Stomatologija. Baltic Dental and Maxillofacial Journal, 23: 114-20, 2021

Simvastatin in polymer bioscaffold for bone regeneration. An *in vitro* and *in vivo* analysis

Swati Gupta[1], Pratibha Gopalkrishna[1], Usha Yogendra Nayak[2], Kishore Ginjupalli[3], Thayyil Sivaraman Hrishi[4], Chetana Chandrashekar[5], Raghu A R[5], Pallavi K[6], Lakshmi P[1]

SUMMARY

Objective. The study aimed to fabricate and test the biocompatibility of a polylactic-co-glycolic acid (PLGA) based guided tissue regeneration membrane impregnated with 'simvastatin' to promote sustained drug delivery near osseous defects and evaluate the regenerative potential of the membrane histologically.

Materials and methods. We tested the mechanical properties and cytotoxicity of an indigenously fabricated PLGA membrane incorporated with simvastatin (1 mg/cm^2). An animal study evaluated the regenerative potential of the membrane. Twenty-four adult Wistar rats, approximately 175 g in weight, were used in this study. The rats were divided randomly into four groups based on the postoperative healing periods into ten days, 1, 3, and 6 months. Within each time group, six rats were divided into three subgroups: Subgroup A – sham surgery controls; Subgroup B – PLGA without Simvastatin; Subgroup C – PLGA with simvastatin tests. The radiographic examination intervals were ten days, 1 and 3 months, while the histological assessment was around 1, 3, and 6 months.

Results. Simvastatin content was distributed uniformly in all the prepared membranes and was equivalent to 1 mg/cm^2. 100 mg PLGA membrane with simvastatin demonstrated uniform drug release over time, excellent mechanical properties, and biocompatibility. The rat models in Subgroup C had better bone tissue formation radiographically and histologically.

Conclusion. The study suggested that 'PLGA with Simvastatin' has the requisite properties to serve as a third-generation barrier membrane with the potential for local drug delivery.

Key words: barrier membrane, local drug delivery, polymer, regeneration, simvastatin.

INTRODUCTION

Periodontitis is a chronic infection with a concomitant immune-inflammatory imbalance resulting in tooth loss, disability, masticatory dysfunction, and low nutritional status (1). Hence, treatment of periodontal disease is essential and includes both non-surgical and surgical periodontal therapy (2). Periodontal regeneration as a part of surgical periodontal treatment is extensively studied. This therapeutic concept entails complete replication of the original architecture and function of the periodontal attachment (3). Research involving pharmacological compounds like teriparatide (4), bisphosphonates (5), and desferrioxamine (6) have demonstrated the upregulation of the periodontal regenerative process through the activation of specific and intricate biochemical pathways. Statins are another group of drugs that show the ability to modulate the process of bone regeneration. These drugs belong to the family of HMG-CoA inhibitors, which possess bone anabolic activity by preventing bone resorption and enhancing osteopromotion (7). Simvastatin is antagonistic to pro-inflammatory activity and up-

[1]*Department of Periodontology, Manipal College of Dental Sciences, Manipal, Manipal Academy of Higher Education, India*
[2]*Department. of Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal, Manipal Academy of Higher Education, India*
[3]*Department of Dental Materials, Manipal College of Dental Sciences, Manipal, Manipal Academy of Higher Education, India*
[4]*Department of Periodontics, Government Dental College, Kozhikode, Kerala, India*
[5]*Department of Oral Pathology and Microbiology, Manipal College of Dental Sciences, Manipal, Manipal Academy of Higher Education, Manipal*
[6]*Department of Pharmaceutics, Yenepoya Pharmacy College and Research Centre, Yenepoya University, Mangalore, Karnataka, India*

Address correspondence to Pratibha Gopalkrishna, Dept. of Periodontology, Manipal College of Dental Sciences, Manipal, Manipal Academy of Higher Education, India.
E-mail address: pratibha.pk@manipal.edu

regulates the expression of osteoinductive factors (8), thereby exerting a host modulatory effect. We can presume that an optimal dose of simvastatin would have an osteopromotive effect. Hence, the present study intended to determine the optimal dosage of simvastatin in a polymer vehicle necessary for increasing bone mineral density and aimed at fabricating and testing the biocompatibility of a polylactic-co-glycolic acid (PLGA) based guided tissue regeneration membrane incorporating simvastatin, which would promote sustained drug delivery at the endosseous site. The membrane would thus serve as a local drug delivery vehicle while facilitating bone regeneration via barrier function.

