COMPARITIVE EVALUATION OF CLINICAL AND RADIOLOGICAL PARAMETERS OF INTRABONY DEFECTS TREATED WITH SIMVASTATIN LOADED COLLAGEN MEMBRANE / COLLAGEN MEMBRANE ALONE – 12 MONTHS RANDOMIZED CONTROLLED CLINICAL STUDY

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CERTIFICATE

This is to certify that **Dr. SARANYA S,** Post Graduate student in the Department of Periodontics, J.K.K. Nattraja Dental College and Hospital, Komarapalyam has done this dissertation titled **"COMPARITIVE EVALUATION OF CLINICAL AND RADIOLOGICAL PARAMETERS OF INTRABONY DEFECTS TREATED WITH SIMVASTATIN LOADED COLLAGEN MEMBRANE / COLLAGEN MEMBRANE ALONE – 12 MONTHS RANDOMIZED CONTROLLED CLINICAL STUDY"** under my direct guidance during her post graduate study period 2012-2015.

This dissertation is submitted to **THE TAMILNADU Dr. MGR MEDICAL UNIVERSITY** in partial fulfillment of the degree of **MASTER OF DENTAL SURGREY, BRANCH II – Periodontics.**

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Periodontitis is an immuno inflammatory disease of supporting tissues of the teeth, caused by specific microorganisms resulting in the progressive destruction of periodontal ligament, alveolar bone with pocket formation.[1]The primary clinical features of periodontitis include clinical attachment loss (CAL), alveolar bone loss (BL), periodontal pocketing, and gingival inflammation.[2] The purpose of periodontal therapy is to eliminate the inflammation of the periodontal tissues and to regenerate the periodontal attachment apparatus including cementum, functionally oriented periodontal ligament and alveolar bone.[3]

Conventional treatment procedures such as scaling and root planning (SRP) are highly effective in repairing disease related defects and halting the progression of periodontitis. These treatments typically result in the development of long junctional epithelium between the root surface and gingival connective tissue rather than regrowth of tissue that restores the architecture and function.[4] For decades, a number of surgical procedures have been advocated which includes open flap debridement with bone grafts or bone substitutes and guided tissue regeneration. Open flap debridement may also result in the formation of long junctional epithelium which is more susceptible to microbial invasion and is thought to be less stable attachment. Thus bone grafting become common regenerative therapy.[5]

The use of bone grafts result in the regrowth of alveolar bone and formation of new attachment which would be stimulated either by osteogenesis, osteoconduction or osteoinduction.[6] Autogenous bone grafts have been adopted as gold standard procedure since there is possibility to retain cell viability and no possibility of disease transmission.[7] The major disadvantages of autografts are donor site morbidity, technique sensitivity which have led to the development of

allografts.[8] Allografts, xenografts, alloplasts do not possess inherent osteogenic properties and act only as a substrate for cell migration and proliferation.

Several studies on periodontal wound healing following different treatments performed in the later years revealed that neither the flap procedures alone or in combination with grafting with bone or bone substitutes would result in the formation of a true new attachment. These findings and further studies on periodontal wound healing led to the development of guided tissue regeneration (GTR) therapy.[10]

Guided tissue regeneration with barrier membranes has been demonstrated to be effective in preventing epithelial and gingival connective tissue cells from migrating into the blood clot about the instrumented root surface. Cementum, periodontal ligament and alveolar bone are expected to form. The treatment rationale of applying guided tissue regeneration in deep intrabony defects arises from the need to increase the periodontal support of the involved teeth. Non resorbable and resorbable membranes are widely used for guided tissue regeneration. Since there is a need for second surgery to remove the non resorbable membranes, resorbable membrane are widely used.

Collagen is commonly used resorbable membrane in guided tissue regeneration. Collagen membrane is generally regarded as non – allergic, nontoxic, and non – irritating material and is used as a sustained release vehicle for therapeutic drugs.[11] Besides being a physical barrier for tissue separation a regenerative membrane should be endowed with multiple functions to facilitate desirable regeneration. So in the recent years regenerative membrane is loaded with many

bone substitutes and pharmacological compounds to favor potential periodontal regeneration[12].

Statins, such as simvastatin (SMV), lovastatin, and pravastatin, were first introduced as cholesterol lowering drugs through the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Because statins also inhibit the isoprenoid intermediates of the mevalonate biosynthetic pathway, many recently discovered effects of these drugs extend beyond the lowering of cholesterol. Statins modify the inflammatory cascades through pleiotropic actions at multiple levels, such as changing inflammatory mediators, inducing hemeoxygenase, altering leukocyte–endothelial cell interaction, and reducing expression of major histocompatibility complex-II. It was also reported that statins augmented vascular endothelial growth factor, which is known to stimulate bone formation.[13]

These so-called pleiotropic effects, as well as their potential mechanisms, have received much recent attention and include vasodilative, antithrombotic, antioxidant, anti-inflammatory, and anti - proliferative effects. In addition, statins can raise the expression of bone morphogenetic protein-2 (BMP-2), a potent stimulator of osteoblast differentiation and activity, and can promote bone formation by cultured osteoblasts.[14] In the present study simvastatin was loaded in type I collagen membrane and its efficacy in periodontal therapy was determined.

The aim of the study is to evaluate the regenerative potential of simvastatin loaded on collagen membrane compared with collagen membrane alone in intrabony defects both clinically and radiographically during 6, 12 months post-operative period.

The aim of this present study is to compare simvastatin loaded collagen membrane (Group1) with collagen membrane alone (Group 2) in human periodontal intrabony defects. The clinical and radiological parameters were evaluated after a period of 6 and 12 months in intrabony defects.

1. To compare changes in probing pocket depth following therapy of both the groups.

2. To estimate the change in clinical attachment level following therapy of both the groups.

3. To compare and quantify the amount of bone fill in both the groups following therapy.

Periodontal regeneration:

The goal of regenerative therapy is to promote healing and regeneration of tissue structure and function. Regenerative treatment modalities include the use of three dimensional biomaterial scaffolds or matrices to support the regeneration of lost tissues.

John Hunter's in 18th century did experiments in transplanting teeth and restores the lost fibrous attachment due to periodontitis.

Melcher AH (1976)[15] suggested that cells of periodontium could produce new cementum, alveolar bone and periodontal ligament, provided that these cells are in closer affinity to periodontal wound area. This can be achieved by blockage of epithelial cells and gingival fibroblasts.

Nyman S, Lindhe J et al (1982)[16] proposed the basis of guided tissue regeneration. In this, the exclusion of soft fibrous connective tissue is thought to enhance the regeneration of periodontal wounds by cells derived from periodontal ligament and bone.

Many treatment modalities are available to achieve the goal periodontal therapy. This includes nonsurgical periodontal therapy, such as scaling and root planning alone, or SRP combined with local antimicrobial/anti – inflammatory agents and surgical periodontal therapy. Regeneration may be accomplished by the use of autogenous bone grafts, allografts, bone substitues, guided tissue regeneration, pharmacological agents, growth factors, and stem cells.

Autogenous bone grafts:

The use of bone grafts for reconstructing osseous defects produced by periodontitis, dates back to **Hegedus** in 1923 and was reviwed by **Nabers CL and O'Leary TJ et al (1965)**[17]. Numerous bone grafts have been used to improve regeneration, among them autogenous bone grafts have been regarded as the gold standard for bone regeneration. Autogenous bone grafts provide conditions essential for angiogenesis and migration of cells with osteogenic potential. In contrast to both allograft and xenografts, autogenous bone grafts has osteoinductive and osteoconductive properties and its immunologic free.

Sangeetha S (2010)[18] conducted a study to evaluate the regenerative potential of intra – oral autogenous bone grafts in the treatment of intrabony defects in patients with generalized periodontitis. The study concluded that autogenous bone grafts produced a significant probing pocket depth reduction and bone fill at 6 months.

Xenografts:

Brunsvold MA and Melloning JT (2000)[19] evaluated bovine derived xenograft in the treatment of intrabony defects. Four patients with radiographic evidence of vertical bone loss were selected and grafted with bovine derived xenograft. After 6months, block sections were taken. Histologically they found that formation of new bone, cementum, and periodontal ligament coronal to the base line.

Guided tissue regeneration

A physical barrier (membrane) is placed to cover the surgically treated area and the barrier is properly shaped and positioned to form a space around the bony defect. In the space under the barrier, cells from periodontal ligament and bone accumulate in the blood clot, and favors regeneration.

Blumenthal NM (1993)[20] compared the use of collagen and expanded polytetrafluoroethylene (ePTFE) in human mandibular Class II furcations. He found that the collagen membrane evoked a lower inflammatory response; the material is pliable when moist and conforms well to the surgical area. The collagen provides a thrombogenic surface that is sealed coronally to the root surface by a fibrin clot.

Tonetti MS, Pini-Prato G, Cortellini P et al (1993)[21] investigated the factors which might affect the healing response in intrabony defects treated with guided tissue regeneration.They suggested that the total depth of the intrabony component and the radiographic defect angle significantly affected the amount of tissue gain. Seventy-five percent (75%) of the variability of regenerated probing attachment level and bone fill was explained in terms of tissue gain under the membrane, radiographic width of the defect angle, full mouth bleeding score, and presence or absence of flap coverage of the newly formed tissue.

Cortellini P, Pini Prato G, Tonetti MS et al (1995)[22] compared the clinical efficacy of titanium reinforced membranes, conventional ePTFE barrier membranes and access flap procedure for the treatment of deep intrabony defects. He concluded that significantly greater amount of CAL gains were noticed in titanium reinforced membranes compared with other control groups.

Cortellini P, Pini Prato G, Tonetti MS et al (1996)[23] conducted a clinical study to compare the clinical efficacy of 3 treatment modalities, such as bioresorbable Membranes, conventional ePTFE barrier Membranes, access flap procedure (MWF) for the treatment of deep interproximal intrabony defects. He concluded that clinically significant CAL gains can be obtained with GTR procedures using both bioresorbable and non-resorbablemembranes. Patients' morbidity, however, was lower in the group treated with bioresorbable membranes.

Hans Falk, Lars Laurell et al (1997)[24] conducted a study comprised of 143 patients, consisted of 208 patients with 3-, 2-, and 1-walled defect treated with bioabsorbale barrier matrix. He concluded that GTR treatment of intrabony defects of 4mm in periodontal practice may result in clinical attachment level gain and bone fill.

Bouchard P, Giovanno JL et al (1997)[25] conducted a study comprising of 30 patients with mandibular buccal class II furcation defects. The test group received a bioabsorbablepolyglycolic-polylactic membrane and the control group received a non-resorbable expanded polytetrafluoroethylene membrane (ePTFE group). The results of this study suggest that 12 months after initial surgery, similar clinical improvements can be obtained in GTR therapy of buccal class II furcation lesions, regardless of whether bioabsorbable PGA/PLA membranes or non-resorbable ePTFE membranes are used.

Christgau M, BaderN et al (1998)[26] conducted a split mouth study to compare the clinical and radiographic healing results in intrabony periodontal defects 12 months after GTR therapy with 2 different bioresorbable barriers. The 2

defects of each patient were randomized for treatment either with polylactic acid membranes or with polyglactin 9-10 membranes. They concluded that both polylactic acid membrane and polyglactin membrane favor regeneration in deep intrabony defects. No statistical difference was found between these two membranes.

Christgau M, Bader N et al (2002)[27] conducted a study to compare the clinical, radiographic, and microbiological healing results in deep intrabony defects following GTR therapy with two different bioresorbable membranes (Polydioxanon membrane or a Polylacticacid matrix) barrier in a split mouth study. They concluded that both these membranes favor similar regeneration in deep intrabony defects.

Slotte C, Asklow B et al (2007)[28] conducted a study to evaluate the outcome of combined use of guided tissue regeneration (GTR) barriers and bovine bone in advanced periodontal defects. In each of 24 patients, one defect was surgically exposed, debrided, filled with bovine bone, and covered with a bioresorbable barrier. They concluded that advanced periodontal defects can be successfully treated with the combined use of GTR barriers and bovine bone to substantially reduce PPD and achieve a stable, long-term gain of CAL.

Pharmacological compounds:

These compounds accelerate the necessary autogenous growth factors in the wound site to stimulate bone growth, and they are considered as alternate substance to correct osseous defects. Bone morphogenic proteins (BMPs) is one among the growth factor, provided promising results[29]. Bisphosphonates like alendronate were commonly used drugs, which inhibit bone resorption by blocking the mevalonate

pathway. Some of the products of this pathway are involved in osteoclast maturation and activation and leads to inhibition of bone resorption. Another group of drugs widely used is statins such as simvastatin, atorvastatin, cerivastatin, etc.

