Effect of tetracycline blended polylactic and polyglycolic acid membrane on the healing of one wall intrabony defects in beagle dogs

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July 2003

감사의 글

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그리고, 논문이 완성되기까지 지도 편달해주신 치주과 김종관 교수님, 조규성 교수님, 최성호 교수님, 김창성 교수님께 진심으로 감사드립니다. 바쁘신 와중에도 예심 본심 종심까지 관심있게 지켜봐주신 구강병리학 김진 교수님, 구강미생물학 유윤정 교수님께도 고마움을 전합니다.

김태균 선생님을 비롯한 치주과 의국원 여러분들께도 많은 도움을 받아 논문을 완성할수 있었습니다. 감사합니다.

제가 일하는 밀레니엄 연세치과 직원 여러분께도 감사드립니다.

짧은 영어 손봐주신 이윤 선생님께도 감사드립니다.

논문 쓰는 데 적극적으로 방해한 철없는 두 아들 우주, 태주.

논문 내용보다 공개 발표시 의상에(?) 더 신경써주신 사랑하는 나의 어머니.

박사학위 격려금을 열심히 준비하시는 든든한 나의 아버지, 학위 축하 여행을 보내주신 장인 장모님,

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김 일 영

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Abstract

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Guided tissue regeneration (GTR) is an accepted therapeutic modality for the treatment of periodontal destructive lesions. However, incomplete regeneration or infection of surgery site is often noted after GTR therapy.

The purpose of this study was to evaluate the regenerative effects of TC-PLGA and PLGA barrier membrane implanted to the preclinical one wall intrabony defects surgically created in beagle dogs. 4×4 ㎜ defects were created in bilateral mandibular second and fourth premolars. The surgical control group received flap operation only. The subjects were sacrificed at eight weeks after operation, and the comparative histological examination of their healing results yielded the following conclusion.

1. In comparison of extension of junctional epithelium migration, a significant difference showed between the surgical control and TC-PLGA membrane group ($P<0.05$), and also between membrane and TC-PLGA membrane group ($P<0.05$)

2. In comparison of connective tissue adhesion, no significant difference was apparent between the groups.

3. Cementum regeneration amounted to 2.00 ± 0.70 ㎜ (49.5% of the depth

of defects), 3.16 ± 0.37 ㎜ (62.5 %), and 3.72 ± 0.53 ㎜ (73.8%) respectively in the surgical control, membrane, and TC membrane group. Both the TC membrane group and the membrane group showed a significant difference from the control group ($P<0.05$).

No significant difference was seen between PLGA membrane group and TC-PLGA membrane group.

4. Alveolar bone regeneration amounted to 1.46 ± 0.68 ㎜ (24.8% of the depth of defects), 2.39 ± 0.52㎜ (47.2%), and 2.88 ± 0.66㎜ (57.1%) respectively in the surgical control, PLGA membrane, and TC-PLGA membrane group. Both the TC-PLGA membrane group and the PLGA membrane group showed a significant difference from the control group ($P<0.05$). No significant difference was seen between PLGA membrane group and TC-PLGA membrane group ($P<0.05$).

The above results demonstrate the beneficial effect of TC-PLGA membranes to the preclinical one wall intrabony defects of beagle dogs. The inhibited apical migration of epithelium and the increase in new bone and new cementum suggest the potency of TC-PLGA membrnae in inducing periodontal tissue regeneration.

Key word : TC-PLGA membrane, PLGA membrane, one wall intrabony defect, Guided tissue regeneration, new bone, new cementum, periodontal ligament, long junctional epithelium.

Effect of tetracycline blended polylactic and polyglycolic acid membrane on the healing of one wall intrabony defects in beagle dogs

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I. Introduction

Periodontal regeneration means the restoration of the various components of the periodontium lost in periodontal disease in their appropriate locations, amounts and relationships to each other (Aukhil. 1991).

To achieve the regeneration of lost peridontal attachment, different regenerative therapies, including the use of bone graft or bone substitutes, acid conditioning of the root surface, the application of growth factors, occlusive membranes, etc, have been advocated (Gottlow. 1994). Guided tissue regeneration (GTR) is an accepted therapeutic modality for the treatment of periodontal destructive lesions (Becker et al. 1988). The goal of GTR therapy is not only to make a physical barrier, but also to endow multiple functions to facilitate desirable regeneration (Slots et al. 1990). However, it has been suggested that the

limited extent of periodontal regeneration in the case of GTR therapy may be associated with barrier placement, which creates the avascular walls (root surface and the barrier) adjacent to the defect (Gottlow 1994). So, incomplete regeneration or infection of surgery site is often noted after GTR therapy.

In order to prevent early wound healing problems and to provide more predictable regeneration results, investigations of different resorbable membrane system on the optimal membrane design and material composition are necessary.

Various topical and systemic antimicrobial agents have been used to reduce infectious complications in barrier membrane assisted periodontal therapy (van Winkelhoff et al. 1996). Tetracycline is a potentially valuable antibiotic in barrier membrane assisted periodontal therapy because of its broad-spectrum of activity against numerous periodontal pathogens. And it also can be used as a root demineralization agent, and it inhibits human collagenase and bone resorption (Wikesjo et al. 1986; Golub et al. 1984; Roflom et al. 1993). In addition it is associated with bone formation(Marby et al. 1985; Sasaki et al. 1992). Also, it has been suggested that clinical attachment gain of intrabony defects following GTR was favorable with repeated local administration of minocycline ointment (Yoshinari et al. 2001).

It would be ideal if the GTR membrane can deliver the antibiotic locally avoiding the systemic use of antibiotics and possible side-effects. Chang and Yamada(2000) suggested that 25% doxycycline-loaded resorbable membrane have a beneficial effect on osteogenesis to favor

periodontal regeneration. Also Kurtis et al.(2002) suggested that PLGA membranes with and without metronidazole may have a beneficial effect on periodontal regeneration..