MATERIALS AND METHODS

Preparation of polymeric film of polylactic-co-glycolic acid (PLGA) containing simvastatin

The solvent casting method enabled the preparation of polylactic-co-glycolic acid membranes (PLGA membrane) [PURASORB® PDLG 5004, Purac Biochemicals, Netherlands]. We dissolved pre-weighed quantities of PLGA 50:50 (100 mg, 200 mg, and 300 mg) in a pre-measured volume of dichloromethane. Simvastatin at a concentration of 1 mg/cm^2 was added to the solution gradually, and the mixture was stirred, which was then poured into a rectangular glass mould (8×2 cm^2). The solvent and polymer were kept on an ice bath (2-8ºC) for 48 hours to ensure slow evaporation of the solvent with the formation of a porous and bubble-free film, followed by drying under vacuum, at room temperature for 24 hours. Finally, the biodegradable films were peeled from the glass mould and cut into dimensions of 1×1 cm^2. F4, F5, and F6 indicated the PLGA membranes with 100 mg, 200 mg, and 300 mg of the polymer.

Characterization of the membrane

Identification of Simvastatin

Simvastatin estimation utilized the UV-visible spectrophotometric method (UV-1601PC, Shimadzu Corporation, Japan) between 400-800 nm wavelengths. The wavelength of 243 nm was selected, and a calibration curve was prepared based on absorbance values of the drug at different concentrations.

Drug content uniformity

The drug content uniformity was quantified by placing a 1×1 cm^2 area of the membrane on a slide in isotonic phosphate buffer at a pH of 7.4. The solution was filtered, and drug content was then estimated after proper dilution at 243 nm using a UV spectrophotometer.

In vitro drug release

The drug-release was evaluated by placing 'PLGA membranes containing simvastatin' in glass vials containing 5 ml phosphate buffer saline (pH 7.4). Samples of 1 ml were withdrawn at regular intervals of two hours up to six hours, and 24 hours, 48 hours, 72 hours, 120 hours, 168 hours, 216 hours, 264 hours, 360 hours, and 720 hours. At each sample withdrawal, an equal quantity of fresh phosphate buffer saline (pH 7.4) was added, and then analyzed by spectrophotometry.

Mechanical properties

Tensile strength of the prepared specimens was measured using a universal testing machine (Instron Model 3366, United Kingdom). The experiment was conducted at room temperature using films of 2.0 mm thickness with a gauge length of 10.0 mm and at a tensile jig crosshead speed of 0.5 mm/min. A digital readout indicated the maximum load and tensile strength of the films.

The membrane having 100 mg (F4) of the polymer was found superior to the membranes with 200 mg and 300 mg of the polymer when considering the drug release potential and mechanical properties. Hence, the F4 membranes were chosen for the biocompatibility test and in vivo studies.

Biological properties

Assessment of biocompatibility utilized cell proliferation assay and the cytotoxicity assay. The PLGA membranes were placed in sterile culture plates of 6×3 cm^2 in dimensions. Two lakh human skin fibroblasts, developed in the Department of Radiobiology and Toxicology, were cultured in Dulbecco's modified eagle medium (DMEM). The cells were observed for proliferation and adhesion to the membrane using a light microscope. The cytotoxicity assay depended on the numbers of cells present and the mitochondrial activity per cell. The cleavage of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to purple coloured formazan derivative by living cells is the basis of the assay.

Animal study

The institutional Animal Care and Use Committee provided ethical clearance for the pre-clinical, in vivo study on Wistar albino rat models (18.5.2012; IAEC/KMC/48/2012). Twenty-four adult Wistar rats were kept individually in cages at the institutional 'Animal House' under monitored conditions. Water and 'rat food' in pellets were supplied to the animals, which were kept under observation for any adverse events throughout the study.

