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Root surface biomodification

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قَالُوا۟ سُبْحَـٰنَكَ لَا عِلْمَ لَنَآ إِلَّا مَا عَلَّمْتَنَآ ۖ إِنَّكَ

أَنتَ ٱلْعَلِيمُ ٱلْحَكِيمُ ﴿٣٢﴾

سُورَةُ البَقَرَةِ

Certification of the Supervisor

I certify that this project " **Root surface biomodification**" was prepared by "**Ameer Nafie** " under my supervision at the College of Dentistry/University of Baghdad in partial fulfillment of the graduation requirements for the Bachelor Degree in Dentistry.

Supervisor: Assist. lecturer Mohamed Saeed

Dedication

To my father, my mother, I could never done this without your faith, support, and constant encouragement .

Thank you for teaching me to believe in myself, in God, and in my dreams.

To my supervisor Dr. Mohamed who believed in my abilities and was always there for me whenever I needed.

To all people who supported and encourage family, friends, teachers and colleague.

Many thanks to all of you.

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Introduction

To achieve new attachment on the diseased root surfaces affected by periodontal disease it is necessary to eliminate calculus, bacterial plaque and other cytotoxic substances on or within the root surface. Scaling and root planing are the most widely used techniques in periodontal therapy. Scaling is instrumentation to remove dental plaque and dental calculus from the surface of a tooth apical to the gingival margin accumulated in periodontal pockets, or from the surface coronal to the gingival margin. While root planing is a technique of instrumentation by which the softened cementum is removed and the root surface is made hard and smooth. This is usually done by hand instrumentation using sharpened blades **(Eaton and ower, 2015).**

The search for the appropriate root coverage technique has taken many different approaches. Various surgical options have been developed to achieve the above goals and include the use of subepithelial connective tissue grafts (SCTG),free gingival grafts laterally sliding flaps ,coronally advanced flaps ,double papillae flaps guided tissue regeneration, and acellular dermal matrix allografts **(Mlachkova and popova, 2014).**

Among these surgical options, variations of SCTG procedures demonstrated a high percentage of root coverage with high predictability and without significant postsurgical complications.

Also, the root coverage gained with SCTG procedures was reported to be stable over the long term.

So far, a concerted effort has been made in the field of root conditioning to improve the outcome of regenerative periodontal therapies by favoring the attachment of the

regenerated periodontal structures. Mechanical instrumentation (scaling and root planing) leaves a smear layer **(Alparslan, 2010) .**

The formation of a smear layer consisting of dentinal debris and some bacteria occurs after scaling and root planing

The removal of this smear layer assumes clinical importance due to the fact that the smear layer prevents attachment of connective tissue to the root surface **(Theodoro LH et al, 2010)** .

Therefore, chemical conditioning of the roots has been performed to remove the smear layer and improve biocompatibility. After smear-layer removal, the dentinal collagens are exposed, and they are supposed to be a chemoattractant for periodontal fibroblasts. Besides these surgical options, various adjunctive agents have been applied to promote healing and further enhance clinical outcomes. These include root conditioners (e.g., citric acid, tetracycline HCI, EDTA) phosphoric acid, and hydrogen peroxide enamel matrix proteins, recombinant human growth factors, platelet-rich plasma, and dentin bonding conditioner.

In addition to chemical conditioning the applicability of different laser systems, such as the CO,Nd:YAG diode, and Er:YAG laser in the removal of the smear layer has been demonstrated **(Alparslan, 2010)** .