Polylactic and polyglycolic acid Electro-spun non woven membrane can be used as an GTR membrane because of its biocompatibility and abilities of space maintenance. In present study used Electro-spun Non woven membrane can exclude using of the plasticizer which induced foreign-body reaction and control the resolving time by the spun form of membrane preventing early resorption of the PGA.

The hypothesis of this study is that newly developed tetracycline blended polylactic and polyglycolic acid (TC-PLGA) barrier membrane may decrease the risk of infection during membrane therapy and result in increased gain of clinical attachment. The purpose of this study was to compare the histological effects of TC-PLGA membranes and non-blended membranes (PLGA) and controls.

II. Materials & Methods

A. Materials

1. Animals

six male beagle dogs, 18 to 24 months old and weighing about 15kg, were chosen. The animals had intact dentition and healthy periodontium. Animal selection and management, surgical protocol, and preparation followed routines approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. Defects were created in left and right mandibular second and fourth premolars.

2. Tetracycline blended polylactic and polyglycolic acid (TC-PLGA) barrier membranes* and non blended polylactic and polyglycolic acid (PLGA) barrier membranes*

PLGA chips are melted in heat plate at 240 ℃, and then they are put through high pressure and voltage to anneal them through the nozzle. The annealed PLGA of about 10 ㎛ in size was compressed on the rotating drum (Fig 1).

In brief, the mixing ratio of PLGA and the tetracycline* powder was 100 (10g): 1 (0.1g). TC-blending PLGA solution was sprinkled into the polyvinyl alcohol solution in the high speed centrifuge to make TC-PLGA, polymers of microsphere type were used, and their average size was 50 ㎛. And then microsphere type TC-PLGA were chemically evaporized onto the electrospun non woven PLGA membrane.

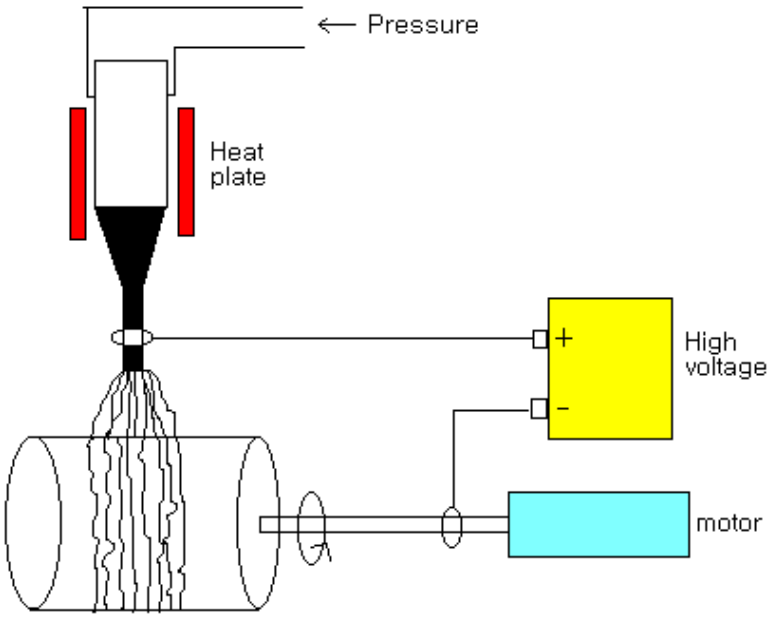

Fig 1. Schematic diagrams of Electro-spun non woven process

a) Melting the PLGA chips at 240℃
b) Increasing the voltage between the nozzle and the drum
c) Radiating PLGA were compressed over the rotating drum
d) Taking up the non woven PLGA wound over the drum

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† TC-PLGA membrane, Chunbuk Univ, Textile Engineering., Chunbuk, Korea
‡ PLGA membrane, Chunbuk Univ, Textile Engineering., Chunbuk, Korea
* Tetracycline, Kwangmyung Pharmaceutical Ind. Co., LTD. Seoul, Korea

B. Methods

1. Experimental design

Animals were divided in three groups. Animals in the surgical control group were given the flap operation only. In the tetracycline blended polylactic and polyglycolic acid (TC-PLGA) membrane and non blended (PLGA) membrane groups, TC-PLGA and PLGA were used during the flap surgery, respectively.

2. Surgical protocol

The surgical procedure was performed under the general anesthesia induced by the intravenous injection of atrophin† (0.04㎎/㎏) and the intramuscular induction with the compound of xylazin†† and ketamin†††, followed by the application of enflurane†††† through inhaling. Routine dental infiltration anesthesia (2% lidocaine hydrochloride with 1/80,000 epinephrine) was used at the surgical sites. The mandibular third premolars had been extracted in advance of the experimental surgeries, and the extraction sites had been allowed to heal for 8 weeks.

At reconstructive surgery, buccal and lingual mucoperiosteal flaps were elevated and 4×4 ㎜ one wall intrabony defects were created at the mesial and distal aspect of the mandibular second premolars and the mesial aspect of the mandibular fourth premolars (Fig 3).

Following root planing, a reference notch was made with 1/4 round bur on the root surface at the base of the defect. Bilateral mandibular defects each received one out of three experimental conditions: TC-PLGA (1 site),

PLGA (1 site), and surgical control (1 site). Experimental conditions were rotated between defect sites in subsequent animal.

The defects of control group were treated with only root planing. In the TC-PLGA group, TC-PLGA membrane was implanted over the defect and sutured with 4-0 vicryl. (Fig 4) The flaps were repositioned and sutured with the 4-0 vicryl. The PLGA group was operated by same ways. Sutures were removed after 7 days. Postsurgery management included intramuscular administration of antibiotics[#], soft diet, and daily topical application of a 0.12 % chlorhexidine solution[##] for the entire healing period.

