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Review Article

RECENT ADVANCEMENT IN PERIODONTICS

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ABSTRACT
Curiosity is the mother of invention; the more curious work more to develop and evolve giving rise to new science and technology. How can Periodontology still untouched by these changes? From Neuman flap Microsurgery, from clinical detection of disease in the oral cavity to molecular basis of detection and so on. There are numerous changes that have been introduced since earliest times to date. These changes are opening a new paradigm in understanding the disease and in its treatment. In this article, we are trying to enumerate as many changes that have been introduced in the field of Periodontology in the last few decades.

KEY WORDS: Curiosity, Neuman flap, Microsurgery, Periodontology.

INTRODUCTION

Periodontal disease has been the biggest problem in our society since ages, its incidence is increasing these days because of the changes in eating habits and oral hygiene practices in the population. Earlier the diagnosis was made using probes and clinical signs of the disease along with radiographs. Nowadays there are numerous advancements introduced in the probing system which could help in identifying the exact measurements of pocket depth and clinical attachment loss. In accordance with the advancement in the probing system, there are various radiographic techniques developed which provide the three-dimensional view of the defect present in periodontal disease which helps in better treatment planning and hence treatment of the disease. We could now identify the individual susceptible to the disease way before the disease expresses itself and could also vaccinate the individual for the same. However, the gold standard for treating periodontal disease has been scaling and root planing but since last few decades newer methods in adjunct with scaling and root planing has been introduced which are not just eliminating the disease but also providing regeneration of the lost periodontal tissue, instead of just repair as was achieved by flap surgeries, for normal functioning of the masticatory apparatus. The various advances introduced in diagnosis and treatment of periodontal disease are enumerated under following headings.

1. The newer generation of probing systems
2. Biomarkers for the diagnosis of the disease
3. Vaccine for the disease
4. Probiotics in the treatment of periodontal disease
5. Lasers in the treatment of periodontal disease
6. Microsurgery for periodontal treatment
7. Photodynamic therapy in the treatment of periodontal disease
8. Tissue engineering in treatment of periodontal disease
9. Nanotechnology in treatment of periodontal disease
10. Newer drugs developed for treating periodontal disease
11. Ozone therapy in periodontology
12. New classification system for periodontal disease.

Newer generation of probing system

There are five generations of the probe that has been introduced in the field of Periodontology. The first generation includes the conventional probe like William's probe which was introduced by Charles H.M. Williams in 1936. These have a thin, blunt tip at the end of diameter 1 mm and length of 13 mm and markings 1, 2, 3, 5, 7, 8, 9, 10 mm.

Then was introduced the second generation probe which was pressure sensitive by Hunter in 1994. These probes have a disposable hemispheric tip of 0.5mm diameter with a visual guide used sliding scale and two indicator lines that meet at a specified pressure. van der Velden and de Vies introduced a pressure sensing probe and with a cylinder and piston attached to the air pressure system. In 1980 Polson introduced an electronic pressure sensitive probe which allowed for controlled insertion pressure.

Then came the third generation probes to answer the limitations of the second generation probes. Foster-Miller probe is the standard probe for this generation introduced by Jeffcoat *et al*. in 1986 with controlled probing pressure and automated detection of the cement enamel junction. It has the following components a pneumatic cylinder, linear variable differential transducer (LVDT), force transducer, accelerator, and the probe tip. This probe can automatically detect the position of the cementenamel junction with controlled acceleration and pressure, the abrupt pressure changes are seen only when the tip of the probe reaches the cementoenamel junction and base of the pocket.

The fourth generation probe is still under construction this probe aims at recording the sequential probe position with gingival sulcus to provide a 3D image of the defect site.

Then is the fifth generation probe these probes are designed to provide a 3D image and are being non-invasive. The Ultra Sonographic probe uses the ultrasonic wave to detect, image and map the upper boundary of the periodontal ligament to mark the periodontal disease.

There are further research going on to provide even better tool for periodontal disease diagnosis this includes Optical Coherence Tomography (OCT) introduced by Huang et al in 1991 works on principle of coherence near infrared region. It gives real-time 3D tomographic image with resolution of 5-15µm and penetration depth of 1-2mm. Mota *et al*. used two OCT systems with 930 and 1325nm wavelength operating in Fourier domain to do the structural analysis of periodontal tissue in jaws of porcine and concluded that the operating system with 1325nm wavelength had better performance than 930nm.

Fernandes *et al*. used OCT technology to measure gingival sulcus depth of anterior teeth at three sites (total 445 sites) in healthy individuals and compared it with North Carolina manual probe (UNC-15) and Florida automated probe. Results showed that sulcus depth values obtained by OCT were significantly lower as compared to North Carolina manual probe and Florida automated probe where as usage of OCT was non-invasive and produced no pain and discomfort compared to other two.

