

Evaluation of cell sheet application on one wall bone defect in *Macaca nemestrina* through periostin expression

This content has been downloaded from IOPscience. Please scroll down to see the full text.

2017 J. Phys.: Conf. Ser. 884 012039

(<http://iopscience.iop.org/1742-6596/884/1/012039>)

View [the table of contents for this issue](#), or go to the [journal homepage](#) for more

Download details:

IP Address: 152.118.148.226

This content was downloaded on 06/09/2017 at 04:20

Please note that [terms and conditions apply](#).

You may also be interested in:

[Accuracy of measurements of potassium content of monkeys by in vivo body counting as compared to chemical analysis](#)

A M Kodama, N Pace and S J Parot

[Assembly of cells and vesicles for organ engineering](#)

Tetsushi Taguchi

[ALP gene expression in cDNA samples from bone tissue engineering using a HA/TCP/Chitosan scaffold](#)

N Stephanie, H Katarina, L R Amir et al.

[Repairing a bone defect with a three-dimensional cellular construct composed of a multi-layered cell sheet on electrospun mesh](#)

Zhiwei Ren, Shiqing Ma, Le Jin et al.

[Shaping tissues by balancing active forces and geometric constraints](#)

Jasper Foolen, Tadahiro Yamashita and Philip Kollmannsberger

[Generation of tissue constructs and cell patch seeding for cellularization](#)

P R Anil Kumar, H K Varma and T V Kumary

[Fabrication of a thermoresponsive cell culture dish: a key technology for cell sheet tissue engineering](#)

Jun Kobayashi and Teruo Okano

[Comparison of mesenchymal stem cells released from poly\(N-isopropylacrylamide\) copolymer film and by trypsinization](#)

Lei Yang, Fang Cheng, Tianqing Liu et al.

Evaluation of cell sheet application on one wall bone defect in *Macaca nemestrina* through periostin expression

R Y Tamin¹, Y Soeroso¹, L Amir² and E Idrus^{2*}

¹Department of Periodontics, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

²Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

*E-mail: erik.idrus31@ui.ac.id

Abstract. Chronic periodontitis is an oral disease in which the destruction of periodontal tissue leads to tooth loss. Regenerative therapy for attachment cannot be applied to one wall bone defects owing to the minimal existing healthy bone. Tissue engineering in the form of cell sheets has been developed to overcome this limitation. In a previous study, cell sheet application to a one wall bone defect in *Macaca nemestrina* showed good clinical results. To evaluate the effectiveness of cell sheet application histologically, the level of periostin expression in the gingival crevicular fluid (GCF) of *M. nemestrina* was determined. Periostin is a 90-kDa protein that regulates coordination and interaction for regeneration and tissue repair. A laboratory observation study was performed to see the differences in periostin levels in samples collected from *M. nemestrina*'s GCF, where a cell sheet was applied to the bone defect. Gel electrophoresis with SDS-PAGE was performed to detect periostin expression based on its molecular weight and to compare the expression band between the cell sheet and the control at 1, 2, and 3 weeks after treatment. The gel electrophoresis result shows different thicknesses of the protein band around the molecular weight of periostin between the cell sheet groups.

1. Introduction

Periodontal disease is the second-most common oral health problem in Indonesia after dental caries [1]. Periodontitis is a chronic inflammation of periodontal tissue that is caused by a group of specific microorganisms; this disease leads to the destruction of the periodontal ligament and alveolar bone. Periodontitis appears in the form of pocket formation and/or gingival recession. Progressive periodontitis with inadequate treatment could lead to a mobile tooth and tooth loss [2,3]. Most chronic periodontitis cases involve a horizontal bone defect or one wall bony defect that involves almost all of the bone surface. Conventional periodontal treatment, including surgical debridement, is usually performed for repair rather than for regeneration. The ultimate objective of periodontal regenerative therapy is to form new bone and cementum with supportive periodontal ligaments. However, currently available periodontal regenerative therapies cannot achieve this goal [4]. Bone graft application cannot be used to reconstruct large defects in periodontal tissue because of the absence of a healthy bone wall to stabilize the graft until osteogenesis is completed. A previous study showed the potential of mesenchymal stem cells for tissue engineering to reconstruct large defects in periodontal disease. Four important components that are crucial to periodontal regenerative therapy are cells, scaffolds, signaling molecules, and blood supply [5]. Cell sheet technology (CST) is a stem cell cultured with



temperature-sensitive substrates. Cell sheets with periodontal ligament cells can be applied on defect areas to promote osteogenesis. Although CST shows promise as a mesenchymal stroma cell carrier that can be used as regenerative material, its loose structure has limited its function. It is difficult to maintain its adherence to the root surface. To overcome this limitation, it is modified via the application of RGD and chitosan [6]. Periodontal tissue regeneration using CST was clinically and radiographically evaluated on *Macaca nemestrina*. To support this study series, experimental molecular evaluation is needed to describe the progression of regeneration during the short term. Periostin is a 90-kDa extracellular protein that can be used as a biomarker for periodontal ligament and bone regeneration. As a prior study for molecular evaluation, this study will evaluate the protein band expression detected in SDS-PAGE gel around the molecular weight of periostin (90 kDa) in a control group (chitosan only), chitosan-cell sheet group, and RGD-chitosan cell sheet group in the gingival crevicular fluid (GCF) of *M. nemestrina*.