3. Histologic procedures

The animals were sacrificed at 8 weeks by intravenous injection of concentrated sodium pentobarbital[§]. Tissue blocks, which include teeth, bone, and tissue, were removed, rinsed in saline, and then fixed in 10% buffered formalin for 10 days. Next, the block sections were decalcified in 5% nitric acid for 7-8 days and were embedded in paraffin. 5㎛ thick serial sections were made in a mesiodistal direction at interval of 80㎛. The 4 most central sections from each block were stained with hematoxylin/eosin for examination by light microscopy.

4. Histological analysis

A PC-based image analysis system[‡] was used to observe the experimental sites with regards to junctional epithelium migration, formation of new bone and new cementum, absorption of implanted materials, regeneration of the attachment apparatus, arrangement of the

collagen and periodontal ligament fiber, root resorption, and ankylosis.

5. Histometric analysis

For the histometric analysis, the cementoenamel junction (CEJ) and the notch were used as reference points. The alveolar crest (AC) point is gained by subtracting from CEJ the distance between the CEJ and AC measured at the experiment. The histometric parameters and method of measuring were as follows:

1) Defect height (DH): distance from alveolar crest to the base of the reference notch (bN).
2) Junctional epithelium migration (JE): distance from alveolar crest to the apical extension of the junctional epithelium.
3) Connective tissue adhesion (CT): distance from the apical extension of the junctional epithelium to the coronal extension of cementum regeneration.
4) New cementum regeneration (NC): distance from the base of the reference notch to the coronal extension of newly formed cementum on the root surface. Cementum regeneration is measured in two ways:
   ① Infrabony cementum regeneration (IBC): distance from the base of the reference notch to the regenerated cementum that is lined with cementoblasts or has perpendicularly inserted collagen fiber.
   ② Suprabony cementum regeneration (SBC): distance from the infrabony cementum regeneration to the collagen fiber has paralleled to the new cementum and/or where cementoid tissue and cementum are not easily

distinguishable by light microscopy.

5) New alveolar bone regeneration (NB): distance from the base of the reference notch to the coronal extension of newly formed alveolar bone along the root surface.

6. Statistical analysis

Histometric recordings from the four sections from each defect were used to calculate mean scores for each animal. The data in each group was statistically analyzed with Kruskal-Wallis test. For the comparison between groups, Mann-Whitney U test was used. In addition, root resorption and ankylosis were dischotomously scored present if observed in one or more of the four sections for each tooth.

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† atrophin, Kwangmyung Pharmaceutical Ind. Co., LTD. Seoul, Korea
†† Rompun, Bayer Korea Co., Seoul, Korea
††† Ketara, Yuhan Co., Seoul, Korea
†††† Gerolan, Choongwae Pharmaceutical Co., Seoul, Korea
# Tetracyclin HCl, Chongkundang Pharmaceutical Co., Seoul, Korea
Hexamedin, Bukwang Pharmaceutical Co., Seoul, Korea
§ Entobar®, Hanlim Pharmaceutical Co., Seoul, Korea
‡ Image-Pro Plus, Media Cybernetics, Silver Spring, MD., USA

III. Results

A. Histologic Observation

1. Surgical control group

It was observed that the junctional epithelium has a tendency to migrate apically and laterally in the surgical control group than the other groups (Fig 5, 6). The collagen fibers were observed in parallel arrangement beneath the junctional epithelium. There was little or no inflammatory cell infiltration (Fig 5). The periodontal ligament fibers were usually oriented in a parallel direction but were perpendicular to the root surface where new bone was present adjacent to a thick cementum layer. Above the apical notch, a little amount of new cementum and bone was formed along the root surface (Fig 7). No resorption bays on the root surface were observed in all of the teeth. No site had signs of ankylosis (Fig 7).

2. Non Blended Polylactic and polyglycolic acid non woven (PLGA) Membrane group

The extension of junctional epithelium appeared irregular: the restraint in general was significant in most of the defects. Although the formation of new cementum and new bone tissue increased significantly, compared with the surgical control group, the extension of long junctional epithelium appeared slightly irregular and had migrated apically. It has tendency to be more apical than TC-PLGA membrane group and more coronal than

surgical control group (Fig 8). In the arrangement of periodontal ligament fibers, root resorption, and inflammatory cell infiltration, the results were similar to those of the TC-PLGA membrane group (Fig 8). There is no evidence of inflammation sign like infiltration of polymorphonuclear cells on the connective tissue where the membrane applied (Fig9).

3. Tetracycline Blended Polylactic and polyglycolic acid (TC-PLGA) Membrane group

Marked bone regeneration, greater than that in the surgical control, was observed in sites receiving TC-PLGA membrane. The extension of junctional epithelium was restrained. A significant amount of new cementum formed along the indentation of the notch and root surface to the level of the junctional epithelium, and new bone was also seen along with it above the alveolar bone (Fig 11). Both new cementum and new bone, however, increased in thickness apically (Fig 12). The cementoblasts aggregated on the root surface linearly in the notch surface (Fig 13). Neither the evidence of ankylosis nor root resorption was detected (Fig 11). There is no evidence of inflammation sign like infiltration of polymorphonuclear cells on the connective tissue where the membrane applied (Fig 14).

B. Histometric analysis (Table 1, Figure 2)

1. Junctional epithelium migration

Junctional epithelium migration amounted to 1.20 ± 0.27 ㎜ (27.6% of the entire depth of defects) in the surgical control group, 1.21 ± 0.26 ㎜ (23.9%)

in the PLGA membrane group, and 0.66 ± 0.17 ㎜ (13.1 %) in the TC-PLGA membrane group. A significant difference showed between the surgical control and TC-PLGA membrane group ($P<0.05$), and also between PLGA membrane and TC-PLGA membrane group ($P<0.05$).

