의학석사 학위논문

Evaluation of biodegradable plate and screw systems for fixation of mandible bone fracture fixation in a rabbit model

가토 하악골절 모델에서의 흡수성 골접합용판의 안전성 및 생체적합성에 대한 연구

2013 년 2 월

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ABSTRACT

Introduction: Currently, titanium alloy systems have been used for the treatment of bone fracture. This device has many advantages such as the reduction of healing time and the convenience in operation process. However, concerns about infection, exposure, transcranial migration, and growth restriction when using metal devices were reported in studies. To overcome these problems with the conventional metallic system, a variety of bioabsorbable materials have been developed for the treatment of bone fracture. Plate and screw fixation techniques for fracture healing had been boosted with the development of new biocompatible materials. The aim of this study is to evaluate the effects and safety of recently developed bendable bioabsorbable plate and screw device system in mandibular fracture in a rabbit model. The success of this system in mandibular ramus, which is known for high-load-bearing site, might suggest the usefulness of given bioabsorbable plates and screws.

Methods: We investigated the efficacy and safety of recently developed bendible bioabsorbable plates and screwwhich are made of PLGA (polylactic-*co*-glycolic acids) and 100% poly (L-lactic acid) only in a rabbit model. *In vivo* mandibular fracture model in rabbit was introduced to evaluate the efficacy and biocompatibility of the each fixation system. Twenty-five New Zealand white rabbits for each system were randomly assigned for each system. At 4, 6, 8 and 10 weeks after implantation, tissue specimens were taken from the implanted sites of the rabbits and histological analysis was performed for the each of the specimen.

Results: Until 6 weeks, both devices of fixation system showed similar result. After 4 weeks, plates and screws were covered by amorphous connective tissues and overwhelming severe active chronic inflammation in soft tissue has observed. After 6 weeks, the inflammation decreased and some of the specimens exhibited new bone formation around the end of a fracture line. After 8 and 10 weeks, in the case of PLGA made plates and screws, new bone formation was observed with all samples without severe inflammation, implying the healing statue of the bony fracture. Meanwhile, rabbits with PLA plates and screws showed incomplete bone remodeling although new bone formation and increase of bone thickness were observed.

Conclusion: Given by these, it could be suggested that biodegradable plate and screw systems that we evaluated in this work be effective for treatment of mandibular fracture, one of the sites under a high load-bearing condition. The adjustment process and long-term follow-up study is in progress for clinical application of this plate and screw system.

Keywords: bioabsorbable plate and screw, bone fixation, PLGA (polylactic-*co*-glycolic acids), PLA(polylactic acid), biocompatibility

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INTRODUCTION

Nowadays, the titanium alloy systems for bone has have been used for the treatment of facial bone fracture. These systems have many advantages, such as, the reduced time of bone healing and operational convenience. However, concerning about infection, exposure, transcranial migration, and growth restriction when using metal devices were reported by studies. Schnidt et al (1998) reported that 11% of Le Fort I osteotomy patients had secondary removal of plates due to infection and plate exposure[1]. Mosbah et al (2003) reported the cases of the 16 orthognathic patients who had to have their plates removed: 9 removals were due to infection, 4 due to pain, 1 due to denture discomfort, and 2 due to palpability[2]. In the field of orthopedics, it has been reported that the rigid metallic plate fixation system can be obstacles for rapid formation of a primary callus[3]. To overcome these problems with the conventional metallic system, a variety of bioabsorbable materials have been developed for the treatment of bony fracture. Plates and screws internal fixation techniques for fracture healing had been boosted with the new development of new biocompatible materials[4]. The leading advantages of resorbable plates and screws are (i) No need of operation for removal, (ii) Minimal restriction of bony growth due to the gradual fall off in mechanical strength, (iii) Less risk of osteoporosity followed by stress-shielding on account of excessive high strength (iv) No tissue reaction caused by metallic corrosion, and (v) No generation of visible artifacts on computed tomography[5]. According to Data Monotir®, the market value of bio-absorbable plates and screws has grown up from 60 million dollars in 2000 to 90 million dollars in 2006. With the increase of biocompatible material use and internal bone fixation system, several companies have developed improved screws and plates. First, this study introduced bioabsorbable plates and screws made of **polylactic acid (PLA)** alone. Although PLA plates and screws are strong enough to support bone fracture until bone healing, they need long periods of time to be degraded. Therefore, poly lactic-co-glycolide (PLGA) has been extensively studied for the development of resorbable bone replacements with controllable properties. As crystallinity, strength and degradation are controlled by the ratio of PLA and PGA, PLGA has been found be suitable for supporting bony stability until healing in orthopedic fields. Accordingly, as the second part of this study, we investigated plates and screws made by PLGA..

This article documents the biocompatibility and efficiency of PLA and PLGA bone fixation system through *in vivo* experiments.

MATERIALS AND METHODS

1. Bending test

Plates and screws used in this study were made of 100% PLA or PLGA (Figure 1). One fixation system pair consists of one plate and two screws which satisfy the standard of medical devices. The dimension of plates and screws is shown in Table 1. The length of plate and number of screws can be modified according to the region they are applied to. Bending test performed according to guidance of ASTM (American Society for Testing and Materials) F2502 rule as written below.

(1) Attach specimen to fixture and align with pilot hole.

(2) Apply 1-5r/min torsional load for 4 rotations (1440°) and 1.14kg or less axial load to maintain screw driver bit in the screw head.

(3) Record max load in N m.

(4) Compare a variety of specimen sizes.

2. Elution test

To determine whether plates and screws would cause acute systemic toxicity, USP (United States Pharmacopeial) and ISO (International Organization for Standardization) systemic toxicity study was performed. A single extract of the test particle was prepared using single strength MEMS (Minimum Essential Medium) supplemented with 5% serum and 2% antibiotics (1X MEM) and agitated at 37 °C for 72 hours. By the extract, six kinds of measurement were performed: morphology, change of pH compared to MEM itself, absorption spectrum to identify the presence of pollutants, KMnO test to detect dissolved harmful organics, evaporation residue and color of heavy metal compared to control. A grade of reactivity was assigned based on macroscopic or microscopic observation of the control and test extract cultures.

3. Surgery

Under approval from Seoul National University Bundang Hospital (SNUBH) **Institutional Animal Care and Use Committees** (IACUC) (BA1011-072/059-01), 50 male New Zealand white rabbits weighing from 2.5kg-3.0kg were recruited for assessment. The certified staff in animal laboratory examined the animals on a daily basis for evidence of adequate feeding, activity, and signs and symptoms of distress. The animals randomly divided into 5 groups for 5 time points of biopsy (2, 4, 6, 8, and 10weeks) for each fixation system (PLA system and PLGA system). Each plate and screw set was fixated to its correspondent mandibular ramus fracture site (Figure 2).