2. Materials and Methods

The study design is a descriptive laboratory experiment on *M. nemestrina*. A split-mouth design was performed on four regions of the lateral incisors of *M. nemestrina*. The alveolar bone in each region was intentionally destroyed to imitate the one wall defect of chronic periodontitis with the open flap procedure. In each region, different treatment was applied to the destruction site: chitosan only as a control group, cell sheet with chitosan, and cell sheet with RGD-chitosan. Cell sheet preparation was done before the flap procedure with periodontal ligament cells extracted from the central incisor. CST filled with mesenchymal stroma cells was adhered to the chitosan and applied to the defect area, and in the other group, CST was adhered to chitosan and RGD was used to adhere the cell sheet-chitosan to the root surface to enhance the attachment.

GCF was collected from the gingival sulcus of each region of the lateral incisor by using some paper points every week. Paper points filled with GCF were placed in 100 μ l of phosphate buffered saline in Eppendorf tubes and stored at -80 °C in a refrigerator. A Bradford assay (ThermoScientific™ Pierce™ Coomassie Plus Protein Bradford kit) was performed to determine the protein concentration of each sample by using an ELISA Reader. An electrophoresis assay was performed using SDS PAGE (BioRad™) to detect protein in a sample based on its molecular weight.

3. Results and Discussion

3.1 Results

The initial bone regeneration could be evaluated from the periostin expression. Periostin was detected based on its molecular weight by using SDS PAGE. The molecular weight of periostin is 90 kDa, and some form of periostin could be detected at 87 kDa (Rios, Padial-Molina, and Volk 2015). In this study, some protein band was seen at 70–100 kDa in the cell sheet and chitosan group 2–3 weeks after application. Another protein band appeared at 70–100 kDa in the RGD-chitosan cell sheet group 1–2 weeks after application and faded after three weeks. This protein band can be assigned to periostin. In the chitosan group, no protein band was seen at 70–100 kDa in the first three weeks.

In the cell sheet chitosan group, no protein band appears in the first week, but there is a light protein band in the second week and a thicker one in the third week at around the molecular weight of periostin. In the RGD cell sheet and chitosan group, there is a protein band at 70–100 kDa area in the first two weeks. This detection of the periostin protein band could indicate that healing and bone regeneration has already occurred in the first two weeks, which is earlier than in the cell sheet chitosan group.

3.2 Discussion

The goal of periodontal therapy is periodontal regeneration. Developments in tissue engineering have been aimed at realizing regeneration, especially in one wall bone defects. To achieve regeneration, scaffold, cells, and growth factor along with the blood supply are crucial. Chitosan has been used as a scaffold for many years, and it shows good results in healing [7,8]. Mesenchymal stromal cells can

regenerate the cementum, bone, and periodontal ligament. Recent studies in a canine model have shown that the transplantation of PDL-d derived cells can regenerate periodontal tissue [9,10].

Periostin can usually be found in periodontal ligament and the periosteum. It is important for tissue stability and tissue response to inflammation. Periostin binds to extracellular molecules such as type 1 collagen and tenascin-C and increases the collagen fiber diameter, resulting in greater tissue strength. A previous study has shown that periostin levels are elevated after the surgical treatment of periodontal disease [11]. The periostin level increased over time, and this increase was correlated with the healing process in healthy tissue and in periodontal disease [12]. The presence of periostin can improve periodontal wound healing and regeneration owing to its stabilizing effect on the extracellular matrix. In this study, for evaluating cell sheet application to *M. nemestrina*, the detection of periostin in its protein band was used for histologic evaluation. The periostin band appears in the second and third weeks in the cell sheet and chitosan group, indicating that healing and regeneration began in the second week. Furthermore, it appears in the first two weeks in the RGD cell sheet and chitosan group, indicating that healing and regeneration began earlier than in the cell sheet and chitosan group. The chitosan group did not show any periostin band in the first three weeks, indicating that periostin has not yet started to play a role in healing.