Kakizaki *et al.* showed in his study on human periodontally healthy subjects that gingival thickness and biological width can be measured using OTC. However due to the high scattering property of light in deeper tissues which is used in OCT this new modality need reformations for more accurate measurements of periodontal pocket.

Other technology like Endoscopic capillaroscopy that images microcirculation of periodontal pocket. Townsend & D'Aiuto showed the usage of fiber-optic probes in visualizing directly into the periodontal pocket wall and through its microcirculation and measuring the changes in number and diameter of blood vessels connected with periodontal disease. The root of the system is made up of a fiber-optic image probe of 950 µm that is inserted in the gingival sulcus or periodontal pocket. 520 nm wavelength gree light is used for illumination that is absorbed by both oxygenated and deoxygenated blood. Thus, blood vessels with red blood cells will appear dark against the green background. From this study the Authors concluded that the combination of capillaroscopy and optical fiber technology could produce high-resolution imaging of the periodontal pocket microcirculation.

Another technology is the Photoacoustic imaging. Photoacoustic (PA) imaging is a combination of the high contrast of optical imaging with the high resolution of ultrasound imaging. This imaging technique is based on the PA effect, first appreciated by Alexander G Bell in 1880. In PA imaging, there is an absorption of optical energy by either an endogenous chromophore such as hemoglobin, melanin, etc, or exogenous contrast agents such as organic dyes, this gives rise to thermoplastic expansion and thus leading to generation of acoustic (ultrasound) waves. These ultrasound waves can be identified and changed to electric signals that are then processed for imaging. Lin *et al.* in his study showed the usage of PA imaging for measuring periodontal pocket depth in porcine jaw model by artificially creating pocket with the help of a scalpel. Then to these artificial pockets were added food-grade cuttlefish ink as a contrast medium and a comparison was made between PA pocket depth measurement and gold standard periodontal probing method, results showed that PA imaging technique could visualize the periodontal pocket with 0.01 mm precision and hence suggested that PA imaging technique can be used for periodontal pocket imaging and measurement as a diagnostic tool with the added advantage of non-invasive nature of modality.

Diamond probe is another technology with additional benefit of measuring volatile sulfur content. It is plastic instrument with black bands for measurement of pockets apart from that it also measures the volatile sulfur content within the sulcus thus predicting the disease site for the clinician.

Biomarkers in detection of periodontal disease

Since long periodontal disease detection is being carried out using periodontal probes and radiograph but now it can be detected even using biomarkers at an early stage before the disease has produced optimal destruction to the periodontium. In 1988, the National Institutes of Health Biomarkers Definitions Working Group defined Biomarkers as the characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to therapeutic intervention. The term "biomarker" was given, in 1980. The term "biological marker" was introduced in 1950s. Various media are being used for procuring biomarkers these media are urine, saliva, serum, gingival crevicular fluid. The various biomarkers can be classified under following headings proteomic biomarkers, genetic biomarkers, microbial biomarkers, and others biomarkers. Biomarkers like alkaline phosphatase, Aminopeptidase, Lactoferrin, Translactoferin, IgM, MMP-13, MMP-8, MMP-9 are proteomic biomarker; IL-1 polymorphisms, IL-10 polymorphisms, Tumor necrosis factor, Polymorphisms are genetic biomarkers; Aggregatibacter actinomycete mcomitans, Campylobacter rectus, Mycoplasmas, Porphyromonas gingivalis, Prevotella intermedia, Peptostreptococcus are microbial biomarkers; and Calcium, Cortisol, Hydrogen sulfide, Methyl mercaptan, Pyridine are other biomarkers.

The various chair side kit available for detection of biomarkers for periodontal disease are BANA (N-benzoyl-DL-arginine-2) periodontal test (detects bacterial trypsin like proteases), Periocheck (detects neutral proteinases like collagenase), Perioscan (detects activity of bacteria like *A. actinomycetemcomitans*, *T. forsythus*, *P. gingivalis*), Evalusite (detects antigen of *A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis* with the help of antibodies), Prognostic (detects serine proteinases and elastases), Biolise (detects elastase), Periogard (detects aspartate aminotransferase, TOPAS (Toxic oral pathology assay - used for detection of toxins derived from anaerobic

metabolism and measures protein levels in gingival crevicular fluid).

Biosensors these are devices which detect and measure chemical and biological reactions by generating signals when it comes in contact with the analyte. These devices are now being used to detect biomarkers for periodontal and peri-implant diseases. In 2016 Mohseni et al. used Carboxymethyldextran hydrogel sensor chip with immobilized monoclonal MMP-9 antibodies to detect Matrix metalloproteinases (MMP-9) to diagnose chronic periodontal disease. In 2017 Ritzer et al**.** used Diagnostic chewing gum in his study to detect Matrix metallo-proteinases (MMP-1, MMP-8, MMP-9) for diagnosis of peri-implant diseases. All these authors suggested further clinical trials for the usage of biosensors in wider population.

