INTRODUCTION

Increasing aesthetic demand of the modern era has converted modern dentistry to pink and white esthetic dentistry[1.] Modern aesthetic dentistry involves not only the restoration of lost teeth and their associated hard tissues, but increasingly the management and reconstruction of the encasing gingiva[2.] One of the most difficult goals in the regeneration of the soft tissues is the reconstruction of interdental papilla [1]. Periodontal plastic surgery consists of a broad range of procedures aiming at correcting or eliminating anatomical, developmental and/or traumatic deformities of the gingiva or alveolar mucosa. One among such problems is open interproximal spaces or interdental papilla loss [2].

Interdental papilla also known as the interdental gingiva, is the part of the gingiva that occupies the gingival embrasure, which is the interproximal space beneath the area of tooth contact [3]. The common reasons for papilla loss are plaque-associated lesions, abnormal tooth shape and orthodontic anomalies, and traumatic oral hygiene procedures. The loss of papilla can lead to phonetic problems, lateral food impaction and cosmetic deformities (so called “black triangle disease”).

“Black triangles” or the interproximal spaces are one of the most troubling dilemmas in dentistry. The most common reason for black triangle in the adult population is gingival inflammation, attachment loss, and interproximal bone resorption[2].

The various approaches for management of black triangles are categorized as non-invasive and invasive. The non-invasive approaches include correction of traumatic oral hygiene procedure, orthodontic approach and repeated curettage of the papilla. The invasive approaches are further categorized as non-surgical and surgical. The local injection of fillers like hyaluronic acid gel and cultured and expanded autologous fibroblast comprises the non-surgical or minimally invasive approaches. The surgical approaches includes pedicle flap, semilunar coronally repositioned flap, envelop type flap, autogenous osseous and connective tissue grafts. As the interdental papilla region is characteristically narrow, surgery becomes difficult and limits the blood supply to the associated soft tissue[4.] Hence less invasive technique such as the application of hyaluronic acid gel injection can be considered as a suitable alternative.

Hyaluronic acid (HA) is a natural polysaccharide from the glycosaminoglycan family. The molecules composed of repeated sequence of non-sulfated disaccharide units of N acetylglucosamine and D-glucuronic acid (high molecular weight glycosaminoglycan) making it non-immunogenic. The currently available injectable hyaluronic acid gels are polymers that are formulated by cross-linking hyaluronic acid produced by Streptococcus species, and their durability is often enhanced by improving their biodegradability in skin using advanced cross-linking technology [4].Hyaluronic acid participates in many physiological processes as angiogenesis, wound healing and promotes the formation of fibroblasts and osteoblasts (Aslan et al,2006). It also stimulates the migration and proliferation of endothelial cells (Slevin , et al 2002).Hyaluronic acid hydrogel provides a 3 D space under the periosteum which leads to migration and differentiation of the stem cells, derived from bone morphogenic protein-2, and formation of mature bone (Park, 2011). The cross-linked hyaluronic acid shields the wound area from penetration of bacteria, accelerates wound healing, improves tissue regeneration, and increases osteoblasts formation [5]. These properties collectively make hyaluronic acid, a material of choice for interdental papilla reconstruction.

Previous studies[4,6,7,8]have used the hyaluronic acid gel injection as a treatment modality for papilla reconstruction in class I and II interdental papilla loss there by establishing hyaluronic acid gel as the safe material to significantly decrease the interdental black triangle in the aesthetic zone.

However there is lack of published literature regarding the efficacy of hyaluronic acid gel injection as a treatment approach for papilla reconstruction in class III interdental papilla loss. Hence the present study is sought to assess the effect of hyaluronic acid gel injection for possibly reducing or eliminating deficient papilla adjacent to teeth. In the aesthetic zone, the use of a commercially available hyaluronic acid gel will be evaluated as a possible method for enhancing deficient papillae.

AIM:

- The aim of the study is to evaluate the efficacy of hyaluronic acid gel injection as a minimally invasive approach for reconstruction of interdental papilla.

OBJECTIVES:

- To assess the effect of hyaluronic acid gel injection on reconstruction of interdental papilla in Class I, II, III interdental papilla loss by using Nordland & Tarnow classification system.
- To assess the effect of hyaluronic acid gel injection on reconstruction of interdental papilla in Class I, II, III interdental papilla loss by using the image analysis system to examine the percentage reduction in surface area of visible black triangle.
- To assess the Patient Related Outcome using Questionnaire and Visual Analogue Scale (VAS), pre and post treatment.

Interdental space is the physiologic space between two adjacent teeth. It is composed of four pyramidal embrasures, viz. cervical, occlusal/incisal, buccal, and lingual/palatal. The apex of each pyramid ends at the contact point between two teeth. The space is occupied by the interdental papilla, is composed of dense connective tissue. Its shape is defined by (a) the contact relationship between teeth, (b) the width of proximal tooth surfaces, and (c) the course of the CEJ's. Thus in anterior regions of the dentition, the interdental papilla assumes a pyramidal or conical shape. In the premolar/molar region, the apex of the papilla is separated by a concavity known as the 'col'. It is the only non-keratinized portion of the gingival and is most susceptible to trauma. Though anatomically small, it plays a huge role in face esthetics due to its strong association with the patient smile as a result of its anterior positioning. Gingival black triangles (GBTs) are cosmetic deformities which refer to an absence of papilla resulting in black spaces or open embrasures which impair esthetic features, phonetics problems and food accumulation.

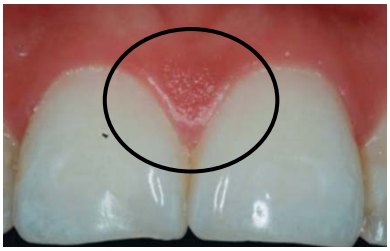
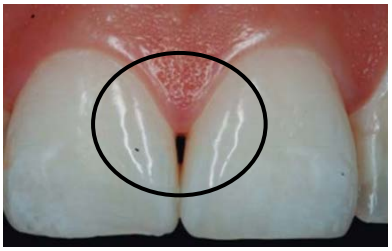
A.  B. 

Fig 1. A. Interdental papilla. B. Interdental papilla loss or Black triangle

Etiology of interdental papilla loss

The etiology of interdental papilla is multi-factorial. Patients may present with one or more etiologic factor; thus, managing each patient requires an individual assessment and treatment plan. The major causes of interdental papilla loss are explained below:

I. **Interproximal space between teeth** - Interproximal space between teeth represents the distance between the proximal surfaces of the adjacent teeth. Horizontal dimensions of interdental space can affect the shape and maintenance of the

interdental soft tissue. While a greater width may enable improved blood supply to papillae tip, which may be helpful to maintain full papilla, a very wide interdental width increases the risk of the presence of a GBT possibly due to stretching the papilla. Teeth with root proximity (less than 0.5 mm interdental distance) possess very thin bone. In return, thin cancellous bone has a greater risk for resorption, decreasing the interproximal bone height and implicitly the papillary disappearance.

II. **The distance between proximal contact point to crest of alveolar bone** - The most crucial factor for the loss of interdental papillae is the distance between interproximal contact point and alveolar bone crest. The presence or absence of GBT by estimating the vertical distance.

III. **Diverging roots and root angulation** - Root divergence of adjacent teeth is highly associated with open gingival embrasures. This either occurs naturally or is caused by improper bracket placement during orthodontic treatment. Bracket placements results in relaxation of stretched transeptal fibers which aids in filling the gap. Prior bracket fixation it is important to radiographically review the positioning of the bracket which should be placed perpendicular to the tooth long axis and not parallel to the incisal edges of tooth. Therefore, the severity of occurrence of open gingival embrasures in case of root angulation can be reduced by connecting the brackets in such a way it lies perpendicular to the tooth long axis which allowing root convergence without causing open embrasures.

IV. **Traumatic interproximal oral hygiene procedures** - There is sufficient evident from literatures to prove that brushing is an efficient way of removing plaque and prevention of gingivitis and periodontitis. However, its efficacy depends upon the physique and mental attitude of an individual, type of toothbrush, condition of the bristles. Gingival recession has been related to increased brushing frequency with a hard toothbrush. Traumatic flossing may also contribute to open embrasures, in this case interproximal cleaning should be discontinued to allow tissue recovery.

V. **Abnormal crown forms and tooth morphology** - The basic tooth forms: circular, square or triangular, determine the degree of gingival scallop. Circular or square teeth produce a shallower gingival scallop; while triangular teeth form have a pronounced scallop. The latter predisposes to GBTs especially with a thin biotype which has a propensity for recession. Furthermore, triangular teeth have divergent roots with thicker interproximal bone, resulting in reduced vertical bone loss compared with square teeth. However, squarer teeth yield better interproximal papilla maintenance due to a smaller interproximal distance from the osseous crest to the contact point.

VI. **Gingiva biotype** - The periodontal biotype has been classified as scalloped-thin and flat-thick biotype. Scalloped-thin tissue is more prone to develop recession following trauma or inflammation, while flat-thick tissue is more likely to develop deeper periodontal pockets. Limited blood supply is believed to be one of the major reasons of unpredictable regeneration of interdental papilla. Thick tissues respond more favorably due their increased vascularity and extracellular matrix volume. Therefore, flat-thick biotype has been considered more favorable for achieving optimal esthetics.

VII. **Patient's age** - Aging results in thinning of oral epithelium, decreased keratinization, reduced papilla height and bone loss which are risk factors of open embrasures. It was found that papillary tissue height decreased by 0.012 mm/ year of age.

VIII. **Periodontal disease and loss of attachment** - Periodontitis is defined as an inflammatory disease of the supporting tissues of teeth and it is caused by the microorganisms which have the ability to invade and colonize the periodontal tissues. Periodontal disease has been associated with loss of interdental papilla due to the loss of alveolar bone. The foundation for the gingival support is the underlying contour of the osseous crest. If the distance from the alveolar crest to interdental contact point exceeds 5 mm, it is more likely that the papilla is insufficient to fill the embrasure.

Classification Of The Interdental Papilla Loss

A classification system to identify and describe the severity of papillae loss is useful during the patient examination and facilitates monitoring of papillary augmentation techniques.

Jemt classification (1997) based on the Relationship between the papilla and the interdental area, where:-

Grade 0 – No papilla is present.

Grade 1- Less than half of the height of the papilla is present.

Grade 2- Half or more of the height of the papilla is present.

Grade 3- The papilla fills up the entire proximal space, harmonic gingival contour.