The surgical procedures were performed under anesthesia using 0.6mg/kg mixed solution (Zoletil: Xylazie, 1:1) administered intramuscularly. In addition, 1% lidocaine (Dai Han Pharm.Co.Ltd, Korea) was injected to reduce the local pain. Once anesthetized, whole mandibular regions were shaved and cleansed with Betadine (Sungkwanpharm, Korea). A 5.0 cm sized incision was made along the inferior border of the mandibular body at right side with #15 bladed scalperl. The periosteum was incised for the operation field. Once the periosteum was elevated free from the proximal part of the mandibular bone, the two prong retractions were used to provide retraction of the soft tissue. Using electrical saw system, mandibular body osteotomy was performed (Figure 2). Each bone fracture was fixed to the mandibular angle using each plate and screw, followed by copious irrigation. After periosteum was tied over the screw heads with Vicryl3-

0 (Ethicon, USA), the skin was closed with 4-0 nylon suture (Woorhi medical, Korea). Then animals were delivered to recover room.

4. Histological study

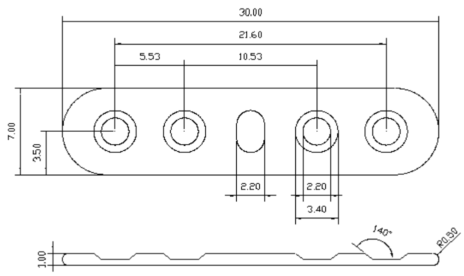
For each time points (2, 4, 6, 8, and 10 weeks), euthanasia was performed using ketamine 40mg/kg intramuscularly, followed by extirpation of whole mandible. The plates and screws were inspected for evidence of bony consolidation, soft tissue positioning, absorption and displacement. When removing the experimental site, the electrical saw blade system was used. The specimens were immediately fixed with 4% buffered paraformaldehyde. After decalcification using formic acid-sodium citrates samples were embedded in paraffin. Plates and screws were removed during this process for the convenience of sectioning. Using microtome, each specimen was sectioned for 10μm intervals. The histological sections were stained with hematoxylin-eosin (H&E) solution. Specimens were examined in a blinded fashion by two examiners under routine light microscopy and evaluated for the presence of inflammation and the degree of healing in bony region.

Table1. The dimension of plates and screws

Material		Length(mm)	Width(mm)	Thickness(mm)
PLA*	Plate	31.0	4.5	0.50
	Screw	3.0	1.4	-
PLGA**	Plate	30.88	4.57	0.50
	Screw	3.0	1.4	-

PLA*(Polylactic acid)
PLGA**(Poly lactic-co-glycolide)

(A)



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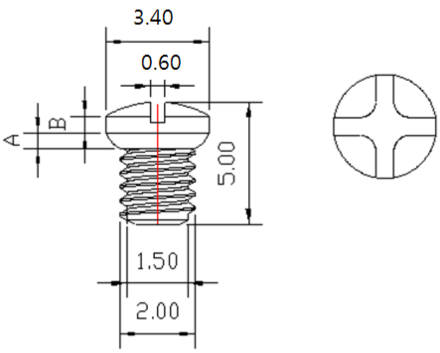
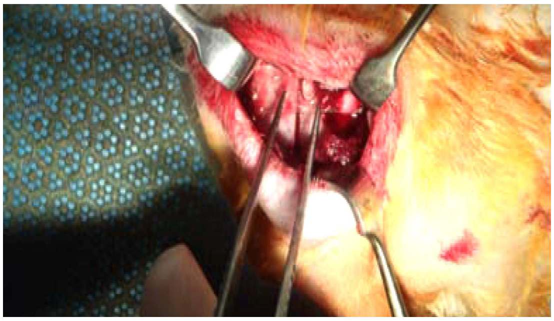


Figure 1. Schematic drawing of bone fixation system

(**A**) Plate. (**B**) Screw

(A)



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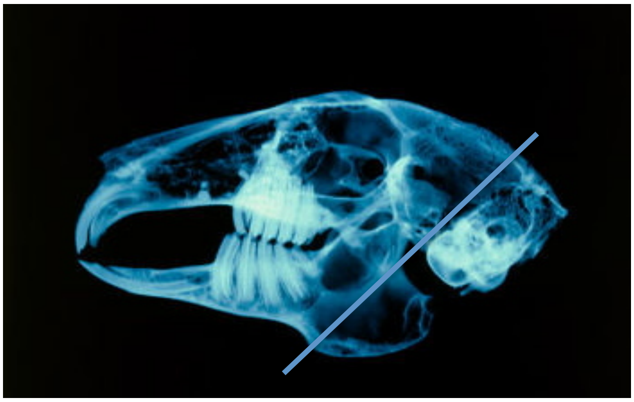


Figure 2. Placement of plate and screw

(A) Placement of PLA plate and screw in rabbit mandible fracture model. (B) Rabbit skull image indicating the position of osteotomy and implantation (blue line).

RESULTS

Bending test

The ultimate strength of PLA plates was determined averagely to be 41.8 N/cm^2. This value is similar to the value of synthetic metalic plate, which is conventional titanium plate (Table 2).

Elution test

There was no mortality or evidence of significant systemic toxicity over the 72hour test period from the test article extracts compared to the control blank extracts. The result of extract test for each test is given in Table 3. Both of PLA and PLGA bone fixation evaluated in this study satisfied all standards for biomedical devices.

In vivo study (PLA plate and screw system)

3 weeks after surgery, one rabbit was found dead. Except that case, all animals survived until euthanasia and maintained normal activities. The significant change in the pattern of chewing, eating and sucking behaviors was not detected. In the first biopsy group, two rabbits showed inclusion cysts around rear neck but not relative to plates and screws.

By macroscopic view, 4 weeks after surgery (the first biopsy), plates and screws were discriminated obviously in operational field. After 6 weeks, screws were not to be found. The plates also seemed to be absorbed. First cases of broken plates and absorbed screws were detected. After 8 and 10 weeks, however, all plates were observed and they were covered by connective tissue. Healing profess was found in all fractures after 6 weeks. At biopsy of 10 weeks, all fractures found to be healed.

From microscopic views, indicating histological analysis, at 4^{th} weeks after surgery, the severe active chronic inflammation in bone marrow and adjacent soft tissue was observed. Especially, extensive foreign body reaction and granuloma were detected. Under the microscope, bone healing was discovered in all slides. New bone formation was observed but not noticeably (Fig 3A). At 6^{th} weeks after surgery, active inflammation in bone marrow was disappeared. Chronic inflammation with fibrosis was observed in adjacent soft tissue. Foreign body reaction decreased while new bone formation was prevailed around periostium (Fig 3B). At 8^{th} and 10^{th} weeks after surgery, new bone formation was observed and the thickness of bone increased. Bone remodeling was not completed. 10 weeks after surgery, severe fibrosis and mild chronic inflammation were detected in all slides (Fig 3C).