Chapter one : Review of literature

1.1 Root Surface Changes in Periodontal Disease

Periodontitis produces substantial changes on the root surface, and the root is commonly referred to as "Pathologically Exposed." The normal root is rich in collagen, with extrinsic and intrinsic fibers that form a renewable connection to the adjacent alveolar bone. Plaque- induced inflammation destroys these Sharpey's fibers allowing down growth of junctional and pocket epithelium. Thus, the root surface becomes exposed to the periodontal pocket and oral environment With loss of collagen the root surface becomes
'Hyper mineralized'. The bacterial plaque and calculus penetrate the cementum and or dentin of the root. The exposed root surface, as a result of periodontitis, will undergo substantial alterations and may no longer serve as an appropriate substrate for cell attachment and fiber development. These alterations include :

- Loss of collagen fiber insertion
- Contamination of the root surface by bacteria and or endotoxins
- Alterations in mineral density and composition.
- Also the pathologically exposed root surface may lack the necessary chemotactic stimuli for migration of cells capable of producing periodontal regeneration **(Akilesh H, 2016) .**

The apical migration of the junctional epithelium along the root surface over the connective tissue following surgical therapy appears to preclude regeneration by acting as a physical barrier between the gingival connective tissue and the root surfaces.

1.2 Definition of Root Surface Biomodification

Root biomodification refers to procedures which are done to detoxify, decontaminate and demineralize the root surface, thereby removing the smear layer and exposing the collagenous matrix of dentin and cementum . The oldest and the most conventional methods include scaling and root planing which are primarily aimed at the gross removal of microbes from the root surface **(Vamsi Lavu et al.,2015)** .

Rationale for Root Bio-modification

The factors influencing successful periodontal therapeutic outcomes (regeneration) include :

1. Clot stability.
2. Cell migration towards the root surface.
3. Cell attachment.
4. Cell proliferation and differentiation **(vamsi lavu et al, 2015).**

1.3 Classification of root surface bio-modification agents

Various chemicals have been used on the root surface to remove the smear layer, thus promoting healing and further enhancing clinical outcomes. Along with this, application of growth factors to the root surface to enhance regeneration has also been the focus of research **(Nitin Saroch , 2020)** .

The root surface bio-modification agents are broadly classified into following categories **(chauhan G& parkash S, 2016) .**

1. Root surface conditioners**:**

Citric acid.

Tetracycline HCI.

EDTA.

Fibronectin.

Laminin.

Doxycycline.

Minocycline.

Polyacrylic acid.

Chlorhexidine.

Bile salts and plasma fractions.

2. Dentin bonding conditioners.

3. Enamel matrix proteins.

4. Platelet-rich plasma.

5. Hyaluronic Acid (HA)

6. Recombinant human growth factors

7. Plasma rich fibrin

8. Lasers

1.3.1 Root surface conditioners

1) Citric Acid:

Root surface demineralization with citric acid has been suggested to be used as a part of regenerative procedures because of the ability of citric acid to modify the root surface **(A.L. Dumitrescu, 2011)** .

The actions of citric acid that have been reported are as follows:

1.It removes the smear layer and may open dentinal tubules, thus allowing cementum to form within these tubules creating the blunderbuss effect and produce cementum pins. This could be associated with accelerated cementogenesis

2. It has also been shown to expose collagen fibers on the root surface, which may splice with the collagen fibers of a soft tissue graft or flap (called collagen splicing), resulting in collagen adhesion without cementum formation and accelerated healing.

3. Epithelium does not migrate apically because of the accelerated healing either by connective tissue attachment or a collagen adhesion may occur before epithelium migrates.

4.Finally, citric acid may demineralize small bits of residual calculus, disinfect the root surface and aid in removing endotoxins **(Shantipriya Reddy, 2018)** .

Technique for Application:

Topical application on root surface for 2–3 minutes after scaling and root planing **(Rameshwari.2017)**.

Disadvantages:

The major disadvantage associated with citric acid is induction of acidic environment around which can lead to tissue necrosis or delay in wound healing thus delaying regeneration. Low pH of the acid has been known to cause cell death when it comes in contact with periodontal cells **(Bhushan K et al,.2016)**

Fig1: Root biomodification with citric acid Ph 1.0 for 3 minutes.

Figure 2: Citric acid 30 ml syringe.