Statins are specific competetive inhibitor of 3- hydroxyl – 2-methyl-glutaryl coenzymeA (HMGCoA) reductase. These agents are widely used to lower cholesterol, and they provide an important and effective approach for the treatment of hyperlipidemia and arteriosclerosis. However, statins also appear to modulate bone formation, inflammation, and angiogenesis[14].

Effects of statins:

Mundy G, Garret R, Harris S et al. (1999)[30] investigated more than 30000 compounds and tested the effects of compounds on BMP -2 gene expression. They concluded statins, hydroxyl methylglutaryl coenzyme A (HMGCoA) reductase inhibitors, which block the cholesterol production pathway, as the natural products in this compounds that specifically induced BMP – 2 cells.

Davingon and Laaksonen (1999)[31] unlocked the pleotropic properties of statins. They also analyzed the functions of simvastatin like 1) nitric oxide mediated improvement of endothelial dysfunction and up regulation of endothelial -1 expression 2) Antioxidant effects 3) Anti-inflammatory properties 4) Inhibition of cell proliferation with anticarcinogenic actions in animals 5) stabilization of atherosclerotic plaques 6) Anti-coagulant effects 7) Inhibiton of graft rejection after kidney and heart transplantation.

Sugiyama M, Kodama T et al (2000)[32] analyzed that simvastatin like compactin (another statin) also activated the BMP-2 promoter, whereas pravastatin

did not. The statin mediated activation of BMP -2 promoter was completely blocked by mevalonate (downstream metabolite of HMG- CoA reductase), suggests that activation was the result of inhibition of the enzyme. The authors suggest that, if statins are selectively targeted to bone, they have positive effects in the treatment of osteoporosis or bone fracture.

Animal studies:

Mundy G, Garret R, Harris J et al (1999)[30] stated that osteoporosis and other diseases of bone loss are a major public health problem. The authors concluded that statins treated rats showed enhanced new bone formation. This effect was correlated with increased expression of the BMP-2 gene in bone cells. Lovastatin and simvastatin when injected subcutaneously over the calvaria of mice, it showed increased bone formation and increased cancellous bone volume when orally administered to rats. Thus, in appropriate doses, statins have therapeutic use in the treatment of osteoporosis.

Ricky WK, Wong A et al (2005)[33] studied the early histological and ultra-structural pictures of bone defect healing with and without statin. The results indicate that new bone was formed on day 5 in the defects grafted with statin. No cartilage stage was detected in statin group. The bone defects on the day 14 revealed that an abundance of bone formation in the statin group and osteocytes were identified ultra-structurally. The authors concluded statin induced and accelerated bone formation locally.

Shamiul A, Koichiro U, Kiyomasa N et al (2009)[34] examined the effect of BMP-2 expression after implantation of a statin and recombinant human BMP -2

(rhBMP-2) in the rabbit nasal bone and evaluated the bone regeneration capability of these substances by immune histologic methods. They revealed that statins showed BMP -2 expression and osteoinductive activity similar to rhBMP- 2.

Human studies:

Chung YS, lee MD, lee SK et al (2000)[35] concluded that simvastatin has a number of pleiotropic effects. The authors suggested that statins possess anabolic effects on bone, in addition to its anti resorptive action. These effects are confirmed in the form of increased bone mineral density in diabetes mellitus patients who were administered statins systemically for the correction of increased cholesterol levels.

Chan KA, Andrade SE, Boles Metal (2000)[36] conducted a case control study in women aged 60 years or older and found that regular statin use was associated with a more than 50% reduction in the risk of pathologic fracture.

Wang PS, Solomon DH, Mogun H et al (2000)[37] found a significantly decreased risk of hip fractures in elderly individual after being given statins orally for a period of 3 months to 3 years. The authors concluded that there is a positive association between statin use and risk of hip fracture in elderly individuals.

Cunha-Cruz J, Saver B, Maupome G et al (2006)[38] studied the effects of statin on tooth loss by chronic periodontitis patients. The results showed that any form of statin use during 3 years was not associated with tooth loss rate. Regular statin use during 3 years was associated with a non-significant 37% reduced tooth loss rate in the year subsequent to the 3-year study period. Any form of statin use during the first 3 years after the initial periodontal examination was associated with a 48% decreased tooth loss rate in year 4 and subsequent years.

Saxlin T, Suominen-Taipale L, Knuuttila M et al (2009)[39] reviewed the association between statin medication and periodontal infection in an adult population. They revealed a weak negative association between statin medication and periodontal infection among subjects with dental plaque or gingival bleeding. They also revealed in patients with no gingival bleeding, statin medication was found to be associated with an increased likelihood of having deepened periodontal pockets. They concluded that statin medication appears to have positive effect on the periodontium that is dependent on the inflammatory condition of the periodontium.

Masahiko M, Tetsunari N, Kazuya M et al (2010)[40] reviewed statins acts as inducer for promoting bone formation. They suggest that statin family increases bone mineral density in humans and it decreases the risk of fractures in osteoporotic patients and elderly individuals. It also, enhances mRNA protein expression levels of BMP-2 and vascular endothelial growth factors (VEGF) to stimulate osteoclastic function. Statins up regulated the gene expressions of extra cellular matrices such as osteocalcin, bone sialoprotein, collagen and proteoglycans. Statins are found to induce bone formation locally, triggers early development of growth factors, regulate angiogenesis by VEGF, and therefore causes bone mineralization.

Effects of simvastatin:

Various studies showed that SMV assists in bone regeneration as well as the anti-inflammatory when delivered topically or locally.

In- vitro studies:

Maeda T, Kawane T, and Horiuchi N et al (2003)[41] examined statin effects on vascular endothelial growth factor (VEGF) expression in osteoblastic

cells. They revealed that simvastatin markedly increased VEGF mRNA in non-transformed osteoblastic cells (MC3T3 E1). Simvastatin (10^{-6}M) time dependently increased VEGF mRNA expression in MC3T3 E1 cells by without altering mRNA stability. These authors suggested that simvastatin promotes osteoblastic differentiation by stimulating VEGF expression in osteoblasts via reduced protein prenylation and the phosphatidylinositide -3- kinase pathway.

Sakoda K, Yamamoto M, Negishi Y et al (2006)[42] reviewed the effect of simvastatin on IL -6 and -8 production in a cultured human epithelial cell line (KB cells) in response IL -1. Simvastatin decreased the production of IL-6 and 8, an effect that was reversed by adding mevolonate but not farnesyl pyrophosphate. Simvastatin reduced nuclear kappa B and activator protein-1 promoter activity in KB cells, indicating an anti-inflammatory effect for simvastatin on human oral epithelial cells, apparently involving Rac1 GTPase (a hydrolase enzyme that can bind and hydrolyze guanosine triphosphate) inhibition.

Seto H, Ohba H, Tokunaga K et al (2007)[43] showed that in cultured rat calvarial cells simvastatin maintained high alkaline phosphatase activity and it also shows increased bone nodule formation in a dose dependent manner.These results suggest that simvastatin increased and maintained a high level of osteoblastic function.

Animal studies:

Ayukawa Y, Okamura A, Koyano K (2003)[44] evaluated the effects of simvastatin on the promotion of osteogenesis around titanium implants. Ten 30-week old rats received pure titanium implants in both tibiae, and then were divided

into experimental and control groups. The experimental group was administered simvastatin daily. 30 days later, all animals were sacrificed and then specimens were prepared. The results indicated that in experimental groups, new bone formation could be seen around implants, which was indirect contact with the implant surface. The authors concluded that the administration of simvastatin increases the value of both bone contact ratio to the implant and implant density. By this simvastatin may have the potency to improve the nature of osseo integration.

Stein D, Lee Y, Marian J et al (2005)[45] estimated the effect of simvastatin doses and cyclooxygenase (COX) synthase inhibitors on tissue inflammation and bone growth in rats and gene expression in mice. By administering 0.5 mg of simvastatin, it was found that there was significant up regulation in procollagen, fibronectin, and matrix metallo proteinase -13 genes. They concluded that reducing simvastatin dose from 2.2 to 0.5 mg reduced inflammation to a more clinically acceptable level. But COX associated inflammation appears to be necessary for bone growth in vivo.

Nyan M, Sato D, Oda M et al (2007)[46] conducted a study to identify whether simvastatin stimulates bone regeneration when combined with calcium sulfate as a carrier or not. Specific sized bone defects in rat calvaria were treated with calcium sulfate alone or with combination of 1 mg simvastatin. In the combination group, although the least inflammation was observed at 2 and 4 weeks, remarkable bone formation was evident at 8 weeks. The combination of simvastatin and calcium sulfate stimulated bone regeneration in spite of the inflammatory response.

Ozec I, Kilic E, Gumus C and Goze F (2007)[47] examined the effect of local simvastatin application on 3mm bone defects in mandible. Radiologic assessment of newly formed bone by peripheral quantitative computed tomography showed significantly increased density in the experimental group.

Houshmand B, Hassanizade R, Eslami B et al (2010)[48] investigated whether injection of statins could lead to ectopic bone formation in rats. Bone formation was also evident in lovastatin treated area in one rat and simvastatin treated area in another after six weeks. They concluded that subcutaneous injection of simvastatin and lovastatin could induce ectopic bone formation.

In – vivo studies:

Lee Y, Schmid MJ, Marx DB et al (2008)[49] investigated the effect of local simvastatin delivery strategies on mandibular bone formation in vivo. Less invasive and more flexible injection protocol was studied to evaluate the bone inducing effects compared to surgical implantation. Bone formation rate, short – and long term bone augmentation histology, and mechanical properties were evaluated to characterize the new bone rat bone in a rat bilateral mandible model. Compared to controls, bone formation in rate was significantly higher on the simvastatin side, especially in the dome. The study concluded that multiple injections of a dose 0.5 mg dose of simvastatin gel could induce an accumulative effect in new bone formation with minimal soft tissue swelling.

Suthanthiran TK, Elavarasu S, Naveen D et al (2012)[50] showed that simvastatin enhanced cell metabolism dose dependently at 24-hr time and the maximum effect was obtained at a concentration of 1.5 mg of simvastatin. These

results indicate that collagen with 1.5 mg SMV exhibits positive effect on cell metabolism of human osteoblast-like SaOS-2 cells.

Effects of simvastatin on the periodontium:

Thylin MR, McConenell JC, Schmid MJ et al.(2002)[51] done a study to test if similar bone stimulation could be induced by 2 single dose drug delivery systems of appropriate to periodontal therapy. They concluded that a single high dose of simvastatin gel could stimulate murine cranial bone apposition, particularly when delivered under an occlusive membrane.

Yazawa H, Zimmermann B, Asami Y et al (2005)[13] analyzed the effect of simvastatin on cell proliferation and osteoblastic differentiation in periodontal ligament cells. The result shown that simvastatin enhanced cell proliferation and metabolism in dose dependent manner. Simvastatin also stimulated ALP activity of human periodontal ligament cells dose dependently, and maximum effect was obtained at the concentration of 10^{-8}M. These results suggest that at a low concentration, simvastatin exhibits positive effects on proliferation and osteoblastic differentiation of periodontal ligament cells, and these effects may be caused by the inhibition of the mevalonate pathway.

Joen JH, Piepgrass WT, Lin YL et al (2008)[52] evaluated the effect of localized delivery of simvastatin hydroxy acid enhanced osteoblastic activity in vitro, the objective of this investigation was to determine bioactivity of the delivery system in vitro. Devices for sustained or intermittent release of simvastatin hydroxy acid were formed using a blend cellulose acetate phthalate and a poly ethylene oxide copolymer, and they were implanted directly over the calvarium of young male rats.

The results shown that devices delivering hydroxy acid was associated with 77.5% to 13.3% increase in new woven bone thickness compared to control devices without a drug. They concluded by saying that these devices for the intermittent delivery of simvastatin favors additional osteogenic response.

Lindy O, Suomalainen K, Makelam et al (2008)[53] did a retrospective study to evaluate the association of statin use and clinical markers of periodontitis. The results shown that periodontitis patient taking statins had a 37% lower number of pathological periodontal pockets than those without statin medication.

Animal studies:

Vaziri H, Roodsari RN, Fahadan NT et al (2007)[54] evaluated the efficacy of locally injected simvastatin in human sized periodontal defects and the oral adverse events associated with local simvastatin application in the periodontium. The result showed that the periodontal tissues tolerated the simvastatin well in the dog gingiva and mucosa. Evidence of new cementum coronal to the base of the defects occurred over time for most of the intra-bony defects. There was a trend towards more new cementum formation for intra-bony defects that received simvastatin. They concluded that multiple injections of simvastatin are not appropriate for the treatment of intra-bony or furcation defects.