Grade 4- The papilla are hyperplastic, covering too much tooth structure, irregular tissue contour possible discolouration.

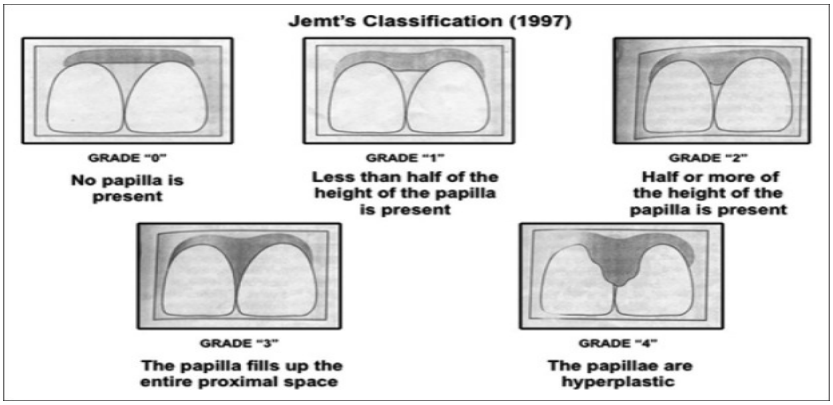


Fig 2. Jemt's classification

Nordland and Tarnow classification system (1998), where:-

Class I: The tip of the interdental papilla is located between the contact point and the most coronal extent of the interproximal cemento-enamel junction (CEJ).

Class II: The crest of the papilla is at or apical to the interproximal CEJ but coronal to the facial CEJ.

Class III: Crest of the papilla is at or apical to the facial CEJ.

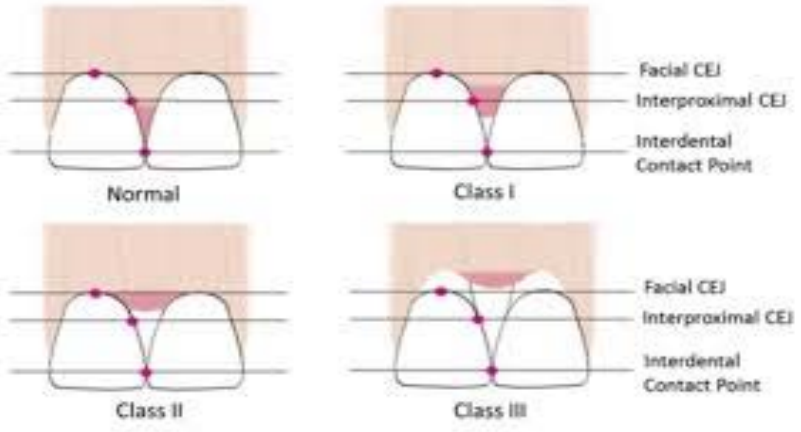


Fig 3. Nordland and Tarnow classification system for interdental papilla loss.

Palacci & Ericsson (2001), a classification system based on the loss of hard and soft tissues, divides implant sites into four classes according to the vertical and horizontal dimensions of tissue loss, respectively.

Vertical loss

Class I- Intact or slightly reduced papillae

Class II- Limited loss of papillae (less than 50%)

Class III- Severe loss of papillae

Class IV-Absence of papillae (edentulous ridge).

Horizontal loss

Class A- intact or slightly reduced buccal tissues

Class B- Limited loss of buccal tissues

Class C- Severe loss of buccal tissues

Class D- Extreme loss of buccal tissue,often in combination with a limited amount of attached mucosa.

Combinations of the different classes can exist, as shown below:

VERTICAL LOSS	HORIZONTAL LOSS
Class I	Class A
Class II	Class B
Class III	Class C
Class IV	Class D

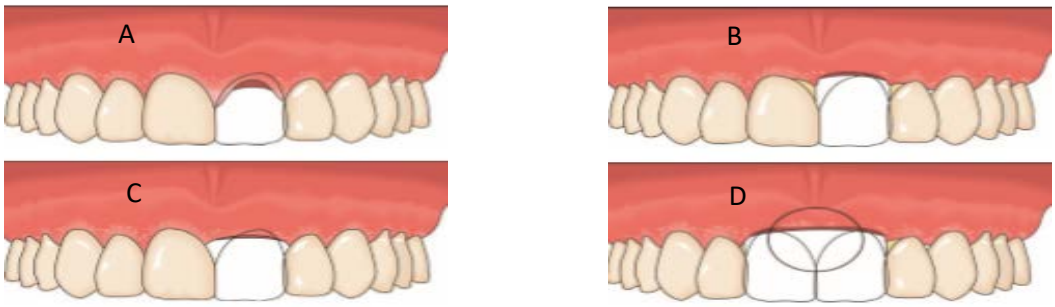


Fig 4. Vertical loss A.Class I, B. Class II, C. Class III, D. Class

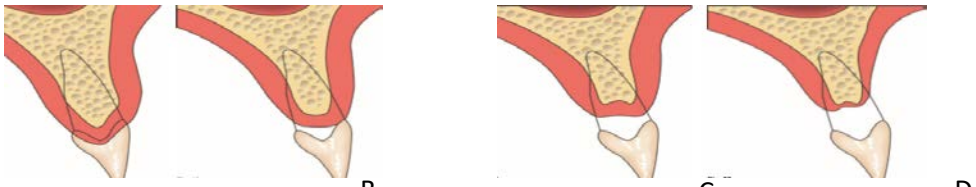


Fig 5. Vertical loss A.Class A, B. Class B, C. Class C, D. Class D

Nemcovsky (2001), introduced a classification system as a papillae index score (PIS) based on a comparison with adjacent teeth, four different index scores were used to measure papillae:

PIS 0: Presence of no papilla and no curvature of the soft tissue contour.

PIS 1: Present papillae height less than half the height of the papilla in the proximal teeth and a convex curvature of the soft tissue contour.

PIS 2: Presence of at least half the height of the papilla in the proximal teeth, but not in complete harmony with the interdental papilla of the proximal teeth.

PIS 3: papillae able to fill the interproximal embrasure to the same level as in the proximal teeth and in complete harmony with the adjacent papillae.

Cardarpoli et al. (2004), proposed a scoring system as papillae presence index (PPI) positional relationship among the papilla, CEJ, and adjacent teeth to assess interproximal papillary level:

PPI 1: The papilla is completely present and at the same height of the adjacent tooth.

PPI 2: The papilla is not completely present but interproximal CEJ is not visible.

PPI 3: The papilla is not completely present and interproximal CEJ is visible.

PPI 4: The Papilla lies apical to both interproximal and buccal CEJ.

Miller (2018), proposed a classification for types of Interdental Papilla:

Type A –

There is no interdental bone loss.

The papilla extends 5 mm coronal to the interdental bone crest.

The papilla is $\geq$3 mm wide at its base at the level of adjacent CEJs.

Complete root coverage is expected

Type B –

There is no interdental bone loss.

The papilla extends 5 mm coronal to the interdental bone crest.

The papilla is a3 mm wide at its base at the level of adjacent CEJs.

The predictability of complete root coverage is compromised.

Type C –

There is interdental bone loss, or the tooth is extruded.

If there is interdental bone loss, the papilla may or may not fill the interdental space.

The papilla may be s3 mm wide at its base at the level of adjacent CEJs.

Complete root coverage is not expected but may be achieved under certain circumstances.

Treatment modalities

It is said that 'Soft tissue always follows the hard tissue.' This is undoubtedly evident in active periodontal disease where bone loss leads to loss/absence of the interdental papilla. In such cases, complete reconstruction is generally not achieved. However, if the damage is mainly due to soft tissue alone reconstructive techniques may be useful in restoring the papilla completely. Interdental papilla may be restored either by surgical approach or Non-Surgical approach.

Surgical Approaches

Surgical techniques may be used to re-contour, preserve or re-construct the interdental papilla.

Recontouring of the papilla - Recontouring the papilla may be required in cases of gingival enlargement or in cases of localized gingival lesions, viz. peripheral giant cell granuloma. In such cases, excess soft tissue is eliminated by gingivectomy associated with a free gingival graft in order to remodel the soft tissue architecture.

Fig 6. Gingivectomy

Papilla preservation - Periodontal defects may be corrected by various flap procedures. In order to preserve the interdental soft tissues for maximum soft tissue coverage following surgical interventions like the papilla preservation, modified papilla preservation technique and simplified papilla preservation technique. Cortellini and Tonetti further improved the results by using a microsurgical approach which has advantage of improved illumination, access, and magnification of the surgical field. Dissection is extremely accurate; defect debridement is perfected, control over membrane positioning and stabilization is achieved and primary closure is best obtained.

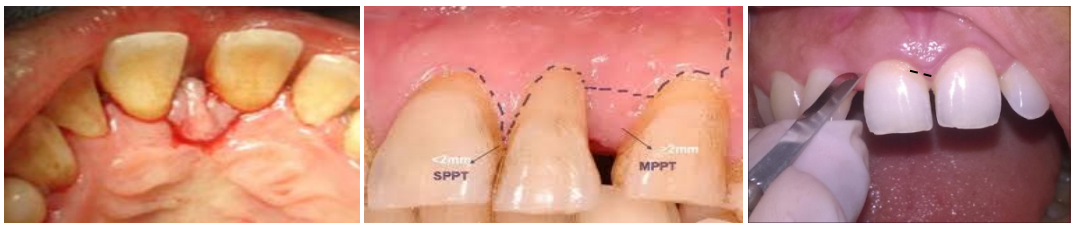


Fig 7. Papilla preservation, Modified papilla preservation technique and Simplified papilla preservation technique.

Reconstruction of the Interdental papilla - various procedures have been described to reconstruct the papilla like pedicle graft, semilunar coronally positioned papilla, use of a connective tissue graft, Acellular micronized dermal graft or Prf.

In all these techniques the blood supply to the recipient site might get jeopardized, as they used releasing incisions and restricted space in which to operate make surgical grafting procedures difficult. Placing releasing incisions close to the connective tissue graft, bone graft are barrier membrane can cause tenting of the papilla, which can result in exposure of the connective tissue graft, bone graft, and membrane. Apart from resulting in scarring releasing incisions given in patients with thin gingival biotype increases the risk of failure.