2) Tetracycline HCl:

Tetracyclines are a group of bacteriostatic antimicrobials effective against a wide range of microorganisms. The unique property of drugs of this group is their ability to modulate the host response. This anti-microbial group of drugs has been shown to have matrix metalloproteinase inhibitory and anti-inflammatory properties. Along with this, tetracycline hydrochloride inhibits microbial attachment and has root surface conditioning properties . It has been demonstrated that tetracycline conditioning of the root surfaces not only removes the surface smear layer, but also inhibits collagenase activity and bone resorption . In a comparative study, the effect of tetracycline root conditioning and flap surgery was compared with flap surgery alone. The histological analysis revealed 0.27mm of average increase in connective tissue attachment and also cementogenesis was seen in tetracycline treated sites Thus, in brief.

the properties of tetracycline hydrochloride, which make it a suitable root surface bio-modification agent are,

1.It enhances attachment and growth of gingival fibroblasts, thus facilitating regeneration.

2.It has anti-collagenase activity.

3.It has anti-inflammatory properties.

4.It has high substantively.

5.It inhibits parathyroid hormone-induced bone resorption **(Nitin Saroch, 2020) .**

Effect of citric acid and tetracycline hydrochloride on fibroblast Behavior :

Studies have demonstrated that they can also influence fibroblast behavior thereby improving their attachment and migration on the root surface. The proposed mechanisms by which these influence fibroblast behavior are **(Bhushan K1 et al,2016) .**

• Induced cementogenesis

• Collagen splicing

• Fibronectin fibrin-collagen binding thereby inhibiting epithelial apical migration

• Enhanced fibroblast chemotaxis, migration and attachment

Figure3: Root biomodification with Tetracycline HCL

Figure4 : Tetracycline fibers.

Application:

Ideal concentration for their usage is 0.5% solution at a pH of 3.2 and the application time is generally 2-3 mins as above that they have been proved to cause impaired periodontal healing **(Penmatsa T et al ,.2013) .**

Disadvantages:

The only disadvantages associated with the use of tetracycline is that sometimes the patient may elicit allergic reactions to their topical application.

3) Ethylenediaminetetraacetic acid (EDTA):

Use of acidic agents to demineralize the root surface had a drawback of adversely affecting the surrounding tissues. So, a chemical agent that could remove the smear layer and demineralize the tooth surface at neutral pH was required. EDTA is a

chelating agent which is widely used during endodontic treatment. It exerts its demineralizing effect through chelating divalent cations at neutral pH**) (Nitin Saroch.2020).**

Application:

The concentration of EDTA that is used ranges from 12%-24% for 30s to 3mins**(Rezende ML et al ,.2011) .**

Disadvantage

No disadvantage has been seen with the use of EDTA. However, it has been noted that they

lack the ability of initial clot stabilization and adhesion. As it is the first step in initiation of periodontal healing. Lack of this step may lead to a delayed healing process. Limited regeneration has been seen with its use alone **(Preeja C et al ,.2013)**

Fig5: Application of 24% of EDTA to root surface

Figure 6: EDTA syringe 24%

4) Fibronectin:

Fibronectin is a high molecular weight extracellular matrix glycoprotein with a molecular weight of approximately 440 KDa. This glycoprotein exists in two main forms:

1. as an insoluble glycoprotein dimer that serves as a linker in the ECM (extracellular matrix) .
2. as a soluble disulphide linked dimer found in the plasma (plasma FN) **(Nitin Saroch .2020)** .

Action:

1. Helps in tissue repair, embryogenesis, blood clotting, and cell migration/adhesion **(K.REKHA RANI &PMVIDS.2021)**
2. Stabilizes the clot between exposed root and new fibers .
3. Chemo attractant for fibroblasts .

4. Aids in adherence of fibroblast cells on the root surface **(Rameshwari, 2017) .**

Application:

The optimum concentration of usage is 0.38 mg/ml saline

(mirnalini &nintin.2020).