Dalacio R, Menezes MA et al (2013)[14] evaluates the effect of simvastatin on rats subjected to experimental periodontal disease. Treatment with simvastatin improved alveolar bone loss within all of the parameters of periodontitis, thus demonstrating anti-inflammatory and antioxidant activity. Simvastatin reduced expression of iNOS, MMP-1 and -8, RANK, and RANKL and increased BMP-2 and

OPG levels in the periodontal tissue. Simvastatin (30 mg/kg) increases total alkaline phosphatase activity on day 11 compared with the saline group. They that simvastatin prevents inflammatory bone resorption in experimental periodontitis, which may be mediated by its anti-inflammatory and antioxidant properties.

In vivo studies:

Pradeep AR and Thorat MS (2010)[55] investigated the effects of simvastatin 1.2 mg, in a biodegradable controlled-release gel as adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis. They concluded that there was a greater decrease in gingival index and PD and more CAL gain with significant intrabony defect fill at sites treated with SRP plus locally delivered simvastatin.

Pradeep AR, Priyanka N, Kalra N et al (2012)[56] investigated the effect of 1.2-mg simvastatin as a local drug delivery system as an adjunct to scaling and root planning (SRP) for the treatment of Class II furcation defects. They concluded that there was a greater decrease in gingival index and PPD, with significant bone fill with locally delivered simvastatin in patients with Class II furcation defects.

Pradeep AR, Rao NS, Bajaj P et al (2012)[57] investigated the effectiveness of 1.2% SMV in an indigenously prepared, biodegradable, controlled-release gel as an adjunct to scaling and root planing (SRP) in the treatment of patients with type 2 diabetes and chronic periodontitis. There was a greater decrease in modified sulcus bleeding index and PPD and more CAL gain with significant intrabony defect fill at sites treated with SRP plus locally delivered SMV in patients with type 2 diabetes and chronic periodontitis.

Method of administration:

Jun- Beom P (2008)[58] reviewed on simvastatin and summarized various in vitro and in vivo studies. The effects of simvastatin from different method of administration, dosage and carriers were described. He concluded that abundant information about simvastatin indicates their possible beneficial effect on bone, available both in the preclinical and clinical field, there have been some conflicting results on the effects of simvastatin. This is because the effects of simvastatin's may be influenced by a range of factors including the method of administration, duration of exposure, experimental animal model and bioavailability.

Evaluation of collagen properties

Locci P, Calvititti M, Belcastro S et al (1997)[60] compared the degree of biocompatibility between collagen and polytetrafluroethylene (PTFE). Using 3H-thymidine, it was shown that fibroblasts grown on collagen significantly increased 3H- thymidine incorporation, while fibroblasts grown on PTFE membrane decreased 3H-thymidine incorporation, compared to plastic used as a control. Moreover, the PTFE membrane induced a marked decrease of GAG accumulation both in cellular and in extracellular matrix pool. These findings suggest that collagen is most suitable to stimulate both cellular proliferation and ECM macromolecule accumulation.

Bunyaratavej Pand Hom- Lay wang (2001)[59] using 3H- thymidine auto radiography, observed cell kinetics during periodontal healing with and without collagen membrane on rat model. Histological observations at day 1 on the experimental side demonstrated regenerated epithelium opposed to the collagen

membrane with an intervening layer of necrotic tissues and / or fibrous exudate. There was no observable proliferation of regenerated epithelium toward the root apex. These histological and auto radiographic findings suggest that atelocollagen membrane inhibits apical migration of regenerating epithelium and accelerates connective tissue reattachment.

Simvastatin membrane preparation:

Simvastatin at 1.5 mg concentration was loaded in collagen membrane by ELISA coating method.

In vitro release kinetics of simvastatin membrane by HPLC

HPLC analysis was carried on a Shimadzu LC-10A series system (Kyoto, Japan) equipped with a Phenomenex AQUA C18 reverse phase column (150 mm x 4.6 mm, 5 mm; Torrance, USA). In order to evaluate the kinetics of drug-release, samples were taken from the air-dried collagen membranes and soaked in absolute EtOH to facilitate degradation of membrane.

Five Pieces were weighed individually and crushed in mortar. An accurately weighed quantity of powdered membrane (1.4 mg) was extracted with phosphate buffer and the solution was filtered through 0.45µ membrane. The release characteristic of test and reference formulation of Simvastatin was determined by USP XXIII dissolution apparatus at 50 rpm. The dissolution media used for Simvastatin was 0.1 N hydrochloric acid pH 1.2 and phosphate buffer pH 7.4 containing for 24 hours maintained at 37°C. Dissolution tests were performed on the five collagen membranes loaded with Simvastatin. Five ml of the samples were withdrawn at 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0,10, 12.0, 18.0 and 24.0 hours' time intervals. Equal quantity of the dissolution medium was replaced to the dissolution jar after each sampling. The amount of the drug released was estimated by optimized and validated HPLC methods. Percentage drug release and cumulative release at various time intervals were calculated and compared. (Table No.1 and Graph No.1)

A randomized split mouth, single evaluator; 12 months prospective clinical study was conducted to compare and evaluate the clinical and radiographic parameters in periodontal intra bony defects using simvastatin incorporated collagen membrane and collagen membrane alone. Patients were instructed about the utility and design of this clinical trial and informed consent were obtained. The study design was explained to institutional ethical board and clearance was obtained. Patients were selected from outpatient Department of Periodontics, J.K.K. Nattraja dental college and Hospital, Komarapalayam based on the following selection criteria.

Inclusion criteria:

1. Patients age limit of 20-50 years of both genders.

2. Probing depth of > 5mm as assessed by William's graduated probe.

3. Patients with minimum of two contralateral intra bony defects.

Exclusion criteria:

1. Patients with known systemic diseases, short and long term therapies.

2. Previous periodontal therapy.

3. Known drug allergy.

4. Teeth with traumatic occlusion.

5. Smokers.

6. Pregnancy and lactating women.

STUDY DESIGN:

A split mouth design was planned, in which two contralateral sites with > 5 mm probing pocket depth and radiographic evidence of bone loss at baseline were chosen. Probing pocket depth was standardized with acrylic stent in all the selected areas.

GROUP CRITERIA

Group 1 : Intrabony defects treated with simvastatin incorporated collagen membrane.

Group 2 : Intrabony defects treated with collagen membrane.

CLINCAL PARAMETERS:

The following variables were measured at baseline, 6 months, and 12 months post-operative period.

1. Gingival index.
2. Plaque index.
3. Oral hygiene index (simplified).
4. Probing pocket depth – deepest probing depth was measured.
5. Clinical attachment level.

Probing pocket depth:

Pocket depth was measured at desired sites with William's periodontal probe. The probe was inserted parallel to the tooth surface until resistance was felt and the

readings were recorded to the nearest millimeter marking from the gingival margin to the base of the pocket. Acrylic stents were used to standardize the path of insertion and angulations of the probe.

Clinical attachment level:

The level of attachment is the distance between the base of the pocket and cemento enamel junction (CEJ). The distance from the CEJ (if CEJ is not clinically detected, the coronal border of the stent was used) to the base of the pocket was measured. The readings were noted.

Occlusal stents were fabricated with cold cure resin on patient model cast for positioning and measuring probe markings were fabricated. Notch was made on the stent to permit and standardize the entry of periodontal probe into the pocket. The occlusal stent was made to cover the occlusal surfaces of the tooth being treated and occlusal surface of one tooth in mesial and distal directions. The stents were also extended apically on the buccal and lingual surfaces to cover the coronal third of teeth involved.

Radiographic parameters:

Intraoral radio visual radiograph (RVG) of each defect was exposed. Bone density area of group 1 and group 2 radiographs were determined. To calculate bone density area, three reference points were used 1) alveolar crest 2) cemento enamel junction 3) bone defect depth. These three points were united to form a triangle and the area of triangle was calculated by using the formula ½ × bh mm^2, where b is the base of the triangle, h is the vertex opposite to the base of the triangle. The images were displayed on adobe photoshop 7.0 and bone density area was calculated.

Pre surgical therapy:

For all the selected patients, routine blood investigations were taken. Initial therapies consisted of scaling and root planing, oral hygiene instructions, diet counselling and medications. Three weeks following phase I therapy, re – evaluation was performed.

Surgical procedures:

Following pre surgical phase periodontal surgical procedures were performed. The patient as anaesthetized using lignocaine 2% with 1; 1, 00,000 epinephrine. Using Bard parker blade number 15, buccal and lingual sulcular incisions were made to elevate the mucoperiosteal flaps. Pocket epithelium and degranulation tissue from the inner surface were removed gently. Thorough soft tissue debridement and root planing were accomplished with Hu – Friedy curettes and washed with saline.

Surgical procedure:

Group 1

Simvastatin incorporated collagen membrane was cut into desired shape and placed in the intrabony defect. Then the flaps were repositioned to accomplish complete inter proximal closure. Then the flaps were approximated with simple interrupted suture using 3-0 non absorbable silk thread. Periodontal dressing (coe – pak) was given. Post-surgical instructions were given to the patient and recalled after one week for suture removal and further follow up.

Group 2

Collagen membrane (Healiguide) was cut into desired shape and palced in the intrabony defect. Then the flaps were repositioned to accomplish complete inter proximal closure. Then the flaps were approximated with simple interrupted suture using 3-0 non absorbable silk thread. Periodontal dressing (coe – pak) was given. Post-surgical instructions were given to the patient and recalled after one week for suture removal and further follow up.

APPENDIX - 1

Instructions to the patient

- Patients are advised to report immediately on developing any untoward reactions like pain, swelling, hypersensitivity, and drug allergies.
- Patient should report to the dentist, if secondary bleeding persists within 24 hours.
- Patients are advised to avoid hot and hard foods.
- Patient was advised to take antibiotic every 8 hours for 3 days and take analgesic every 12 hours.
- Patients are instructed to avoid brushing in the surgical site for 1 week from the day of surgery; use cotton tip applicator to clean the area.
- Patients are instructed not to use dental floss and tooth picks at the surgical site.
- Patients are instructed to use 0.2% chlorhexidine mouth rinse twice daily.
- Follow up visits have to be done in 24 and 48 hours.
- Patients were asked to perform regular oral hygiene habits by appropriate brushing technique using toothbrush and tooth paste.
- Patients were instructed to report on the subsequent appointment.

APPENDIX – 2

PROFORMA

PATIENT NAME: O.P. NO:

AGE: SEX:

ADDRESS: PHONE NO:

CHEIFCOMPLAINT:

SITE SELECTED:

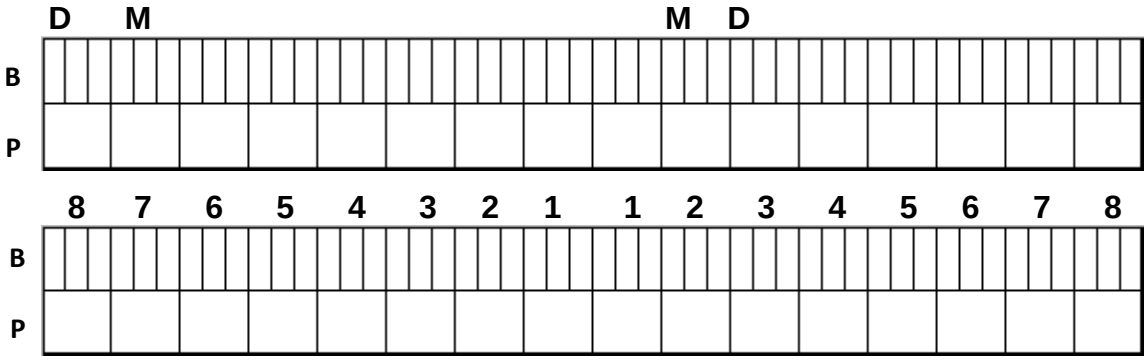
GROUP 1: (COLLAGEN MEMBRANE WITH SIMVASTATIN) ☐

GROUP 2: (COLLAGEN MEMBRANE) ☐

INDICES

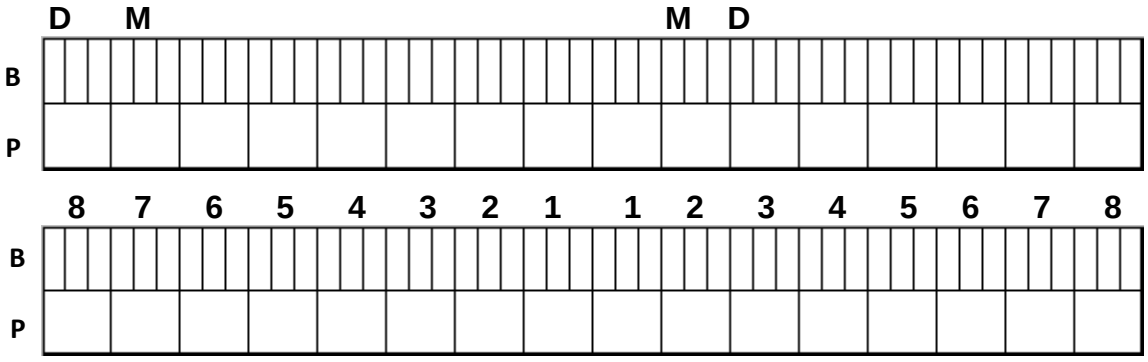
GINGIVAL INDEX

BASELINE:



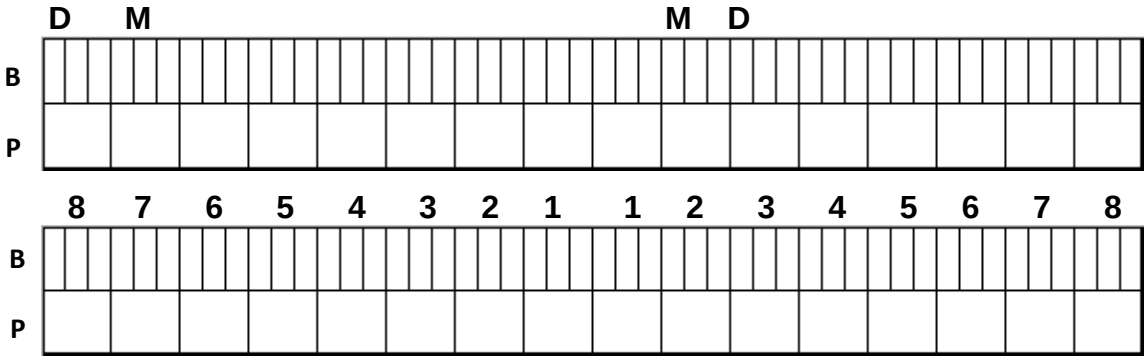
SCORE ☐

6 MONTHS



SCORE ☐

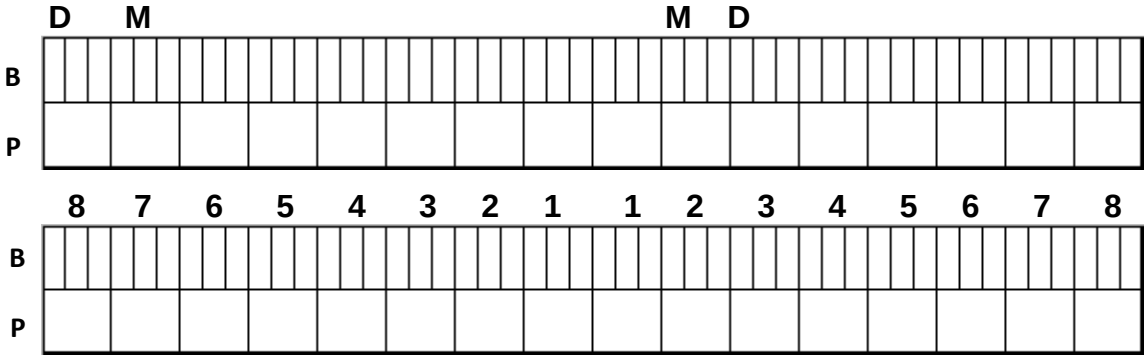
12 MONTHS



SCORE ☐

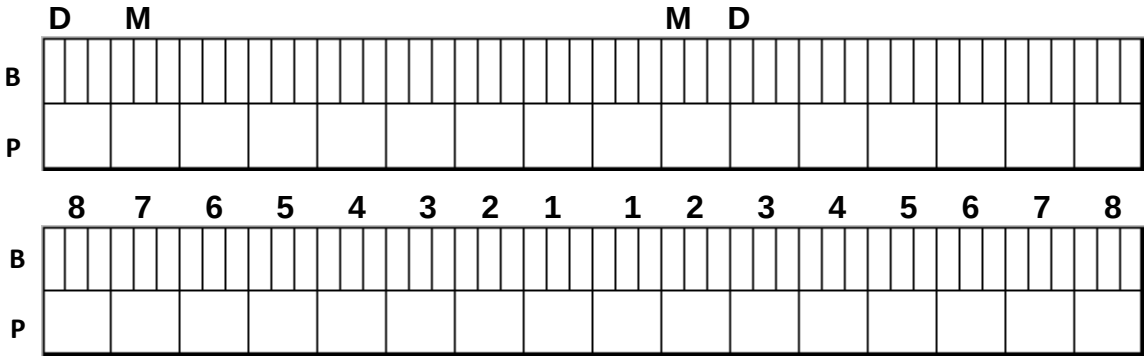
PLAQUE INDEX

BASELINE:



SCORE ☐

6 MONTHS



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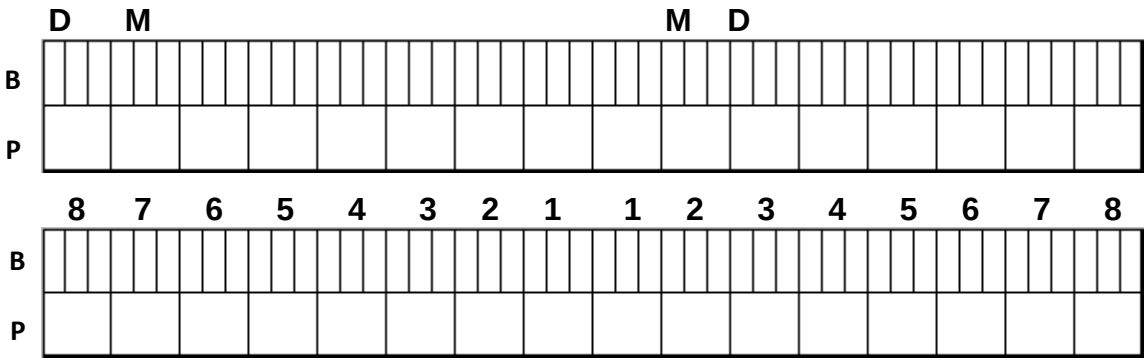
12 MONTHS



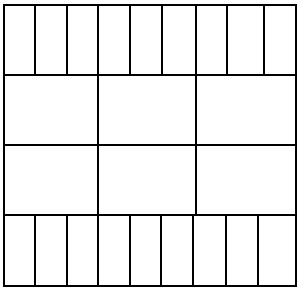
SCORE ☐

ORAL HYGIENE INDEX

Debris index (DI)

BASELINE:

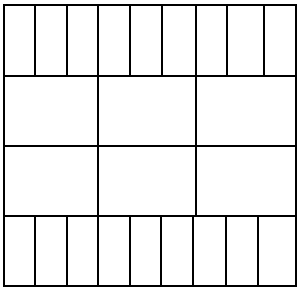
16 11 26



46 31 36 **SCORE** _____

6 MONTHS:

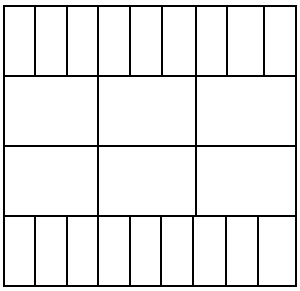
16 11 26



46 31 36 **SCORE** _____

12 MONTHS:

16 11 26

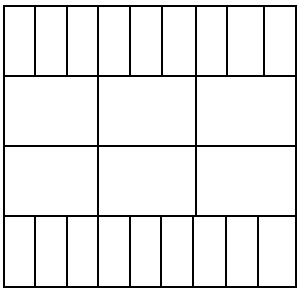


46 31 36 **SCORE** _____

Calculus index(CI)

BASELINE:

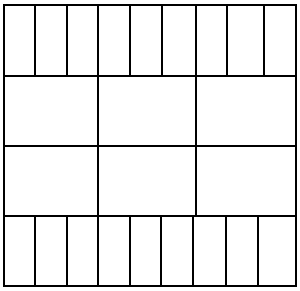
16 11 26



46 31 36 **SCORE** ____

6 MONTHS:

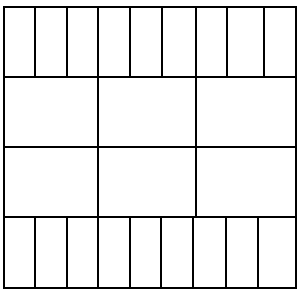
16 11 26



46 31 36 **SCORE** ____

12 MONTHS:

16 11 26



46 31 36 **SCORE** ____

OHI SCORE (DI+CI) =

INTERPRETATION

CLINICAL PARAMETERS:

DATA	BASELINE		6 MONTHS		12 MONTHS	
	GROUP 1	GROUP 2	GROUP 1	GROUP 2	GROUP 1	GROUP 2
PROBING POCKET DEPTH (mm)						
CLINICAL ATTACHMENT LEVEL (mm)						

RADIOGRAPHIC FINDINGS:

DATA	BASELINE		6 MONTHS		12 MONTHS	
	GROUP 1	GROUP 2	GROUP 1	GROUP 2	GROUP 1	GROUP 2
BONE FILL						

INFORMED CONSENT OBTAINED FROM THE PATIENT

Department of Periodontics, J.K.K. Nattraja Dental College & Hospital,

Komarapalyam, Namakkal District.

PATIENT NAME:

I have been explained about the nature and purpose of this study in which, I have been asked to participate. I understand that I am free to withdraw my consent and discontinue at any time without prejudice to me or effect on my treatment.

I have been given the opportunity to question about the material and study. I have also given the consent for photographs to be taken at the beginning, during and end of the study. I agree to participate in this study.

I hereby have given the consent to be included in "COMPARITIVE EVALUATION OF CLINICAL AND RADIOLOGICAL PARAMETERS OF INTRABONY DEFECTS TREATED WITH SIMVASTATIN LOADED COLLAGEN MEMBRANE / COLLAGEN MEMBRANE ALONE – 12 MONTHS RANDOMIZED CONTROLLED CLINICAL STUDY".

Station SIGNATURE OF PATIENT

Date SIGNATURE OF PROFESSOR

APPENDIX – 3

ARAMAMENTARIUM

MATERIALS AND ISTRUMENTS USED FOR PERIODONTAL FLAP SUREGRY

- Gloves
- Mask
- Patient apron
- Chair apron
- Head cap
- Sterile cotton rolls
- Sterile gauze
- Saline
- Kidney tray
- Betadine solution
- Lignocaine
- Injection syringe

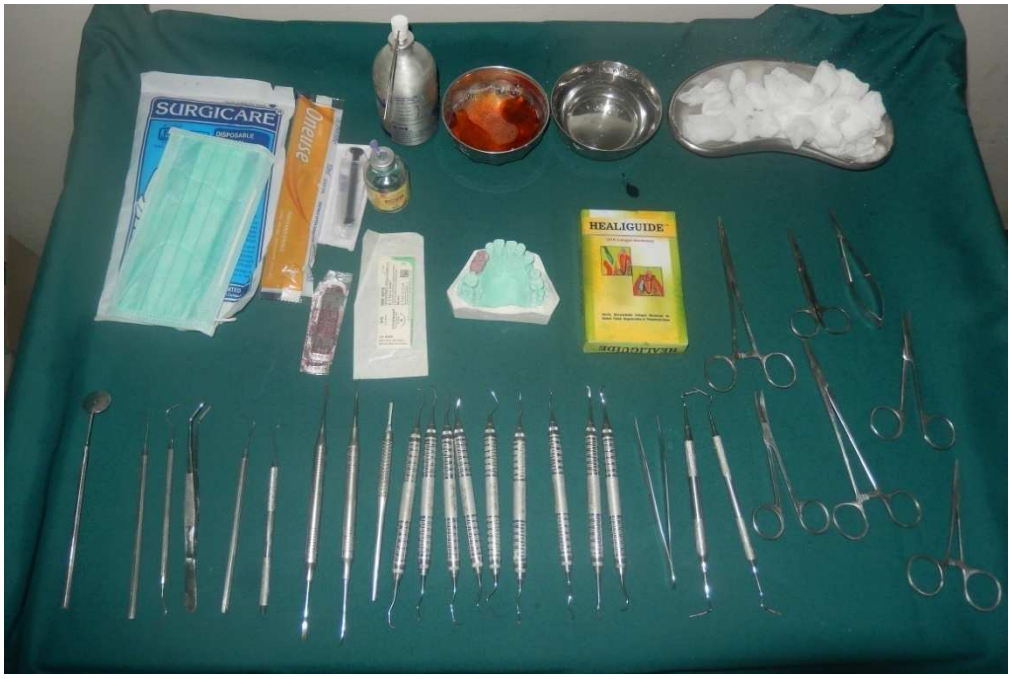
DIAGNOSTIC INSTRUMENTS

- Mouth mirror
- Straight probe
- Explorer
- Tweezer

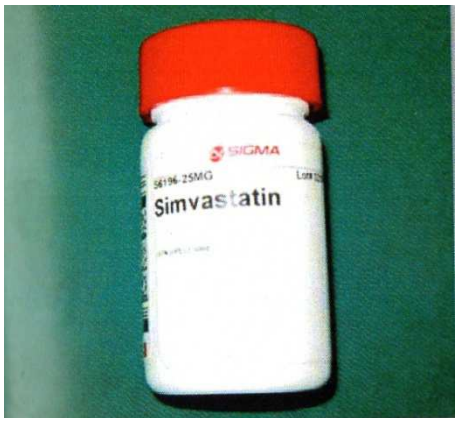
SURGICAL INSTRUMENTS

- Bard parker handle.
- Bard – parker blade number 11 and 15.
- Periosteal elevator.
- Hu – Friedy Gracey curettes.
- Hu – Friedy universal scaler.
- Hu – Friedy cumin scaler.
- Tissue holding forceps.
- Schugler bone file.
- Scissors.
- 3- 0 non - absorbable silk suture.
- Simvastatin powder (Sigma Aldrich – Bangalore)
- Collagen membrane (Advanced Biotech Products (P) Ltd. India)
- Plastic spatula.
- Non-eugenol coe – pak.