In vivo study (PLGA plate and screw system)

All 25 animals underwent the general anesthesia and surgical procedures. Any significant change in the pattern of chewing, eating and sucking was not observed.

Gross examination revealed the absorbability of plates and screws we prepared. 4 weeks after surgery, plates were observed obviously while screws were dismantled. After 6weeks, plates were seemed to start being absorbed, followed by furthermore absorption at 10th week. Moreover, all mandible bone fractures were found to heal after 10weeks post-operation.

Under light microscopic examination, decrease in the degree of inflammation and bone healing process were observed as the time passed. At 4 week after surgery, the severe acute inflammation was detected, followed by reduction of acute inflammation and that of foreign body reaction at 6th week. After 8weeks, acute inflammation around surgical site was faded away. At 10th week, healing of fracture and fibrosis and complete remodeling of bone were observed (Fig4).

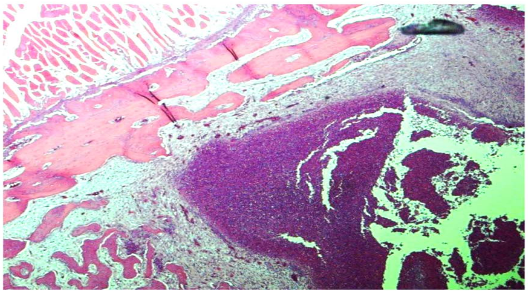
Table2. Bending stiffness of bone fixation system

Bone fixation plate	Bending stiffness (N/cm^2)
Inion plate	53.2
Synthes titanium plate	41.3
Glotech plate	41.8

Table3. The result of elution test

Measurement	PLA		PLGA	
	Result	Standard	Result	Standard
Morphology	Transparent No alien material			
pH	1.47	≤ 1.5	0.07	≤ 1.5
Absorption spectrum in ultraviolet rays	0.061	≤ 0.1	0.0516 (250-350nm)	≤ 0.1
KMnO reducing agent	0.3 mL	≤ 2.0 mL	0.4 mL	≤ 2.0 mL
Evaporation residue	0.1mg	≤ 1.0 mg	0.5 mg	≤ 1.0 mg
Heavy metal	No darker than control			

(A)



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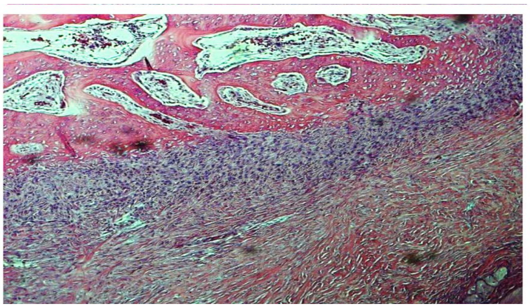


(C)

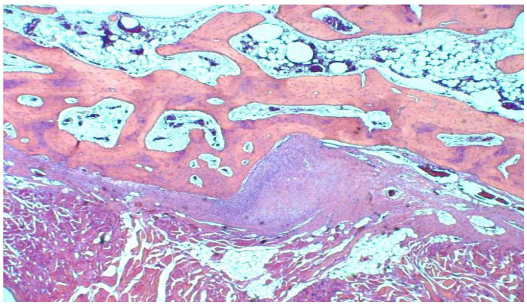
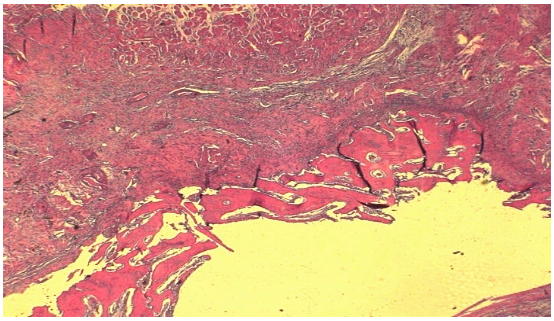


Figure 3.The histological evaluation of specimens under H&E staining of PLA system (X4)

A magnification of the specimen 4th weeks post operation (A), 6th weeks after operation (B), and 10th weeks after surgery (C).

(A)



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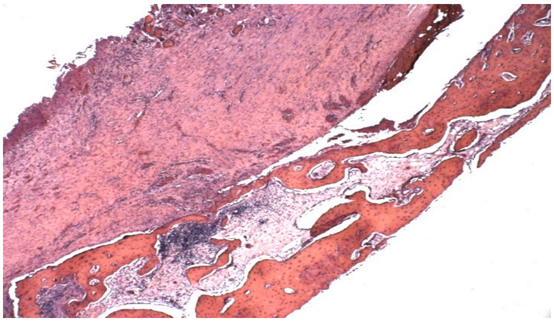
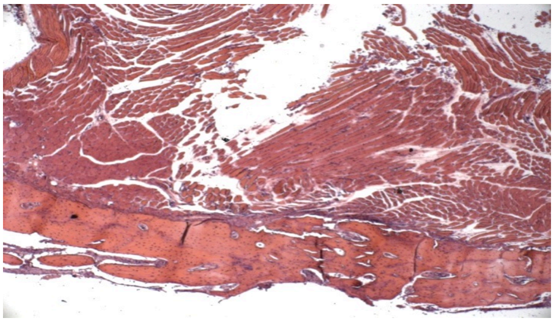


(C)



(D)

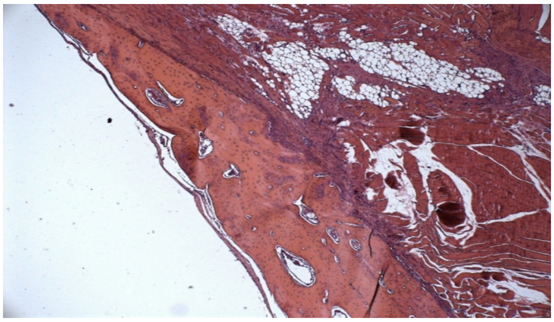


Figure4. The histological image of specimens under Hematoxylin-Eosin staining of PLA system

A magnification of the specimen 4th weeks post operation (A), 6th weeks after operation (B), 8th weeks after surgery (C) and 10th weeks after surgery (D).

DISCUSSION

Recently, biodegradable plate and screw system has been arising the method for osteofixation in craniofacial reconstructive surgery. With this expanded studies of biomaterials, bioresorbable polymeric devices have replaced traditional metallic system in internal osteofixation especially in craniofacial reconstructive surgery. These devices have overcome the disadvantages and complications of traditional metal devices such as stress shielding, growth restriction and inconvenience of reoperation[6]. Such bioabsorbable system is expected to have following features: afford stability similar to that of titanium during the first six weeks, regarded as the reossification period, not compromise bone growth, reduce complication rates (neurocir)and proper polymer degradation rate which lines between the clinical effects of being “too fast or too slow”[7].

