Archives of Oral Biology 63 (2016) 15–21

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Efficacy of doxycycline release collagen membrane on surgically created and contaminated defects in rat tibiae: A histopathological and microbiological study

Esma Kütan[a,*], Gonca Duygu-Çapar[b], Ceyda Özçakir-Tomruk[c], Ozkan Cem Dilek[d], Fatma Özen[e], Özge Erdoğan[f], Ipek Özdemir[g], May Kroachi[h], Aydin Gürel[i]

[a] Department of Oral Implantology, Faculty of Dentistry, Yeditepe University,Göztepe, Istanbul, Turkey
[b] Department of Oral and Maxillofacial Surgery, Trakya University, Edirne, Turkey
[c] Department of Oral and Maxillofacial Surgery, Yeditepe University, Göztepe, Istanbul, Turkey
[d] Department of Oral Implantology, Faculty of Dentistry, Yeditepe University, Göztepe, Istanbul, Turkey
[e] Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, Kayışdağı, Istanbul, Turkey
[f] Department of Pathology, Faculty of Veterinary, Istanbul University, Avcilar, Istanbul, Turkey
[g] Department of Biological Sciences and Bioengineering, Faculty of Engineering and Natural Sciences, Sabanci University, Tuzla, Istanbul, Turkey
[h] Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, Kayışdağı, Istanbul, Turkey
[i] Department of Pathology, Faculty of Veterinary,Istanbul University, Avcilar, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 20 November 2014
Received in revised form 29 June 2015
Accepted 2 November 2015

Keywords:
Doxycycline
Rat tibiae
Guided bone regeneration
Porphyromonas gingivalis

ABSTRACT

Background: The effects of systemic antibiotics on controlling infective pathogens after guided bone regeneration(GBR) procedures especially in membrane exposures are limited. However, local administrations of antibiotics are rare in GBR techniques.
Aim: The aim of this study was to investigate the osteogenesis potential and the antibacterial effect of a doxycycline releasing collagen membrane in surgically created and contaminated defects in rat tibiae.
Material and methods: Defects were created in 20 rats that wer randomly divided in to two groups: control group (defect contaminated by *Porphyromonas gingivalis*, filled with bone graft and covered by collagen membrane); test group (defect contaminated by *P. gingivalis* filled with bone graft and covered by collagen membrane containing 1 mg/cm^2 doxycycline. Animals were sacrificed post surgically on the 14th day for microbiologic evaluation and on the 28th day for histopathological evaluation.
Results: The degree of osteogenesis in the test group was seen to be significantly higher than control group (p: 0.011; $p < 0.05$). Furthermore in test group, no bacterial growth was observed. The bacteria counts were determined between 1×104 and 268×104 CFU/g with a median of 1.32×104 for control group.
Conclusions: Within the limitations of this study, the results of the present study suggests that the use of a doxycycline releasing membrane has a positive effect on contaminated GBR procedures for limiting *P. gingivalis* infections leading to bone formation following GBR procedures in a rat model.

1. Introduction

Many studies on guided bone regeneration (GBR) have evaluated the use of different types of grafts, membranes or their combinations. However there is limited information on the potential use of drug releasing membranes (Retzepi & Donos, 2010).

Barrier membranes that successfully prevents the migration of cells, like epithelial or fibroblasts, are mostly used in GBR for both the regeneration of periodontal tissue and pre-implant augmentation of the bone. Various types of bony defects are successfully filled with bone substitutes and covered with membranes that allows mesenchymal differentiation into osteoblasts in deficient alveolar ridges (Zohar et al., 2004). Dura mater, poly-L-lactic acid, polyglycolic acid, polyurethane and collagen have been introduced as biodegradable membranes which advantageously eliminate the need for an additional membrane removal procedure (Khojasteh, Soheilifar, Mohajerani, & Nowzari, 2013). This procedure not only diminishes the cost and time but is also preferred by patients.

* *Corresponding author at: Bagdat Cad. No: 238 34728 Goztepe, Istanbul, Turkey.
E-mail address: esmakutann@gmail.com (E. Kütan).

http://dx.doi.org/10.1016/j.archoralbio.2015.11.001
0003-9969/

Collagen, which is generally accepted as an ideal material for barrier preparation,promotes progenitor cell adhesion, chemotaxis, homeostasis, and physiologic degradation with low immunogenicity (Zohar et al., 2004). It is also the fundamental component of connective tissue which provides structural support for tissues of the whole body and a good reservoir for drug delivery (Hitti & Kerns, 2011).

Collagen membranes in GBR procedures have been widely reported (Hitti & Kerns, 2011), and there are many factors that are reported to influence the success of GBR techniques. These include: wound dehiscence, uneventful plaque accumulation, periodontal infections and smoking (Cheng, Wu, Chen, & Hung, 2013). Furthermore, the predominant factor affecting bone formation, is believed to be due to bacterial colonization (Cheng et al., 2013; Donos, Kostopoulos, & Karring, 2002; Yoshinari et al., 2001). Therefore, the effective control of bacterial contamination has been identified as a major point in the regeneration procedure (Cheng et al., 2013).