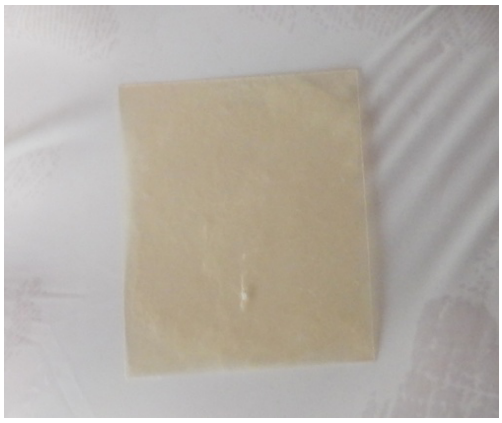
In-Vivo Study – Surgical Instruments



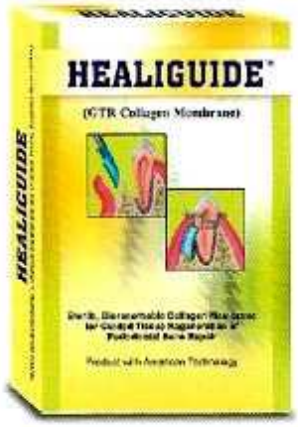
Simvastatin Powder



Simvastatin loaded collagen membrane



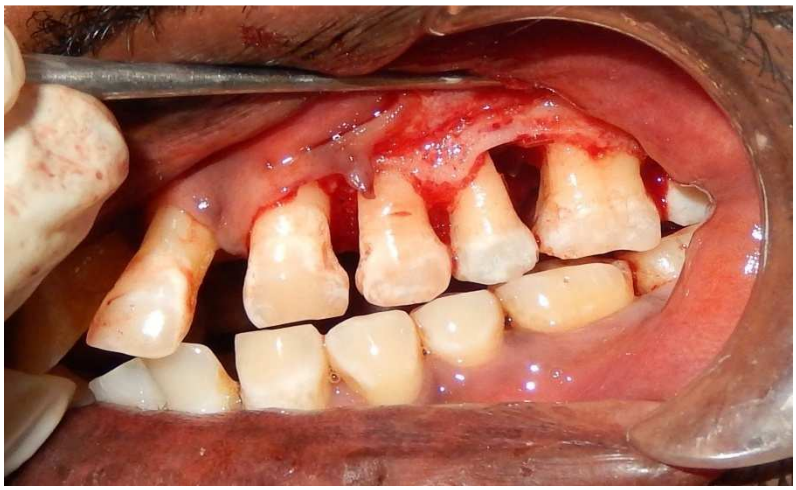
Collagen Membrane



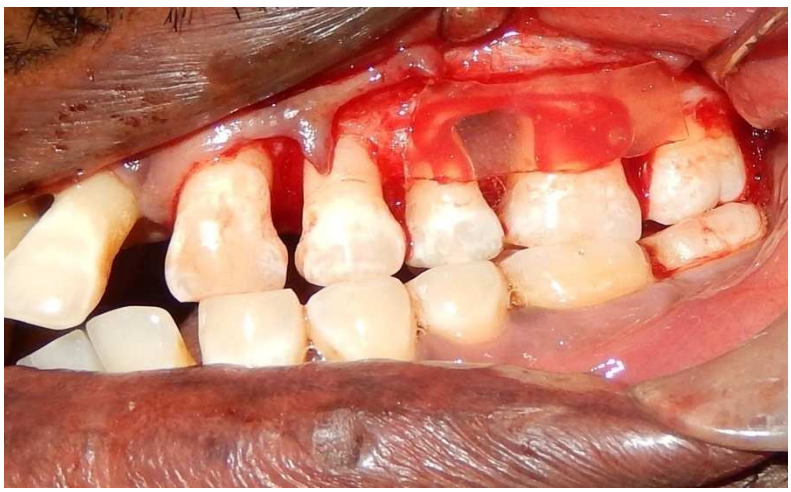
GROUP – 1

PRE-OPERATIVE VIEW

OPERATIVE VIEW



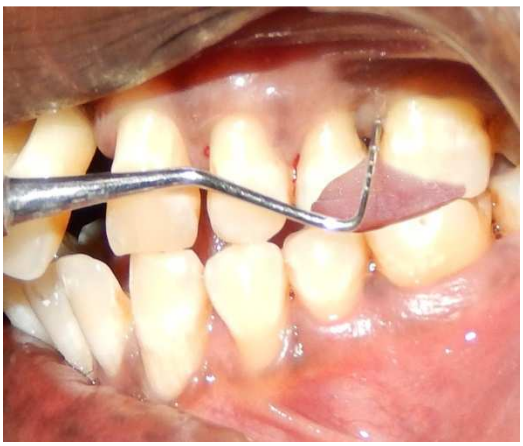
SIMVASTATIN MEMBRANE IN POSITION



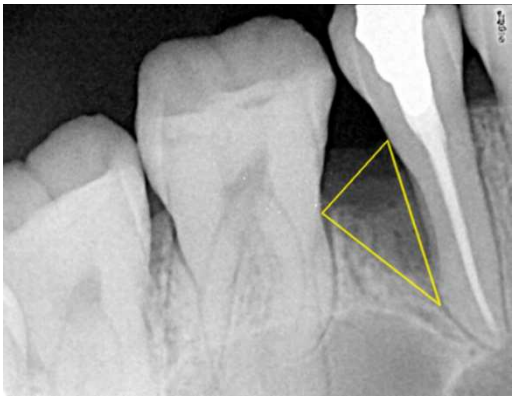
GROUP – 1

PRE-OPERATIVE VIEW

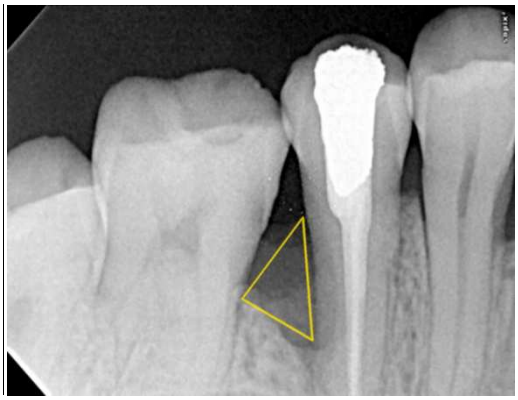
12 MONTHS POST OPERATIVE VIEW



PRE-OPERATIVE RVG

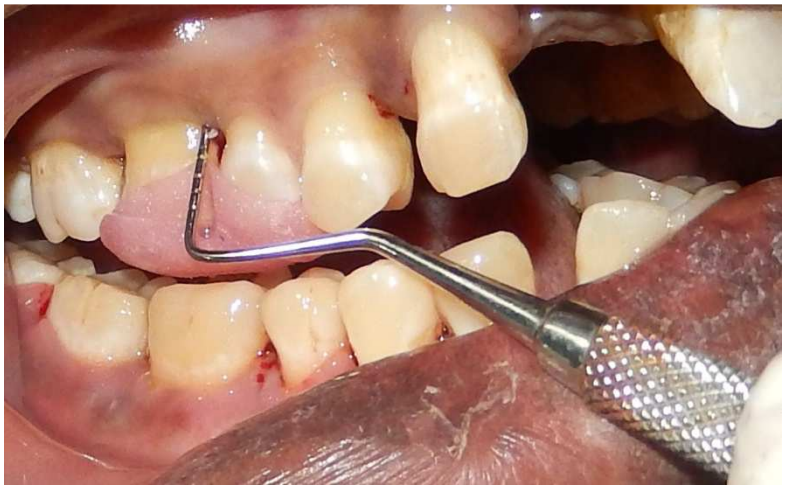


POST OPERATIVE RVG 12 MONTHS

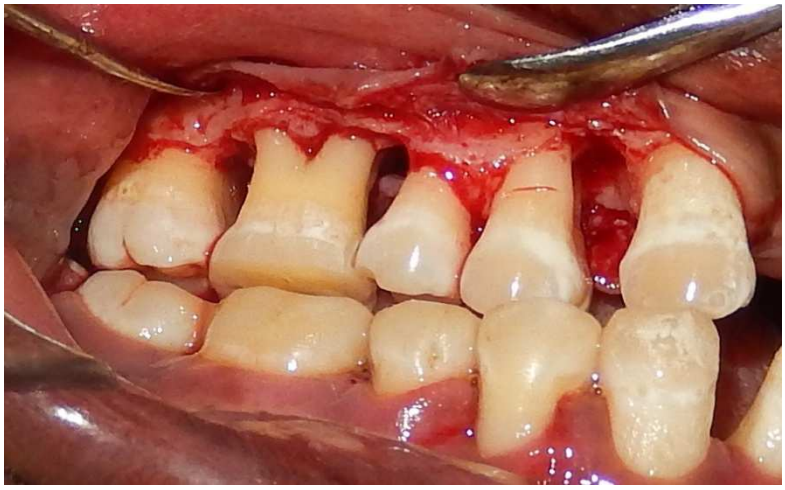


GROUP – 2

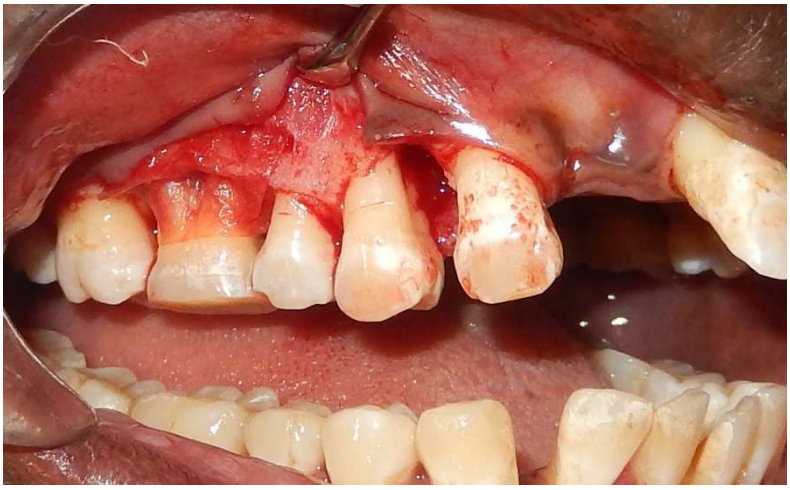
PRE-OPERATIVE VIEW



OPERATIVE VIEW



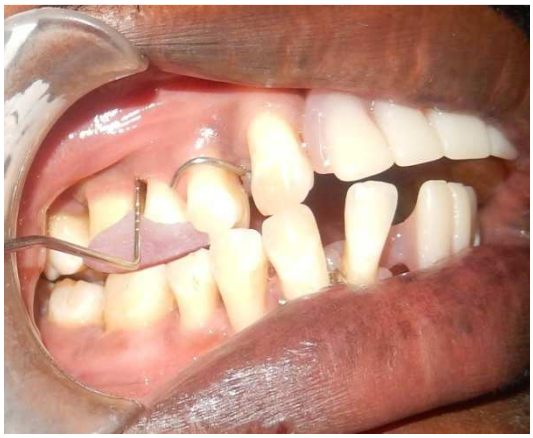
COLLAGEN MEMBRANE PLACED



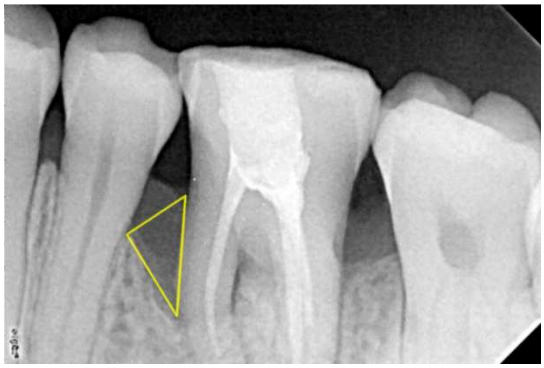
GROUP – 2

PRE-OPERATIVE VIEW

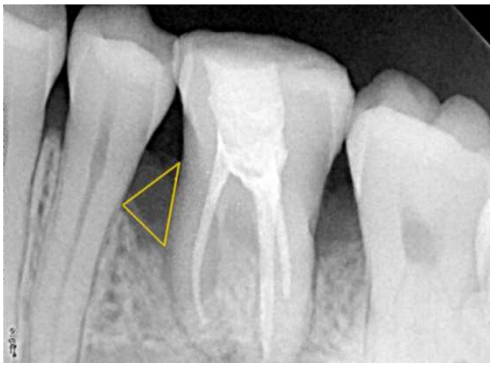
12 MONTHS POST OPERATIVE VIEW



PRE-OPERATIVE RVG



POST OPERATIVE RVG 12 MONTHS



The results obtained were analyzed statistically and comparisons were made with each group using paired student t- distribution test at different time intervals. The paired t- distribution test is used when the sample size is less than 30 and when the standard deviation is unknown.