Since 1970s, in vivo and in vitro studies on biodegradable fixation systems have been reported[8]. Cutright, et al. (1971) reported the successful recovery of mandible fractures in monkeys using PLA plates and screws[9] and Hunsuck(1972) made advantage of PLA to repair orbital cone defects in monkeys[10]. Wittenberg, et al. (2011) suggested that PLA polymeric screws could function effectively to stabilize bones, confirming there was little difference between titanium screws and polymeric ones[11].Haers, et al. (1998) demonstrated that the use of biodegradable self-reinforced poly-L/DL-lactide plates and screws in bimaxillary surgery leads to a predictable short-term pattern of skeletal stability that is comparable to the standard of titanium plates and screws[9].

Currently, PLA and PGA are commonly available for fixation system in the field of osteology[12]. PLA is the most commonly used one due to its highest mechanical strength among all existing polymers[13]. This study was designed to investigate the efficiency and safety of bioabsorbable plates and screws made of 100% PLA in mandible fracture healing process.

From gross investigative views, except that one plate was broken and the certain amount of plates seemed to be disrupted at 6th weeks, all plates were detected obviously. Other than that, the plates were not moved and sustained fracture part, helping reossification.

The plates were maintained in intended site without any additional devices or treats. We used 4-hole plates with 1.5 mm in thickness and screws with 2.00 mm in diameter and 3.00 mm in length. The length of these screws is easily modifiable, unlike other bioabsorbable ostheosynthesis materials. However, the certain technical difficulty was detected during the operation. The length of the screws was 3 mm and it was short for fixation. This made screw placement difficult on occasion. It would be more desirable if the length of screws were 6 mm for the operation for operational convenience.

From histological views, new bone formation was detected obviously at 6th weeks after surgery. Despite the incomplete remodeling, the thickness of bone increased after 10th weeks from the operation. Furthermore, although there was severe inflammation at 4th week followed by fibrosis and chronic inflammation at 10th week, it seemed that the surgical treatment was not necessary unlike in titanium fixation systems.

Although PLA plate and screw fixation system proved to be effective, the length of time to biodegrade PLA should be noted. While the high mechanical strength is necessary for bone fixation system, the PLA loss of strength is quite slow, only 25% over the first three months and 100% at one year[14]. Complete degradation also does not take place until four to five years after implantation due to hydrophobicity and crystalline. This slow absorption can result in stress shielding and growth restriction as non-bioabsorbable fixation system[15]. In the case of PGA, it degrades too fast and accumulation of break down materials can cause the local adverse effect. Furthermore, due to the lack of time for supporting bone fracture, mechanical failure can occur. As a solution, the appropriate blending of these two materials has been accepted, satisfying desirable conditions for bioabsorbable fixation systems. It has been reported that PLGA fixation system degrades predictively with few clinically apparent foreign body reaction[16]. Being resorbed by 24 months' time, which is much shorter than that of PLA alone system yet still long enough for supporting the bone fracture until complete healing, PLGA devices could solve the absorption rate problem. Moreover, with the final result of carbon dioxide and water, this system has been proved to be safe and biocompatible.

Here, we developed plates and screws made of 82: 18 L-lactic acid: glycolic acid. *In vitro* experiments in terms of toxicity tests, neither cytotoxicity nor the genotoxicity was observed, indicating the safety of these plates and screws. Based on these results, we used absorbable plates and screws system to stabilize mandibular bony fracture where high strength over natural bone and retention is required to achieve bony fix fracture due to high loading[17]. And since the osteotomy was performed at the site on mandibular ramus, experiment could be performed in the independent condition from mouth behavior.

From histological views, the low inflammatory response indicated that the biologic response to the procedure was favorable to osteotomy. The absence of a severe inflammatory reaction in our in vivo models is consistent with previous studies using this material as and osseous fixation device. And the complete bone healing in such a high loading region suggests the possible application of this system in various osteotomies.

The present study showed that bioresorbable plates and screws provide for excellent biocompatibility and stability at the healing process on mandibular ramus bony fracture in a rabbit model. It is expected that a further study about histological analysis in depth would confirm the results of this study. Based on our findings and evaluations, it can be concluded that with the adjustment process and long-term follow-up study, clinical application of the plate and screw system would be available.

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국문초록

서론: 최근까지, 안면골절 치료를 위하여 티타늄 소재의 골절합용 판이 사용되어왔다. 이러한 합용판의 경우 회복기간이 짧고 수술과정이 용이하다는 장점이 있다. 하지만 감염과 노출, 결찰사의 이동과 골재생의 방해 등이 여러 연구에 걸쳐 문제점으로 지적되어왔다. 이러한 전통적인 금속 재질 골접합용 판의 단점을 보완하기 위하여 많은 종류의 생체흡수성 물질들이 골절치료를 위하여 개발되고 있다. 이 연구의 목적은 최근 개발된 변형이 용이한 생체 흡수성 골접합용 판의 효용성과 생체적합성을 하악골절 가토모델에서 평가하는 것이다. 가장 하중이 강한 곳인 하악에서 골접합용 판의 성공적인 적용은 곧 이 생체흡수성 골접합용 판의 충분한 효용성을 보장한다고 할 수 있겠다.

방법: 이번 연구는 PLGA 또는 100% PLA로 만들어진 두 가지의 골접합용 판을 가지고 실험하였으며, 각 골접합 시스템 당 25마리의 뉴질랜드산 흰색 가토가 배정되었다. 술 후 4,6,8,10주되는 시점에 바이옵시가 진행되었으며 골접합 스크류와 플레이트를 포함한 하악부근의 조직을 드러내어 골절치유 정도 및 생체적합성을 평가하였다.

결과: 6주까지 두 가지 골접합시스템은 비슷한 결과를 보였다. 4주가 지난 시점에서 골접합용 판 주위로 결합조직과 심한 만성염증반응이 관찰되었다. 6주후, 염증정도는 조금 가라앉고 골막부근으로 새로운 골형성이 관찰되었다. 8주와 10주 후 , PLGA 골접합용 판을 사용한 가토군에서는 심한 염증반응 없이

모든 골절이 치료가 된 것을 확인 한 반면, PLA 골접합용 판을 사용한 경우 아직 골재생이 완전히 이루어 지지 않았으나 이 경우에도 새로운 골형성과 골두께 증가가 관찰되었다.

결론: 실험결과, 평가된 두가지 종류의 골접합용 판 모두 가장 하중이 많이 걸리는 하악부분의 골절에 유용한 것으로 나타났다. 장기간의 연구와 임상연구가 현재 진행계획중에 있다.