Nowadays technologies like lab-on-a-chip (LOC) are being used as point-of-care testing device to detect various biomarkers for periodontal disease. LOC work on the principle of immunoassay for example, Christodoulides *et al***.** developed a LOC platform that uses a microfluidic chip with a fluorescence-based optical system to quantify three salivary biomarkers (MMP-8, IL-1b, and C-reactive protein) for periodontitis diagnosis. The results were compared with the results obtained using standard ELISA testing. Comparison stated that LOC device can achieve a sensitivity of 20ng/ml for MMP-8 and 10pg/ml for IL-1b and C-reactive protein. Another immunoassay-based LOC device, called the integrated microfluidic platform for oral diagnostics (IMPOD), was developed to detect various salivary proteins in a small sample volume (10 ml) but with high sensitivity. Recently, for rapid detection of periodontal pathogens a PCR chip has also been devised. The device consists of two components: a microfluidic cartridge containing all the PCR reagents and a component that is used to drive and control the working process. The basic elements of the drive consist of a rotary PCR chip and a thermo cycling device which is forned by six fan-shaped heating blocks, three for achieving denaturation, annealing, and elongation, and others three for rapidly changing the temperature inside the blocks with samples and PCR reagents are mixed in a chip during the detection process, which then rotates on the thermo circulator to conduct PCR. In every cycle, a fluorescence detector quantifies the fluorescence signal at 72 to detect the amplification. This device can measure different bacterial strains in clinical sample.

Vaccine for periodontal disease

Vaccination induces immunity by forming antibodies against the injected dead or attenuated. The concept of vaccination for periodontal disease came from the fact that it's bacterial in origin along with other factors. The theory of vaccination was introduced by Edward Jenner in 1798. Vaccins work on principle of active immunization (in which entire bacterial cell or it's subunit or synthetic peptides are introduced in host as antigen), passive immunization (in which murine monoclonal antibodies, plantibodies are introduced in host for immunization) and genetic immunization (plasmid vaccine, live and viral vector vaccine are introduced for immunization).

In the 20th century, the periodontal vaccine came into the picture with Vancott's vaccine and Inava endocarp vaccine. The bacterias which are mainly responsible for periodontal disease are *P. gingivalis*, Aggregatibacter. actinomycetemcomitans and *T. forsythia*. These vaccines have the potential to prevent from these pathogenic bacteria of periodontitis and also help greatly in enhancing the quality of life for people who cannot afford periodontal treatment. There are still researches going on to find an antigenic component from various organisms to decrease the load of subgingival microflora.

Huang *et al*. in his study used cell-free protein synthesis (CFPS) to produce vaccinable targets suitable for testing in a *P. gingivalis*-induced murine oral bone loss model. The protein was generated using Recombinant *P. gingivalis* minor fimbriae protein (Mfa1), RgpA gingipain hemagglutinin domain 1 (HA1), and RgpA gingipain hemagglutinin domain 2 (HA2) combination in equivalent doses in adjuvants and injected intramuscularly to immunize mice. Then Serum levels of protein-specific antibody were detected by ELISA, and oral bone levels were defined by morphometrics. Recombinantly generated *P. gingivalis* proteins possessed high accuracy and induced protein-specific IgG formation following immunization. Importantly, immunization with the vaccine cocktail protected from *P. gingivalis* elicited oral bone loss. Puth *et al*. in their study developed a divalent mucosal vaccine that consists of a combination of FlaB-tFomA and Hgp44-FlaB fusion proteins targeting virulence factors of bacteria *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, respectively. They Introduced a peptide linker between FlaB and antigen which improved the stability and immunogenicity of engineered vaccine antigen. Results showed that intranasal immunization with divalent vaccine induced protective immune responses inhibiting alveolar bone loss initiated by *F. nucleatum* and *P. gingivalis* infection.

Probiotics in the treatment of periodontitis

Elie Metchnikoff Ukrainian Nobel prize laureate at the beginning of 20th century discovered some beneficial bacteria that have a good effect on health and suggested that these bacteria can be used to reduce the number of harmful bacteria in human body. Probiotics can be defined as "Live microorganisms that once administered in adequate amounts confer a health advantage on the host" (Guarner *et al*., 2005).

The mechanism of action for the Probiotics are as follows; 1) it either compete for space and aggregate, inhibiting adhesion of pathogenic bacteria or inhibiting its growth and other effects on dental plaque ecology, 2) or it competes for nutrients and growth factors by producing antimicrobial compounds like acids thus inhibiting growth of pathogen, 3) or it enhance host immune response by increasing the production of IgA and defensins, or it inhibits the production of pro-inflammatory cytokines thus influencing local and systemic immune response. All these mechanism acts as antagonist against pathogenic bacteria leading to reduction in tissue inflammation and destruction.