5) Laminin:

It is the most abundant component of the basement membrane. It plays an important role in directing the different cells. It is also a glycoprotein of high molecular weight. Various actions have been seen that are promoted by laminin these include:

1. Stimulating cell adhesion

2. Cell growth

3. Cell differentiation

4. Cell migration

The mineralized surface is attractive for laminin however they favour epithelial growth contrast to which fibroblast attracts fibroblast and favour their attachment and they have added advantage of acting on their surface. This process is known as reciprocal utilization of biologic mediators. **(Lavu V et al .,2015) .**

Disadvantage:

As it acts as a chemoattractant for epithelial cells thus, they may lead to formation of long junctional epithelium rather than new attachment thus is not a favoured agent for root biomodification. **(Lavu V et al .,2015) .**

6) Doxycycline:

Doxycycline belongs to the tetracycline group of drugs. It is an effective antimicrobial agent against periodontal pathogens. Along with this, it has anti enzymatic properties. Topical application of doxycycline has shown a long-lasting substantively on periodontally diseased root surfaces. It has been demonstrated that the anti-bacterial effect of doxycycline persists on the conditioned root surface up to 14 days **(Nitin Saroch .2020) .**

Application of doxycycline (pH 2.2). Doxycycline HCl solution (100 mg/ml) was made by mixing doxycycline HCl powder (100 mg) in sterile water (1 ml) **(Gurparkash Singh Chahal& Kamalpreet, 2014)**

7) Minocycline:

It is a semi-synthetic tetracycline having good bacteriostatic potential. Minocycline has a low pH in concentrated solution. It acts as a calcium chelator and its application results in enamel and root surface demineralization and removal of endotoxins invading untreated periodontally diseased roots. **(Nitin Saroch, 2020).** It possesses anti-collagenase activity and promotes fibroblast attachment to the root surface. Various studies done to evaluate the effects of minocycline on root surface when used as root surface bio-modification agent have demonstrated its efficacy comparable to other members of this group.

8) Polyacrylic acid:

It is considered as a weak acid and hence its use has been studied for its use as a root biomodification. Its mechanism of action is mostly that it removes the smear layer from the root surface thus increasing the healing capacity of the periodontium **(Mirnalini & nitin, 2020).**

9) Chlorhexidine

Chlorhexidine has a wide variety spectrum of activity encompassing gram negative and positive bacteria, viruses, yeast, fungi, dermatophytes. It shows different effects at different concentrations, at low concentration, bacteriostatic whereas at higher concentration is bactericidal. It has been one of the potent antiseptic agents that can be used as a chemical plaque control. It is effective against preventing biofilm formation. However, its use has not proved to be beneficial for root biomodification. It has the capacity to prevent smear layer formation. However, they do not show prolonged effect, so their use has been limited **(Mirnalini & nitin, 2020)** .

10) Bile salts and plasma fractions:

Bile salts such as sodium deoxy cholate and plasma fractions such as plasma fraction Cohn IV. They have the capability to dissociate endotoxin into subunits and thus were believed to have properties to detoxify the root surface **(Mirnalini & nitin, 2020) .**

1.3.2 Dentin bonding conditioners:

The dentin bonding conditioners have also been used for root surface biomodification. In one scanning electron microscope study, the surface morphology of roots treated with dentin bonding conditioner was compared to that of routinely used root surface demineralization acid and citric tetracycline agents, hydrochloride. The results of this study indicated morphological similarities between surfaces obtained with the dentin conditioning agent and other acidic materials that are used routinely in periodontal regenerative therapy There is relatively insufficient clinical research done on dentin bonding conditioners as root surface bio-modification agents and their regenerative potential. More comparative studies are required to investigate the behavior of fibroblasts towards the root surface treated with dentin bonding conditioners as compared to traditional root surface conditioners **(Nitin Saroch, 2020)** .

1.3.3 Enamel matrix protein (EMP)

Tooth development takes place through a series of reactions and various factors play an important role in this process. Mineralization is a key step in the tooth formation which is generally carried out by the organic matrix present. Enamel matrix proteins are generally involved in the formation of enamel, cementum, periodontal ligament and alveolar bone. It is generally composed of 90% Amelogenins and 10% Non-amelogenins. Non-amelogenins are composed of tuftelin, sheathlin, enamelin, enamel proteases, serum proteins and sulphated enamel proteins. Since, they are naturally present at the time of development, they provide

a biologic environment for the cells to migrate and function when used for root modification. Various actions of EMD are noted they include:

1. Increase in cell attachment
2. Increase in cell proliferation
3. Expression of various growth factors, cytokines, extracellular matrix components.
4. Increased bone and cementum forming cell differentiation
5. Accelerated wound healing
6. Antibacterial property

They have been proved to have increased influence on the periodontal fibroblast compared to gingival fibroblast and epithelial cells. Thus, they are one of the most successful agents that can cause regeneration. They are commercially available as Emdogain. Extracted from embryonal enamel of porcine origin **(Bhushan K et al,.2016)** .