주요어 : 생체흡수성 골접합용 판, 골고정, PLGA, PLA, 생체적합성

학 번 : 2011-21883

ABSTRACT

Introduction: Currently, titanium alloy systems have been used for the treatment of bone fracture. This device has many advantages such as the reduction of healing time and the convenience in operation process. However, concerns about infection, exposure, transcranial migration, and growth restriction when using metal devices were reported in studies. To overcome these problems with the conventional metallic system, a variety of bioabsorbable materials have been developed for the treatment of bone fracture. Plate and screw fixation techniques for fracture healing had been boosted with the development of new biocompatible materials. The aim of this study is to evaluate the effects and safety of recently developed bendable bioabsorbable plate and screw device system in mandibular fracture in a rabbit model. The success of this system in mandibular ramus, which is known for high-load-bearing site, might suggest the usefulness of given bioabsorbable plates and screws.

Methods: We investigated the efficacy and safety of recently developed bendible bioabsorbable plates and screwwhich are made of PLGA (polylactic-*co*-glycolic acids) and 100% poly (L-lactic acid) only in a rabbit model. *In vivo* mandibular fracture model in rabbit was introduced to evaluate the efficacy and biocompatibility of the each fixation system. Twenty-five New Zealand white rabbits for each system were randomly assigned for each system. At 4, 6, 8 and 10 weeks after implantation, tissue specimens were taken from the implanted sites of the rabbits and histological analysis was performed for the each of the specimen.

Results: Until 6 weeks, both devices of fixation system showed similar result. After 4 weeks, plates and screws were covered by amorphous connective tissues and overwhelming severe active chronic inflammation in soft tissue has observed. After 6 weeks, the inflammation decreased and some of the specimens exhibited new bone formation around the end of a fracture line. After 8 and 10 weeks, in the case of PLGA made plates and screws, new bone formation was observed with all samples without severe inflammation, implying the healing statue of the bony fracture. Meanwhile, rabbits with PLA plates and screws showed incomplete bone remodeling although new bone formation and increase of bone thickness were observed.

Conclusion: Given by these, it could be suggested that biodegradable plate and screw systems that we evaluated in this work be effective for treatment of mandibular fracture, one of the sites under a high load-bearing condition. The adjustment process and long-term follow-up study is in progress for clinical application of this plate and screw system.

Keywords: bioabsorbable plate and screw, bone fixation, PLGA (polylactic-*co*-glycolic acids), PLA(polylactic acid), biocompatibility

Student number: 2011-21883

CONTENTS

LIST OF TABLES AND FIGURES

INTRODUCTION

Nowadays, the titanium alloy systems for bone has have been used for the treatment of facial bone fracture. These systems have many advantages, such as, the reduced time of bone healing and operational convenience. However, concerning about infection, exposure, transcranial migration, and growth restriction when using metal devices were reported by studies. Schnidt et al (1998) reported that 11% of Le Fort I osteotomy patients had secondary removal of plates due to infection and plate exposure[1]. Mosbah et al (2003) reported the cases of the 16 orthognathic patients who had to have their plates removed: 9 removals were due to infection, 4 due to pain, 1 due to denture discomfort, and 2 due to palpability[2]. In the field of orthopedics, it has been reported that the rigid metallic plate fixation system can be obstacles for rapid formation of a primary callus[3]. To overcome these problems with the conventional metallic system, a variety of bioabsorbable materials have been developed for the treatment of bony fracture. Plates and screws internal fixation techniques for fracture healing had been boosted with the new development of new biocompatible materials[4]. The leading advantages of resorbable plates and screws are (i) No need of operation for removal, (ii) Minimal restriction of bony growth due to the gradual fall off in mechanical strength, (iii) Less risk of osteoporosity followed by stress-shielding on account of excessive high strength (iv) No tissue reaction caused by metallic corrosion, and (v) No generation of visible artifacts on computed tomography[5]. According to Data Monotir®, the market value of bio-absorbable plates and screws has grown up from 60 million dollars in 2000 to 90 million dollars in 2006. With the increase of biocompatible material use and internal bone fixation system, several companies have developed improved screws and plates. First, this study introduced bioabsorbable plates and screws made of **polylactic acid (PLA)** alone. Although PLA plates and screws are strong enough to support bone fracture until bone healing, they need long periods of time to be degraded. Therefore, poly lactic-co-glycolide (PLGA) has been extensively studied for the development of resorbable bone replacements with controllable properties. As crystallinity, strength and degradation are controlled by the ratio of PLA and PGA, PLGA has been found be suitable for supporting bony stability until healing in orthopedic fields. Accordingly, as the second part of this study, we investigated plates and screws made by PLGA..

This article documents the biocompatibility and efficiency of PLA and PLGA bone fixation system through *in vivo* experiments.

MATERIALS AND METHODS

1. Bending test

Plates and screws used in this study were made of 100% PLA or PLGA (Figure 1). One fixation system pair consists of one plate and two screws which satisfy the standard of medical devices. The dimension of plates and screws is shown in Table 1. The length of plate and number of screws can be modified according to the region they are applied to. Bending test performed according to guidance of ASTM (American Society for Testing and Materials) F2502 rule as written below.

(1) Attach specimen to fixture and align with pilot hole.

(2) Apply 1-5r/min torsional load for 4 rotations (1440°) and 1.14kg or less axial load to maintain screw driver bit in the screw head.

(3) Record max load in N m.

(4) Compare a variety of specimen sizes.

2. Elution test

To determine whether plates and screws would cause acute systemic toxicity, USP (United States Pharmacopeial) and ISO (International Organization for Standardization) systemic toxicity study was performed. A single extract of the test particle was prepared using single strength MEMS (Minimum Essential Medium) supplemented with 5% serum and 2% antibiotics (1X MEM) and agitated at 37 °C for 72 hours. By the extract, six kinds of measurement were performed: morphology, change of pH compared to MEM itself, absorption spectrum to identify the presence of pollutants, KMnO test to detect dissolved harmful organics, evaporation residue and color of heavy metal compared to control. A grade of reactivity was assigned based on macroscopic or microscopic observation of the control and test extract cultures.

3. Surgery

Under approval from Seoul National University Bundang Hospital (SNUBH) **Institutional Animal Care and Use Committees** (IACUC) (BA1011-072/059-01), 50 male New Zealand white rabbits weighing from 2.5kg-3.0kg were recruited for assessment. The certified staff in animal laboratory examined the animals on a daily basis for evidence of adequate feeding, activity, and signs and symptoms of distress. The animals randomly divided into 5 groups for 5 time points of biopsy (2, 4, 6, 8, and 10weeks) for each fixation system (PLA system and PLGA system). Each plate and screw set was fixated to its correspondent mandibular ramus fracture site (Figure 2).