Nonsurgical Approaches

Several surgical and nonsurgical treatment options are available, there is no golden standard set due to lack of large scale clinical trials nor long terms clinical outcomes. When

compared to surgical techniques which are less predictable and painful, nonsurgical techniques are preferred due to their cost effectivness, less stressful and achieve immediate results with high satisfaction rate. Nonsurgical approaches include correction of traumatic oral hygiene procedure, restorative techniques, orthodontic movement, repeated scrapping of the papilla and tissue volumizers.

Correction of Traumatic Oral Hygiene - Procedure Toothbrush abrasion leads to cement-enamel wear and damage of supporting gingival tissues leading to recession and papilla loss. Usage of flat trim toothbrush bristle, end-rounded filaments, rubber bristles, interdental cleaner are recommended to reduce gingival abrasion. Improper use of dental floss may damage the interdental papilla. Traumatic interproximal hygiene procedures must be initially discontinued and successively modified. Re-epithelialization of the traumatic lesion can restore the papilla completely.

Restorative and Prosthetic Approaches - Prosthetic approaches for reproduction of gingival tissue include porcelain, acrylics, silicone-based soft materials, or co-polyamide and composite resin. Composite resin is available in pink shades for gingival reproduction. It can be used on restorations to replace missing soft tissue and considered to be more realistic than pink porcelain in similar situations. Although pink porcelain can mask the loss of interdental papilla, porcelain shades and optical properties are limited. Hygiene training is strongly recommended by dentist to patients who seek restoration therapy to improve the performance of prosthesis.

Fig 8. Restorative and prosthetic approaches

Orthodontic Approach - Orthodontic movement has several applications in reducing the GBTs. Closing the interdental contacts by conventional orthodontic movement of adjacent teeth creates a new contact point thereby reducing diastema and subsequent creeping of gingival tissues towards interdental space. In conjunction with orthodontic treatment, interproximal reduction (IPR) of enamel is one of orthodontic approach to achieve contact point between two adjacent teeth. Coronal movement of tooth by application of gentle, continuous pressure results in closure of interdental space. The effects are alterations within the supporting structures, causing changes in bone level and the soft tissue contours and thereby creating new papillae.

Fig 9. Orthodontic approach

Repeated Scrapping of the Papilla - Repeated curettage every 15 days for 3 months recreate papillae which were previously destroyed by necrotizing gingivitis this instrumentation induce a proliferative hyperplastic inflammatory reaction of the papilla. About 9 months after initial treatment, regeneration of interdental papillae was observed.

Tissue Volumizing - Among several minimally invasive techniques proposed, the local injection of various fillers like hyaluronic acid gel and biological preparations like cultured and expanded autologous fibroblast comprises the non-surgical or minimally invasive approaches has been studied for papilla reconstruction. Long used as dermal fillers, recent findings have suggested its use to treat interdental papilla loss.

Hyaluronic acid

Hyaluronic acid (HA) is also known as hyaluronan or hyaluronate. It is a large molecule, non-sulphated glycosaminoglycan present in connective tissues like skin and cartilage, vitreous of the eye, synovial joint fluid, disc nucleus, and umbilical cord. Under physiological conditions, it contributes to tissue hydrodynamics, by binding to water to provide elasticity and stability resulting in tissue regeneration and healing. [10,11]

It has unique and incomparable chemical-physical properties, and it is characterized by numerous biological functions.[2] Its non-immunogenic, biocompatibility, biodegradable, mucoadhesive and bacteriostatic properties enhance its clinical significance. Long used as dermal fillers, recent findings have suggested its use to treat interdental papilla loss.[10]

Structure and properties - HA (as well as its sodium salt) is a linear polyanionic bio-polysaccharide, belonging to the class of glycosaminoglycans. It consists of repeating disaccharide units composed of N acetyl-D glucosamine and D-glucuronic acid linked bya β-1,4 glycosidic bond, whereas the disaccharides are linked by β-1,3 glycosidic bonds. HA exists with different molecular weights, which determine its chemical-physical and biological properties.[11]

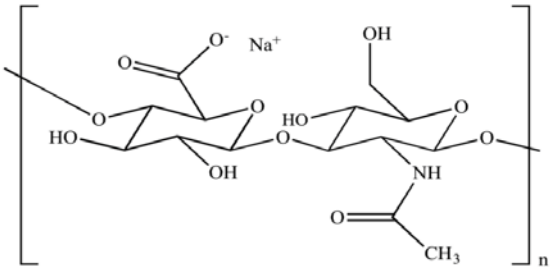


Fig 10. Structure of hyaluronic acid

In aqueous solution, HA forms an extended hydrogen bonded system, intramolecularly but also inter-molecularly. Because of these H-bonds, but also of hydrophobic interactions that arise from HA hydrophobic patches, the polymer arranges a self-aggregate and, more precisely, a physical cross-linked system. These intra- and inter-chain interactions are weak and are responsible for the formation of a temporary network structure, that is, of a reversible gel. These are the basis of the gel shear-thinning and self-healing properties; the gel undergoes a reversible destructuration, upon a shear, of the transient polymer network. Upon cessation of the shear, the hydrogel is able to autonomously reassemble and recover its initial viscosity. The rheologic properties of HA solutions make HA solutions ideal for pharmaceutical, medical, and cosmetic uses (e.g., DFs). Indeed, these characteristics allow a simple application of the product and its rapid immobilization at the target site.[11]

When HA is incorporated into aqueous solution, hydrogen bonding occurs between adjacent carboxyl and N-acetyl groups; this feature allows hyaluronic acid to maintain conformational stiffness and to retain water. One gram of hyaluronic acid can bind up to 6 L of water. As a physical background material, it has functions in space filling, lubrication, shock absorption, and protein exclusion.[12]

The viscoelastic properties of the material may slow the penetration of viruses and bacteria, a feature of particular interest in the treatment of periodontal diseases. Hyaluronan as a viscoelastic substance assists in periodontal regenerative procedures by maintaining spaces and protecting surfaces. Through recognition of its hygroscopic and viscoelastic nature, hyaluronic acid can influence the cell function that modify the surrounding cellular and the extracellular micro and macro environments.[12]

Hyaluronan has many structural and physiological functions within tissues, including extracellular and cellular interactions, growth factor interaction and in the regulation of osmotic pressure and tissue lubrication, which help maintain the structural and homeostatic

integrity of tissues.[12] Its hygroscopic properties, hyaluronan significantly influences hydration and the physical properties of the extracellular matrix. Hyaluronan is also capable of interacting with a number of receptors resulting in the activation of signaling cascades that influence cell migration, proliferation, and gene expression (Turley et al., 2002; Taylor et al., 2004).[13]

Hyaluronic acid accelerates the bone regeneration by means of chemotaxis, proliferation and successive differentiation of mesenchymal cells. Hyaluronic acid shares bone induction characteristics with osteogenic substances such as bone morphogenetic protein-2 and osteopontin.[12]

HA in tissues is digested by macrophages in blood or lymphatic system and broken HA reaches bloodstream to get disintegrated in liver for excretion. HA is usually eliminated through urine at a very minute quantity. HA acts as an antioxidant by scavenging reactive oxygen species, which helps in the regulation of immune response implying its anti-inflammatory properties. HA's anti-inflammatory response and rheology properties make it ideal for biomedical usage.

Chemical modified hyaluronic acid preparations degrade more slowly than biological HA (due to crosslinkage and decreased water solubility) extending its clinical efficacy by 6-12 months. Such preparations are used as fillers which are usually manufactured from animal sources and more recently Streptococcus species of bacteria was used to extract gel form of hyaluronic acid which was chemically cross-linked with butanediol diglycidyl ether, stabilized and suspended in neutral phosphate buffered saline.

Mechanism of action - Although the predominant mechanism of HA is unknown, in vivo, in vitro, and clinical studies demonstrate various physiological effects of exogenous HA. Hyaluronic acid can reduce nerve impulses and nerve sensitivity associated with pain. In experimental osteoarthritis, this glycosaminoglycan has protective effects on cartilage (Akmal

et al., 2005); exogenous hyaluronic acid is known to be incorporated into cartilage (Antonas et al., 1973). Exogenous HA enhances chondrocyte HA and proteoglycan synthesis, reduces the production and activity of proinflammatory mediators and matrix metalloproteinases, and alters the behavior of immune cells. These functions are manifested in the scavenging of reactive oxygen-derived free radicals, the inhibition of immune complex adherence to polymorphonuclear cells, the inhibition of leukocyte and macrophage migration and aggregation (Balazs and Denlinger, 1984) and the regulation of fibroblast proliferation. Many of the physiological effects of exogenous HA may be functions of its molecular weight (Noble, 2002; Uthman et al, 2003; Hascall et al., 2004; Medina et al., 2006).

Hyaluronan is highly hygroscopic and this property is believed to be important for modulating tissue hydration and osmotic balance (Dechert et al., 2006). In addition to its function as a passive structural molecule, hyaluronan also acts as a signaling molecule by interacting with cell surface receptors and regulating cell proliferation, migration, and differentiation. Hyaluronan is essential for embryogenesis and is likely also important in tumorigenesis (Kosaki et al., 1999; Camenisch et al., 2000). Hyaluronan functions are diverse. Because of its hygroscopic properties, hyaluronan significantly influences hydration and the physical properties of the extracellular matrix. Hyaluronan is also capable of interacting with a number of receptors resulting in the activation of signaling cascades that influence cell migration, proliferation, and gene expression (Turley et al., 2002; Taylor et al., 2004)

CLINICAL APPLICATIONS OF HYALURONIC ACID IN PERIODONTICS

Hyaluronan has been identified in all periodontal tissues in varying quantities, being more prominent in the non-mineralized tissues, such as gingiva and periodontal ligament, compared to mineralized tissues, such as cementum and alveolar bone. In addition, due to the high levels of hyaluronan in circulating blood serum, it is constantly present in gingival crevicular fluid (GCF) as a serum overload factor.[15] Natural hyaluronic acid is an extremely

hydrophilic polymer; it exists as a viscous gel and does not per se have the structural features required for use as a surgical product. An ester of hyaluronic acid synthesized by esterification of a carboxyl group with benzyl alcohol is less water soluble and thus more stable. Because of its unique molecular structure, hyaluronic acid can be assembled into various molecular weights and lyophilized or esterified into a variety of different structural configurations such as sponges and membranes. The rate of biodegradation of these materials can be manipulated by altering their degree of lyophilization or esterification. Thus, hyaluronic acid may be of benefit as a resorbable grafting material in regenerative surgical procedures..[16]

Hyaluronic acid has a multifunctional role in periodontics:

1. **Topical application of subgingival hyaluronic acid gel can be used as an antimicrobial agent as an adjunct to scaling and root planing**.