This novel study examines periostin expression in cell sheet applications as a histological evaluation. A previous study of periostin in normal and periodontitis patients showed increased periostin over time that was correlated with the healing process, as found from an ELISA analysis [12]. In this study, the appearance of the periostin protein band in the cell sheet group shows that periostin may play a greater role in promoting healing and bone regeneration. A recent study with RGD conjugation also showed significant mineralization of mesenchymal stem cells after 14 days of osteogenic culture. In combination with chitosan scaffolds, this provides a favorable environment for mesenchymal stem cells to grow and differentiate into fibroblasts and osteoblasts, thus enhancing reattachment and regeneration compared to that in the case of chitosan scaffolds without RGD [13,14]. This finding matches the one in this study, where the RGD-chitosan cell sheet group showed earlier detection of periostin protein band expression.

4. Conclusion

The periostin protein band could be detected in the cell sheet groups with chitosan alone or RGD-chitosan at different appearance times. Cell sheet application may enhance the role of periostin in promoting healing and bone regeneration. The combination of the chitosan scaffold with RGD results in earlier healing compared to the other groups.

Acknowledgment

This research was supported by PITTA grant No. 1947/UN2.R12/HKP.05.00/2016. The authors declare no conflicts of interest with this article.

References

- [1] Notohartoyo I and Sihombing M 2015 Faktor risiko pada penyakit jaringan periodontal gigi di Indonesia (RISKESDAS 2013). Government Document. Edited by Badan Penelitian Dan Pengembangan Kesehatan Kementerian Kesehatan R I Tahun 2013. *Bull. Penelitian Sistem Kesehatan*. **18** 87–94. Available from: <http://ejournal.litbang.depkes.go.id/index.php/hsr/article/view/4274>.
- [2] Nield-Gehrig J and Willmann D D 2008 Disease of Periodontium. *Foundations of Periodontics for the Dental Hygienist*. (Philadelphia: Lippincott) p 137-77.
- [3] Hinrichs J and Novak J 2012 Classification of Diseases and Conditions Affecting the Periodontium. Ed. Fermin Carranza *Clinical Periodontology* 11th edition. (Missouri: Elsevier) p 34-54.
- [4] Sanz A R, Carrión F S and Chaparro, A P 2015 Mesenchymal stem cells from the oral cavity and their potential value in tissue engineering. *Periodontol. 2000*. **67** 251-67. Available from: doi: 10.1111/prd.12070.

- [5] Rao R, Zhu Y B, Alinejad T, Tiruvayipati S, Thong K L, Wang J and Bhassu S 2015 RNA-seq analysis of *Macrobrachium rosenbergii* hepatopancreas in response to *Vibrio parahaemolyticus* infection. *Gut Pathogens*. **7** 1-16.
- [6] Hansson A, Hashom N, Falson F, Rousselle P, Jordan O and Borchard G 2012 In vitro evaluation of an RGD-functionalized chitosan derivative for enhanced cell adhesion. *Carbohydr. Polym.* **90** 1494-500. Available from: doi:10.1016/j.carbpol.2012.07.020.
- [7] Chen F M, Zhang J, Zhang M, An Y, Chen F and Wu Z F 2010 A review on endogenous regenerative technology in periodontal regenerative medicine. *Biomaterials*. **31** 7892-927.
- [8] Soeroso Y, Bachtiar EW, Bachtiar BM, Sulijaya B, Prayitno SW 2016 The prospect of chitosan on the osteogenesis of periodontal ligament stem cells *Journal of International Dental and Medical Research* **5** 93-7.
- [9] Tobita M, Uysal C A, Guo X, Hyakusoku H and Mizuno H 2013 Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. *Cytotherapy*. **15** 1517-26.
- [10] Akizuki T, Oda S, Komaki M, Tsuchioka H, Kawakatsu N, Kikuchi A, Yamato M, Okano T and Ishikawa I 2005 Application of periodontal ligament cell sheet for periodontal regeneration : a pilot study in beagle dogs. *J. Periodont. Res.* **40** 245-51.
- [11] Rios H F, Bonewald L F and Conway S J 2014 Lessons from the matricellular factor periostin. *J. Dent. Res.* **93** 843-5.
- [12] Padial-Molina M, Volk S L, Rios H F 2015 Preliminary insight into the periostin leverage during periodontal tissue healing. *J. Clin. Periodontol.* **42** 764-72. Available from: doi: 10.1111/jcpe.12432.
- [13] Tsai W B, Chen Y R, Li W T, Lai J Y and Liu H L 2012 RGD-conjugated UV-crosslinked chitosan scaffolds inoculated with mesenchymal stem cells for bone tissue engineering. *Carbohydr. Polym.* **89** 379-87.
- [14] Wang C, Chen B, Zou M and Cheng G 2014 Cyclic RGD-modified chitosan/graphene oxide polymers for drug delivery and cellular imaging. *Colloid. Surf. B-Biointerfaces*. **122** 332-40.