Experimental groups

Animals were divided randomly into four groups based on the postoperative healing period

into ten days, one month, three months, and six months. From each time group, six rats were distributed into three subgroups: Subgroup A assigned sham surgery – control; Subgroup B assigned PLGA without Simvastatin, and Subgroup C assigned PLGA with Simvastatin – test.

Surgery

The animals were prepared for surgery by shaving and disinfecting the mandibular region. Two study investigators administered anaesthesia by injecting ketamine (100 mg/ml intramuscularly). They placed an incision inferior to the angle of the mandible extending deep into the bone, unilaterally. After atraumatic skin and muscle reflection, a critical-sized 5×5 mm defect was created using a slow-speed dental micromotor handpiece and bur, under constant irrigation with sterile saline. Care was taken to avoid a 'through-and-through' defect. The defect was treated considering the subgroup to which each animal was assigned.

After implantation of the membrane in subgroups B and C, the muscles and skin were sutured, followed by monitoring until recovery from anaesthesia.

Radiology

The bone mineral density (BMD) was evaluated at baseline, ten days, one month, and three months utilizing a Computed tomography (CT) scanner, Philips 64 slice, with head and abdomen protocol. The software used was 4.1ebw. The rats were anaesthetized with ketamine (100 mg/ml) to keep them sedated during the procedure. The CT scan parameters used were 80mA, 120 KVp, with a field of view of 52 mm, slice width of 0.67 mm, and exposure time of 6 s. The radiographic density of the 'region of interest' was calculated in Hounsfield units.

Histology

Six rats in each group, i.e., two from each subgroup, were sacrificed at one month, three months, and six months for histologic assessment of healing at the defect site. The specimens were placed in 10% buffered formalin solution up to 24 hours after collection and then decalcified in formic acid for one week. After rinsing in tap water, the specimens were dehydrated with ascending ethyl alcohol concentrations, cleared in xylene, and infiltrated with paraffin. The inferior 'two millimetres' of the facial surface of the mandible was evaluated. A microtome (Thermo Scientific Shandon Finesse ME Microtome) was used to obtain 7μm serial-sections from the centre of each defect. An investigator, who was blind to the groups to which each histologic section was assigned, conducted the histologic evaluation. The investigator examined the 'hematoxylin and eosin' stained slides for new bone formation, residual GTR membrane, fibrin clot, soft tissue elements, and inflammatory reaction using polarized light microscopy (Microscope: Olympus BX41) at 10X.

.

Statistical analysis

The Kruskal Wallis test estimated the difference in the bone mineral density within each group, while the Mann Whitney U test compared the bone mineral density between the groups. A p-value of <0.05 was considered significant.

RESULTS

Drug content uniformity

A standard plot calibration curve prepared to assess the drug content uniformity showed that the simvastatin content in 100 mg membrane was 0.96 mg, 0.95 mg in 200 mg of PLGA, and 0.95 mg in 300 mg PLGA. Thus, the simvastatin content was uniformly distributed in all three samples and was equivalent to 1 mg/cm^2.

In vitro drug release

In the 100 mg PLGA membrane with 1 mg simvastatin, the drug release was initially slow (13.76% release in 4 hours). 28% release occurred in 24 hours, 58.23% in five days, followed by a sustained release of the drug in minimal amounts. A similar sustained drug release pattern ensued in the 200 mg PLGA membrane with 11.87% in 4 hours, 27.54% in 24 hours, and 53.21% of the drug release in 5 days. In 300 mg of PLGA, 9.9% release was seen in 4 hours, 28.39% in 24 hours, and 47.65% of drug release in five days. The increased polymer concentration led to a decrease in drug release rate. Therefore, there is a slower diffusion of the drug from films containing a higher polymer concentration. PLGA 100 mg with Simvastatin (F4) showed a constant drug release over time (Figure 1).