A randomized controlled clinical trial was conducted to evaluate the clinical and radiographic efficacy of simvastatin loaded collagen membrane in intrabony defects.

The present study aimed comprised of 10 patients with 20 intra bony defects that were randomly selected and divided into two groups (group 1 and group 2). Group 1 patients received collagen membrane loaded with simvastatin (1.5mg) and group 2 patients received collagen membrane in intrabony defects. Clinical parameters such as probing depth, clinical attachment level,and radiographic measurements were recorded.

Plaque index:

The mean plaque index score at baseline was 1.99±0.260, reduced to 1.00±0.211 at 6 months and further reduced to 0.63±0.226 at 12months post-operative period. The values at 6 month and at 12 months were statistically significant when compared to baseline, with a p- value < 0.05 as shown in table no.2 and graph No.2.

Oral- hygiene index – simplified:

The mean oral hygiene index –simplified score at baseline was 1.59±0.074, reduced to 0.69±0.074 at 6 months and further reduced to 0.044±0.052 at 12 months

post – operative period. Compared to baseline, the values at 6 months and at 12 months were statistically significant with a p- value < 0.05 as shown in table no .2 and graph no. 2.

Gingival index:

The mean gingival index score at baseline was 2.14±0.386, reduced to 1.37±0.46 at 6 months and further reduced to 1.32±0.238 at 12 months post-operative period. The values at 6 month and at 12 months were statistically significant when compared to baseline, with a p- value < 0.05 as shown in table no.2 and graph no.2.

Probing pocket depth:

In group1, at baseline the mean probing pocket depth was 7.30±0.483, reduced to 3.60±0.516 at 6 months and 3.25±0.483 at 12 months post-operative period. In group 2, at baseline it was 7.40±0.516, reduced to 4.6±0.516 at 6 months and 4.4±0.516 at 12 months post –operative period as shown in table no.3 and graph.no.3. In group 1 the percentage of PPD reduction was 50.68±0.07%,at 6 months and 53.48±0.00% at 12 months. In group2 it was 37.84±0.00 at 6 months and 40.54±0.00% at 12 months as shown in table no.6 and graph .no.6. On comparison between the groups, the mean value of PPD reduction was higher in group 1 and group2 when compared with the baseline. On comparing the group 1 and group 2, the mean value of PPD reduction was higher in group 1, with a statistically significant p-value of >0.05 at 6 and 12 months post- operative period.

Clinical attachment level:

In group 1, at baseline the mean clinical attachment level was 7.4±0.527, increased to 3.70±0.483 at 6 months and further increased to 3.71±0.432 at 12 months postoperative period. In group 2, at baseline it was 7.50±0.527,increased to 4.50±0.527 at 6 months and 4.4±0.517 at 12 months as shown in table no. 4 and graph no.4. In group 1, the percentage of CAL gain was 50.00±0.006% at 6 months and 52.56±0.54 at 12 months respectively. In group 2, it was 40.00±0.00% at 6 months and 41.33±0.00% at 12 months which was shown in table no.6 and graph.no 7. From baseline, the mean value of CAL gain was higher in both group 1 and group 2. On comparing between group 1 and group 2, the mean value of CAL gain was higher in group1 with a statistically significant p-value of < 0.05 at 6months and 12 months post-operative period.

Radiographic bone fill:

In group 1, at baseline the mean defect was 7.34±0.793 and the bone fill measured was 6.15±0.622 at 6 months and 5.74±0.381 at 12 months post – operative period. In group2, at baseline it was 7.13±0.868, and the measured bone fill was 6.09±0.568 at 6 months and 5.67±0.482 at 12 months, as shown in table .no.5 and graph no.5.In group 1, the percentage of bone fill was 16.21±0.22% at 6months and 21.80±0.52 % at 12 months post-operative period. in group 2, it was 14.59±0.35% at 6months and 20.48±0.52 at 12 months as shown table.no.6 and graph no.8. From baseline, the mean value of bone fill was higher in both group 1 and group 2. On comparing bone fill in group 1 and group 2, there is no statistical difference was noted among the groups.

TABLE -1

In vitro release profile of simvastatin from collagen membrane by HPLC

CUMULATIVE RELAESE OF SIMVASTATIN (%)	TIME IN HOURS
10%	3.0
20%	6.0
30%	10.0
40%	18.0
50%	24.0
90%	72.0

TABLE – 2

Comparison of mean gingival index scores, plaque index scores, oral hygiene index scores at baseline, 6 months and 12 months

PARAMETERS	BASELINE	6 MONTHS	12 MONTHS	p - value
Gingival index	2.14 ± 0.386	1.37 ± 0.346	1.32 ± 0.238	< 0.05*
Plaque index	1.99 ± 0.260	1.00 ± 0.211	0.63 ± 0.226	< 0.05*
Oral hygiene index	1.59 ± 0.074	0.69 ± 0.074	0.44 ± 0.052	< 0.05*

* p- value between baseline, 6 months and 12 months is <0.05 denotes statistically significant at 5%

TABLE – 3

Inter group difference in mean probing pocket depth (PPD)

at baseline 6 months and 12 months

Probing pocket depth (mm)	GROUP 1 (Mean±SD)	GROUP 2 (Mean±SD)	p - value
Baseline	7.30 ± 0.483	7.40 ± 0.516	> 0.05**
6 months	3.60 ± 0.516	4.6 ± 0.516	< 0.05*
12 months	3.25 ± 0.483	4.4 ± 0.516	< 0.05*

** p- value between the group 1 and 2 at baseline is >0.05 denotes statistically insignificant at 5% level.

*p- value between baseline, 6 months and 12 months is >0.05 denotes statistically insignificant at 5% level.

TABLE – 4

Inter group difference in mean clinical attachment level (CAL)

at baseline, 6 months and 12 months

Clinical Attachment Level (mm)	GROUP 1 (Mean±SD)	GROUP 2 (Mean±SD)	p - value
Baseline	7.4 ± 0.516	7.5 ± 0.527	> 0.05**
6 months	3.70 ± 0.483	4.50 ± 0.527	< 0.05*
12 months	3.71 ± 0.432	4.4 ± 0.517	< 0.05*

* p- value between baseline, 6 months and 12 months is <0.05 denotes statistically significant at 5% level.

** p- value between the group 1 and 2 at baseline is >0.05 denotes statistically insignificant at 5% level.

TABLE – 5

Inter group difference in mean Radiographic bone density at baseline, 6 months and 12 months

Radiographic bone density $(mm)^2$	GROUP 1 (Mean±SD)	GROUP 2 (Mean±SD)	P Value
Baseline	7.34 ± 0.793	7.13 ± 0.868	> 0.05**
6 months	6.15 ± 0.622	6.09 ± 0.568	< 0.05*
12 months	5.74 ± 0.381	5.67 ± 0.402	< 0.05*

* p- value between baseline, 6 months and 12 months is <0.05 denotes statistically significant at 5% level.

** p- value between the group 1 and 2 at baseline is >0.05 denotes statistically insignificant at 5% level.

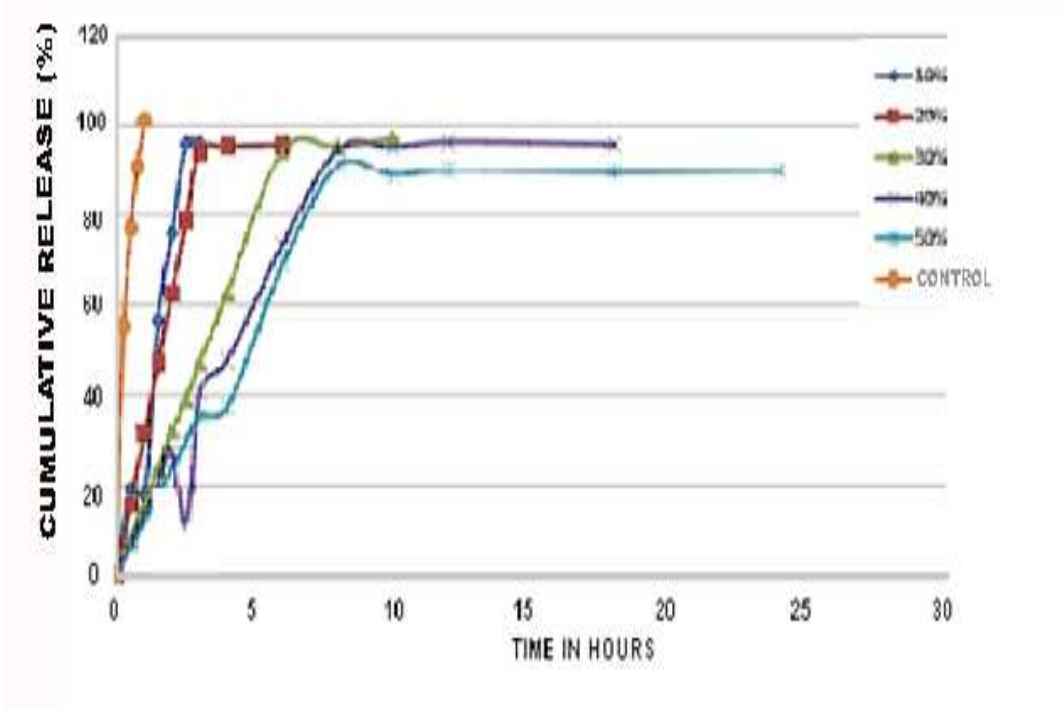
TABLE - 6

Inter group difference in percentage (%) of PPD reduction, % of CAL gain, and % of bone fill at baseline 3months and 6 months

PARAMETERS	GROUP 1		GROUP 2	
	6 months	12 months	6 months	12 months
% of PPD reduction	50.68 ± 0.07	55.48 ± 0.00	37.84 ± 0.00	40.54 ± 0.00
% of CAL gain	50.00 ± 0.06	50.00 ± 0.54	40.00 ± 0.00	41.33 ± 0.02
% of Radiographic bone density	16.21 ± 0.22	21.80 ± 0.52	14.59 ± 0.35	20.48 ± 0.54

