The Influence of Citric Acid Bone Surface Etching and Bone Substitute Intra-Osseous Defect Fill on Crevicular Fluid BMP-2 Release Profile

(Randomized Clinical Trial)

A Thesis

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LIST OF ABBREVIATIONS

AA	Amino Acids
AAP	American Academy of Periodontology
ALP	Alkaline Phosphatase
BMP	Bone Morphogenic Protein
CAL	Clinical Attachment Level
CDMP	Cartilage-Derived Morphogenetic Protein
CEJ	Cemento Enamel Junction
DFDBA	Demineralized Freeze-Dried Bone Allograft
EDS	Energy Dispersive Spectrometry
EDTA	Ethylene Diamine Tetra-acetic Acid
EMD	Enamel Matrix Derivative
ePTFE	Expanded Polytetrafluorethylene
FDBA	Freeze-Dried Bone Allograft
FGF	Fibroblast Growth Factor
GBR	Guided Bone Regeneration
GCF	Gingival Crevicular Fluid
GDF	Growth/Differentiation Factor
GI	Gingival Index
GTR	Guided Tissue Regeneration
HA	Hydroxyapatite
IGF	Insulin like Growth Factor
NSAID	Non-Steroidal Anti-Inflammatory
OP-1	Osteogenic Protein 1
PD	Probing Depth
PGA	Poly-Glycolic Acid
PHEMA	Polyhydroxylethylmethacrylate
PI	Plaque Index
PLA	Poly-lactic Acid
PMMA	Polymethylmethacrylate
PRF	Platelet-Rich Fibrin
PRP	Platelet-Rich Plasma
Rh-BmP-2	Recombinant Human Bone Morphogenic Protein 2
SEM	Scanning Electron Microscope
TCP	Tricalcium Phosphate
TGF-b	Transforming Growth Factor Beta

REVIEW OF LITERATURE

Periodontitis is defined as loss of periodontal attachment due to microbially associated host mediated inflammation. This would lead to the activation of host derived proteinases that results in the destruction of the marginal periodontal ligament fibers as well as the apical migration of the junctional epithelium allowing the bacterial biofilm to undergo apical widespread along the root surfaces of teeth. **(Tonetti et al 2018).**

Recently, Periodontitis has been classified by **Caton et al** based on a multi-dimensional staging and grading system where staging depends on initial severity of the periodontal disease on presentation and complexity in its management while grading depends on the rate of progression of the periodontal disease as well as the response to anticipated treatment. Staging includes four categories based on the amount of clinical attachment loss, probing depth, amount of bone loss, present of vertical infra-bony defects, presence of furcation involvement, tooth mobility and lost teeth due to periodontitis. Grading involves three grades based on the rate of progression of the disease, general health status and other exposure factors such as smoking **(Caton et al 2018).**

Cross-sectional epidemiological studies found that advanced periodontitis affected 10-15% of the adult population and that moderate periodontitis affected 80% of the population while 5-10% were periodontally healthy **(Heitz-Mayfield et al 2002). Genco & Borgnakke** reported that there are risk factors which increased the susceptibility of individuals to periodontitis. Among these risk factors are genetics, race, smoking, medical condition and stress **(Genco & Borgnakke 2013)**

Treatment of chronic periodontitis includes controlling the inflammatory state that is associated with it by non-surgical mechanical instrumentation in order to remove dental plaque and calculus and disrupt the plaque biofilm. Successful periodontal treatment is evident when there is reduction in pocket depth, maintenance or gain in the clinical attachment level and decrease in bleeding on probing **(Heitz-Mayfield et al 2002).**

One of the common features that occurs in chronic periodontitis is the destruction of the supporting structure of teeth. This includes the cementum, the periodontal ligament and the alveolar bone. Alveolar bone destruction may lead to formation of either horizontal defects or vertical defects according to the extent and location of subgingival plaque **(Papapanou & Tonetti 2000)**.

Papapanou & Wennstrom reported higher risk of periodontal disease progression and tooth loss with vertical bony defects. Vertical defect could be defined radiographically whenever the distance between the top of the alveolar crest and the most apical part of the alveolar crest is more than or equal to 2.0 mm **(Papapanou & Wennstrom 1988).**

Infra-bony pockets were classified by **Goldman and Cohen** based on the number of remaining osseous walls into three walls, two walls and one wall infra-bony pockets. **(Lindh et al 2008)**