Mechanical property

The tests to determine the tensile strength of the membranes showed that membranes F4 (100 mg) withstood a load of 5.4 N, F5 (200 mg) withstood a load of 9.3 N, and F6 (300 mg) withstood a load of 28.4 N. The tensile strength measurements indicated an extension of 12.4 mm in F4 before the break, F5 membrane showed an extension of 18.6 mm and F6 membrane demonstrated an extension of 11.5 mm before the break. Comparison of maximum load to the load extension ratio, F4 (100 mg) with simvastatin 1 mg/cm^2, demonstrated adequate tension at

the 'maximum load,' suggesting better tensile strength properties (Table 1).

Biocompatibility tests

In the cell proliferation assay, day '2' was characterized by fibroblasts' adhesion to the membrane margins. The microscopic picture at high magnification on the day '4' demonstrated proliferation and multiplication of the fibroblasts, with an evident increase in the number of fibroblasts compared to the day '2.' The cytotoxicity (MTT) assay revealed 100% cell viability in the 'control well,' which contained Dimethyl sulfoxide (DMSO) in the concentration of 200 microliters. The 'test' well with the cells cultured in the presence of 'PLGA membrane with simvastatin' revealed that a concentration of 0.1 µg/ml simvastatin showed cell-viability of 96.7%. As the concentration increased to 200 µg/ml, the cell-viability was 90%.

Animal study

The CT scan showed a variation in defect healing at ten days. Subgroup A and Subgroup B seemed to show no 'healing' whereas, in Subgroup C, there was an increase in radio-density in the centre of the defect, noticeable in all the specimens of subgroup C scanned at ten days (Figure 2).

CT scan analysis

Statistical analysis showed that there was a significant difference in bone mineral density in the subgroups at thirty days as compared to ten days postoperatively, whereas, at ninety days, the difference in the density was not statistically significant (Table 2). Mann Whitney test to compare differences between the three subgroups established a statistically significant difference between Subgroups A and C (Table 3).

Histomorphometric analysis

Histological examination of the defect site at one month showed more osteoblastic surfaces with increased mineralization in Subgroup C. At three months, Subgroup B demonstrated more woven bone with inflam-