Machtei et. al., reported an overall incidence of membrane exposure in 60% of GBR procedures. These had a negative effect on the healing process.Furthermore, experimental studies on guided tissue regeneration (GTR) in monkeys have shown that bacterial accumulation caused by membrane exposure, decreased the level of new bone formation (Machtei, 2001; Sander & Karring, 1995).

The use of systemic antibiotics for infection control following GBR surgical procedures are limited in their efficiency (Demolon, Persson, Ammons, & Johnson, 1994; Vest et al., 1999). Different topical antibiotic application methods including tetracycline and amoxicillin have previously been evaluated in GBR treatment with promising outcomes, including reduced bacterial contamination and increased clinical attachment (Yoshinari et al., 2001; Frandsen, Sander, Arnbjerg, & Theilade, 1994; SanderL, randsen, Arnbjerg, Warrer, & Karring, 1994; Zucchelli, Sforza, Clauser, Cesari, & De Sanctis, 1999; Markman, Fracalanzza, & Novaes, 1995; Zarkesh, Nowzari, Morrison, & Slots, 1999; Goodson, 1994). Little is known about drug release membranes in guided bone regeneration around implants when membranes exposed during healing.

Previous studies have found high proportions of periodontal pathogens such as *Porphyromonas gingivalis*,*Actinobacillus actnomycetemcomitans* and *Peptostreptococcus micros* in exposed membranes. Adversely, no periodontal pathogens were detected in submerged healing (Nowzari, London, & Slots, 1995). When controlled degradation process is obtained for the collagen membrane, this may be active against a wide range of gram-positive and gram-negative organisms. Furthermore, collagen aids in the recovery process (Albu, Ghica, Ficai, Titorencu, & Popa, 2010; Titorencu et al., 2010; Walker, Pappas, Tyler, Cohen, & Gordon, 1985; Kulkarni, Lee, Aitken, Birek, & McCulloch, 1991). To the authors knowledge, the osteogenic potential and antibacterial efficacy of a doxycycline releasing collagen membrane in a rat model with induced contaminated defects has not been previously reported.

The aim of this study was to evaluate both the osteogenic potential and the antibacterial effect of a doxycycline releasing collagen membrane in surgically created and contaminated defects in rat tibiae.

2. Materials and methods

Twenty, three months old rats (Rattus norvegicus albinus, Wistar), weighing between 400 and 450 g were used. The animals were kept in temperature-controlled rooms and took water and food ad libitum. All animals were kept according to the guidelines for laboratory animal treatment and care and all protocols were approved by the local animal welfare committee (363/13.12.2013). The animals were randomly allocated into two experimental models.

2.1. Experimental model

- Control group: the defect was contaminated with *P. gingivalis* ATCC 3744, filled with Xenograft and covered with a membrane consisted of only one layer of collagen with thickness of about 0.2 mm which contain 5 mg collagen sterilized with gamma radiation, 25 kGy (Albu et al., 2010; Ghica, Albu, TitorencuI Albu, & Popa, 2012).
- Test group: the defect was contaminated by *P. gingivalis* ATCC 3744, filled with Xenograft and covered with a membrane consisted of only one layer of collagen with thickness of about 0.2 mm which contain 5 mg collagen and 1 mg/cm^2 doxycycline hyclate sterilized with gamma radiation, 25 kGy (Albu et al., 2010; Ghica et al., 2012).

2.2. Surgical procedure

All surgical procedures were performed under strict aseptic conditions. Thirty rats were weighed before subcutaneously injecting with ketamine (Ketasol %10, Richter Pharma Ag, Wels, Austria) (20 mg/kg) and xylazine (10 mg/kg) (Rompun %2, Bayer

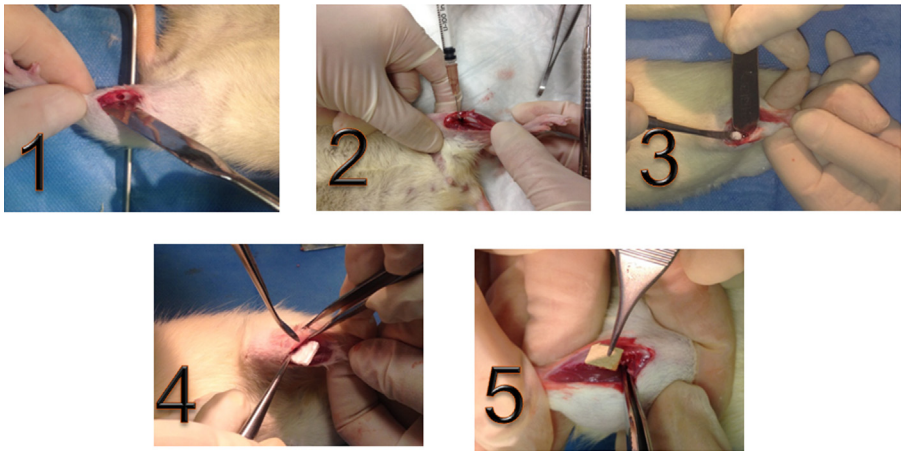

Fig. 1. Surgical procedures of specimens (1) Defect. (2) Microbial injection. (3) Grafting. (4) Collagen covering. (E) Doxycycline released collagene covering.