Three walls infra-bony pockets are defects that have one osseous wall missing. The missing osseous wall could be either the buccal, lingual, mesial or distal wall. Two walls infra-bony pockets are defects that have two osseous walls missing. The missing osseous walls could be buccal and proximal wall or lingual and proximal walls, in these cases there is a curtain

of soft tissue covering the destructed osseous walls. Two walls infra-bony pockets could also occur where the missing osseous walls are the proximal walls while the buccal and lingual walls are intact, in this case it is called intraosseous interproximal crater. An interproximal crater is a cup or bowl shaped defect that affects two adjacent root surfaces in a similar degree which is attributed to the spread of periodontitis simultaneously on both roots. One wall infra-bony pockets are defects that have only one osseous wall remaining while the other osseous walls are destroyed. The most common osseous wall remaining is usually one of the proximal walls while other walls are destroyed. **(Lindh et al 2008)**

Infra-bony pockets usually do not occur as one definite form but rather as a combination of different forms, where the apical part of the pocket could have more walls than the coronal part. In order to successfully treat infra-bony pockets, the etiology should be taken into consideration e.g. The tooth anatomy and position relative to the alveolar housing as well as occlusal forces. Any attempt for treatment without elimination of the causative factors will usually lead to failure on the long term. **(Lindh et al 2008)**

Currently the aim of Infra-bony pockets treatment is to regenerate the lost part of alveolar bone. Regeneration is defined by the American academy of periodontology as the reproduction or reconstitution of a lost or injured part to restore the architecture and function of the periodontium. In order for a material or technique to be considered as a regenerative modality, it has to show histologically the formation of bone, cementum and a functional periodontal ligament. **(American Academy of Periodontology 2001)**

Although the primary objective of periodontal therapy is to achieve complete regeneration, it is hard to be achieved in numerous situations due to

complex biological events, factors, cells, and mediators involved in the healing process which makes periodontal regeneration not constantly predictable. **(Wang et al 2005)**.

Healing of the periodontium is considered more complicated than other types of tissues in the body, this is attributed to the need of different types of cells for periodontal ligament, alveolar bone and cementum formation. Also the presence of avascular root surface which not only reduces the blood supply in the area but is sometimes contaminated with bacteria and their toxins that can reduce the healing potential. **(Alpiste et al 2006).**

Kornman and Robertson reported that the treatment outcome of infra-bony defects after using periodontal regenerative materials was variable and several factors affected it. Among these factors are patient related factors such as Age, genetics, medical condition and patient's ability to maintain plaque control during the healing phase after surgery. Also site characteristic factors such as defect architecture, teeth anatomy and occlusion, as well as factors regarding the surgical protocol such as operator skill, surgical approach and the regenerative material used. (**Kornman & Robertson 1985).**

Regarding the defect architecture, **Klein et al** found that favorable healing was related to the depth and the angle of the defect as well as the amount of the remaining residual bony walls. Defect angulation could be determined radiographically by measuring the angle between a line drawn from the CEJ of the affected tooth to the base of the defect and another line drawn from the base of the defect to the alveolar bone crest, **Klein et al** also stated that narrow defects less than 26° showed increased clinical attachment level gain after six and twenty four months follow-up periods when compared to wider defects that were treated by GTR using expanded poly-tetrafluroetheylene (ePTFE) **(Klein et al 2001).**

Another study by **Eickholz et al** also reported that narrow defects less than 37° showed better clinical attachment level gain compared to wider defects after twenty four months follow-up period when treated by GTR using non-resorbable and bioabsorbable barrier membranes **(Eickholz et al 2004)**.

Polimeni et al stated that deep narrow infra-bony defects showed better healing potential than wide shallow infra-bony defects, also three walls infra-bony defects tended to show better regeneration compared to two walls and one wall infra-bony defects. This was attributed to the abundance of blood supply and cells in the environment surrounding the defect area in case of deep, narrow and three walls defects compared to shallow, wide and two or one wall defects **(Polimeni et al 2006).** The defect site and morphology was found to be influenced by the anatomy of the alveolar process as well as the occlusal forces acting on the teeth. **(Manson & Nicholson 1974).**

Various regenerative materials have been used for the treatment of infra-bony defects, these include barrier membranes, bone grafts, enamel matrix derivative (EMD), and growth factors **(Darby I 2011)**.

Barrier membranes are materials that has been used to treat infra-bony defects through guided tissue regeneration (GTR) where it prevents epithelial and fibroblast migration into the defect and provides space that allows for periodontal regeneration **(Gottlow 1993).**

Barrier membranes should be biocompatible, it should provide and maintain space to permit tissue regeneration, and it should also be easily handled, trimmed and placed. Barrier membranes can be classified into three generations. First generation membranes are non resorbable membranes that were used early for periodontal regeneration. These include cellulose acetate (Millipore), expanded polytetrafluoroethylene (e-PTFE), titanium reinforced

ePTFE, high-density-PTFE. The main problem with the first generation is the need for second surgery in order to remove the membrane. Second generation membranes are resorbable membranes which could be natural such as collagen or synthetic which are made from polyesters such as poly-glycolic acid (PGA), poly-lactic acid (PLA) and their copolymers. Problems of resorbable membranes include the difficulty to control degradation rate and possible tissue interaction with degradation products of the membrane. Third generation membranes are membranes that act as barrier and as a delivery vehicle for local agents such as antibiotics and growth factors. **(Sam & Pillai 2014)**