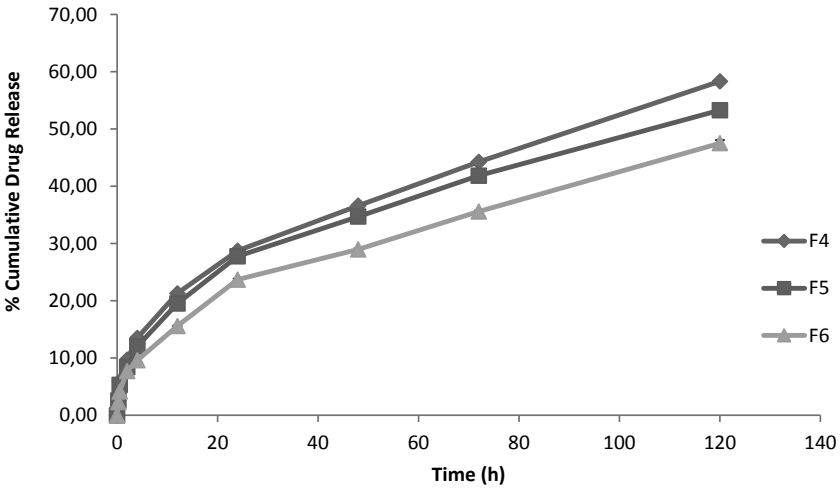

Fig. 1. In vitro drug release of Simvastatin from PLGA membrane

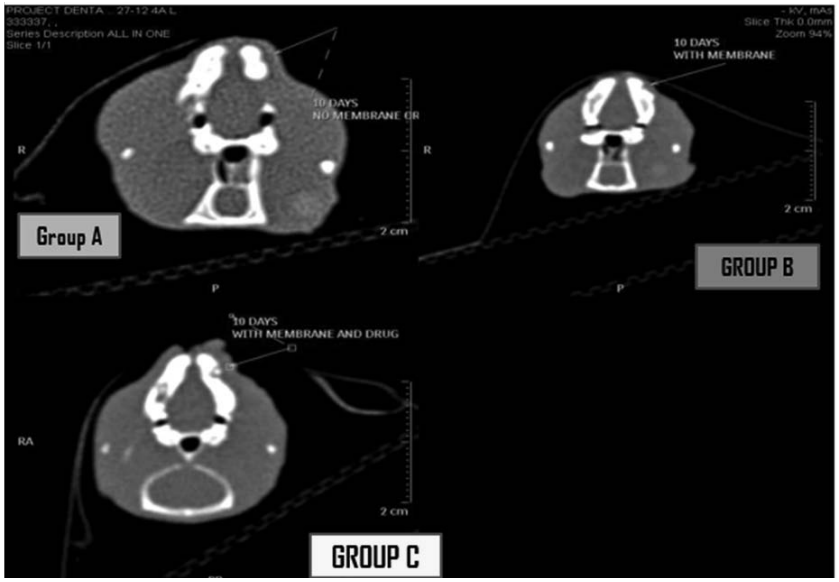

Fig. 2. CT Image on Day 10

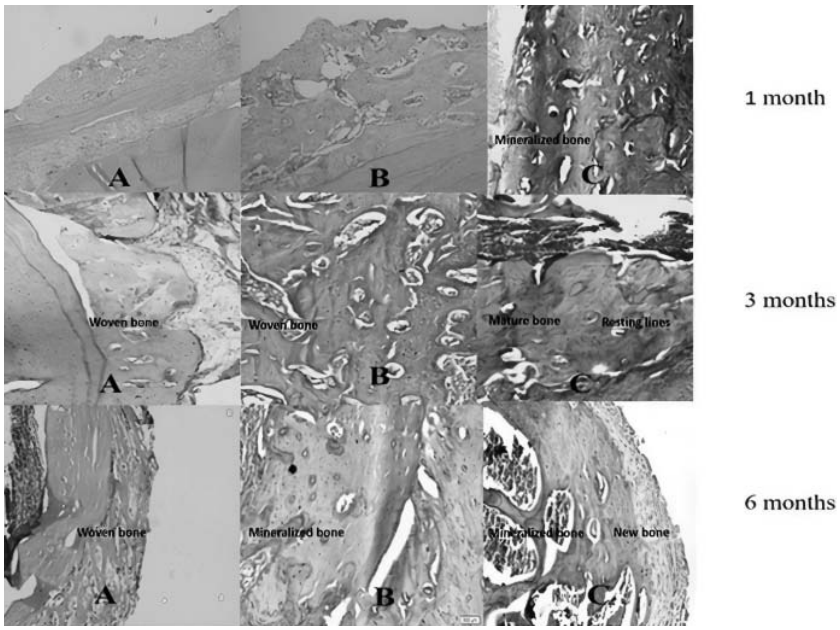

Fig. 3. Histology at 1, 3 and 6 months. A – Subgroup A, B – Subgroup B, C – Subgroup C.

matory cells and high vascularization. Subgroup C showed more mineralization, resting lines, and more mature bone. There is a difference between the groups concerning 'bone formation' at six months. In Subgroup A, a wide area of woven bone was seen, demonstrating continued bone remodelling. In Subgroup B, the mineralized bone was distinctly evident, while in Subgroup C, well-mineralized bone with distinct staining, outlined with new bone was observed (Figure 3).

DISCUSSION

Regeneration of the periodontal apparatus involves a complex series of events that require the synchronized existence of optimal environmental and molecular factors. Modulating the host environment, and creating and maintaining space for the regenerative process, is a prerequisite for achieving restitutio ad integrum (9). Simvastatin, a lipid-lowering drug, (10) can additionally downregulate the destructive effect of the inflammatory process (11). Simvastatin facilitates the production of 'Bone Morphogenetic Protein 2' (BMP2) in addition to 'vascular endothelial growth factor' (VEGF), osteocalcin, and 'bone sialoprotein' (BSP) and thus promotes osteoblast differentiation and bone regeneration at the molecular and cellular levels (12).

