8.00 % at before and after treatment, respectively (P>0.05).

Considering the baseline difference in the yield strength and tensile strain for each material, the changes between before and after were calculated as percentage (Fig. 1). The changes of yield strength were 107.0 ± 31.8 %, 101.7 ± 63.6 %, and 75.5 ± 36.4 % for flatwise spun silk mat, TDI, and OssGuide, respectively. When compared the difference among groups, there was no significant difference (P>0.05). The changes of tensile strain were 158.7 ± 27.4 %, 142.9 ± 65.6 %, and 52.5 ± 30.3 % for flatwise spun silk mat, TDI, and OssGuide, respectively. When compared the difference among groups, there was statistically significant difference (P=0.006). In post hoc test, the tensile strain of OssGuide was significantly lower than that of flatwise spun silk mat and TDI (P=0.010 and 0.021, respectively).

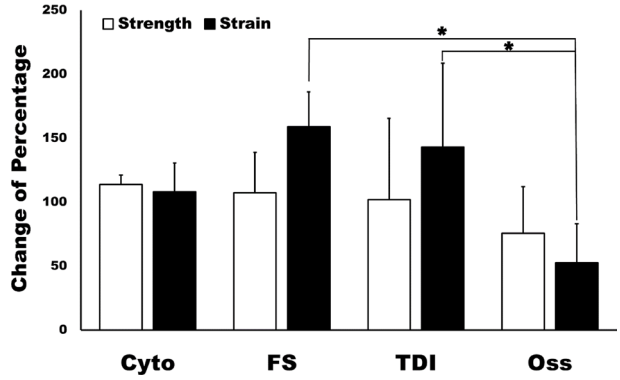

Fig. 1. The changes of yield strength and tensile strain as percentage compared to untreated control (Cyto: Cytoplast, FS: flatwise-spun silk mat, Oss: OssGuide). There was no significant difference in yield strength and tensile strain changes between FS and TDI. However, Oss group showed significantly lower tensile strain changes compared to those of FS and TDI groups (*P<0.05).

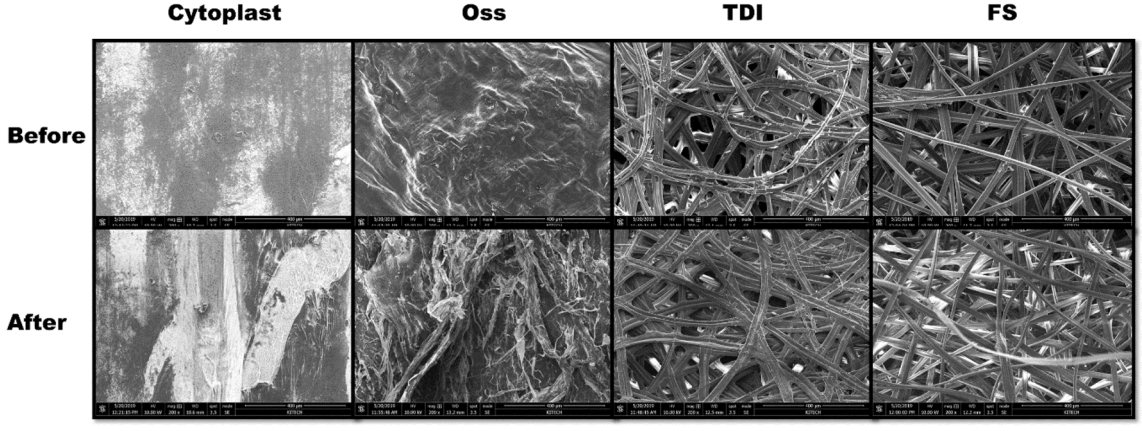

Fig. 2. SEM images before and after treatment. There were no significant changes in Cytoplast, TDI, and flatwise spun silk mat after 2 weeks' treatment. However, Oss showed damaged surface after 2 weeks' treatment (FS: flatwise-spun silk mat, Oss: OssGuide).

The SEM images before and after treatment were shown in Fig. 2. There were no significant changes in Cytoplast, TDI, and flatwise spun silk mat after 2 weeks' treatment. However, OssGuide showed damaged surface after 2 weeks' treatment.