The surgical procedures were performed under anesthesia using 0.6mg/kg mixed solution (Zoletil: Xylazie, 1:1) administered intramuscularly. In addition, 1% lidocaine (Dai Han Pharm.Co.Ltd, Korea) was injected to reduce the local pain. Once anesthetized, whole mandibular regions were shaved and cleansed with Betadine (Sungkwanpharm, Korea). A 5.0 cm sized incision was made along the inferior border of the mandibular body at right side with #15 bladed scalperl. The periosteum was incised for the operation field. Once the periosteum was elevated free from the proximal part of the mandibular bone, the two prong retractions were used to provide retraction of the soft tissue. Using electrical saw system, mandibular body osteotomy was performed (Figure 2). Each bone fracture was fixed to the mandibular angle using each plate and screw, followed by copious irrigation. After periosteum was tied over the screw heads with Vicryl3-

0 (Ethicon, USA), the skin was closed with 4-0 nylon suture (Woorhi medical, Korea). Then animals were delivered to recover room.

4. Histological study

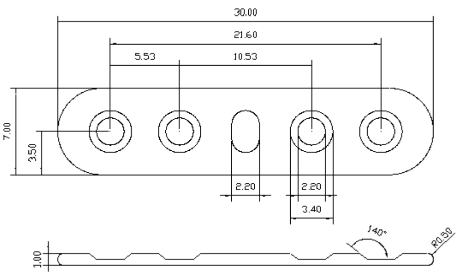
For each time points (2, 4, 6, 8, and 10 weeks), euthanasia was performed using ketamine 40mg/kg intramuscularly, followed by extirpation of whole mandible. The plates and screws were inspected for evidence of bony consolidation, soft tissue positioning, absorption and displacement. When removing the experimental site, the electrical saw blade system was used. The specimens were immediately fixed with 4% buffered paraformaldehyde. After decalcification using formic acid-sodium citrates samples were embedded in paraffin. Plates and screws were removed during this process for the convenience of sectioning. Using microtome, each specimen was sectioned for 10μm intervals. The histological sections were stained with hematoxylin-eosin (H&E) solution. Specimens were examined in a blinded fashion by two examiners under routine light microscopy and evaluated for the presence of inflammation and the degree of healing in bony region.

Table1. The dimension of plates and screws

Material		Length(mm)	Width(mm)	Thickness(mm)
PLA*	Plate	31.0	4.5	0.50
	Screw	3.0	1.4	-
PLGA**	Plate	30.88	4.57	0.50
	Screw	3.0	1.4	-

PLA*(Polylactic acid)
PLGA**(Poly lactic-co-glycolide)

(A)



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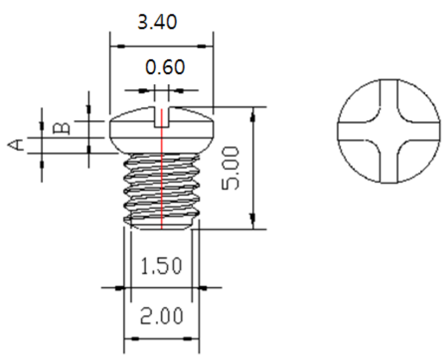
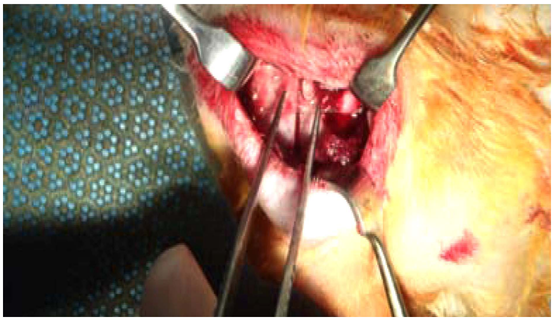


Figure 1. Schematic drawing of bone fixation system

(**A**) Plate. (**B**) Screw

(A)



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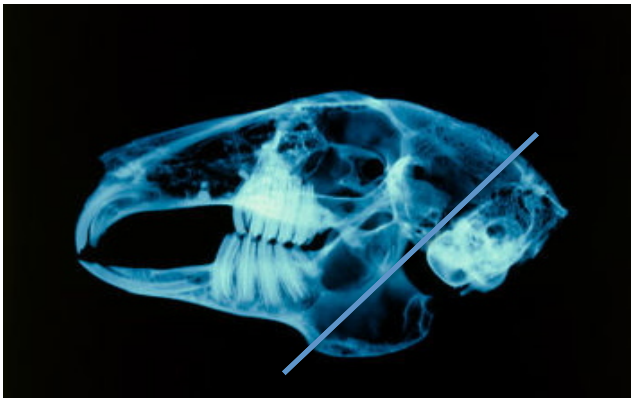


Figure 2. Placement of plate and screw

(A) Placement of PLA plate and screw in rabbit mandible fracture model. (B) Rabbit skull image indicating the position of osteotomy and implantation (blue line).

RESULTS

Bending test

The ultimate strength of PLA plates was determined averagely to be 41.8 N/cm^2. This value is similar to the value of synthetic metalic plate, which is conventional titanium plate (Table 2).

Elution test

There was no mortality or evidence of significant systemic toxicity over the 72hour test period from the test article extracts compared to the control blank extracts. The result of extract test for each test is given in Table 3. Both of PLA and PLGA bone fixation evaluated in this study satisfied all standards for biomedical devices.

In vivo study (PLA plate and screw system)

3 weeks after surgery, one rabbit was found dead. Except that case, all animals survived until euthanasia and maintained normal activities. The significant change in the pattern of chewing, eating and sucking behaviors was not detected. In the first biopsy group, two rabbits showed inclusion cysts around rear neck but not relative to plates and screws.

By macroscopic view, 4 weeks after surgery (the first biopsy), plates and screws were discriminated obviously in operational field. After 6 weeks, screws were not to be found. The plates also seemed to be absorbed. First cases of broken plates and absorbed screws were detected. After 8 and 10 weeks, however, all plates were observed and they were covered by connective tissue. Healing profess was found in all fractures after 6 weeks. At biopsy of 10 weeks, all fractures found to be healed.

From microscopic views, indicating histological analysis, at 4^{th} weeks after surgery, the severe active chronic inflammation in bone marrow and adjacent soft tissue was observed. Especially, extensive foreign body reaction and granuloma were detected. Under the microscope, bone healing was discovered in all slides. New bone formation was observed but not noticeably (Fig 3A). At 6^{th} weeks after surgery, active inflammation in bone marrow was disappeared. Chronic inflammation with fibrosis was observed in adjacent soft tissue. Foreign body reaction decreased while new bone formation was prevailed around periostium (Fig 3B). At 8^{th} and 10^{th} weeks after surgery, new bone formation was observed and the thickness of bone increased. Bone remodeling was not completed. 10 weeks after surgery, severe fibrosis and mild chronic inflammation were detected in all slides (Fig 3C).