Polymer membranes, which are non-toxic and biocompatible materials, have been used in studies of periodontal regeneration. PLGA polymer, which can form an optimally porous, biodegradable, innocuous membrane (13), finds its application as a scaffold for the drug and a barrier for space maintenance and exclusion of epithelial cells from the periodontal defect sites. Thus, the present study utilized 'simvastatin' in PLGA membranes, demonstrating osteopromotion capacity. This membrane can serve as an economic local drug delivery system for host modulation. 100 mg PLGA membrane was selected over 200 mg and 300 mg membranes based on its mechanical, in vitro drug release, and biocompatibility properties. The PLGA membrane showed maximum load and extension before rupture, demonstrating required tensile strength properties and moldability. We were able to adapt the membrane onto the prepared critical-sized defect in vivo. However, the membrane lacked the stiffness desirable for space maintenance. Placement of the overlying flap would probably reduce the tenting effect. Pirhonen *et al.* used an innovative technique to overcome this limitation by treating PLGA with N-methyl pyrrolidone (NMP) for Inion (InionGTR™) membrane, improving its conformability and shape conservation properties and providing desired porosity for cell ingrowth (14).

In the present study, the PLGA membrane facilitated sustained drug delivery in an optimized manner as only an ideal dose of the drug can stimulate the cells to express BMP-2 without bringing about the inflammatory response (15).

Table 1. Tensile strength assessment

Membrane	Maximum load, N	Tensile extension at break (standard), mm
F4	5.46718 (0.73)	12.31254 (1.39)
F5	9.31016 (0.61)	18.69502 (1.504)
F6	28.94971 (1.786)	11.53360 (0.58)

Table 2. CT scan-comparison of variation in the Bone Mineral Density in the 3 subgroups

Subgroup	Mean change in Bone Mineral Density (Day 10 to Day 30)		Mean change in Bone Mineral Density (Day 10 to Day 90)	
	Mean (SD)	#P-value	Mean (SD)	#P-value
Subgroup A	246 (218)	0.05	780 (205)	0.52
Subgroup B	429 (204)		793 (119)	
Subgroup C	572 (138)		882 (161)	

– Kruskal Wallis test.

Table 3. CT scan-comparison of variation in the Bone Mineral Density between the 3 Subgroups

Subgroup	#P-Value
Subgroup A Vs. Subgroup B	0.10
Subgroup B Vs. Subgroup C	0.26
Subgroup A Vs. Subgroup C	0.02

– Mann Whitney test.

Simvastatin was used at a concentration of 1 mg/cm^2, considering research by Stein *et al.*, where the optimal concentration of 'simvastatin' associated with beneficial effects without inflammation was 0.5 mg. Nevertheless, concentrations of 0.5 mg to 2 mg are suggested to promote bone formation (16). A 'systematic review and meta-analysis,' by Gupta *et al.*, states that the optimal dosage of simvastatin for bone regeneration is between 1 mg/cm^2 to 3 mg/cm^2 (17).

The present study used a rat mandible model for assessing bone regeneration. Rat models prove advantageous for assessing microbial and host responses and determining the subtleties of soft and hard-tissue interfaces relevant to periodontal regeneration and oral inflammatory conditions (18). A critical size defect of 5×5 mm dimension was prepared

in the anteroinferior border of the mandible in the study. The defect-size was determined based on evidence regarding critical-size defects in rats (19).

In the present study, CT scans verified the bone density at various time intervals. Studies suggest that variations in Hounsfield units can indicate bone quality alterations (20). The time points selected for assessing bone density in the present study were as per Stein *et al.* (16), who observed osteoblastic activity at ten days and osteoclastic action by 24 days. Considering that complete bone remodelling would require nearly three months, bone formation was re-assessed at ninety days in the present study. Subgroups A and C showed significantly different bone-mineral densities (BMD), attributed to the combined effects of simvastatin and barrier effects of PLGA (15). Although complete regeneration of periodontal attachment apparatus with statin use may be moot, bone regeneration is evident (21). Studies on subgingival delivery of 'simvastatin' and 'atorvastatin' in intra-bony defects have shown a gain in clinical attachment level with the combined use of both drugs as compared to a placebo. However, 'atorvastatin' was found more effective in the treatment of periodontal defects with better regenerative potential (22). Another study that used simvastatin gel in osseous defects has shown reduced gingival index, probing depth, and increased clinical attachment level with increased bone fill in the intraosseous defect (23). Notably, in the present study, the difference between the bone mineral densities at ninety days was not statistically significant in the subgroups, which could be due to the polymer base degradation. The average degradation period of polymer membranes is usually 4-6 weeks, approximating 28-45 days (24). Degradation of the polymer base, in turn, would result in the dissipation of the drug.