2. Connective tissue adhesion

Connective tissue adhesion amounted to 0.85± 0.43 ㎜ (19.6% of the depth of defects), 0.69 ± 0.17 ㎜ (13.6%), and 0.64 ± 0.10 ㎜ (12.7%) respectively in the surgical control, PLGA membrane, and TC-PLGA membrane group. No significant difference was apparent between the groups.

3. Cementum regeneration

Cementum regeneration amounted to 2.00 ± 0.70 ㎜ (46.1% of the depth of defects), 3.16 ± 0.37 ㎜ (62.5 %), and 3.72 ± 0.53 ㎜ (73.8%) respectively in the surgical control, PLGA membrane, and TC-PLGA membrane group. Both the TC-PLGA membrane group and the PLGA membrane group showed a significant difference from the control group ($P<0.05$).

No significant difference was seen between PLGA membrane group and TC-PLGA membrane group ($P<0.05$).

Infrabony cementum regeneration amounted to 1.48 ± 0.53 ㎜ (34.1% of the depth of defects, 74.0% of the entire new cementum), 2.41 ± 0.21 ㎜ (47.6%, 76.3%), and 2.97 ± 0.31 ㎜ (59.2%, 79.8%) respectively in the surgical control, PLGA membrane, and TC-PLGA membrane group. In the three groups, suprabony cementum regeneration was respectively measured at 0.52 ± 0.21 ㎜ (11.9% of the depth of defects, 26.0% of the entire new

cementum), 0.75 ± 0.13 ㎜ (14.8%, 23.7%), and 0.75 ± 0.24 ㎜ (14.9%, 20.2%). Both the TC-PLGA membrane group and the PLGA membrane group showed a significant difference from the control group ($P<0.05$).

No significant difference was seen between PLGA membrane group and TC-PLGA membrane group ($P<0.05$).

4. Alveolar bone regeneration

Alveolar bone regeneration amounted to 1.46 ± 0.68 ㎜ (33.6% of the depth of defects), 2.39 ± 0.52㎜ (47.2%), and 2.88 ± 0.66㎜ (57.4%) respectively in the surgical control, PLGA membrane, and TC-PLGA membrane group. Both the TC-PLGA membrane group and the PLGA membrane group showed a significant difference from the control group ($P<0.05$). No significant difference was seen between PLGA membrane group and TC-PLGA membrane group ($P<0.05$).

Table 1. Histometric analysis (㎜)

	control group	PLGA membrane	TC-PLGA membrane
	Mean ± SD	Mean ± SD	Mean ± SD
DH	4.34 ± 1.14	5.06 ± 0.35	5.02 ± 0.50
JE	1.50 ± 0.27	1.21 ± 0.26	0.66 ± 0.17 * +
CT	0.85 ± 0.60	0.69 ± 0.17	0.64 ± 0.10
NC	2.00 ± 0.70	3.16 ± 0.37 *	3.72 ± 0.53 *
IBC	1.48 ± 0.53	2.41 ± 0.21 *	2.97 ± 0.31 *
SBC	0.52 ± 0.21	0.75 ± 0.13 *	0.75 ± 0.24 *
NB	1.46 ± 0.69	2.39 ± 0.52 *	2.88 ± 0.66 *

* statistically significant difference compared to surgical control group, $P<0.05$

+ statistically significant difference compared to PLGA membrane group, $P<0.05$

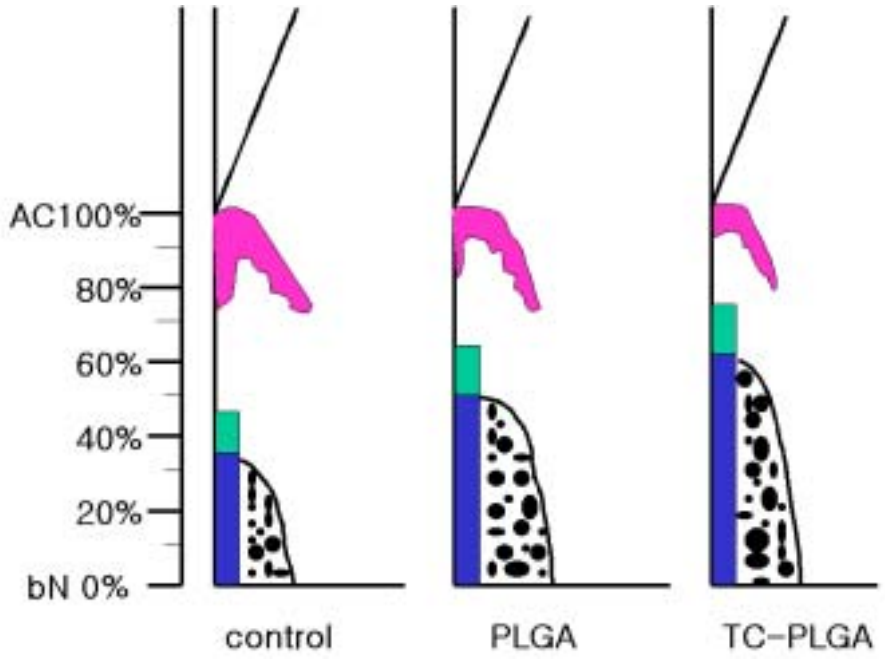

Fig 2. Periodontal healing illustrated in percentage of defect height.
:Rate of New bone and new cementum regeneration
blue stick: intrabony cementum regeneration
green stick: suprabony cementum

IV. Discussion

The ultimate goal of periodontal therapy is to regenerate the destroyed supporting tissue. Though various procedures such as guided tissue regeneration (Kim et al. 1998b; Kim et al. 1998c; Niloofar et al. 1999; Trombelli et al. 1997), autograft (Schallhorn 1972), other bone grafts (Kim et al. 1998a; Kim et al. 1998b; Kim et al. 1998c; Mellonig et al. 1976; Mellonig 1984), and application of a growth factor (Becker et al. 1992; Choi et al. 2002; Lindhe et al. 1995; Park et al. 2003; Wikesjo et al. 1999) have been developed and practiced to help regeneration.