In vivo study (PLGA plate and screw system)

All 25 animals underwent the general anesthesia and surgical procedures. Any significant change in the pattern of chewing, eating and sucking was not observed.

Gross examination revealed the absorbability of plates and screws we prepared. 4 weeks after surgery, plates were observed obviously while screws were dismantled. After 6weeks, plates were seemed to start being absorbed, followed by furthermore absorption at 10th week. Moreover, all mandible bone fractures were found to heal after 10weeks post-operation.

Under light microscopic examination, decrease in the degree of inflammation and bone healing process were observed as the time passed. At 4 week after surgery, the severe acute inflammation was detected, followed by reduction of acute inflammation and that of foreign body reaction at 6th week. After 8weeks, acute inflammation around surgical site was faded away. At 10th week, healing of fracture and fibrosis and complete remodeling of bone were observed (Fig4).

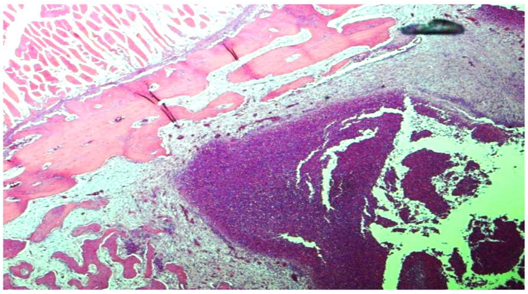
Table2. Bending stiffness of bone fixation system

Bone fixation plate	Bending stiffness (N/cm^2)
Inion plate	53.2
Synthes titanium plate	41.3
Glotech plate	41.8

Table3. The result of elution test

Measurement	PLA		PLGA	
	Result	Standard	Result	Standard
Morphology	Transparent No alien material			
pH	1.47	≤ 1.5	0.07	≤ 1.5
Absorption spectrum in ultraviolet rays	0.061	≤ 0.1	0.0516 (250-350nm)	≤ 0.1
KMnO reducing agent	0.3 mL	≤ 2.0 mL	0.4 mL	≤ 2.0 mL
Evaporation residue	0.1mg	≤ 1.0 mg	0.5 mg	≤ 1.0 mg
Heavy metal	No darker than control			

(A)



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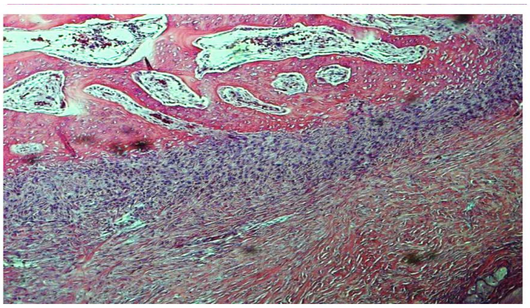


(C)

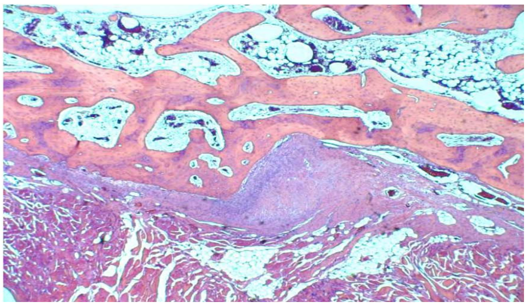
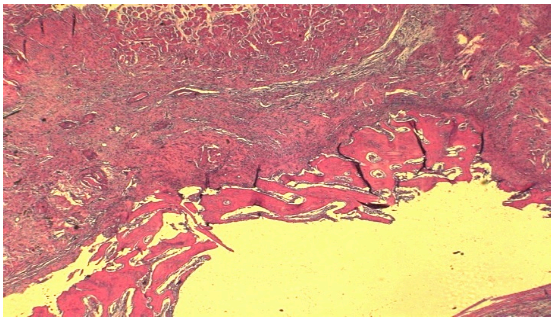


Figure 3.The histological evaluation of specimens under H&E staining of PLA system (X4)

A magnification of the specimen 4^{th} weeks post operation (A), 6^{th} weeks after operation (B), and 10^{th} weeks after surgery (C).

(A)



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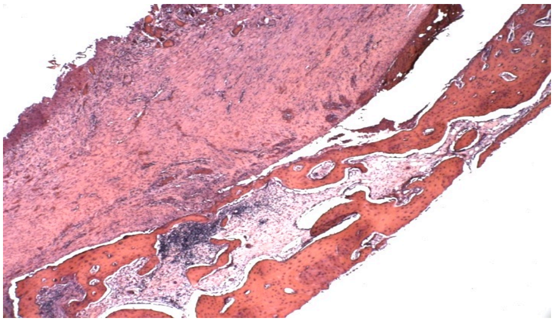
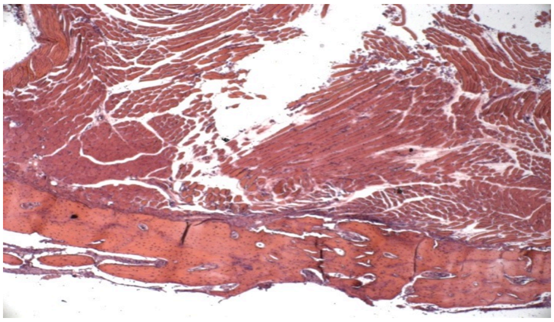


(C)



(D)

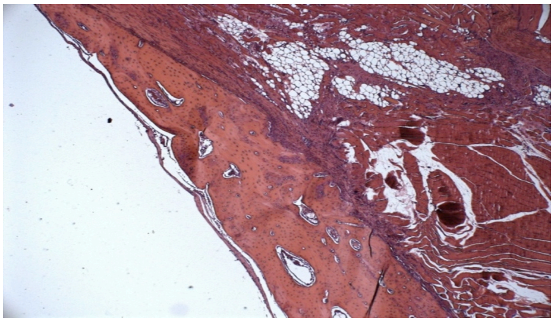


Figure4. The histological image of specimens under Hematoxylin-Eosin staining of PLA system

A magnification of the specimen 4th weeks post operation (A), 6th weeks after operation (B), 8th weeks after surgery (C) and 10th weeks after surgery (D).

DISCUSSION

Recently, biodegradable plate and screw system has been arising the method for osteofixation in craniofacial reconstructive surgery. With this expanded studies of biomaterials, bioresorbable polymeric devices have replaced traditional metallic system in internal osteofixation especially in craniofacial reconstructive surgery. These devices have overcome the disadvantages and complications of traditional metal devices such as stress shielding, growth restriction and inconvenience of reoperation[6]. Such bioabsorbable system is expected to have following features: afford stability similar to that of titanium during the first six weeks, regarded as the reossification period, not compromise bone growth, reduce complication rates (neurocir)and proper polymer degradation rate which lines between the clinical effects of being “too fast or too slow”[7].