The histologic findings correlated with the radiologic observations, with dense bone formation in subgroup C, followed by subgroups B and A. Simvastatin has demonstrated remarkable effects on neovascularization, along with osteogenesis and bone remodelling, and can modulate inflammation, immune response, and bacterial clearance (25).

Future research on larger animal models, soft tissue healing followed by human clinical trials would enable the use of 'PLGA with simvastatin' in periodontal regenerative therapy.

CONCLUSION

The combination of mechanical properties of the polymer, sustained drug release, and osteopromotive properties of simvastatin suggest a potential for this simvastatin-laden PLGA scaffold (1 mg/cm^2) as a third-generation barrier membrane.

STATEMENT OF CONFLICTS OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGEMENTS

We thank Purac biochemical, Netherlands, and Ranbaxy Laboratories Ltd. for providing samples of PLGA and Simvastatin, respectively. We acknowledge the faculty at the School of Life Sciences, for permitting us to use the facilities for cell culture and cytotoxicity assays and at the Department of Radiodiagnosis, for assistance with the CT scans.

REFERENCES

1. Chapple ILC. Time to take periodontitis seriously. BMJ. 2014;348:g2645. doi:10.1136/bmj.g2645
2. Nascimento GG, Dahlén G, López R, Baelum V. Periodontitis phenotypes and clinical response patterns to non-surgical periodontal therapy: reflections on the new periodontitis classification. Eur J Oral Sci. January 2020. doi:10.1111/eos.12670
3. Ivanovski S. Periodontal regeneration. Aust Dent J. 2009;54:S118-S128. doi:10.1111/j.1834-7819.2009.01150.x
4. Bashutski JD, Eber RM, Kinney JS, et al. Teriparatide and osseous regeneration in the oral cavity. N Engl J Med. 2010;363(25):2396-2405. doi:10.1056/NEJMoa1005361
5. Akram Z, Abduljabbar T, Kellesarian SV, Abu Hassan MI, Javed F, Vohra F. Efficacy of bisphosphonate as an adjunct to non-surgical periodontal therapy in the management of periodontal disease: a systematic review. Br J Clin Pharmacol. 2017;83(3):444-454. doi:10.1111/bcp.13147
6. Donneys A, Yang Q, Forrest ML, et al. Implantable hyaluronic acid-deferoxamine conjugate prevents nonunions through stimulation of neovascularization. npj Regen Med. 2019;4(1):11. doi:10.1038/s41536-019-0072-9
7. Muniz FWMG, Taminski K, Cavagni J, Celeste RK, Weidlich P, Rösing CK. The effect of statins on periodontal treatment—a systematic review with meta-analyses and meta-regression. Clin Oral Investig. 2018;22(2):671-687. doi:10.1007/s00784-018-2354-9
8. Yamashita M, Otsuka F, Mukai T, et al. Simvastatin antagonizes tumour necrosis factor-α inhibition of bone morphogenetic proteins-2-induced osteoblast differentiation by regulating Smad signalling and Ras/Rho-mitogen-activated protein kinase pathway. J Endocrinol. 2008;196(3):601-613. doi:10.1677/JOE-07-0532
9. Zhang K, Wang S, Zhou C, et al. Advanced smart biomaterials and constructs for hard tissue engineering and regeneration. Bone Res. 2018;6(1):1-15. doi:10.1038/s41413-018-0032-9
10. van Stee MF, de Graaf AA, Groen AK. Actions of metformin and statins on lipid and glucose metabolism and possible benefit of combination therapy. Cardiovasc Dia-