The use of GTR has proven to be a suitable technique for stimulating regeneration (Becker et al. 1991; Caffesse et al. 1990) and new connective tissue attachment on exposed root surfaces associated with osseous defects (Cortellini et al. 1993; Gottlow et al. 1990).

However, gingival recession during healing following GTR therapy has been described as a frequent complication. In vitro study, where fibroblast attachment to non-resorbable membrane was altered in comparison to the control group (Park et al. 2003). And it appears that resorbable membrane use in GTR such as Guidor® and Resolut® could affect rat gingival fibroblast morphology, and thus alter wound healing (Simain-Sato et al. 1999). Therefore it needs accompanying effects to prevent infection during healing period.

The use of tetracycline HCL as a treatment at other hard/soft tissue boundaries offers the prospect of accelerating the normal healing process (Victor et al. 1986). And in vitro studies have shown that tetracycline

HCL is highly effective against the majority of peridontopathic microorganisms, juvenile periodontitis (Becker et al. 1991; Demolon et al. 1993), and refractory periodontitis (Silverstein et al. 1988).

Bjorvatn et al.(1971) immersed freshly extracted molars of rats in TC solution, which caused a marked stimulation of alveolar bone formation after replantation. And, in similar study high concentrations of locally applied TCs might have an osteogenic action in addition to their antibiotic action (Hars et al. 1972).

The impregnated cellulose membrane can probably be used in GTR acting as a membrane and as a slow release device, liberating the chemotherapeutic agent in concentrations high enough to eliminate periodontopathic microorganism (Cristina et al. 1995). And the use of tetracyline-coated ePTFE barrier membranes can result in additional gain of clinical periodontal attachment, most likely due to the antimicrobial properties of tetracycline during initial healing (Niloofar et al. 1999).

In comparison of the Bioresorbable and the non resorbable membrane, both devices were effective but that the use of the bioresorbable device gave more horizontal probing attachment again and less gingival recession (Lindhe et al. 1995). The necessity of re-entry surgery in non resorbable membrane has disadvantages to the patient and surgeon.

The polymeric components of the PLGA barrier are broken down by hydrolysis and eliminated from the body through the Kreb's cycle as carbon dioxide and water (Caffesse et al. 1997; Hurzeler et al. 1997). The degradation rate is dependent on the pH, the presence of mechanical strain, enzymes, and bacterial infection. It also varies depending on the

composition; modification of poly (L-lactide) by cross-linking or addition of D-lactide or glycolide results in materials that have more rapid degradation, thus diminishing the poly (L-lactide) disadvantage of slow degradation (Bergsma et al. 1995; Marcato et al. 1996; Miller et al. 1977). Poly (glycolic acid) degrades the fastest and poly (L-lactide) is the most stable in vitro. PLGA in present study is composed of 50 : 50 poly (glycolic acid): poly (L-lactide) copolymer.

The use of two different types of resorbable barriers (Resolut® and Guidor®), group of Resolut® which is composed of polylactide-glycolide copolymer promoted new attachment formation over an exposed and instrumented root surface in GTR (Aukhil 1991), but group of Guidor® which is composed of polylactide/citric acid ester (acetyl-tributylcitrate) appeared to be included in a granuloma (Gottlow et al. 1994).

In a review paper by Hutmacher et al. (1996), it was suggested that the biodegradation of a polymer may be markedly affected by the addition of a plasticizer such as citric acid ester. That citric acid is suggested to be induced inflammation and foreign-body reaction.

The advantages of pure electrospun PLGA membrane in present study are the first is porosity to admit blood circulation and tissue integration, the second is to increase exposure surface for the tissue integration, the third is flexibility for the manupulation. the fourth is to reduce the production cost and to make mass production, the fifth is to avoid the citric acid.

The method of making the PLGA membrane is that PLGA chips are melted in heat plate at 240 ℃ and then high pressure and voltage make it

annealed through the nozzle, and the annealed PGA microfilament less than 10㎛ was compressed on the rotating drum. The proper annealing temperature is 130 ℃ by immersion time test because of more stable resorption time and physical properties.

PGA and PLA have crystallization and hydrophilic properties, therefore it induces rapid resolution. In present study used Electro-spun Non woven membrane can exclude using of the plasticizer which induced foreign-body reaction and control the resolving time by the spun form of membrane preventing early resorption of the PGA. Also it can be expected that TC blending membrane display the pharmacological effects of tetracycline in the periodontal wound sites.

Many kind of methods to combine membrane with TC have been introduced such as TC coating over the membrane, soaking the membrane with TC, chemical vapor deposition, or blending. In our study, PLGA is blended with tetracycline 100:1 ratio to make microsphere which is ideal to control the releasing rate by increasing exposure surface, and then microsphere type TC-PLGA is chemically evaporized onto the electrospun non woven PLGA membrane. In pilot study releasing rate of microsphere type TC-PLGA is rapidy increase at the first 7 days than other film matrix type. It can be suggested that TC-PLGA has better releasing properties to protect early infection after surgery.

The use of surgically created defects in the present study was decided based on a previous report showing that the healing process shows no difference between the surgical defects and the attachment loss caused by natural disease or ligation (Wikesjo et al. 1990; Wikesjo et al. 1991a;

Wikesjo et al. 1991b). Such an artificial method helps keep the initial condition of the control and the experimental group almost identical and hence improves the credibility of the experiment (Haney et al. 1993).