Growth factors have also been used for periodontal regeneration. These are signaling molecules which have the ability to stimulate cellular proliferation, migration, differentiation and matrix formation. Growth factors could be either autologous or synthetic (**Darby 2011**). Autologous growth factors are found in platelet concentrates which can be obtained from the patient's own blood and then extracted through centrifugation. Centrifugation causes the separation of blood elements into red blood cells which is discarded and other elements which can be used for regeneration which consists of different growth factors that are released from platelets and leukocytes **(Ehrenfest et al 2014)**

Platelet-rich plasma (PRP) is considered the first generation of platelet concentrates. Problems with PRP was the lack of standardized method for preparation, the use of bovine thrombin which could cause significant immunologic response and the use of anticoagulants which increased the steps needed for PRP preparation. This led to the second generation Platelet-rich fibrin (PRF) which had a simplified standardized method of preparation with no need for anticoagulant or bovine thrombin usage. (**Qiao et al 2016**)

Synthetic growth factors are polypeptides that are made using recombinant technology. Among these growth factors are Bone morphogenic proteins (BMPs), Platelet derived growth factors (PDGFs) insulin like growth factor (IGF), transforming growth factor beta (TGF-b) and fibroblast growth factor (FGF). The Problem with growth factors usage for periodontal regeneration is that it lasts for short duration before degradation that it may not be available when it is most needed. (**Darby 2011**)

Another material that has been used for periodontal regeneration is Enamel matrix derivative (EMD). EMD is a porcine derived material that consists of proteins mainly Amelogenin which forms about 90% of the matrix proteins. **(Esposito et al 2009*)*** It was found that during tooth development the epithelial root sheath of Hertwig produced enamel related matrix proteins which aided in the formation of acellular cementum. It was also postulated that these proteins could also aid in the periodontal and alveolar bone development **(Heijl et al 1997).** Histologic studies showed that EMD was able to induce the formation of cementum, bone and periodontal ligaments It was also shown that EMD had significant gain in the clinical attachment level compared to open flap debridement. (**Koop et al 2012**).

Bone grafts are materials that have been widely used with infra bony defects for periodontal regeneration **(Brunsvold & Mellonig 1993)**. An ideal bone graft material should be biocompatible, it should not cause any toxic or allergic reactions or cause any disease transmission, it should have resorption rate equivalent to that of human bone, and it should maintain space and be easily handled **(Darby 2011)**.

Bone grafts are classified according to their mechanism of action into osteogenic, osteoinductive and osteoconductive. Osteogensis is the ability to form new bone by undifferentiated cells found within the graft itself.

Osteoinduction is the ability to induce undifferentiated mesenchymal cells or osteoprogenitor cells found in the surrounding environment to develop into osteoblasts and form new bone. Osteoconduction is the ability to provide a stable framework that can support osteoprogenitor cells and osteoblasts in the surrounding enviroment in order to allow bone apposition **(Laurencin et al 2006).** Bone grafts are classified into four types according to their origin which are Autogenous, Allografts, Alloplasts and Xenografts **(Wang et al 2005).**

Autogenous Bone are bone grafts which are harvested from the patient where it is taken from one site and transplanted into another site**. (Brunsvold & Mellonig 1993)**. Autogenous bone is considered to be the gold standard material for bone regenerative procedures as it is the only type of graft that can retain the cell viability and does not provoke any immunological response when used. Compared to other bone substitutes Autogenous bone graft was found to be the only one to have osteoconductive, osteoinductive, and osteogenic potential **(Pandit N & Pandit IK 2016)**.

Autogenous bone graft can be harvested from intraoral sites e.g. mandibular symphysis, mandibular ramus, maxillary tuberosity, tori and edentulous areas or from extra-oral sites e.g. iliac crest, calvaria and tibia. Many instruments can be used to harvest autogenous bone such as bone chisels, rotary instruments, bone scrapers or by using Piezo-electric devices. The type of bone harvested could either be cortical, cancellous or corticocancellous. Cortical bone provides structural support and acts mainly as an osteoconductive material with minimal osteogenic and osteoinductive potential. This is due to the high density of the bone that results in slow revascularization and resorption of the graft. Cancellous bone is considered more osteogenic and osteoinductive than cortical bone as they have higher surface area than cortical bone which is attributed to its high porosity. Corticocancellous bone has the advantages of both cortical bone and