betol. 2018;17(1):1-22. doi:10.1186/s12933-018-0738-4
11. Zeiser R. Immune modulatory effects of statins. Immunology. 2018;154(1):69-75. doi:10.1111/imm.12902
12. Yin W, Li Z ZW. Modulation of Bone and Marrow Niche by Cholesterol. Nutrients. 2019;11(6):1394.
13. Iviglia G, Kargozar S, Baino F. Biomaterials, current strategies, and novel nano-technological approaches for periodontal regeneration. J Funct Biomater. 2019;10(1). doi:10.3390/jfb10010003
14. Pirhonen E, Pohjonen T, Weber F. Novel Membrane for Guided Bone Regeneration. The International Journal of artificial organs 2006; 29: 834-40. DOI: 0.1177/039139880602900904)
15. Rojbani H, Nyan M, Ohya K, Kasugai S. Evaluation of the osteoconductivity of α-tricalcium phosphate, β-tricalcium phosphate, and hydroxyapatite combined with or without simvastatin in rat calvarial defect. J Biomed Mater Res - Part A. 2011;98 A(4):488-498. doi:10.1002/jbm.a.33117
16. Stein D, Lee Y, Schmid MJ, et al. Local Simvastatin Effects on Mandibular Bone Growth and Inflammation. J Periodontol. 2005;76(11):1861-1870. doi:10.1902/jop.2005.76.11.1861
17. Gupta S, Del Fabbro M, Chang J. The impact of simvastatin intervention on the healing of bone, soft tissue, and TMJ cartilage in dentistry: A systematic review and meta-analysis. Int J Implant Dent. 2019;5(1). doi:10.1186/s40729-019-0168-4
18. Kantarci A, Hasturk H, Van Dyke TE. Animal models for periodontal regeneration and peri-implant responses. Periodontol 2000. 2015;68(1):66-82. doi:10.1111/prd.12052
19. Chin VKL, Shinagawa A, da Graça Naclério-Homem M. Bone healing of mandibular critical-size defects in spontaneously hypertensive rats. Braz Oral Res. 2013;27(5):423-430. doi:10.1590/S1806-83242013000500006
20. Pauwels R, Jacobs R, Singer SR, Mupparapu M. CBCT-based bone quality assessment: Are Hounsfield units applicable? Dentomaxillofacial Radiol. 2015;44(1). doi:10.1259/dmfr.20140238
21. Bertl K, Steiner I, Pandis N, Buhlin K, Klinge B, Stavropoulos A. Statins in Non-surgical and surgical periodontal therapy. A systematic review and meta-analysis of preclinical in vivo trials. J Periodontal Res. 2018;53(3):267-287. doi:10.1111/jre.12514
22. Martande SS, Kumari M, Pradeep AR, Singh SP, Suke DK. Comparative evaluation of the efficacy of subgingivally delivered 1.2% Atorvastatin and 1.2% Simvastatin in treatment of intra-bony defects in chronic periodontitis: a randomized controlled trial. J Dent Res Dent Clin Dent Prospects. 2017;11(1):18-25. doi:10.15171/joddd.2017.004
23. Ranjan R, Patil SR, Veena HR. Effect of in-situ application of simvastatin gel in the surgical management of osseous defects in chronic periodontitis–A randomized clinical trial. J Oral Biol Craniofacial Res. 2017;7(2):113-118. doi:10.1016/j.jobcr.2017.05.005
24. Sun X, Xu C, Wu G, Ye Q WC. Poly (lactic-co-glycolic acid): Applications and future prospects for periodontal tissue regeneration. Polymers. 2017;9(6):189.
25. Petit C, Batool F, Bugueno IM, Schwinté P, Benkirane-Jessel N HO. Contribution of Statins towards Periodontal Treatment: A Review. Mediators Inflamm. 2019;2019. doi:10.1155/2019/6367402

Received: 08 02 2021
Accepted for publishing: 20 12 2021