In our histometric study, the amount of regenerated new bone and cementum tissue was no significant difference between TC-PLGA and PLGA membrane. but it has tendency to regenerate more new bone and new cementum in TC-PLGA than in PLGA membrane group. And a significant difference in junctional epithelium migration (see Fig 5,8,11.) was observed between the surgical control and PLGA and TC-PLGA groups. TC-PLGA group shows the limited apical migration of long junctional epithelium, that outcome proves that TC-PLGA membrane has an ability of protecting the surgical wound infection and the gingival recession. New bone formation was more extensive in the TC-PLGA group than surgical control group after surgery significantly. And no inflammatory cell like PMN is appeared. Neither the evidence of ankylosis nor root resorption was detected in TC-PLGA and PLGA membrane groups (Fig 8,11).

As described by Ten Cate (1997), the type of cementum which re-forms on these TC-PLGA experimental root surface during successful regeneration is almost cellular cemntum rather than the acellular type. Nearby the new cementum cementoblasts arranged linearly (Fig 13). In the connective tissue field, there is no evidence of infiltration of inflammatory PMN cells for proving safety of this membrane. (Fig 9,14) That proves biocompatibility of newly made TC-PLGA and PLGA membrane.

The increased new bone and new cementum gain in TC-PLGA may be due

to the antimicrobial effect of tetracycline, but could also partly be the result of the antibiotic's enzyme (collagenase) inhibiting and antiinflammatory properties. And other aspects, it is suggested that tetracycline might initiate demineralization on the bone surface layer(Drury et al. 1991), which would probably result in the release of some osteogenic factors, such as transforming growth factor-β (TGF-β), insulin like growth factor (IGF), or bone morphogenetic proteins (BMPs), into the surrounding tissues to trigger the bone induction effects.

The utility of membrane-assisted periodontal therapy remains a subject of controversy (Becker et al. 1999; Mayfield et al. 1998). However, if dentists elect to use the barrier membrane, it may be worthwhile to consider TC-PLGA membrane for reducing the possibility of infection and promoting of new bone formation.

From the above results, it can be inferred that not only TC-PLGA membrane can restrain the migration of epithelium and promote the formation of bone and cementum, but also maintain the wound space without foreign body reaction. In spite of many advantages of newly developed TC-PLGA membrane in our study, it lacks clinical studies.

The details of how it promotes periodontal regeneration, however, still remains unexplained, which calls for future researches. Especially, a longer-term study is necessary to document the process of the formation of bone, cementum, and periodontal ligament and collagen fiber in their structural and functional developments, also to research human clinical study.

V. Conclusion

The purpose of this study was to evaluate the regenerative effects of TC-PLGA and PLGA barrier membrane implanted to the preclinical one wall intrabony defects surgically created in beagle dogs. 4×4 ㎜ defects were created in bilateral mandibular second and fourth premolars. The surgical control group received flap operation only. The subjects were sacrificed at eight weeks after operation, and the comparative histological examination of their healing results yielded the following conclusion.

1. Junctional epithelium migration amounted to 1.20 ± 0.27 ㎜ (29.7% of the entire depth of defects) in the surgical control group, 1.21 ± 0.26 ㎜ (23.9%) in the PLGA membrane group, and 0.66 ± 0.17 ㎜ (13.1 %) in the TC-PLGA membrane group. A significant difference showed between the surgical control and TC-PLGA membrane group ($P<0.05$),and also between membrane and TC-PLGA membrane group ($P<0.05$)

2. Connective tissue adhesion amounted to 0.85± 0.43 (21.0% of the depth of defects), 0.69 ± 0.17 ㎜ (13.6%), and 0.64 ± 0.10 ㎜ (12.7%) respectively in the surgical control, membrane, and TC-PLGA membrane group. No significant difference was apparent between the groups.

3. Cementum regeneration amounted to 2.00 ± 0.70 ㎜ (49.5% of the depth of defects), 3.16 ± 0.37 ㎜ (62.5 %), and 3.72 ± 0.53 ㎜ (73.8%) respectively in the surgical control, membrane, and TC membrane group. Both the TC membrane group and the membrane group showed a significant difference from the control group ($P<0.05$).

Infrabony cementum regeneration amounted to 1.48 ± 0.53 ㎜, 2.41 ± 0.21 ㎜, and 2.97 ± 0.31 ㎜ respectively in the surgical control, PLGA membrane, and TC-PLGA membrane group. In the three groups, suprabony cementum regeneration was respectively measured at 0.52 ± 0.21 ㎜, 0.75 ± 0.13 ㎜, and 0.75 ± 0.24 ㎜. In both, the PLGA and the TC-PLGA group showed a significant difference from the control group ($P<0.05$).

No significant difference was seen between PLGA membrane group and TC-PLGA membrane group.

4. Alveolar bone regeneration amounted to 1.46 ± 0.68 ㎜ (24.8 % of the depth of defects), 2.39 ± 0.52 ㎜ (47.2%), and 2.88 ± 0.66 ㎜ (57.1%) respectively in the surgical control, PLGA membrane, and TC-PLGA membrane group. Both the TC-PLGA membrane group and the PLGA membrane group showed a significant difference from the control group ($P<0.05$). No significant difference was seen between PLGA membrane group and TC-PLGA membrane group ($P<0.05$).

The above results demonstrate the beneficial effect of TC-PLGA membranes to the preclinical one wall intrabony defects of beagle dogs. The inhibited apical migration of epithelium and the increase in new bone and new cementum suggest the potency of TC-PLGA membrnae in inducing periodontal tissue regeneration.

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국문 요약

성견의 1면 골내낭에서 테트라사이클린과 합성된 차단막이 치주조직 치유에 미치는 영향

치주 치료의 최종 목적은 진행되는 치주 질환의 증상을 제거하는 것뿐만 아니라 이미 파괴된 지지조직을 기능적으로 재생시키는데 있다. 이를 위해 조직유도 재생술, 골 이식술과 성장 인자의 적용 등 다양한 치주 재생을 위한 술식이 시행되어져 왔으나, 그 각각이 모두 한계를 나타내고 있다. 특히 차단막을 이용한 치주조직재생술은 오랜기간에 걸쳐 실험되고 임상에 적용되고 있으나 그 재료가 가진 특성과 임상적 적용의 어려움으로 인해 술후 감염과 치은 퇴축 등으로 인한 치주재생조직의 한계성을 나타냈다.