Jentsch H, Pomowski R, Kundt G, Göcke R, 2003[14] evaluated the anti-inflammatory and anti-edematous properties of Hyaluronic acid in a gel formulation for its effect in the treatment of plaque-induced gingivitis. A randomised double-blind study, 50 male subjects with plaque-induced gingivitis were divided into two groups and used a verum or placebo gel twice daily additionally to oral hygiene for a 3-week treatment period. Clinical indices (API, Turesky index, PBI) and crevicular fluid variables (peroxidase, lysozyme) were determined at baseline and after 4, 7, 14 and 21 days, respectively. Results of the study suggested that a hyaluronan containing gel has a beneficial effect in the treatment of plaque-induced gingivitis.

Xu Y, Frentzen M, Jerve- Storm PM, 2004[17] evaluated the clinical effects of hyaluronic acid in local application as an adjunctive to scaling and root planing. In this cross over design study 20 patients with chronic periodontitis were included. Plaque index, sulcus-fluid-flow-rate (SFFR), sulcus bleeding index, probing pocket depth and attachment level were monitored. All patients were treated with full mouth SRP, in addition a HA gel was administered subgingivally in the test site every week for 6 weeks. A statistically significant improvement of

all clinical test parameters was observed in both groups. Thus, they concluded that no post-inflammatory tissue regeneration could be achieved by the adjunctive use of HA gel to SRP in the patients with chronic periodontitis. Due to SFFR a control of local inflammation can be achieved quickly.

Xu Y, Höfling K, Fimmers R, Frentzen M, Jervøe-Storm PM, 2004[18] evaluted the potential benefits of local subgingival application of HA adjunctive to scaling and root planing. Twenty patients with chronic periodontitis were included in this split-mouth study. Sulcus fluid flow rate and sulcus bleeding index were monitored at baseline and after 1, 2, 3, 4, 5, 6, and 12 weeks; probing depth and clinical attachment level were monitored at baseline and 6 and 12 weeks. Results concluded an improvement of all clinical variables in both groups. No difference between test and control sites was seen in the tested microorganisms.

Pistorius A, Martin M, Willershausen B, Rockmann P, 2005[19] evaluted the efficacy of a topical application of hyaluronic acid for treating gingivitis. Sixty non-smoking outpatients in good general condition, with clinical signs of gingivitis, were included in the study. The clinical parameters DMF-T index, approximal plaque index, sulcus bleeding index, papilla bleeding index, and gingival crevicular fluid were measured at baseline, after 3 days, and after 7 days. The results obtained by this study concluded that the topical application of an HA-containing preparation represents a potentially useful adjunct in the therapy of gingivitis, although its use does not diminish the need for plaque reduction as a primary therapeutic measure.

Habiboallah G, Nasroallah S, Mahdi Z, Nasser MS, Massoud Z, Ehsan BN, Mina ZJ, Heidar P, 2008[20] compared the effects of Curcuma longa-ghee formulation and hyaluronic acid on gingival wound healing following surgery. Five healthy 3-year-old male beagle dogs were used in this study. Histological changes were monitored in days 4 and 7 after operation to evaluate the inflammatory and repair stage of healing process. Results showed the significant difference in the inflammatory and repair parameters of the healing process between cases treated with this new formulation and cases of hyaluronic acid application.

Thus, the results concluded a positive potential therapeutic effect on surgical wound healing particularly improvement of periodontal treatment consequences after surgery.

Johannsen A, Tellefsen M, Wikesjö U, Johannsen G, 2009 [21] evaluated the adjunctive effect of the local application of a hyaluronan gel to scaling and root planing in the treatment of chronic periodontitis. Twelve patients with chronic periodontitis were recruited to participate in a study with a split-mouth design. Plaque formation and bleeding on probing were evaluated pre-treatment (baseline) and at 1, 4, and 12 weeks post-treatment. Probing depths and attachment levels were evaluated at baseline and at 12 weeks. A significant reduction in bleeding on probing scores and probing depths was observed in both groups at 12 weeks. Thus, they concluded the local application of hyaluronan gel in conjunction with scaling and root planing may have a beneficial effect on periodontal health in patients with chronic periodontitis.

Rodrigues SV, Acharya AB, Bhadbhade S, Thakur SL, 2010[22] evaluated the efficacy of 0.025% hyaluronan-containing mouthwash in comparison with 0.2% chlorhexidine and a water-based mouthwash and also to evaluate its antibacterial efficacy on isolated strains of perio-donto-pathogens. A single-blinded, parallel design, randomised controlled trial was carried out and the 4-day plaque re-growth model was used to study the efficacy of the three mouthwashes. Effects of the three mouthwashes were tested on the growth of isolated strains of Porphyromonas gingivalis (Pg), Aggregatibacter actinomycetemcomitans (Aa) and Prevotella intermedia (Pi). Results concluded that hyaluronan (0.025%)-containing mouthwash was comparable to chlorhexidine (0.2%) in inhibiting plaque growth in vivo, and it significantly reduced the growth of Aa and Pi in vitro.

Igić M, Mihailović D, Kesić L, Apostolović M, Kostadinović L, Janjić OT, Milasin J, 2011[23] evaluated the efficacy of hyaluronic acid in the treatment of chronic gingivitis in children. The study enrolled 130 children with permanent dentition. All of the examinees were divided into three groups. Assessment of oral hygiene and status of the gingiva and

parodontium was done using the appropriate indexes before and after the treatment. Results concluded that the basic treatment is able to successfully treat chronic gingivitis in children. The use of hyaluronic acid together with the basic treatment can markedly improve the treatment effect.

Sapna N, Vandana KL, 2011[24] evaluated the anti-inflammatory effect of 0.2% hyaluronan gel alone and with mechanical therapy on gingivitis. In each of the 28 gingivitis patients, the four quadrants were subjected to different treatments: scaling, scaling + topical hyaluronan gel, only topical hyaluronan gel, and topical + intrasulcular hyaluronan gel. Clinical parameters were recorded at baseline, and on days 7, 14, and 21. Biopsies were taken from each quadrant, inflammatory infiltrates were graded, and the argyrophilic nucleolar organizer region count was measured before and after treatment.A significant reduction was seen in clinical parameters, inflammatory infiltrates, and the argyrophilic nucleolar organizer region count within the groups. Thus, they concluded that the hyaluronan gel is an effective topical agent for treating gingivitis, along with scaling and intrasulcular application. The argyrophilic nucleolar organizer region count can be used as a histopathological indicator in cases of non-responsive gingivitis to assess the severity of gingival inflammation.

Eick S, Renatus A, Heinicke M, Pfister W, Stratul SI, Jentsch H,2013[25] evaluated the effect on clinical variables, subgingival bacteria, and local immune response brought about by application of hyaluronan-containing gels in early wound healing after scaling and root planing. In this randomized clinical study, data from 34 individuals with chronic periodontitis were evaluated after full-mouth SRP. Probing depth (PD) and clinical attachment level (CAL) were recorded at baseline and after 3 and 6 months, and subgingival plaque and sulcus fluid samples were taken for microbiologic and biochemical analysis. The results concluded that the adjunctive application of hyaluronan may have positive effects on PD reduction and may prevent recolonization by periodontopathogens.

Mallikarjun S, Neelakanti A, Babu HM, Pai SB, Shinde SV, Krishnan S,2016[26] evaluated and compared the effect of 0.2% hyaluronan gel adjunctive to scaling and root planing on the levels of elastase in gingival crevicular fluid. split-mouth study included eighty sites from twenty patients. GCF samples were collected from all the eighty sites; simultaneously, clinical periodontal parameters were recorded. Enzyme-linked immunosorbent assay was used to determine the levels of elastase at baseline and 6 weeks after therapy, following mechanical debridement and subsequent subgingival placement of the experimental drug. There was a mean reduction in the elastase levels from baseline to 6 weeks after therapy in the experimental group. However, the difference between the groups was not statistically significant. Thus, they concluded that the adjunctive use of hyaluronan following mechanical debridement resulted in comparable reduction in the elastase levels, suggesting that this substance has an inhibitory effect on elastase, and subsequent tissue destruction. Further long-term studies are mandatory to validate the results of this study.

2. **Bone regeneration in periodontal bony defects.**

Vanden Bogaerde L,2009 [27] investigated the clinical efficacy of esterified hyaluronic acid for treating deep periodontal defects. Nineteen defects, 18 infrabony and one mandibular molar furcation, were treated. Defects with probing pocket depths (PPDs) of at least 6 mm were consecutively included in the study. The PPD, gingival recession, and clinical attachment level (CAL) were evaluated at each defect before treatment and 1 year after surgery. A full-thickness flap was raised and the roots were accurately planed; hyaluronic acid in the form of fibers was then packed into the defect to completely fill the space. One year after treatment, the mean PPD was reduced by 5.8 mm (range, 0 to 10 mm), gingival recession had increased by 2.0 mm (range, 0 to 6 mm), and attachment gain was 3.8 mm (range, 0 to 7 mm).

Ballini A, Cantore S, Capodiferro S, Grassi FR,2009 [28] studied the osteoinductive effect of the hyaluronic acid (HA) by using an esterified low-molecular HA preparation (EHA) as a

coadjuvant in the grafting processes to produce bone-like tissue in the presence of employing autologous bone obtained from intra-oral sites, to treat infra-bone defects without covering membrane. We report on 9 patients with periodontal defects treated by EHA and autologous grafting with a mean depth of 8.3 mm of the infra-bone defects, as revealed by intra-operative probes. Data were obtained at baseline before treatment and after 10 days, and subsequently at 6, 9, and 24 months after treatment. Clinical results showed a mean gain hi clinical attachment of 2.6mm of the treated sites, confirmed by radiographic evaluation. Such results suggest that autologous bone combined with EHA seems to have good capabilities in accelerating new bone formation in the infra-bone defects.

Briguglio F, Briguglio E, Briguglio R, Cafiero C, Isola G, 2013 [29] examined the use of hyaluronic acid to treat infrabony periodontal defects over a period of 24 months. Forty subjects with a two-wall infrabony defect were selected. The defects were randomly divided into two groups: sites treated with hyaluronic acid (test group) and those treated with open flap debridement (control group). The 12- and 24-month evaluations were based on clinical and radiographic parameters. The results concluded that the treatment of infrabony defects with hyaluronic acid offered an additional benefit in terms of CAL gain, PD reduction, and predictability compared to treatment with open flap debridement.