Discussion

There has been no study for the biodegradability of silk mat. In this study, TDI and FS showed similar pattern of changes in the physical strength until 2 weeks' normal saline treatment (Table 1 and Fig. 1). There were no significant changes in yield strength at 2 weeks' normal saline treatment. However, OssGuide showed decreased yield strength and tensile strain at 2 weeks' normal saline treatment. OssGuide is a collagen membrane and classified as a bio-degradable membrane. When compared the change ratio among groups, OssGuide showed significantly lower tensile strain compared to TDI and FS ($P<0.05$, Fig. 1). Interestingly, the tensile strain was increased in both TDI and FS at 2 weeks' normal saline treatment.

There have been many methods for the evaluation of the biodegradable polymer (Jansen *et al.*, 1995; Sethuraman *et al.,* 2006; Kafedjiiski *et al.,* 2007). The evaluation method is grossly classified as *in vitro* method and *in vivo* method. In case of *in vivo* method, the material is usually grafted into the subcutaneous tissue and evaluated its biodegradability be measuring physical strength or residual weight (Jansen *et al*., 1995; Sethuraman *et al.,* 2006). The disadvantages of *in vivo* measurement are (1) unnecessary animal sacrifice, (2) large variation among individual animals, and (3) difficulty in interpretation of results for human application. Accordingly, *in vitro* method is recommended. For the evaluation of biodegradability, periodic measurements at specified time intervals are required until 104 weeks (FDA, 1996). When the strength has dropped below 20 % of the initial strength, this time is considered as the time for degradation (FDA, 1996). Required time for degradation may be different to the intended purpose of each biomaterial. To be classified as biodegradable suture, its strength should be dropped below 20 % at 2 weeks of normal saline treatment. Accordingly, this experiment was designed to measure at 2 weeks' treatment. OssGuide showed 20 % loss of yield strength and tensile strain at 2 weeks' treatment (Fig. 1). In case of non-biodegradable membrane for GBR, it should be removed at 6 weeks post-operatively (Karapataki *et al.*, 2000). Thus, it should not be degraded within 6 weeks post-operatively. To be classified as non-biodegradable membrane, 6 weeks' treatment might be required for silk mat.

Both TDI and FS are composed mainly of silk fibroin and silk sericin. However, the content of each component is different between TDI and FS (Kang *et al.,* 2019). Approximately 2-folds higher silk sericin is included in FS compared to TDI (Kang *et al.,* 2019). Considering silk sericin is mostly hydrophilic and fragmented in physiologic solution (Jo *et al.,* 2017), FS assumed to be degraded fast compared to TDI. However, there was no significant difference at 2 weeks' treatment. Interestingly, tensile strain was increased in both types of silk mats at 2 weeks' treatment (Fig. 1). In previous study, silk mat in wetting state showed higher tensile strain compared to dry state (Ha *et al.,* 2014). Thus, increased tensile strain might be associated with sericin in silk mat. Though there was no difference between TDI and FS, FS showed higher average value of tensile strain than that of TDI (Fig. 1).

Compared to Cytoplast, the standard deviation of the other 3 groups was big. As collagen and silk mat are originated from natural source, there may be a variation in compositions for each membrane (Grover *et al.,* 2012; Reed *et al.*, 2012; Charlebois *et al.,* 2004). Standardization of natural product for medical application is always difficult. However, there are many advantages of natural product compared to synthetic one. Setting-up for the guideline is required for new natural product. In this study, 5 samples were assigned for each group. Considering big standard deviation, sample size should be increased in future study. When compared average value only, the change of tensile strength after treatment was little in Cytoplast and 2 silk groups (Table 1). Cytoplast is designed for open-membrane technique and shows no bio-degradation (Ha *et al.*, 2014).

In conclusion, both TDI and FS did not have any evidence of biodegradability at 2 weeks' observation in normal saline treatment. However, OssGuide showed more than 20 % decrease in yield strength and tensile strain at 2 weeks' observation in normal saline treatment. OssGuide showed significantly lower tensile strain compared to TDI and FS at 2 weeks' observation in normal saline treatment ($P<0.05$, Fig. 1).

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