Probiotics are available in the form of lozenges, tablets, cheese, yogurt, rinses, capsules and liquid. Various studies

have been done regarding the beneficial effects of probiotics on the patient with generalized chronic periodontitis one such study done by Koll-Klais and co-workers in which they found out that there was a higher prevalence rate for *L. gasseri* and *L. fermentum* in the oral cavity of a healthy individual as compared to patients of chronic periodontitis. In another study done by Riccia and co-workers, to find out the anti-inflammatory role of Lactobacillus brevis in periodontal disease, in the study they used lozenges of *L. brevis* on patients of periodontitis for 4 days and observed a difference in clinical parameters like bleeding on probing, plaque index, gingival index which were markedly reduced. It's a relatively new field and still, a lot of research is required few such studies are mentioned below:

Zupancic *et al.* in their study incorporated autochthonous bacteria a potential probiotics into nanofibers for local treatment. They selected and isolated the strain 25.2.M from the oral microbiota of healthy Volunteers, identified as Bacillus sp. based on 16S rRNA sequence analyses. This strain is non-pathogenic and produces an antimicrobial substance as well as it can grow over the periodontal pathogen Aggregatibacter actinomycete mcomitans in vitro, thus making it a favourable probiotic candidate. The strain 25.2.M was successfully integrated into the nanofibers in the form of spores (107 CFU/mg), the viability of which were good (max. change of 1 log unit) both during the electro spinning and after 12 months of storage. The developed nanodelivery system for administration into periodontal pockets, offers a promising look out for the inhibition of periodontal pathogens with the restoration of the healthy oral microbiota.

Golfre *et al.* in their study used Lactobacillus reuteri Prodentis as a probiotic to treat patients with peri-implant mucositis or periimplantitis who already had periodontitis in combination with non surgical mechanical therapy and found out that there was significant improvement in clinical parameters both in mucositis and periimplantitis around implant.

Lasers in treatment of periodontal disease

The concept of LASER was first given by Albert Einstein. He was the first to describe the stimulated emission of light. Using his theory Maiman an American physicist developed laser with the help of ruby crystal in 1960. The laser was introduced in the field of dentistry by Myers and Myers on 3rd May 1990 after the pioneer work done with laser by Doctor Leon Goldman since 1963. Laser is being used for various purposes in the field of periodontology like in sulcular debridement, soft tissue ablation, curettage, de-epithelialization, incision, desensitization of exposed root surface, second stage implant surgery, osseous ablative surgery, soft tissue crown lengthening, and frenectomy. The use of Laser free running pulsed Nd: YAG for treatment of periodontal disease was proposed by Dr. Robert Gregg and Dr. Delwin McCarthy.

The sulcular debridement by lasers also known as LANAP received its clearance for use as sulcular debridement tool in the year 2004 by FDA after all research-proven data were analyzed. For this procedure a Periolase laser MVP-7 basically an Nd: YAG which operates at a wavelength of 1064 nm was developed. McCawley *et al.* In their study compared the Laser-Assisted New Attachment Procedure (LANAP) with ultrasonic root debridement alone for immediate post-treatment effects on putative bacterial pathogens in deep human periodontal pockets. For this 26 systemically-healthy adults with severe periodontitis were selected. LANAP surgery was performed using, pulsed Nd: YAG laser, with laser energy (4.0W, 150-µs pulse duration, 20-Hz) directed circumferentially around teeth parallel to root surfaces in a coronal-apical direction to probing depth. After ultrasonic root debridement and gingival flap advancement to the alveolar bone crest, a second laser pass (4.0 W, 650-µs pulse duration, 20-Hz) was similarly performed in an apical-coronal direction to thermally initiate a fibrin clot at the tooth-gingival flap junction. Subgingival biofilm specimens were collected before and immediately after completion of the treatments from 2 inflamed periodontal sites with ≥ 6 mm probing depths, and selected periodontal pathogens were identified using established anaerobic culture techniques. Results showed a negative culture for red and orange complex bacteria for the 17 patients out of 20 treated with LANAP where as it was only 1 for 6 treated with ultrasonic debridement.

Another advancement in the laser dentistry is waterlase laser its use was approved by FDA in the year 1998 for cutting of tooth structure. It is basically an Erbium-Chromium doped Yittrium-Selenium-Gallium-Garnet (Er, Cr: YSGG) laser which works on the principle of Hydrophotonics that uses the combination of laser energy and water to perform the various procedure in dentistry. This Waterlase work on a wavelength of 2.78µm.

A dual wavelength soft tissue diode laser has been introduced by Ultra Dent Products, Inc. named Gemini810 +980 diode laser for soft tissue laser surgery it has been approved by FDA for usage in 20 dental procedures including crown lengthening. This system also possess illumination at the tip for surgical assistance.