Santana RB, Santana CM, 2015 [30] evaluated a biological hydrogel of recombinant human Fibroblast Growth Factor type 2 (rhFGF-2) in a hyaluronic acid (HA) carrier applied in periodontal intrabony defects would enhance the clinical parameters of regeneration of the periodontal attachment apparatus. Thirty adult patients were evaluated. Control group were treated by open debridement with the papilla preservation flaps, while the test group also received a topical application of rhFGF-2/HA in the intrabony defect. The parameters evaluated, at baseline and after one year, were, were probing depth (PD), gingival recession (REC), probing attachment level (PAL) and probing bone level (PBL). Test sites exhibited significantly more PD reduction, PAL gains and shallower residual PD than controls. Thus,

they concluded that the application of rhFGF-2/HA significantly improved clinical parameters of periodontal wound healing one year after treatment.

3. Guided Bone Regeneration.

Park JK, Yeom J, Oh EJ, Reddy M, Kim JY, Cho DW, et al, 2009 [31] successfully developed biocompatible and degradation controlled poly(lactic-co-glycolic acid) grafted hyaluronic acid (HA-PLGA) for periodontal barrier applications. The stability of HA-ADH to enzymatic degradation by hyaluronidase increased with ADH content in HA-ADH. The resulting HA-PLGA was used for the preparation of biphasic periodontal barrier membranes in chloroform. According to in vitro hydrolytic degradation tests in phosphate buffered saline, HA-PLGA/PLGA blend film with a weight ratio of 1/2 degraded relatively slowly compared to PLGA film and HA coated PLGA film. Four different samples of a control, OSSIX(TM) membrane, PLGA film, and HA-PLGA/PLGA film were assessed as periodontal barrier membranes for the calvarial critical size bone defects in SD rats. Histological and histomorphometric analyses revealed that HA-PLGA/PLGA film resulted in the most effective bone regeneration compared to other samples with a regenerated bone area of 63.1% covering the bone defect area.

Park JK, Yeom J, Oh EJ, Reddy M, Kim JY, Cho DW, et al, 2016 [32] evaluated the effect of hyaluronic acid (HA) on healing of infected sockets. Six beagle dogs, both mandibular third premolars were hemisected, and the distal roots were extracted. Subsequently, periodontal and endodontic lesions were induced at the remaining mesial root. After communication of the periodontal lesion, an endodontic periapical lesion was observed at 4 months, and the mesial roots of both the right and left sides were extracted. HA was applied into the socket of the test group, and no treatment was administered to the other group (control group). Three months after extraction of the mesial roots, the dogs were sacrificed, and histologic evaluations were performed. The sockets were filled by mineralized bone and bone marrow in the control group. There was a statistically significant difference between the groups. Reversal lines and

a copious lineup of osteoblasts were observed in the middle and apical parts of the sockets in the test group. Thus, they concluded an infected socket shows delayed healing of the socket wound, and HA, because of its osteoinductive, bacteriostatic, and anti-inflammatory properties, may improve bone formation and accelerate wound healing in infected sockets.

4. **Accelerates healing during flap surgeries.**

De Araújo Nobre M, Carvalho R, Malo P, 2009 [33] compared the efficacy of a protocol for irrigating peri-implant pockets using a plastic needle with 0.8% HA or 0.2% CHX, through evaluation of clinical parameters included in the implant success criteria. eighteen clients with one implant presenting probing pocket depth up to 6 mm. Bone loss and bleeding on probing were treated through mechanical debridement, and were randomly allocated to either a treatment with 0.8% hyaluronic acid (AH) or with 0.2% chlorhexidine (CHX) gels for irrigation of the peri-implant pocket. No significant differences were found between the two groups in treatment success. The results obtained in this study favour the adoption of non surgical protocols. The fact that no significant differences were found between both groups supports the research hypothesis in the use of HA in the treatment of pockets up to 5 mm and of CHX for the treatment of pockets up to 6 mm. Thus, they concluded that the use of non surgical therapy is effective, making it possible either to treat peri-implant pathologies with a simple protocol, or to prepare the site for surgical therapy in case of an unsuccessful treatment.

5. **Non surgical treatment of peri-implant pockets.**

De Araujo Nobre M, Cintra N, Malo P, 2007 [34] compared the health status of the peri-implant complex (hard and soft tissues surrounding the implant) during the healing period of immediate function implants, using HA or CHX gels in the patient's maintenance protocol. Thirty complete edentulous patients, with four immediate function Brånemark System implants placed in the mandible (total of 120 implants), were randomly assigned to two groups (HA and CHX) using only these two chemicals in their daily implant self-care. Both groups were followed up for 6 months, with clinical observations on the 10th day, 2 months, 4

months and 6 months post-surgically. HA and CHX produced good results in maintaining a healthy peri-implant complex in immediate function implants for complete rehabilitations in the edentulous mandible. Thus, they concluded that both chemicals are valid tools for implant maintenance.

Trombelli L, Simonelli A, Pramstraller M, Guarnelli ME, Fabbri C, Maietti E, Farina R, 2018 [35] evaluated the post-surgery gingival healing as well as plaque, gingival inflammation and staining levels following the use of a 0.2% chlorhexidine (CHX) solution with or without antidiscoloration system (ADS) and 0.2% hyaluronic acid (HA). After surgery, patients used the assigned mouthrinse (CHX + HA + ADS or CHX) for 21 days. At days 7 and 21, the healing process was evaluated at experimental teeth using a composite index, namely the Gingival Healing Index (GHI). The results concluded that Post-surgery plaque control based on either CHX or CHX + HA + ADS mouthrinses results in optimal plaque control and quality of early gingival healing along with limited tooth and tongue staining.

6. **Peri-implant maintenance of immediate function implants**.

Lopez MA, Manzulli N, D'Angelo A, Lauritano D, Papalia R, Candotto V,2017 [36] evaluated the use of hyaluronic acid as an adjuvant in the management of peri-implantitis. The results of the statistical analysis demonstrated that there was a slight difference between the pocket depth as measured in the treated sites at time 0 (pre-treatment) and time 1 (15 days post-treatment), although the difference was so small as to render it statistically irrelevant. Bleeding on probing as measured at time 0 and time 1 indicated an improvement on both sides, but with no greater improvement noted on the side treated with HA.

Genovesi A, Barone A, Toti P, Covani U, 2017 [37] evaluated the incidence of post-surgical adverse events at submerged implant sites as well as the antiplaque, antigingivitis and antistaining effects in the entire dentition of patients treated with two mouthwashes. 40 patients subjected to dental implant treatment. Two 0.12% chlorhexidine mouthwashes were compared for 15 days: one with 0.1% hyaluronic acid (CHX⊗HYL group) and one without it

(CHX group). Surgical outcome variables, and plaque, gingival, and staining indexes were recorded. Significant differences were found between the two rinses regarding the presence of oedema within 2 days after surgery. The results concluded that the sites of patients subjected to dental implant placement, an additional anti-oedematigenous effect in early healing seemed to be disclosed for 0.12% CHX⊗HYL mouthwash. Regarding antiplaque and antigingivitis activities, HYL seemed to be ineffective.

7. **As autologous cell hyaluronic acid graft gingival augmentation in mucogingival surgery.**

Prato GP, Rotundo R, Magnani C, Soranzo C, Muzzi L, Cairo F, 2003 [38] evaluated an autologous cell hyaluronic acid graft for gingival augmentation in mucogingival surgery. Seven sites from 6 patients were used in this study. The gingival tissue was processed: keratinocytes and fibroblasts were separated and only fibroblasts were cultivated. They were cultured on a scaffold of fully esterified benzyl ester hyaluronic acid (HA) and returned to the periodontal office under sterile conditions. During the gingival augmentation procedure, the periosteum of the selected teeth was exposed, and the membrane containing cultivated fibroblasts was adapted to and positioned on the site. Three months after surgery, an increased amount of gingiva was obtained, and the histological examination revealed a fully keratinized tissue on all the treated sites. Thus, they concluded that tissue engineering technology using an autologous cell hyaluronic acid graft was applied in gingival augmentation procedures and provides an increase of gingiva in a very short time without any discomfort for the patient.

8. **As a carrier for newer molecules in various regenerative procedures**.[40]

Ballini A, Cantore S, Capodiferro S, Grassi FR, 2009 [39] studied the osteoinductive effect of the hyaluronic acid (HA) by using an esterified low-molecular HA preparation (EHA) as a coadjuvant in the grafting processes to produce bone-like tissue in the presence of employing autologous bone obtained from intra-oral sites, to treat infra-bone defects without

covering membrane. 9 patients with periodontal defects treated by EHA and autologous grafting with a mean depth of 8.3 mm of the infra-bone defects, as revealed by intra-operative probes. Data were obtained at baseline before treatment and after 10 days, and subsequently at 6, 9, and 24 months after treatment. Clinical results showed a mean gain hi clinical attachment (gCAL) of 2.6mm of the treated sites, confirmed by radiographic evaluation. Results suggested that autologous bone combined with EHA seems to have good capabilities in accelerating new bone formation in the infra-bone defects.

STUDIES RELATED TO INTERDENTAL PAPILLA LOSS

Animal study

Pi S, Choi YJ, Hwang S, Lee DW, Yook JI, Kim KH, Chung CJ, 2017[40] evaluated the Loss of interdental papilla height and calculated using standardized serial photographs, micro-computed tomography, and histologic sections. Afterward, HA fillers or phosphate-buffered saline (PBS) was locally injected, and changes in the interdental papilla were evaluated. The results of the study after 7 days of space opening, the margin of the interdental papilla between the mandibular incisors gradually became irregular and flat, indicating a condition similar to the open gingival embrasure. Local injection of HA filler induced an augmentation effect of the interdental papilla compared with injection of PBS. Interdental papilla became convex, and inner granules containing HA were detected within the submucosal layer after its injection. Thus, the study concluded that the local injection of HA filler was validated as a meaningful minimally invasive procedure to improve open gingival embrasure.