Disadvantage:

The major disadvantage of using Emdogain is that it is not cost effective and its limited availability**. (Mirnalini & nitin, 2020)**

Figure 7: flap reflection

Figure 8: Application of EDTA gel for 2 min.

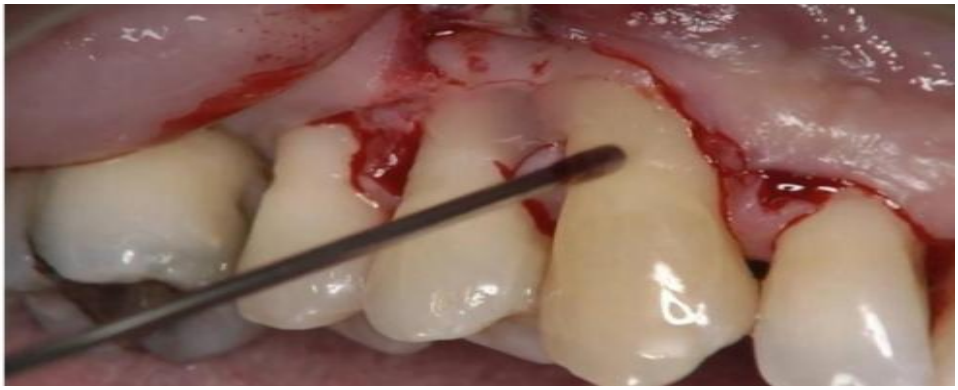

Figure 9: Application of Emdogain

Figure 10: Suturing

Figure 11 : Emdogain syringe

1.3.4 Platelet-rich plasma

Platelet-rich plasma has been used clinically in humans for its healing properties attributed to the increased concentrations of autologous growth factors and secretory proteins that may enhance the healing process on a cellular level **(Foster TE et al,.2009)**

The concept behind PRP application for periodontal regeneration is to obtain high density platelet concentrate from a patient's own blood and then applying this concentrate in the area of periodontal wound healing where regeneration is desired. **(Nitin Saroch, 2020)**

1.3.5 Hyaluronic acid (HA)

It is a natural component of extracellular matrix. It helps in maintaining the structural and functional integrity of the tissues. It has proved to have actions on cell adhesion, migration, and differentiation.

Various actions of hyaluronic acid have been seen these include:

1. Modulates host's inflammatory response and extracellular matrix cell infiltration
2. Prevent periodontal pathogen colonization
3. Induce the production of proinflammatory cytokines by fibroblasts, keratinocytes, cementoblasts and osteoblasts
4. Promotes cell proliferation, migration of matrix cells
5. Accelerates the bone regeneration

6. Low molecular weight HA has marked angiogenic effect. **(Mirnalini & nitin ,2020)**

Disadvantage:

They may sometimes show allergic reactions **(Mirnalini&nitin.2020)**

1.3.6 Recombinant human growth factors:

These are the biological mediators that bring about growth, proliferation, healing, cellular growth, and differentiation. In periodontal regeneration these factors bring about proliferation of fibroblast and favour bone formation. Growth factors determine the fate of the progenitor cells. **(Mirnalini & nitin, 2020)**

Various growth factors play important role in periodontal regeneration these include:

1. Platelet derived growth factors (PDGF)
2. Fibroblast growth factors (FGF)
3. Insulin like growth factor (IGF)
4. Transforming growth factors (TGF)
5. epidermal growth factor (EGF)

a) Platelet derived growth factors:

It is generally released at the time of blood clotting. It promotes chemotaxis and mutagenesis. It can reduce the inhibitory effects of lipopolysaccharides. It enhances demineralization. **(Mirnalini & nitin, 2020)**

b) Fibroblast growth factor:

These are potent factors that initiate mitosis, and promote the action of endothelial cells, fibroblast, osteoblast. They stimulate angiogenesis **(Mirnalini & nitin, 2020) .**

c) Insulin like growth factors:

These are the factors that attract PDL derived cells, stimulate mitosis and protein synthesis, stimulates bone formation and aids in type I collagen biosynthesis **(Mirnalini & nitin.2020).**

d) Transforming growth factor:

It has stimulatory as well as inhibitory action on cell. It has modulatory action on other growth factors as well PDGF, EGF and FGF. It down regulates epithelial cell proliferation and up regulates fibroblast differentiation. Stimulatory as well inhibitory actions have also been seen on osteoblast **(Suchetha A et al 2011).**

1.3.7 Plasma rich fibrin:

Platelet-Rich Fibrin (PRF) is a second-generation platelet concentrate that was introduced by Choukroun. It is thought that PRF can improve tissue regeneration due to its effects on vascularization, capturing the circulating stem cells, immune control, and closure of the epithelium **(Choukroun et al. 2006) .**

PRF has the capacity to conserve open wounds and improve healing due to its ability to attract epithelial cells and facilitate micro vascularization. **(Simonpieri A**

et al 2012) .

Thus, application of PRF (by itself or in combination with connective tissue) for root coverage has become increasingly popular for gingival recession treatments **(Eren G & Atilla G, 2013) .**

Injectable PRF (I-PRF) is the liquid form of PRF. I-PRF is a bioactive agent obtained by low-speed centrifugation, and it has the capacity to stimulate tissue regeneration. At high concentrations, PRF may stimulate the secretion of several growth factors and trigger fibroblast migration **(Miron RJ et al,.2017) .**

I-PRF is generally used in regenerative treatments, with good outcomes **(Mourão CF et al,.2015) .**

One of the components that make up I-PRF is fibronectin which is an extracellular glycoprotein with a high molecular weight (approximately 440 kDa) . Application of fibronectin to root surfaces improves cellular proliferation from the periodontal ligament towards the supracrestal parts . Fibronectin is used as an RSB agent in periodontal surgery **(Bhushan K et al ,.2016)** .

Surgical procedure:

Aseptic and antiseptic techniques were used for every subject. The surgical site was locally anesthetized using 20 mg/mL of lidocaine . The exposed root surfaces were smoothed with curettes .No material was applied to the exposed root surfaces of the patients in the control group. In the experiment group, 20 cc of blood was drawn from each subject, and the samples were centrifuged (for 3 min) to obtain IPRF. I-PRF was applied to the root surfaces for 5 min The exposed root surface was prepared with a no. 15 sharp curette. The enamel-cement border was horizontally incised in the mesial and distal directions which was followed by

vertical incisions to form trapezoidal flaps. The partial thickness flap was elevated and removed with surgical scissors. It was ensured that the periosteum was intact and at least 3–5 mm away from the apex of the exposed root surface **(Simonpieri A et al 2012) .**

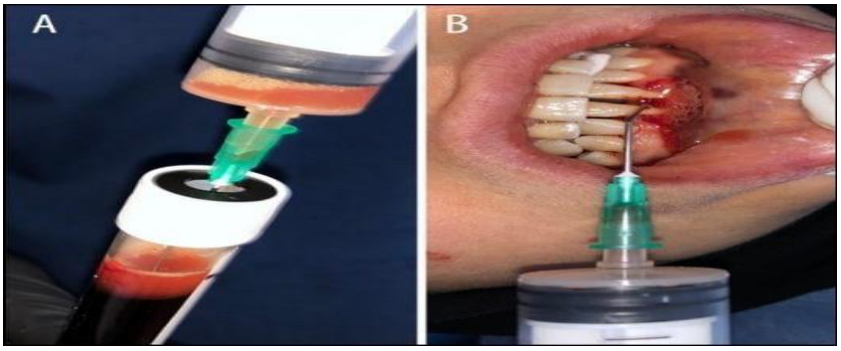

Figure 12: A) PRF obtained. B) Application of I-PRF to the root surface through an injector.