Another laser system SiroLaser Blue has been introduced that works at three wavelengths that is at 970, 660 and 445nm. This system has been newly introduced in United States in September 2018 and promoted by Dr. Smon Suppelt for its cutting efficiency at 445nm. This is manufactured by Dentsply Sirona. This system emits blue light at 445nm.

Microsurgery for periodontal treatment

Microsurgery is defined as the surgical procedure done under the microscope (as defined by Daniel in 1979) for better visualization so that less trauma is rendered to the tissue with better healing results. Magnification in the field of surgery was introduced by Carl Nylen, thus he is considered the father of microsurgery, he used microsurgery for correction of otosclerotic deafness. Apotheker and Jako were first to introduce microscope in the field of dentistry in the year 1978. The use of magnification in the field of Periodontology was introduced in the year 1992. Since then various enhancement in the magnifying power of the system used for microsurgery that is from loupes to surgical microscope has been made. Nowadays the various advancement made in video technology had made it possible to visualize the operating field on the screen in

three dimensions thus excluding the need to actually look into the eyepiece of the microscope.

The introduction of microsurgery was done to increase visibility, minimize trauma and to enhance surgical results. In the field of Periodontology microsurgery is being used I the following procedures perio-aesthetic surgeries like root coverage procedure, papilla reconstruction, aesthetic implant surgery, periodontal flap surgery, alveolar ridge deficiencies management, sinus lift procedure *etc*.

MIS technique that is minimally invasive surgery is one of the outcomes of usage of magnification in periodontal surgery. The term MIS was given by Harrel and Rees in 1995. Tibbetts and Shanelec in 1994 used the microsurgical technique for periodontal soft tissue regeneration and augmentation. Cortellini and Tonetti in 2007 came with the concept of minimally invasive surgical technique and later introduced the concept of space provision for regeneration with the modified MIST 2009 (M-MIST). The procedure is done under magnification with microsurgical instruments. Loups or microscope is being used for the purpose with magnification ranging from 3.5-20. The main advantage of the procedure is less postoperative pain and better and faster healing.

Harrel *et al.* in their case series showed the clinical outcomes from videoscope assisted minimally invasive surgery (VMIS- It is a kind of endoscopy like procedure where the surgical site can be monitored over the screen for better and clear view) at 36 to 58 months. In results at 36 months or more, post-surgery time there was a statistically significant improvement ($P < 0.001$) in mean PD and CAL (PD: 3.80–1.18 mm, CAL: 4.16–1.18 mm) at all surgical sites compared with baseline and there was a mean improvement in soft tissue height (0.36–0.64 mm, $P = 0.03$) too. In most cases, patients reported no postoperative discomfort. In a review by Harrel, the usage of VMIS for peri-implant surgeries is mentioned with positive regenerative outcome.

Photodynamic therapy in the treatment of periodontal diseases

Photodynamic therapy has been introduced as treatment modality thousands of years ago when Egyptian used light activated substance obtained from leaves of parsley and sunlight to treat sunburn. Oscar Raab was first to demonstrate the antimicrobial effect of acridine hydrochloride and visible light on Paramecia caudatum almost a hundred year ago. The term Photodynamic was given by Jodelbaner and Von Tappeiner in the year 1904. With it's targeted action and non-invasive nature this treatment modality has been successfully used for the treatment of many precancerous and cancerous lesion.

Mechanism of action: the photosensitizer get localized to the site of action and upon illumination, with the light of wavelength 630-700 nm the photosensitizer turns into the excited state and leads to two types of reaction Type I and Type II reactions in photodynamic therapy. The Type I pathway involves reaction that require transfer of electron from the photosensitizer triplet state with the involvement of a substrate to produce radical ions that can react with oxygen to produce cytotoxic species. In Type II pathway energy transfer takes place from the photosensitizer triplet state to the ground state molecular oxygen (triplet) to produce excited singlet oxygen species, which can lead to oxidation of biological molecules.

With the advancement in the therapy, many in vitro studies are being undertaken to use this modality for the treatment of periodontal and peri-implant diseases. Some of these studies include Dobson and Wilson (1992) - study done on *Streptococus sanguinis, Porphyromonas gingivalis, Fusobacteriumnucleatum, Aggregatibacter actinomycetemcomitans* using toluidine blue O, methylene blue, aluminum disulphonated phthalocyanine. An effective elimination of all four targeted organism was found with the use of toluidine blue O and methylene blue photosensitizer and 633nm He/Ne Laser light.

Owing to its advantages like localized action, non-invasive nature, accessibility to all the areas, reduction in chances of infection, etc, this modality can be a prospective adjunct to the scaling and root planning procedure to reduce the bacterial load. This animal model studies are being conducted to establish it's used in periodontal and peri-implant disease.