Human study

Duranti F, Salti G, Bovani B, Calandra M, Rosati ML,1998[41] evaluated clinically and histologically safety and efficacy of a cross-linked stabilized non-animal hyaluronic acid gel (Restylane, Q-Med, Uppsala, Sweden) to determine its characteristics, advantages,

disadvantages, and side-effects. 158 patients were treated with facial intradermal implant of hyaluronic acid gel for augmentation therapy of wrinkles and folds, and for lip augmentation and/or recontouring. The results were evaluated in all patients at time 0 and after 1, 2, 4 and 8 months from the procedure. In addition, a smaller histological study was carried out in five volunteer patients for a term of 52 weeks to determine the interaction and duration of the material in human healthy skin. Clinically, both the physicians' and patients' evaluations revealed very satisfactory results, with a global 78.5% and 73.4% respectively of moderate or marked improvement after eight months, independent of the treated area. The photographic evaluation revealed even better results with a 80.4% of moderate or marked improvement after 8 months. The safety evaluation showed a 12.5% of postoperative immediate adverse events, that were localized and transient. They concluded that hyaluronic acid gel presents several advantages in comparison to previously used injectable biomaterials and expands the arsenal of therapeutic tools in the field of soft tissue augmentation.

William Becker, IldorGabitov, Misha Stepano, John Kois, Ami Smidt, Burton E. Becker, 2009[6]conducted a pilot study to evaluate the use of a commercially available hyaluronic acid gel for reducing or eliminating small papillary deficiencies around the implant and the tooth. Eleven patients, seven females and four males, with an average age of 55.8 years (ranging from 25 to 75 years) with 14 treated sites were included in the study. Patients were followed from 6 to 25 months after initial gel application. Results showed that three implant sites and one site adjacent to a tooth had 100% improvement between treatment examinations. Seven sites improved from 94 to 97%,three sites improved from 76 to 88%,and one site adjacent to an implant had 57% improvement. They concluded that the use of an injectable hyaluronic gel to enhance papillary esthetics after implant treatment should be evaluated in a controlled clinical study.

S. Sadat Mansouri, M. Ghasemi, Z. Salmani, N. Shams, 2013[7]conducted a experimental study in Department of Periodontics, Islamic Azad University, School of

Dentistry, to evaluate the clinical application of hyaluronic acid gel for reconstruction of interdental papilla at the esthetic zone.11 patients with 21 interdental papilla deficiencies were included. In this study the procedure was repeated 3 weeks and 3 months later for all the respective areas. The results suggested that in the second follow up, 10% of subjects showed improvement in interdental papilla reconstruction by 50%. In the third follow up (at 6 months) 43% of samples showed 50% improvement or higher, thereby suggesting that application of hyaluronic acid gel was effective for interdental papilla reconstruction and may be used as a non-invasive technique for reconstruction of interdental papilla.

Fatin A. Awartani, Dimitris N. Tatakis, 2015 [8] conducted a prospective clinical trial to examine the clinical and patient outcomes following aesthetic reconstruction of interdental papilla loss in anterior teeth, using anhyaluronic acid gel. Ten systemically healthy adults, with at least one anterior site with class I or II interdental papilla loss were included. Seventeen sites (13 maxillary, 4 mandibular) were treated in 9 females. The injection was repeated twice 21 days later. Patients were seen monthly for follow-up. The result suggests that the changes from baseline to 4 and 6 months represent an average 62 and 41% reduction in black triangle area, respectively. At 4 months, 13 sites had ≥50% reduction in black triangle area with 2 of these sites having complete papilla fill, while at 6 months the corresponding numbers were 8 and 3 sites.

Won-pyo Lee, Hee-jung Kim, Sang-joun Yu, Byunk-ock Kim, 2016[4]conducted a six month clinical evaluation to clinically assess the efficiency of interdental papilla reconstruction with injectable hyaluronic acid gel. 10 patients with 43 sites in maxillary anterior region were included and the hyaluronic acid gel injection was repeated 5 times during 3 week intervals. Patients were followed 6 months after initial gel application. The result of the study was that the 29 sites had complete papilla reconstruction and 14 sites improved from 39-96% of interdental papilla reconstruction rate. The study demonstrated that

interdental papilla reconstruction using an injectable hyaluronic acid gel can be a viable treatment option for interdental papilla deficiencies in small areas.

Lee WP, Seo YS, Kim HJ, Yu SJ, Kim BO, 2016[42] evaluated the clinical efficacy of enhancing deficient interdental papilla with hyaluronic acid gel injection by assessing the radiographic anatomical factors affecting the reconstruction of the interdental papilla. Fifty-seven treated sites from 13 patients (6 males and 7 females) were included. Patients were followed up for 6 months after the initial gel application. The results showed improvement in all sites. Thirty-six sites had complete interdental papilla reconstruction and 21 sites showed improvement ranging from 19% to 96%. The results concluded that the contact point and the bone crest is closely related to the efficacy of hyaluronic acid gel injection for interdental papilla reconstruction.

TanwarJ, Hungund SA , 2016[43] evaluated the reconstruction of the lost interdental papilla by injecting 0.2% hyaluronic acid via nonsurgical approach in the Department of Periodontics, Darshan Dental College and Hospital, Udaipur, Rajasthan, India. The patient was followed-up for 3 weeks and was recalled for a total of 4 times. This study indicated the possible improvements in regenerating lost interdental papilla and removal of black triangle by injecting HA into the lost papilla using a nonsurgical approach.

Mueller A, Fujioka-Kobayashi M, Mueller HD, Lussi A, Sculean A, Schmidlin PR, Miron RJ, 2017[44], examined morphological changes of dentin surfaces following HA coating and thereafter investigate the influence of periodontal ligament (PDL) cell survival, attachment, and spreading to dentin discs. HA was coated onto dentin discs utilizing either non-cross-linked (HA) or cross-linked (HA cl) delivery systems. Morphological changes to dentin discs were then assessed using scanning electron microscopy (SEM). Thereafter, human PDL cells were seeded under three in vitro conditions including (1) dilution of HA (1:100), (2) dilution of HA (1:10), and (3) HA coated directly to dentin discs. Samples were then investigated for PDL cell survival, attachment, and spreading using a live/dead assay,

cell adhesion assay, and SEM imaging, respectively. The results showed that PDL cells were seeded on control and HA coated dentin discs and demonstrated a near 100 % survival rate for all samples demonstrating high biocompatibility of HA at dilutions of both 1:100 and 1:10. Interestingly, non-cross-linked HA significantly increased cell numbers at 8 h, whereas cross-linked HA improved cell spreading as qualitatively assessed by SEM. The results from the present study concluded that both carrier systems for HA were extremely biocompatible and demonstrated either improved cell numbers or cell spreading onto dentin discs. Future in vitro and animal research is necessary to further characterize the optimal delivery system of HA for improved clinical use.

Naorungroj S, 2017[45] presented a case report of a comprehensive esthetic treatment with adhesive tooth-colored restorations in a combination with hyaluronic acid (HA) fillers of diastema in an orthodontic patient with relapse. A 36 year old female patient consulted about 1.5–2mm midline diastema after an orthodontic relapse of replacing missing central incisors with lateral incisors and dark colored gingival tissue as a result of a metal post and core with porcelain fused to a metal (PFM) crown at the left lateral incisor. Restorative treatments included replacing the PFM with all-ceramic material and placing a ceramic veneer on the right lateral incisor. A HA fillers injection was used to fill the remaining open gingival embrasure. Eighteen months after treatment, the interdental papilla remained stable and the patient was satisfied with the result. They concluded that esthetic reconstruction of diastema and open gingival embrasure in this case can be accomplished without orthodontic retreatment. Tooth-colored restorations and HA filler injection appear as a promising modality to address this patient's esthetic concern.

Corte SD et al., 2017[46] presented a clinical case involving interdental papilla reconstruction with HA infiltration. HA infiltration was undertaken into the papilla, every seven days for four weeks. Results obtained in this clinical case using HA to regenerate papilla were favorable. it was observed that papilla covered all the space found underneath the

interproximal contact point, and was found at the same height as adjacent papillae. Thus, they concluded that it would be suitable to further it taking into consideration different factors; conducting them in greater-sized populations with subjects of different ethnicities and gender, and using different infiltration intervals.

Ahila E, Saravana Kumar R, Reddy VK, Pratebha B, Jananni M, Priyadharshini V, 2018[47] evaluated the augmentation of interdental papilla with platelet-rich fibrin. A total of 25 sites from systemically healthy individuals with papillary recession (Nordland and Tarnow class 1 and 2) were recruited in the study. Han and Takei procedure was planned and augmentation was done with platelet-rich fibrin, which were measured at baseline, 3 and 6 months postoperatively. Healing index was measured at the 1st, 2nd, and 3rd week postoperatively. The result of the study showed that mean value of distance from the contact point to the gingival margin was 4.38 mm at baseline and at 6-month postoperatively, it reduced to 0.36 mm. There was an increase in width of the keratinized gingiva which was clinically and statistically significant. The study concludes that the augmentation of the papilla using PRF in the new position was stable when reviewed at 3 and 6 months postoperatively. The use of PRF achieved successful and predictable results in the management of papillary recession.

Armamentarium

- Mouth Mirror.
- Kidney tray.
- Disposable surgical gloves.
- Disposable mouth mask.
- Head cap.
- Cotton Gauze.
- Tweezer.
- Impression trays (Maxillary and Mandibular).
- Alginate Impression Material.
- Dental Stone.
- Piezoelectric Ultrasonic scaler.
- Local anaesthesia (2% Lignocaine hydrochloride with adrenaline)(1:80000).
- Hyaluronic acid gel injection (hyaDENT BG, Bioscience, Germany)(contains Hyaluronic acid (2.0mg), Hyaluronic acid cross-linked (16.0mg), Sodium chloride (6.9mg), water for injection (1.0ml) with Self-aspirating syringe with 27 Gauge needle.
- Digital camera with tripod stand (Canon,EOS1100Dspecifications)(12.2Megapixels).
- Image analysis system (Adobe Creative Cloud).

Source of data

- Clinical examination was carried out on patients reporting to Department of Periodontology, School of Dental Sciences, Sharda University, Greater Noida.
- The nature and outcome of the study was explained to the patients following which a verbal and written consent was obtained.
- The study was approved by the Institutional Ethics Committee.

Method of collection of Data

- A total of 12 subjects both male and female were included in the study.
- These subjects were selected based on the fulfilment of inclusion and exclusion criteria.