Figure 13: Suturing of the free gingival graft to the recipient area with the ePTFE suture.

Figure 14: The appearance of the gingiva after the sutures are removed 10 days after the operation.

1.3.8 Laser:

Chromophores are defined as the substance that can absorb the radiation of specific wavelength. Water and hydroxyapatite are the molecules that act as chromophores and thus therapeutic advantages are only seen in three types of laser that is Erbium (Er: YAG, Er: YSGG), CO2, Holmium:YAG laser. The incident beam of laser induces microexplosions due to rapid thermal expansion caused by incident laser beam **(LavuV et al ,.2015).**

Maximum efficiency was shown by the erbium family as it has various features that add to the advantage. These include, greater absorption by water, hydroxyapatite crystal and negligible damage to the hard and soft tissue.CO2 laser have also shown some effect on the calcified tissues effectively. They usually cause ablation. However, major side effects associated with these lasers are cracking or charring at target sites and pulpal damage. Hence, their use was limited for root biomodification **(Bhasin MT et al ,.2016)** .

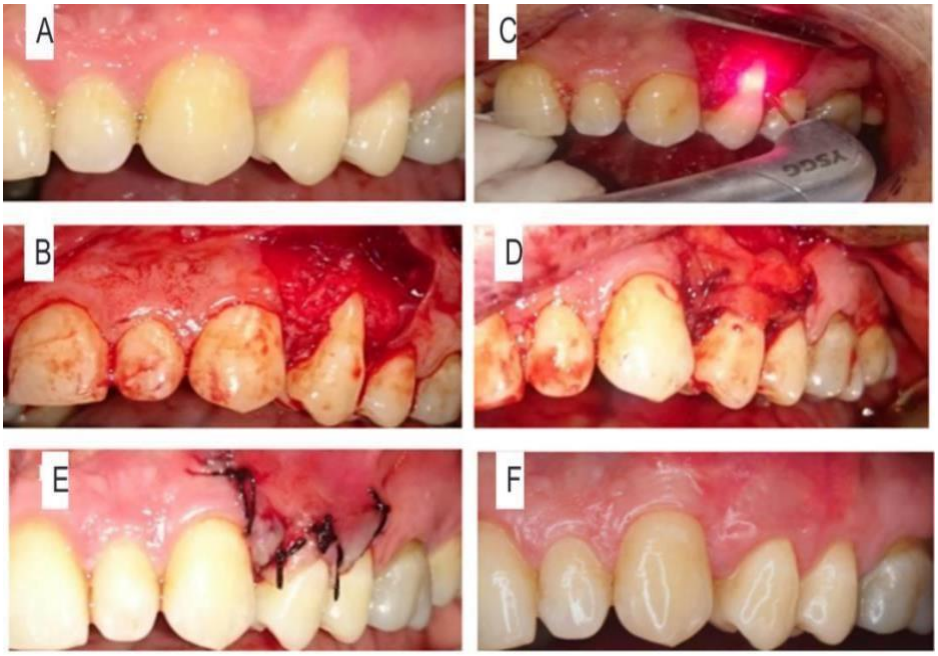

Figure 15: (A) Tooth before treatment. (B)Elevation of a partial thickness flap.

C) Root surface conditioning. D) graft placement at recipient site. E) Suturing the flap.

F) Tooth after treatment.

Pant investigated attachment of periodontal ligament fibroblasts on periodontally involved root surface after conditioning with CO2 laser. He demonstrated better attachment by CO2 irradiation of root surface **(Bhasin MT et al ,2016) .**

Chapter two: Conclusion

The rationale for root surface biomodification is to remove the smear layer on the root surface, uncover and widen the dentin tubules, and unmask the dentin collagen matrix. Many agents have been used for this purpose, which has been described in previous sections. Root surface biomodification is usually combined with other procedures such as guided tissue regeneration and bone grafting to achieve best results. Demineralization has been shown to alter the diseased root surface, creating a more acceptable surface that can influence events in wound healing.

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