Since 1970s, in vivo and in vitro studies on biodegradable fixation systems have been reported[8]. Cutright, et al. (1971) reported the successful recovery of mandible fractures in monkeys using PLA plates and screws[9] and Hunsuck(1972) made advantage of PLA to repair orbital cone defects in monkeys[10]. Wittenberg, et al. (2011) suggested that PLA polymeric screws could function effectively to stabilize bones, confirming there was little difference between titanium screws and polymeric ones[11].Haers, et al. (1998) demonstrated that the use of biodegradable self-reinforced poly-L/DL-lactide plates and screws in bimaxillary surgery leads to a predictable short-term pattern of skeletal stability that is comparable to the standard of titanium plates and screws[9].

Currently, PLA and PGA are commonly available for fixation system in the field of osteology[12]. PLA is the most commonly used one due to its highest mechanical strength among all existing polymers[13]. This study was designed to investigate the efficiency and safety of bioabsorbable plates and screws made of 100% PLA in mandible fracture healing process.

From gross investigative views, except that one plate was broken and the certain amount of plates seemed to be disrupted at 6th weeks, all plates were detected obviously. Other than that, the plates were not moved and sustained fracture part, helping reossification.

The plates were maintained in intended site without any additional devices or treats. We used 4-hole plates with 1.5 mm in thickness and screws with 2.00 mm in diameter and 3.00 mm in length. The length of these screws is easily modifiable, unlike other bioabsorbable ostheosynthesis materials. However, the certain technical difficulty was detected during the operation. The length of the screws was 3 mm and it was short for fixation. This made screw placement difficult on occasion. It would be more desirable if the length of screws were 6 mm for the operation for operational convenience.

From histological views, new bone formation was detected obviously at 6th weeks after surgery. Despite the incomplete remodeling, the thickness of bone increased after 10th weeks from the operation. Furthermore, although there was severe inflammation at 4th week followed by fibrosis and chronic inflammation at 10th week, it seemed that the surgical treatment was not necessary unlike in titanium fixation systems.

Although PLA plate and screw fixation system proved to be effective, the length of time to biodegrade PLA should be noted. While the high mechanical strength is necessary for bone fixation system, the PLA loss of strength is quite slow, only 25% over the first three months and 100% at one year[14]. Complete degradation also does not take place until four to five years after implantation due to hydrophobicity and crystalline. This slow absorption can result in stress shielding and growth restriction as non-bioabsorbable fixation system[15]. In the case of PGA, it degrades too fast and accumulation of break down materials can cause the local adverse effect. Furthermore, due to the lack of time for supporting bone fracture, mechanical failure can occur. As a solution, the appropriate blending of these two materials has been accepted, satisfying desirable conditions for bioabsorbable fixation systems. It has been reported that PLGA fixation system degrades predictively with few clinically apparent foreign body reaction[16]. Being resorbed by 24 months' time, which is much shorter than that of PLA alone system yet still long enough for supporting the bone fracture until complete healing, PLGA devices could solve the absorption rate problem. Moreover, with the final result of carbon dioxide and water, this system has been proved to be safe and biocompatible.

Here, we developed plates and screws made of 82: 18 L-lactic acid: glycolic acid. *In vitro* experiments in terms of toxicity tests, neither cytotoxicity nor the genotoxicity was observed, indicating the safety of these plates and screws. Based on these results, we used absorbable plates and screws system to stabilize mandibular bony fracture where high strength over natural bone and retention is required to achieve bony fix fracture due to high loading[17]. And since the osteotomy was performed at the site on mandibular ramus, experiment could be performed in the independent condition from mouth behavior.

From histological views, the low inflammatory response indicated that the biologic response to the procedure was favorable to osteotomy. The absence of a severe inflammatory reaction in our in vivo models is consistent with previous studies using this material as and osseous fixation device. And the complete bone healing in such a high loading region suggests the possible application of this system in various osteotomies.

The present study showed that bioresorbable plates and screws provide for excellent biocompatibility and stability at the healing process on mandibular ramus bony fracture in a rabbit model. It is expected that a further study about histological analysis in depth would confirm the results of this study. Based on our findings and evaluations, it can be concluded that with the adjustment process and long-term follow-up study, clinical application of the plate and screw system would be available.

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국문초록

서론: 최근까지, 안면골절 치료를 위하여 티타늄 소재의 골절합용 판이 사용되어왔다. 이러한 합용판의 경우 회복기간이 짧고 수술과정이 용이하다는 장점이 있다. 하지만 감염과 노출, 결찰사의 이동과 골재생의 방해 등이 여러 연구에 걸쳐 문제점으로 지적되어왔다. 이러한 전통적인 금속 재질 골접합용 판의 단점을 보완하기 위하여 많은 종류의 생체흡수성 물질들이 골절치료를 위하여 개발되고 있다. 이 연구의 목적은 최근 개발된 변형이 용이한 생체 흡수성 골접합용 판의 효용성과 생체적합성을 하악골절 가토모델에서 평가하는 것이다. 가장 하중이 강한 곳인 하악에서 골접합용 판의 성공적인 적용은 곧 이 생체흡수성 골접합용 판의 충분한 효용성을 보장한다고 할 수 있겠다.

방법: 이번 연구는 PLGA 또는 100% PLA로 만들어진 두 가지의 골접합용 판을 가지고 실험하였으며, 각 골접합 시스템 당 25마리의 뉴질랜드산 흰색 가토가 배정되었다. 술 후 4,6,8,10 주되는 시점에 바이옵시가 진행되었으며 골접합 스크류와 플레이트를 포함한 하악부근의 조직을 드러내어 골절치유 정도 및 생체적합성을 평가하였다.

결과: 6주까지 두 가지 골접합시스템은 비슷한 결과를 보였다. 4주가 지난 시점에서 골접합용 판 주위로 결합조직과 심한 만성염증반응이 관찰되었다. 6주후, 염증정도는 조금 가라앉고 골막부근으로 새로운 골형성이 관찰되었다. 8주와 10주 후 , PLGA 골접합용 판을 사용한 가토군에서는 심한 염증반응 없이

모든 골절이 치료가 된 것을 확인 한 반면, PLA 골접합용 판을 사용한 경우 아직 골재생이 완전히 이루어 지지 않았으나 이 경우에도 새로운 골형성과 골두께 증가가 관찰되었다.

결론: 실험결과, 평가된 두가지 종류의 골접합용 판 모두 가장 하중이 많이 걸리는 하악부분의 골절에 유용한 것으로 나타났다. 장기간의 연구와 임상연구가 현재 진행계획중에 있다.

주요어 : 생체흡수성 골접합용 판, 골고정, PLGA, PLA,
생체적합성
학 번 : 2011-21883