Inclusion criteria

- Systemically healthy Adults (≥18years old).
- Full mouth Gingival index (Loe and Silness,1963) score ≤1
- Full mouth Plaque index (Silness and Loe,1964) score ≤1
- Atleast one maxillary or mandibular anterior interdental space exhibiting class I or II or III interdental papilla loss using Nordland and Tarnow classificationsystem (1998)[9], where:-

Class I: The tip of the interdental papilla is located between the contact point and the most coronal extent of the interproximal cemento-enamel junction (CEJ).

Class II: The crest of the papilla is at or apical to the interproximal CEJ but coronal to the facial CEJ.

Class III: Crest of the papilla is at or apical to the facial CEJ.

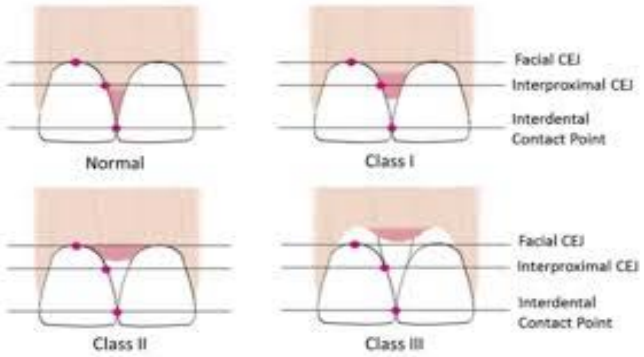


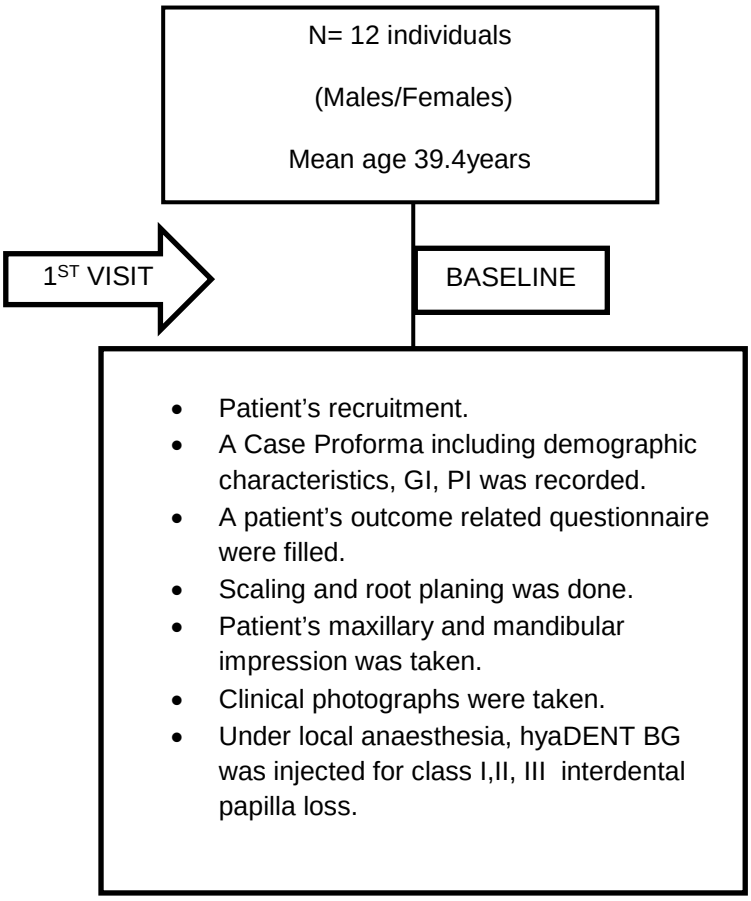
Fig 11. Nordland and Tarnow classification system for interdental papilla loss

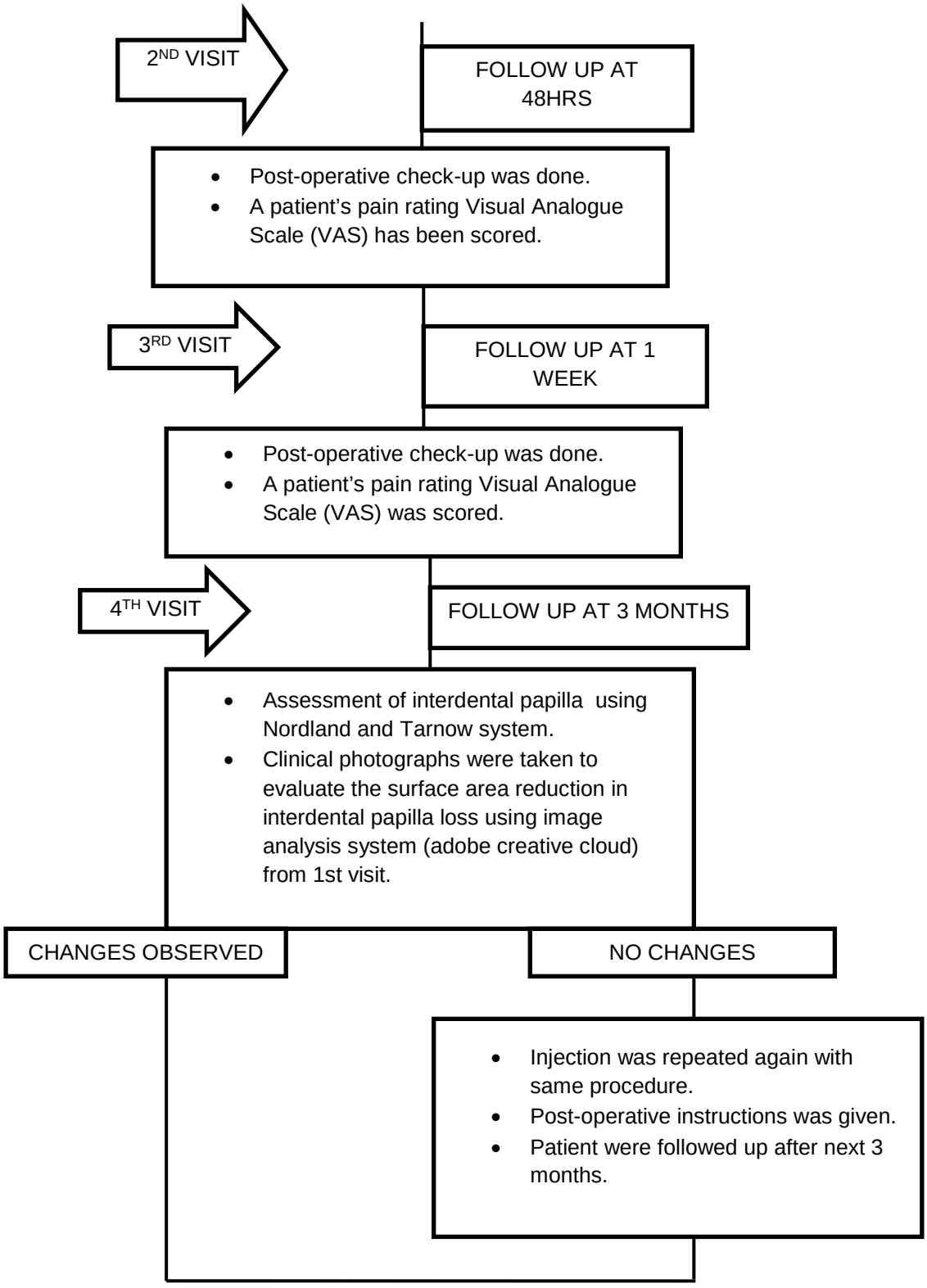
Exclusion criteria

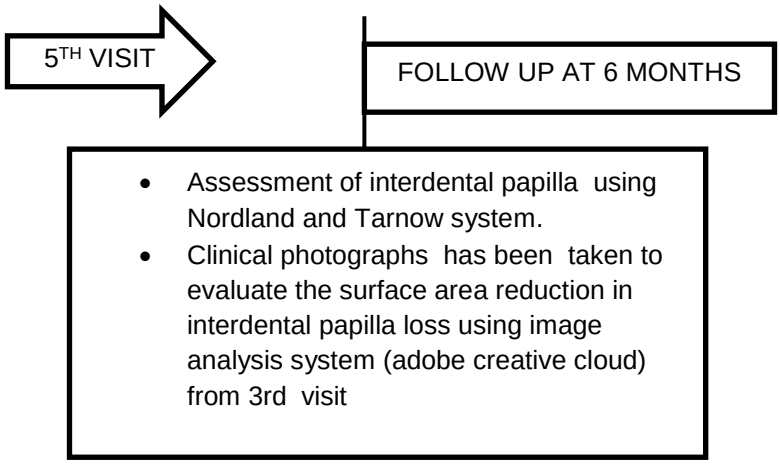
- Any known history of allergic reaction to injectable filler.
- Smokers and tobacco in any form.
- Pregnancy and lactation.
- Medications known to increase gingival hyperplasia.
- Periodontal surgery in the last 12 months on study teeth.
- Carious lesions or fixed restorations on study teeth.
- Patient not willing to give written informed consent form.

STUDY DESIGN

The proposed study was a prospective case series.




2ND VISIT
FOLLOW UP AT 48HRS
Post-operative check-up was done.
A patient's pain rating Visual Analogue Scale (VAS) has been scored.
3RD VISIT
FOLLOW UP AT 1 WEEK
Post-operative check-up was done.
A patient's pain rating Visual Analogue Scale (VAS) was scored.
4TH VISIT
FOLLOW UP AT 3 MONTHS
Assessment of interdental papilla using Nordland and Tarnow system.
Clinical photographs were taken to evaluate the surface area reduction in interdental papilla loss using image analysis system (adobe creative cloud) from 1st visit.
CHANGES OBSERVED
NO CHANGES
Injection was repeated again with same procedure.
Post-operative instructions was given.
Patient were followed up after next 3 months.



Duration of the study

The duration of the study was 1 year 5 months and was conducted between June 2017 to October 2018

METHODOLOGY

1st VISIT (BASELINE)

- Patients were thoroughly informed about the method of conduction of study and a written informed consent was obtained from them.
- A case Proforma was filled including demographic characteristics and confirmation of inclusion criteria with Gingival index (Loe and Silness, 1963) and Plaque index (Silness and Loe, 1964).
- A patient's outcome related questionnaire was filled.
- Scaling and Root Planing was done.
- Patient's clinical photographs were taken with digital camera (with resolution 12.2 megapixel) of study teeth with the patients sitting upright, looking directly ahead (Frankfort plane parallel to the ground), and the camera were held with the lens axis horizontal (parallel to the ground).