Birang *et al*. In their study compared SRP alone with an adjunct to LT (Laser therapy) and PDT (Photodynamic therapy) and found that adjunctive LT or PDT showed more improvement in term of CAL gain as compared to SRP alone (3 months follow-up).A similar study done by Teymouri *et al.* which concluded that laser and PDT reduces the inflammatory mediators (IL-1β and IL-17) and improves the clinical symptoms (PPD and CAL).

Tissue engineering in treatment of periodontal diseases

Tissue engineering is like an opportunity knocking our door to fill all the voids of current periodontal treatment modality. Since years scientists have been trying to recreate lost tissues and organ during the disease process or due to some mishaps. It was when Dr. Charles Vacant, who proposed the idea and concept of tissue engineering to the world. The concept of tissue engineering includes the triad of cells, signaling molecules, and scaffolds. A combination of these three in defect site can induce regeneration.

The concept of tissue engineering was first introduced to the field of Periodontology by Melcher, he was the first to introduce the principle of cell barrier in 1976. Nyman and karring in 1982 introduced guided tissue regeneration in periodontology using barrier membrane which marked the evolution in the field of tissue engineering. Earlier bone grafts were used to treat periodontal defects. Then was introduced Enamel matrix derivative 20 above years back by Lindskog *et al.* Slavkin *et al*. and Lars Hammarstrom. Then came the platelet rich plasma and platelet rich fibrin with growth factors introduced by Whitman and Marx; Choukroun respectively. All these material provide regeneration but not with predictability. A solution was provided by combining the cells, barrier membrane, and growth factor. A systematic review done by Gaubys *et al.* On the usage of Autologous Stem Cells for the Regeneration of Periodontal Defects in Animal Studies. Results showed that Stem cell therapy has a positive influence on periodontal tissue complex regeneration; however such therapy has a greater influence on cementum regeneration than alveolar bone regeneration.

Another systematic review done by Han *et al.* on mesenchymal Stem Cells for Periodontal Tissue Regeneration showed that the that the MSCs-based therapy for periodontal tissue regeneration is effective, however further such analysis may still be required.

Currently, focus has shifted towards development of periodontal ligament (PDL) attachment around dental implants to replace lost teeth. PDL houses various vital cells that play very important role in maintaining a relationship between the tooth and the bone. Thus, ligaplants are now an available option in order to improve the biological performance and to prolong the life of the prosthesis.

Cells procured from pulp, periodontal ligament, gingival connective tissue, etc are being used for various in vitro studies so that their capacity to differentiate into different progenitor cells of periodontium can be established. Similarly, gene therapy (where genes are transferred to the desired site by means of vector which can be viral like Retrovirus, Lentivirus, Adenovirus, Adenoassociated virus or non viral vectors like physical vectors consisting of Electroporation, Gene gun, Ballistic particle delivery; chemical vectors like Cationic polymer diethylamino methyl-dextran, Calcium phosphate co-precipitation , Lipid mediated types like Cationic liposomes , Lipoplexes and as Gene editing tools like Zinc finger nucleases (ZFNs), Transcription Activator Like Effector-based Nucleases (TALENS) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9)) is being used for controlling the release of growth factor to the defect site so that an appropriate amount of factors are released at the appropriate time and in the production of pluripotent cells, called induced pluripotent stem cells. It is also being used to enhance host modulation to resist disease. To hold the cells and growth factor at defect sites scaffolds of different biomaterial and with different preparation technique are being tested for biocompatibility and strength. Still, tissue engineering is a very young technique and many reformations have to take place in future to make this technique feasible.

Currently gene therapy is being employed in antimicrobial therapy to control disease progression. Gene medicines or nucleic acid drugs are categorized on the basis of their therapeutic application as gene inhibitors, gene vaccines and gene substitutes. Gene inhibitors (oligonucleotides, siRNA) drugs silence the defective gene at mRNA level. Gene vaccines are antigens encoding either the genes or RNA of specific pathogen that activate cell-mediated and humoral immune response and leads to production of antibodies.

In anti-apoptotic activity Bcl_2 family of proteins protects against apoptotic activity Utilization of Bcl_2 gene (anti apoptosis gene) with gene activated matrix technology (GAM) when introduced into a localized tissue injury site as seen in periodontal disease, will improve the clinical outcome by means of tissue repair and/or tissue regeneration.

As DNA devices used as selective genetics for the fulfilment of mechanical targeting. Gene therapy being used in implant therapy. Park *et al.* in 2015 evaluated ex *vivo* BMP 2 gene delivery by using canine periodontal ligament stem cells for regeneration of peri-implantitis defects.

Genetic Approach has been used to fight Biofilm Antibiotic Resistance. Study by Mah *et al.* identified gene ndv B encoding for glycosyltransferase which is required for the formation of periplasmic glucans in wild form of *Pseudomonas aeruginosa* RA14 strain. This periplasmic glucans protected them from the antibiotics biocides effect, and disinfectant effect. Using a genetic approach. Researchers have isolated ndv B mutant of *Pseudomonas aeruginosa* still capable of forming biofilm but lacking the characteristic of periplasmic hence rendering them more susceptible to conventional antibiotic therapy.