이에 본 연구에서는 성견의 1면 골내낭에 TC-PLGA와 PLGA membrane을 적용하였을 때 치주조직 재생에 미치는 영향을 평가하기 위하여 비글견의 양측 하악 제 1 소구치 근 원심면과 제 3 소구치 근심면에 근원심폭 4 ㎜, 치조정에서 깊이 4 ㎜의 1면 골결손부를 형성하여 치은박리 소파술만 시행한 부위를 대조군으로, 치은박리 소파술 후 PLGA membrane를 적용한 군을 실험 1군으로, 치은박리 소파술 후 TC-PLGA membrane를 적용한 군을 실험 2군으로 설정하여 실험하고 술 후 8주에 치유 결과를 조직학적으로 비교 관찰하여 다음과 같은 결론을 얻었다.

1. 접합상피의 치근단 이동량은 대조군, 실험 1군, 실험 2군에서 각각 1.20 ± 0.27 ㎜, 1.21 ± 0.26 ㎜, 0.66 ± 0.17 ㎜로 나타났으며, 대조군과 실험 2군간에는 유의성 있는 차이가 있었고 ($P<0.05$), 대조군과 실험 1군과도 유의성

있는 차이가 없었으며, 실험 1군과 실험 2군간에도 유의성있는 차이가 있었다. ($P<0.05$)

2. 결합조직 유착의 길이는 대조군, 실험 1군, 실험 2군에서 각각 0.85 ± 0.43 ㎜, 0.69 ± 0.17 ㎜, 0.64 ± 0.10 ㎜로 나타났으며, 대조군과 실험 1, 2군간에 유의성 있는 차이는 없었다.

3. 신생백악질 형성은 대조군에서 2.00 ± 0.70 ㎜, 실험 1군에서 3.16 ± 0.37 ㎜, 실험 2군에서 3.72 ± 0.53 ㎜로 나타났으며, 대조군과 실험 1군 및 대조군과 실험 2군 사이에 유의성있는 차이를 보였다($P<0.05$).실험 1군과 실험 2군 사이에는 유의성있는 차이가 없었다.

4. 신생골 형성은 대조군, 실험 1군, 실험 2군에서 각각 1.46 ± 0.68 ㎜, 2.39 ± 0.52 ㎜, 2.88 ± 0.66 ㎜로 나타났으며, 대조군과 실험 1군 및 대조군과 실험 2군 사이에 유의성있는 차이를 보였다($P<0.05$). 실험 1군과 실험 2군 사이에는 유의성있는 차이가 없었다.

이상의 결과에서 볼 때, 성견의 1면 골결손부에 TC-PLGA membrane을 사용한 경우 상피 이주 억제 효과가 있었으며, 신생백악질과 신생골 형성도 증가시킬 수 있음을 알 수 있었고, 따라서 TC-PLGA membrane은 치주조직 재생에 효과적인 재료라고 사료된다.

핵심되는 말: TC-PLGA membrane, PLGA membrane, 1면 골내낭, 치주 조직 재생, 신생골, 신생백악질, 치주인대 섬유, 긴 접합상피

Legends

Figure 3. The buccal and lingual alveolar bone was surgically removed to create 4×4 ㎜ 1-wall intrabony defects.

Figure 4. Defect treated with TC-PLGA and PLGA membrane.

Figure 5. A surgical control site showing apical migration of junctional epithelium and minimal regeneration of new bone and new cementum. (H-E: a original magnification×20)

Figure 6. A surgical control site, as in figure 5, showing apical migration of junctional epithelium (arrow) and loose connective tissue. (H-E: a original magnification×40)

Figure 7. A surgical control site, as in figure 5, showing a coronally thinning strip of new cementum with adjacent collagen fibers arranged parallel to the root surface. (H-E: a original magnification×100)

Figure 8. A PLGA membrane site showing dense connective tissue and a moderate increase of new bone and new cementum. (H-E: a original magnification×20)

Figure 9. A PLGA membrane site showing no evidence of inflammation like infiltration of PMN cells (H-E: a original magnification× 400)

Figure 10. A PLGA membrane site showing, as in figure 8, the perpendicular arrangement of periodontal ligament fibers in the areas of new bone and new cementum (arrows) regeneration. (H-E: a original magnification×400)

Figure 11. A TC-PLGA membrane site showing minimal apical migration

of junctional epithelium and noticeable increase of new bone and new cementum, which coronally thin. (H-E: a original magnification×20)

Figure 12. A TC-PLGA membrane site showing thick layers of new cementum with parallel or oblique collagen fiber arrangement and dense connective tissue with many blood vessels. (H-E: a original magnification×100)

Figure 13. A TC-PLGA membrane site, as in figure 12, showing perpendicular insertion of periodontal ligament fibers in new bone and new cementum respectively lined with osteoblasts and cementobalsts (black triangle arrow). (H-E: a original magnification×400)

Figure 14. A TC-PLGA membrane site, as in figure 9, showing no evidence of inflammation like infiltration of PMN cells (H-E: a original magnification×400)

Figures (I)