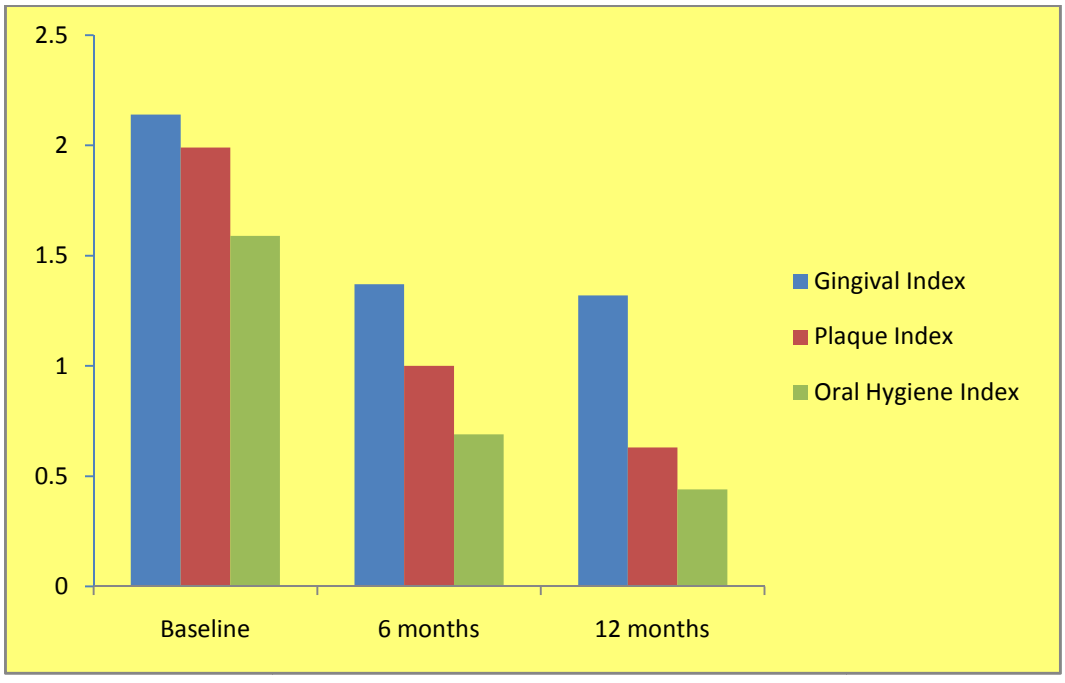
GRAPH - 1

IN-VITRO RELEASE OF SIMVASTATIN



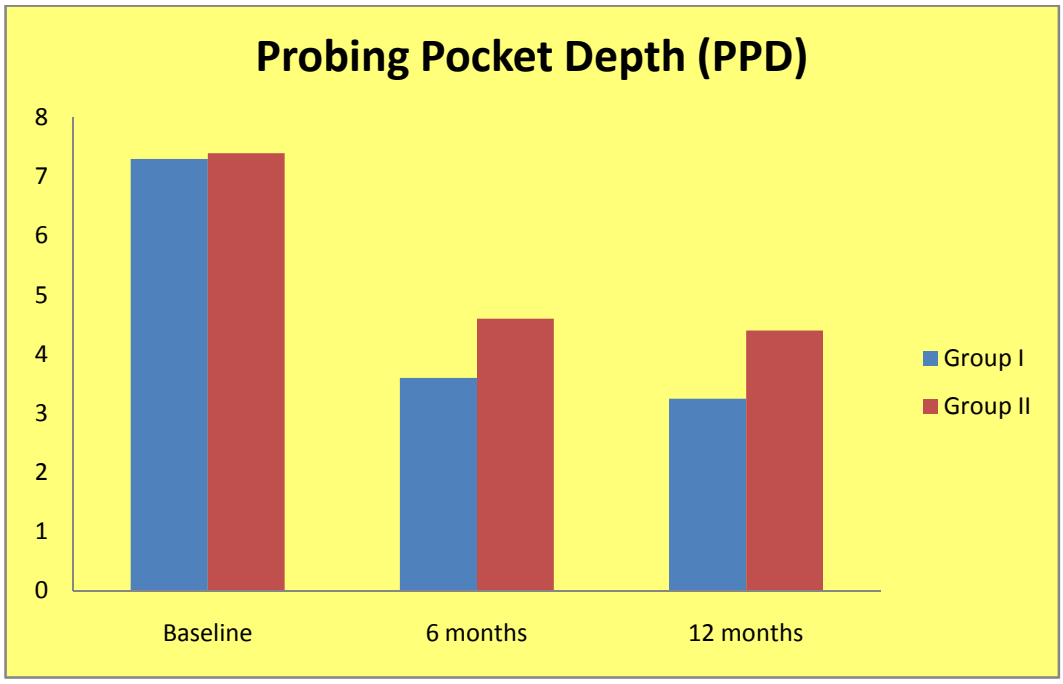
GRAPH - 2

COMPARISON OF MEAN GINGIVAL INDEX SCORES, PLAQUE INDEX SCORES, ORAL HYGIENE INDEX SCORES AT BASELINE, 6 MONTHS AND 12 MONTHS