Nanotechnology in treating periodontal disease

The term "nanotechnology" was given by Tanaguchi. The discovery of nanotechnology has been attributed to the American physicist Nobel Laureate Dr. Richard Phillips Feynman who also presented a paper titled “There is plenty of room at the bottom” on December 29th, 1959 at the annual meeting of the American Physical Society at California Institute of Technology. A nanomaterial is an object with at least one dimension in the nanometer scale (approximately 1 to 100 nm). One billionth of a meter (10^{-9}m) is one nanometer. US government defined nanotechnology as “Nanotechnology is research and technology development at the atomic, molecular or macromolecular level in the length scale of approximately 1-100 nm range, to provide a basic understanding of phenomena and materials at the nanoscale and to form and use structures, devices and systems that have novel properties and functions due to their little and/or intermediate size.”

Nanotechnology has been used in the field of dentistry since early 1970, the era of microfills. Since then various nanomaterials were introduced in the field dentistry including periodontology for proper oral health maintenance. Nanomaterials like Bioactive glass, carbon nanomaterials, Titanium nanotubes coated dental implants, nanoceramics for bone regeneration; nanobiomaterial being used for the preparation of scaffolds for regeneration of periodontium; metallic nanoparticles in the form of toothpaste and mouth rinses for control of oral biofilm and nanoparticles for local drug delivery, Nanorobots for oral analgesia, drug delivery *etc.*

Research is being carried out in the field of nanotechnology for producing better and even modified products for periodontal regeneration with the elimination of side effects and increasing the biocompatibility of the product.

Newer drugs for treatment of periodontal disease

The use of newer drugs to resolve inflammation in periodontal tissue like Resolvin. This drug has been used in pre-clinical models for treatment of asthma, rheumatoid arthritis, inflammatory bowel disease as it lowers the recruitment of neutrophils at the site of inflammation and also reduces the number of cytokines and reactive oxygen species being produced hence helps in reducing the inflammation.

Other than this, various other new drugs like TNF-α inhibitors such as Adalimumab, Golimumab; anti-cytokine

agent like Anakinra, AMG714, Tocilizumab; and RANK/RANKL inhibitor like Denosumab. These drugs are under study however their efficacy is being tested over experimentally induced periodontitis in the animal model. A new antibiotic study by Reed *et al.* named amixicile, a novel inhibitor of pyruvateferredoxin oxidoreductase. A minimal inhibitory concentration ranging from 0.5–1.5µg/ ml inhibited growth and other processes central to virulence in the in-vitro study.

Ozone therapy in periodontology

In 1839, Christian Friedrich Schonbein, first noticed a pungent gas with an electric smell. According to the Greek language, he called it ozone from the Greek word ozein (odorant). Oxygen/ozone therapy has a long history of research with humans along with clinical application. In 1856, just 16 years after its discovery, ozone was started being used in health care setting to disinfect operating rooms and in sterilizing surgical instruments. Its first medical application dates back to 1870 when Dr. C. Lender purified blood in test tubes. By 1929, more than 114 diseases were listed to be treated with oxygen/ozone therapy. In 1930, a German dentist, Dr. E.A. Fisch, used ozonated water as a disinfectant on a regular basis in his dental practice in Zurich, Switzerland, and published numerous papers on the subject. The first ozone generator for medical use was developed by German physicians named Joachim Hansler and Hans Wolff.

Ozone has been used for following purposes: 1. Elimination of pathogens. 2. Restoration of proper oxygen metabolism. 3. Induction of a friendly ecologic environment. 4. Increased circulation. 5. Immune activation. 6. Simulation of the humoral anti-oxidant system. Routes of administration are Gaseous ozone, Ozonated water, Ozonized oil.

Huth *et al.* in 2006, in their study found out that the aqueous form of ozone, act as a potential antiseptic agent, and showed less cytotoxicity than gaseous ozone and established anti microbials (chlorhexidine digluconate [CHX]: 2%, 0.2%; sodium hypochlorite 5.25%, 2.25%; hydrogen peroxide-H 2 O 2 3%) under most of the conditions. Kshitish and Laxman in 2010 performed a randomized, double-blind, split-mouth study on 16 patients suffering from generalized chronic periodontitis. The study period of 18 days was divided into two time-intervals one at baseline-7days followed by second interval of 7 days after a leeway of 4 days. Sub gingival irrigation of each half of the mouth with either ozone or chlorhexidine was done at different time intervals. They observed a higher reduction in plaque index (12%), gingival index (29%), and bleeding index (26%) using ozone irrigation as compared to chlorhexidine.