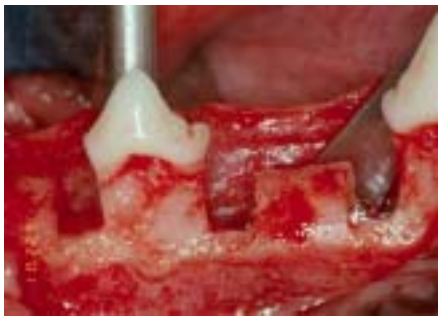

Fig 3

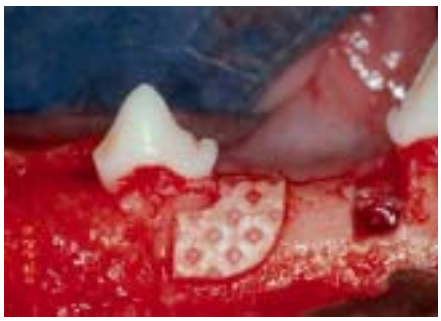

Fig 4

Figures (II)

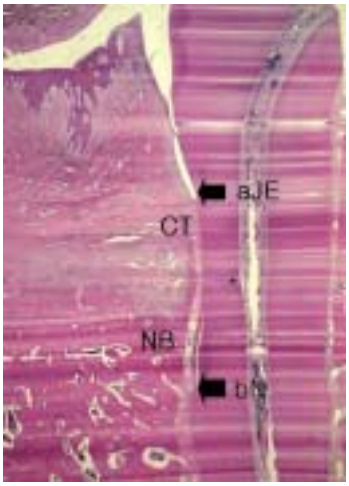

Fig 5

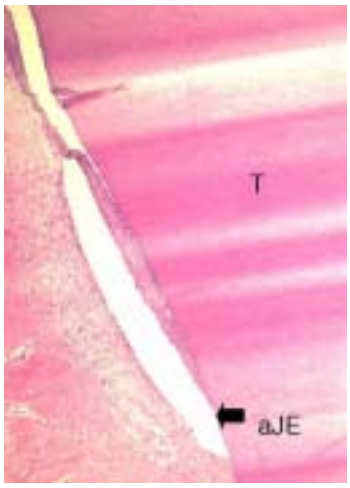

Fig 6

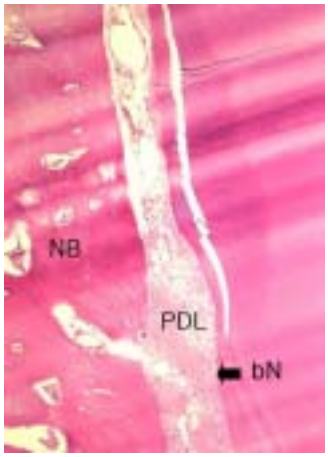

Fig 7

Figures (III)

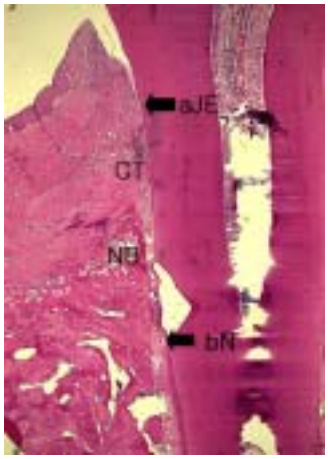

Fig 8

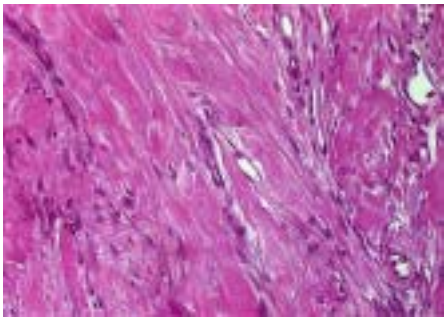

Fig 9

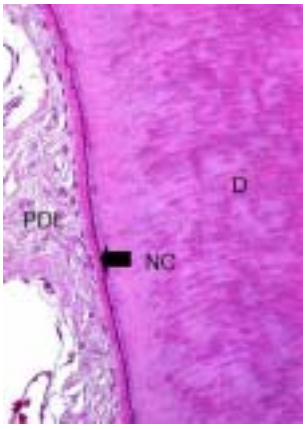

Fig 10

Figures (IV)

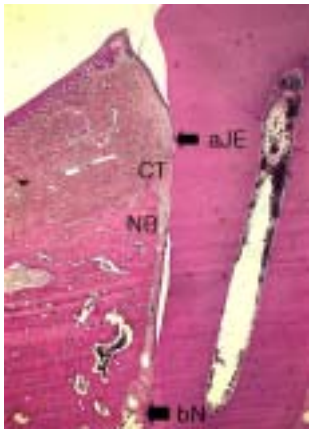

Fig 11

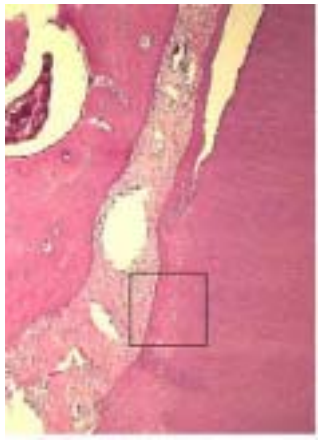

Fig 12

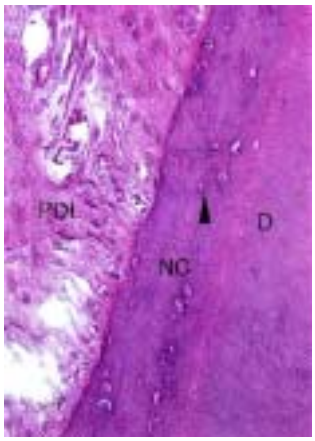

Fig 13

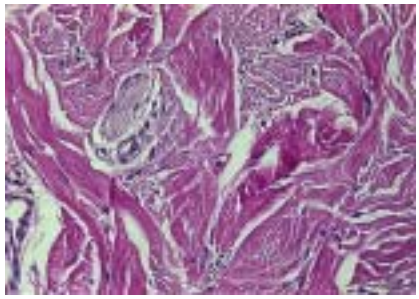

Fig 14