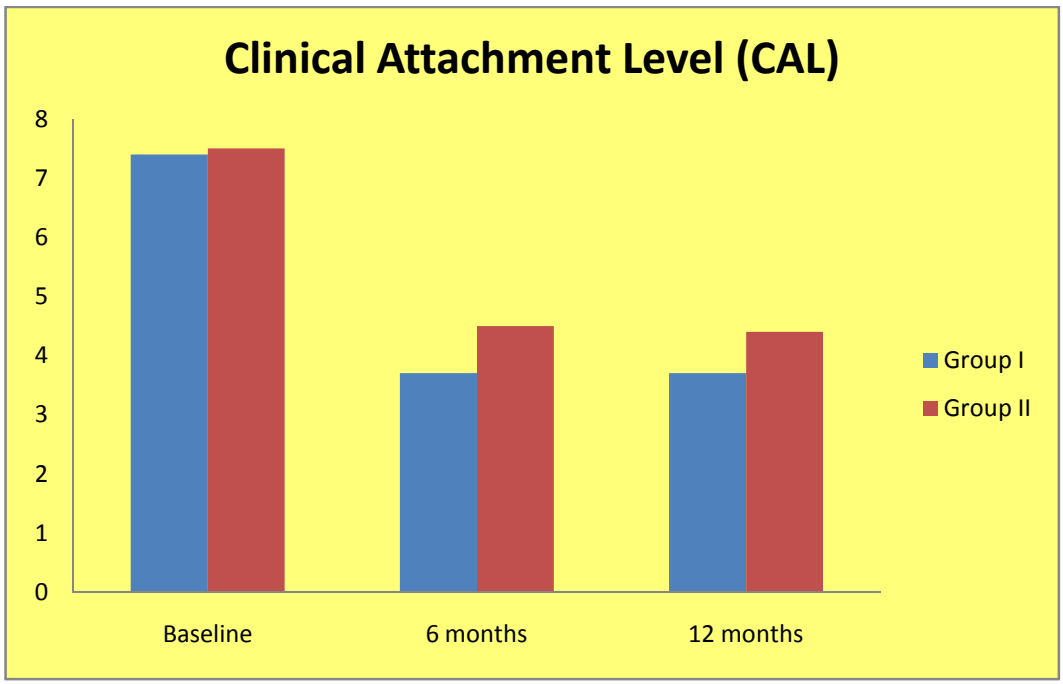
GRAPH - 3

INTER GROUP DIFFERENCE IN MEAN PROBING POCKET DEPTH (PPD) AT BASELINE 6 MONTHS AND 12 MONTHS



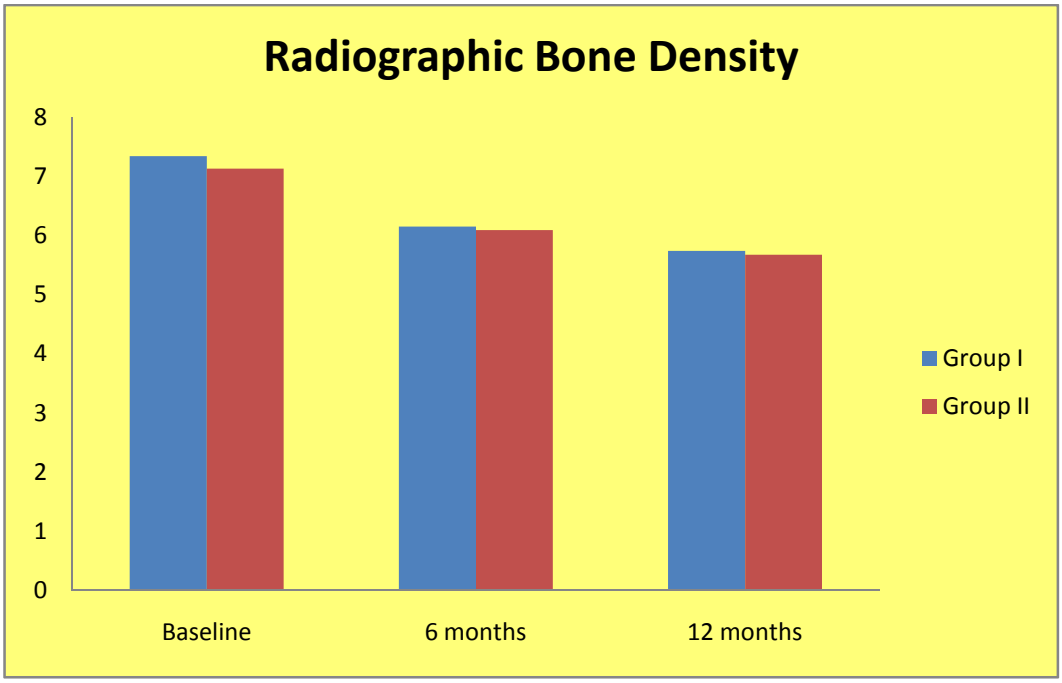
GRAPH - 4

INTER GROUP DIFFERENCE IN MEAN CLINICAL ATTACHMENT LEVEL (CAL) AT BASELINE, 6 MONTHS AND 12 MONTHS



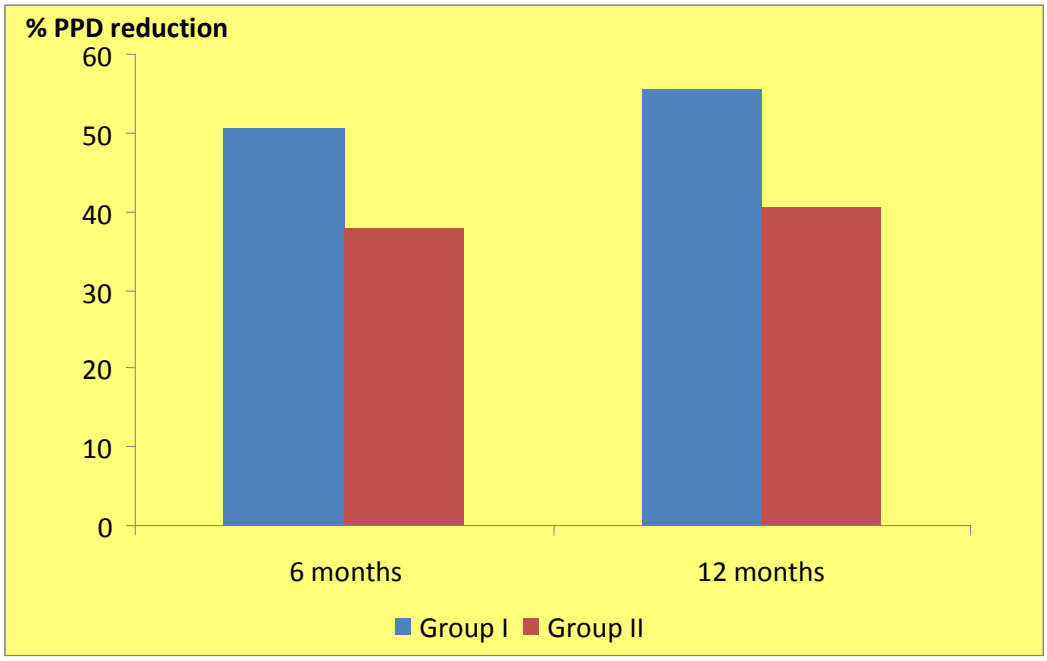
GRAPH - 5

INTER GROUP DIFFERENCE IN MEAN RADIOGRAPHIC BONE DENSITY AT BASELINE, 6 MONTHS AND 12 MONTHS



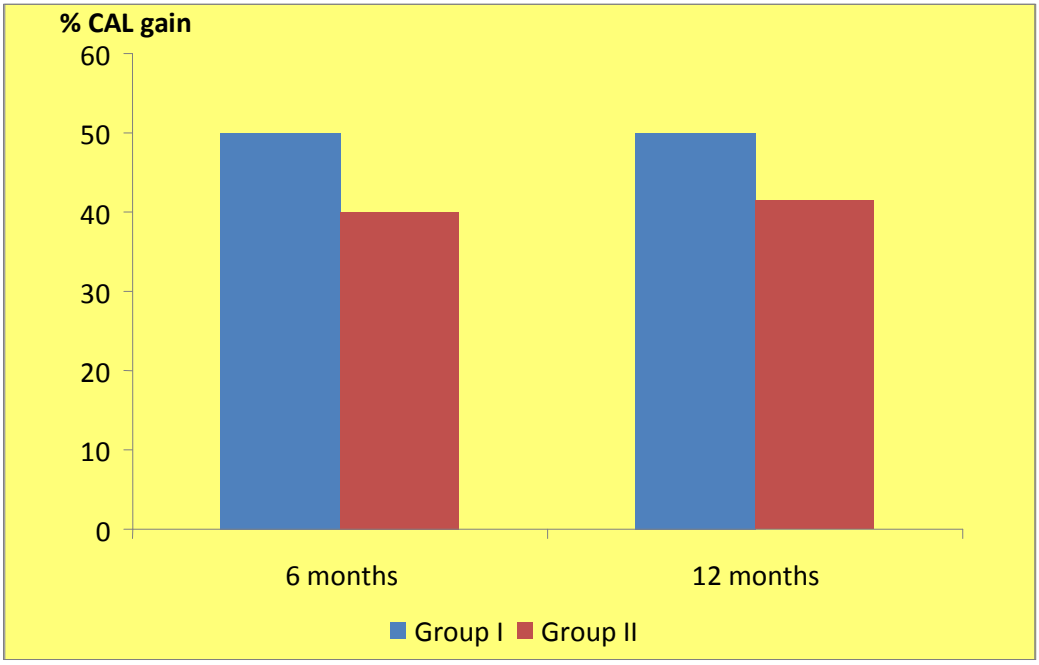
GRAPH – 6

INTER GROUP DIFFERENCE IN PERCENTAGE (%) OF PPD REDUCTION, AT BASELINE 6 MONTHS AND 12 MONTHS



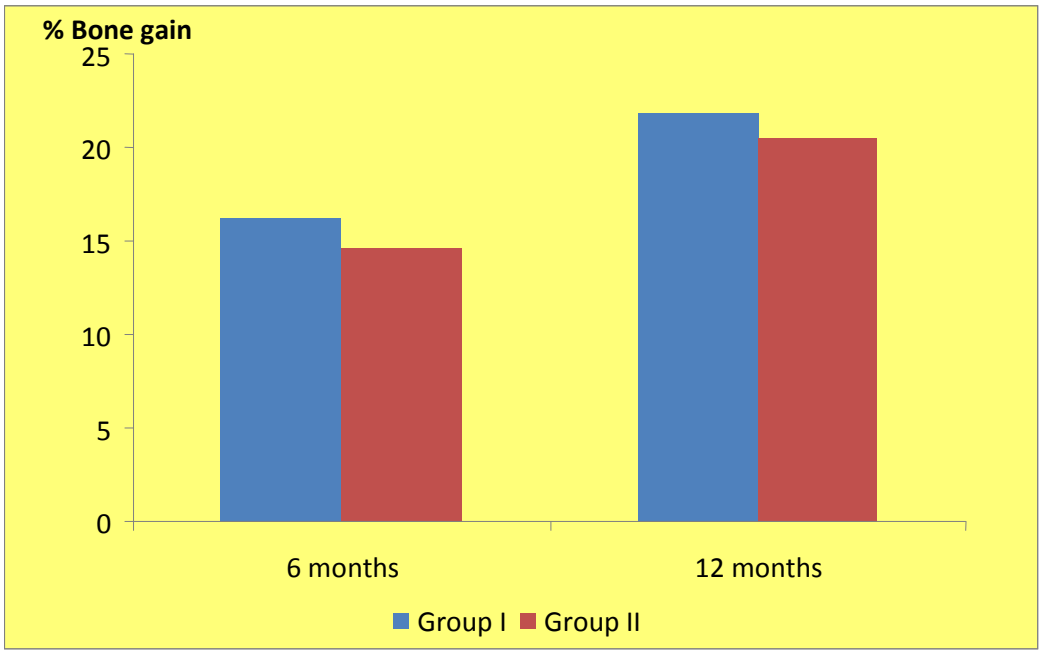
GRAPH – 7

INTER GROUP DIFFERENCE IN PERCENTAGE (%) OF CAL GAIN, AT BASELINE 6 MONTHS AND 12 MONTHS



GRAPH – 8

INTER GROUP DIFFERENCE IN PERCENTAGE (%) OF BONE FILL, AT BASELINE, 6 MONTHS AND 12 MONTHS



The ultimate goal of periodontal reconstructive therapy is to regenerate tissues destroyed by periodontal disease. Guided tissue regeneration offers the possibility of producing a new attachment on teeth which have advanced periodontal destruction. GTR utilizes barrier membranes to isolate the root surface from gingival epithelium and flap connective tissue. This method preferentially allows cells from the PDL and endosteum of bone to repopulate the defect and diseased root surface.

Several investigators have examined type I collagen as a possible membrane for use in GTR procedure. Collagen is absorbable, does not require a second surgical procedure for removal and also possess unique properties when compared with non – absorbable membrane. **Pitaru et al (1996)**[61] explained collagen is a major extracellular molecule of the periodontal connective tissue.

Pharmacological compounds such as statins, a cholesterol lowering drug acts by inhibiting HMG – Co A reductase enzyme. **Mundy G et al (1999)**[30] demonstrated that several statins, especially simvastatin stimulated bone formation when injected over the murine calvaria and also increased expression of BMP – 2 mRNA in osteoblasts. **Sakoda K et al (2006)**[42] demonstrated that simvastatin decreased IL-6 and IL-8 production by cultured human epithelial cell line in response to IL-1a.

In the present study 1.5 mg simvastatin concentration loaded in type I collagen membrane was compared with type I collagen membrane alone in the treatment of human intrabony periodontal defects.

In the present study the mean plaque index, oral hygiene index- simplified and gingival index at baseline was 1.99± 0.260, 1.59±0.074 and 2.14±0.386 respectively. All these values reduced to 1.00±0.211, 0.69±0.074 and1.37±0.346

respectively after 6 months. These values are further reduced to 0.63±0.226, 0.044±0.052 and 1.32±0.238 at 12 months post-operative period. These results concur with the studies done by **Trombelli et al (2010)**[62] who observed marked improvements in the clinical parameters in terms of plaque and gingival index after periodontal therapy. He explained that patients undergoing periodontal therapy will maintain optimal oral hygiene and their compliance led to the improvement in plaque index and gingival index scores.

The baseline mean probing pocket depth in group I was 7.30 ± 0.483 reduced to 3.60±0.516 and 3.25±0.483 at 6 and 12 months respectively. Similar results were observed by **Pradeep AR et al (2010)**[56] who reported simvastatin treated patients exhibited greater probing depth reduction when compared with the placebo group.

In group 2, the mean probing pocket depth reduced from 7.40± 0.516 to 4.6±0.516 at 6 months and 4.4 ± 0.516 at 12 months post-operative period. This is in accordance with **Bouchard P et al (1997)**[25] who showed absorbable PGA/PLA membrane exhibited greater probing pocket depth reduction compared with non-absorbable membranes at the end of 12 months.

In group 1the PPD reduction at the end of 12 months 53.48% and in group 2 it was 40.54%. The overall comparison between group1 and group 2 were statistically significant with p – value< 0.001. This is explained by **Dalacio R et al (2013)**[14] that simvastatin reduces the levels of pro inflammatory cytokines (IL -1 and TNF – α) as well as the neutrophil influx (MPOactivity) in the gingival tissues of rats subjected to periodontal disease. According to **Zhang X et al (2008)**[63] that

statins induce a shift from the production of pro-inflammatory cytokines (IL -2, IL -12, interferon – γ and TNF – α) to the production of T – helper 2 cytokines.

In this study both the study groups 1 and 2 resulted in significant attachment gain at the end of 12 months compared to the baseline. In group 1 the mean gain in CAL was 3.70 mm at the end of 12 months. This was in accordance with **Pradeep AR et al (2012)**[57] who reported simvastatin treated patients exhibited greater clinical attachment gain when compared with the placebo group.

In group 2 the mean gain in CAL was 3.00 mm at the end of 12 months. Similar results were observed by **Mattson SJ et al (1995)**[64] who reported improved clinical attachment level in 21 intrabony defects treated with type I collagen alone.

In group 1 CAL gain at the end of 12 months was 52.56% and in group 2 it was 41.33 %. On comparison between the groups at different time intervals (6 and 12months) group1 had greater CAL gain with a statistically significant p- value of >0.05. This is explained by **Rutledge J et al (2011)**[65] who described simvastatin decreases osteoclast numbers, enhance alkaline phosphatase activity mineralization, increase sialoprotein, osteocalcin, and vascular endothelial growth factors, which all together resulted in marked gain in clinical attachment level in simvastatin treated group.

In group 1 the depth of the defect at baseline was 7.34± 0.793 and the bone defect was 6.15 ± 0.622 the end of 6 months, which further reduced to 5.74±0.381 at the end of 12 months. In the present study the mean bone density area at the end of 12 month was 1.60mm^2. The results concur with previous studies of **Thylin MR et al (2002)**[50] who demonstrated that single dose of simvastatin application

stimulate bone apposition in murine calvaria. **Bradley JD et al (2008)[66]** reported that simvastatin injected onto rat mandibles in methyl cellulose gel stimulate BMP-2 production and bone formation at the site of injection.

In group 2, the depth of the defect at baseline was 7.13±0.868 and it was reduced to 6.09 ± 0.568 at 6 months and further reduced to 5.67±0.402 12 months post operatively. The mean bone density area was at 1.46 mm^2. Similar results were shown by **William Becker et al (1993)[67]** who reported a mean bone fill in the intrabony defects treated with ePTFE barrier membranes at the end of 12 months.

In the present study, group 1 resulted in 21.80 % of bone density area and in group 2 it was 20.48% at the end of 12 months. On comparison there was no statistical significance among the groups (p – value >0.05). This is explained by **Aukhil I et al (1986)[68]** that the limited extent of periodontal regeneration in the case of GTR therapy may be associated with the barrier placement, which creates two adjacent avascular surfaces (root surface and the barrier).

In group 1 there was 16.21% bone fill at 6 months and 21.80% at the end of 12 months post- operative period. This is in accordance with **Park JB et al (2009)[69]** who explained simvastatin induce bone growth by stimulating BMP – 2 induced osteoblast differentiation through antagonizing TNF – α to Ras/p/ mitogen activated protein kinase and augmenting Smad signaling pathways. **Barter MJ et al (2010)[70]** showed that simvastatin significantly inhibited IL-1 plus oncostatin M (OSM)–induced collagen degradation; this outcome was accompanied by a marked decrease in collagenase and gelatinase activity. Statins also significantly decreased MMP-1 and MMP-13 expression in human articular cartilage and chondrocytes stimulated

with IL-1 plus OSM and blocked the activation of critical proinflammatory signaling pathways required for MMP expression. **Dalacio R et al (2013)[14]** showed that simvastatin markedly reduced the expression of RANK and RANKL expression. Simvastatin was also observed to increase OPG expression in periodontal tissue.

Thus the regeneration of the periodontal attachment apparatus in the present study had a favorable clinical and radiological outcome in both the study groups. Among the groups compared group 1 had greater clinical and radiological parameters when compared with group 2 at 6 and 12 months post operatively.

The present study was involved a comparative clinical and radiographic evaluation of regenerative osseous surgery with simvastatin loaded collagen membrane and collagen membrane alone in intrabony defects. The study population comprised of 10 patients and all the patients returned for maintenance visits. A total of 20 intrabony defects were treated and post-operative healing in the treated areas was satisfactory. The following clinical parameters like plaque index, gingival index, oral hygiene index- simplified, probing pocket depth and clinical attachment level were assessed at baseline, 6 months and 12 months. Hard tissue evaluation was made by RVG.

Within the frame work of this study, the following conclusions have been elucidated:-

1. Both Simvastatin and collagen membrane yielded favorable clinical results in periodontal intrabony defects.

2. Probing pocket depth and gain in attachment level were significant in both the groups when compared to pre-operative level. But probing pocket depth and clinical attachment level gain was greater in simvastatin with a statistically significant p- value of <0.001 than the collagen membrane alone at 6 and 12 months post operatively.

3. Both the groups exhibited significant amount of bone fill than the pre-operative levels and the mean bone fill was higher in simvastatin treated group. But there is no statistical significance in between the two groups at 6 and 12months post-operative period.

The results clearly indicate that simvastatin has the potential to promote predictable periodontal regeneration in the treatment of intra osseous defects. It also indicates that simvastatin showed greater probing pocket depth reduction, clinical attachment level gain and radiographic bone fill when compared with collagen membrane alone.

However, limitations of this study include a small sample size and bone fill was estimated in terms of bone density area which still needs justification. In order to evaluate the regenerative potential of the bone substitutes, a study design of larger sample size with 2 years or longer follow up is needed. Surgical reentry and immuno histological assay of treated sites would provide accurate data.

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