- Patient's impressions (maxillary and mandibular) were made to prepare stone cast for future reference.
- Under local anaesthesia following steps were performed:-

For Class I and Class II interdental papilla loss treatment

- hyaDENT BG were injected directly into the middle of the papilla, 3–4 mm apical to the tip of the papilla, using a -27 gauge needle.
- Gentle massage of the area for 1 min was done.
- Droplets of hyaDENT BG were applied topically to the injected papilla to enhance the healing environment.

For Class III interdental papilla loss treatment

- Multiple (3–4) droplets of hyaDENT BG with 27 gauge needle 3-4 mm below the papilla using the three-step technique were injected.

  Step 1: Injection of hyaDENT BG into the marginal gingiva.

  Step 2:Injection into the attached gingiva

  Step 3:Injection 2 mm below the highest point of the papilla to stabilize the papilla itself.
- Gentle massage with the droplets was done for 1 min.
- One or more droplets of hyaDENT BG were topically applied to the injected area to enhance the healing environment topically.

- Postoperative instructions were given to the patients which are as follows:
- 24hrs abstinence from mechanical plaque control in the area.
- The use of soft toothbrush after the first 24 hrs.
- Resumption of routine mechanical oral hygiene after 2weeks.

2nd VISIT(AT 48 HRS)

- Post-operative check-up was done.
- A patient's pain rating Visual Analogue Scale (VAS) was scored.

3rd VISIT(AT 1 WEEK)

- Post-operative check-up was done.
- A patient's pain rating Visual Analogue Scale (VAS) was scored.

4th VISIT (AT 3 MONTHS)

- Patient's clinical photographs were taken with same digital camera under same lightening conditions of study teeth (frontal view with Frankfort plane).
- If changes were seen clinically in the interdental papilla loss then patient was followed up for next visit after next 3 months.
- If no changes were observed then hyaDENT injection was injected again with the same procedure mentioned above and patients were followed up after next 3months.
- Post-operative instructions were given.
- A patient's outcome related questionnaire was filled including Visual Analogue Scale (VAS), post treatment procedures

5thVISIT (AT 6 MONTHS)

- Patient's clinical photographs were taken with same digital camera under same lightening conditions of study teeth (frontal view with Frankfort plane) to see the changes in the interdental papilla loss.
- A patient's outcome related questionnaire was filled.

PHOTOGRAPHIC EVALUATION

- Clinical photographs were used to measure the surface area of lost papilla using image analysis software (Adobe creative cloud).
- Area of interest were calculated at baseline, 3months and 6months which were calculated by measuring the surface area of visible black triangle using the formula-

  Area=0.5 x height (mm) x base (mm)

  And percentage reduction in black triangle by (at 3,6 months)-

  (baseline-postoperative area) x 100/baseline area

MEASUREMENT OF CLINICAL PARAMETERS

- Interdental papilla loss assessment by Nordland and Tarnow system (1998).
- Gingival index (GI) of Loe and Silness (1963).
- Plaque index (PI) of Silness and Loe (1964).

For Full mouth Gingival Index (GI) (Loe H and Silness, 1963) was recorded on four sites per tooth with the following criteria :-

0=Normal gingiva

1=Mild inflammation, slight color change, slight edema, no bleeding on palpation

2=Moderate inflammation, redness, edema, bleeding on probing

3=Severe inflammation, marked redness & edema, tendency to spontaneous bleeding.

For Full mouth Plaque Index (PI) (Silness &Loe, 1964) were recorded on four sites of each tooth with the following criteria :-

0=No plaque at gingival area

1=A film of plaque on gingival margin and/or adjacent tooth surface, recognized only by running a probe across tooth surface.

2=Moderately soft deposits at margin and/or adjacent tooth surface that can be seen by naked eye.

3=Abundant soft matter at margin and adjoining surface.

Calculation of Sample size

The sample size was calculated using the Open epi Version 3 software. The sample size was calculated using the difference in Interdental papilla loss (black triangle area; in mm^2) before and after intervention. The mean difference was found to be 0.52±0.58.

The power of the study was taken to be 80% and Confidence Interval (C.I.) of 95% was taken. The sample size was calculated to be 10.

The sample size calculation was as follows:

The standard normal deviate for $\alpha = Z_\alpha = 1.960$
The standard normal deviate for $\beta = Z_\beta = 0.842$
$A = 1.000$
$B = (Z_\alpha+Z_\beta)^2 = 7.849$
$C = (E/S(\Delta))^2 = 0.816$
$AB/C = 9.62$

Infection control

Several sets of instruments such as self-aspirating syringe, mouth mirror and a tweezer, ultrasonic tip of the scaler were packed in separate sterilization pouches each and autoclaved. These autoclaved instruments in the sterilization pouches were used by the examiner for assessment and treatment of the interdental papilla loss of the patients. One set each of a disposable syringe, disposable mask, disposable head cap and disposable examination gloves were also used for each individual for adequate infection control.

Statistical Analysis

Descriptive statistics was performed by calculating mean and standard deviation for the continuous variables. Categorical variables are presented as absolute numbers and percentage. Nominal categorical data between the groups were compared using chi-square goodness-to-fit test.

The software used for the statistical analysis were **SPSS (statistical package for social sciences) version 21.0 and Epi-info version 3.0**.

The statistical tests used were:

- **One-way ANOVA (Analysis of Variance) test** for comparison of difference between mean values of more than 2 groups when the data follows normal distribution.
- **Post-hoc tests** are run to confirm where the differences occurred between groups, or they are used for the inter-group comparisons. Post hoc tests attempt to control the experimentwise error rate (usually alpha = 0.05) in the same manner that the one-way ANOVA is used instead of multiple t-tests.
- **Unpaired or Independent t-test** is used for comparison of mean value between 2 groups when the data follows normal distribution.
- **Paired or Dependent t-test** was used for comparison of 2 mean values obtained from a same group or a pair of values obtained from the same sample when the data follows normal distribution.
- **Chi-square test** is used to investigate whether distributions of categorical variables differ from one another.
- The **p-value** was taken significant when less than 0.05 **($p<0.05$)** and Confidence interval of 95% was taken.

1. Armamentarium

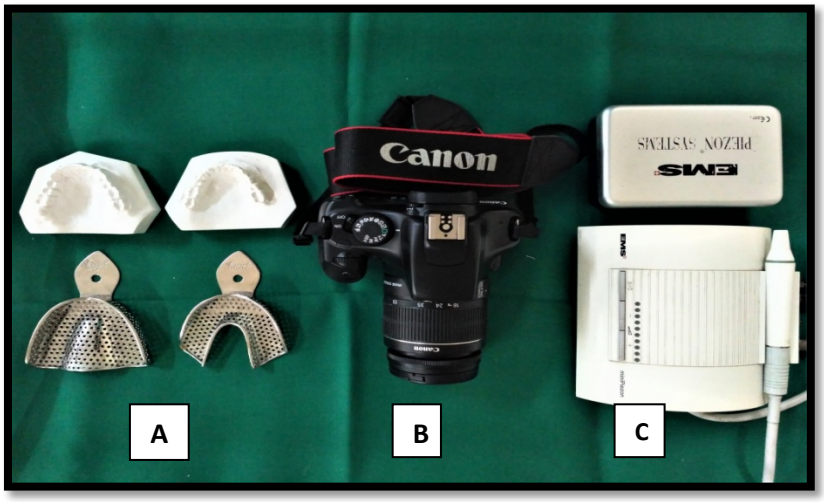


Fig 1(a): (A) Impression trays & stone cast (Maxillary and Mandibular), (B) Digital camera, (C) Piezoelectric Ultrasonic scaler, (from left to right)

Fig 1(b): (A) Mouth Mirror & Tweezer in sterile pouch, (B) Hyaluronic acid gel injection (hyaDENT BG, Bioscience, Germany) with Self-aspirating syringe with 27 Gauge needle, (C) Disposable mouth mask & Head cap, (D) Kidney tray with Cotton & Gauze, (E) Local anaesthesia (F) Disposable surgical gloves (from left to right)

2. Technique for hyaluronic acid injection

Fig 2 (a) Injection technique for class I and class II

Fig 2 (b) Injection technique for class III

Step 1: Injection of hyaDENT BG into the marginal gingiva.

Fig 2 (c) Injection technique for class III

Step 2:Injection into the attached gingival

Fig 2 (d) Injection technique for class III

Step 3:Injection 2 mm below the highest point of the papilla to stabilize the papilla itself.

3. Class I interdental papilla loss

Fig 3 (a) Baseline

Fig 3 (b) 3 months

Fig 3 (c) 6 months

4. Class II interdental papilla loss

Fig 4 (a) Baseline

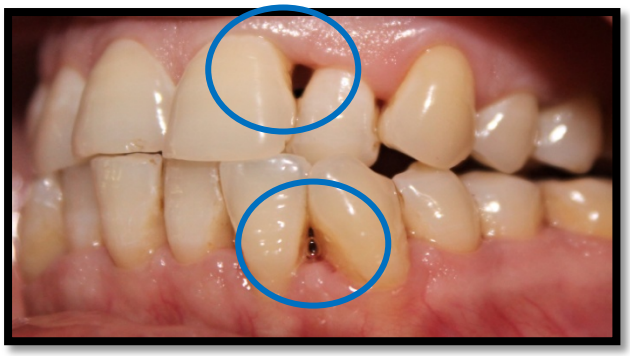


Fig 4 (b) 3 months

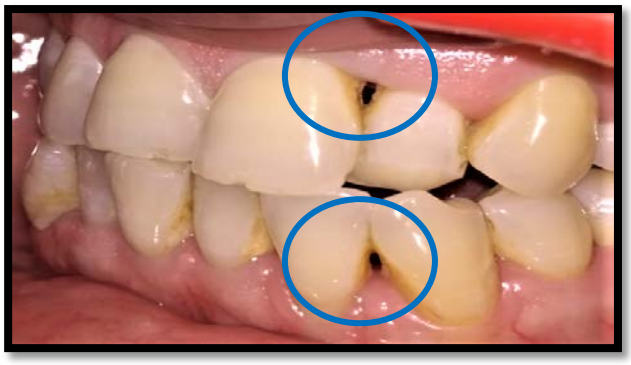


Fig 4 (c) 6 months

5. Class III interdental papilla loss

Fig 5 (a) Baseline

Fig 5 (b) 3 months

Fig 5 (c) 6 months