Huth, *et al.* in 2011 compared the effectiveness of ozone with that of the of antiseptic CHX, against periodontal microorganisms. There were no significant differences observed in the effectiveness of aqueous ozone (20µg ml [−1]) or gaseous ozone (≥4 g [−3]) compared with 2% CHX but the effectiveness varied with 0.2% CHX which was less effective.

Uraz *et al.* (2018) in their study used ozone as adjunctive therapy in chronic periodontitis patients. In results there was a Statistical significant improvement in all clinical parameters along with a reduction in microbiological and biochemical parameters in both treatment groups. SRP treatment resulted in a significant reduction of *Porphyromonas gingivalis* (Pg) at 1st month and Tannerella forsythia (Tf) and *Prevotella intermedia* (Pi) at 3 months. SRP treatment resulted in significant reduction in the interleukin (IL)-8 levels at 1 month.

Marino *et al.* (2018) in this study, investigated the efficacy of treatments with ozone in water and gaseous ozone against attached cells and microbial biofilms of three food borne species, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Listeria monocytogenes*. Biofilms were formed on AISI 304 stainless steel coupons from a combination of three strains (one reference and two wild strains) of each microbial species and were subjected to three types of treatment for increasing times: (i) ozonized water (0.5 ppm) by immersion in static condition, (ii) ozonized water under flow conditions, and (iii) gaseous ozone at different concentrations (0.1–20 ppm). Treatment with aqueous ozone under static conditions resulted in a reduction of viability to 1.61–2.14 Log CFU/cm2 after 20 min, while the reduction values were higher (3.26–5.23 Log CFU/cm^2) for biofilms treated in dynamic conditions *S. aureus* was the most sensitive species to aqueous ozone than to dynamic conditions. Gaseous ozone, at low concentrations (up to 0.2 ppm), resulted in inactivation of 2.01–2.46 Log CFU/cm^2 after 60 min, whereas at the highest concentrations a complete inactivation <10 CFU/cm^2) of the biofilms of *L. monocytogenes* and the reduction of 5.51 and 4.72 Log CFU/cm^2 for *P. fluorescens* and *S. aureus* respectively after 60 and 20 min were achieved. Considering the results achieved, ozone in water form might be used for daily sanitation protocols.

Wang *et al.* in their study examined the effects of ozone exposure on the production of collagen type-1 and inflammatory cytokines in primary human gingival fibroblasts (HGFs) in an *in vitro* condition using enzyme-linked immunosorbent assays. No cytotoxic effect of the ozone ointment was observed at concentration of 0.5ppm, where as cell viability was attenuated at the dose of 5ppm. When ozone ointment was used at the non-cytotoxic concentration of 0.5ppm, a significant enhancement in type 1 collagen production by HGFs was observed for 24 hours. Secretion of the pro-inflammatory cytokines interleukin (IL)-6 and IL-8 by HGFs treated with lipopolysaccharide (LPS) was decreased when ozone ointment was presented in the medium.

Contraindications for ozone therapy Pregnancy, Autoimmune disorder, Hyperthyroidism, Anemia, Myasthenia gravis, Alcohol intoxication, CVD, Myocardial infarction, Ozone allergy, Hemorrhage.

New classification of periodontal disease

The 1989 workshop classification was based on distinct clinical presentations, different ages of onset and rates of progression. Based on these variables periodontitis was categorized as prepubertal, juvenile (localized and generalized), adult, and rapidly progressive. The 1993 European Workshop classification proposed grouping periodontitis into two major headings: adult and early onset periodontitis.

The 1996 workshop participants didn't find sufficient new evidence to change the classification. Major changes were carried out in the 1999 classification of periodontitis which has been in use for the last 19 years. Periodontitis was reclassified as chronic, aggressive (localized and generalized), necrotizing and as a manifestation of systemic disease. Since the 1999 workshop, there were substantial new information that has emerged from various population studies, basic science investigations, and the evidence from prospective studies evaluating environmental and systemic risk factors. The analysis of this evidence has lead to the 2017 workshop to develop a new classification for periodontitis.
The outline of the classification is as follows:
Periodontal diseases and conditions are classified under following headings:

1. Periodontal health, gingival diseases and conditions.
2. Periodontitis
3. Other conditions affecting the Periodontium
4. Peri-Implant disease and conditions

Periodontal health, Gingival diseases and conditions are further classified into Periodontal health and gingival health, Gingivitis: Dental biofilm-induced, and Gingivitis: Non-dental biofilm-induced
Periodontitis is further classified into Necrotizing periodontal diseases, Periodontitis, and Periodontitis as a manifestation of systemic disease.
Other Conditions affecting the periodontium are further classified into Systemic diseases and conditions affecting the periodontal supporting tissues, Periodontal abscesses and Endodontic-Periodontal lesions, Mucogingival deformities and conditions, Traumatic occlusal forces and Tooth and prosthesis related factors.
Peri-Implant diseases and conditions are futher classified into Peri-Implant health, Peri-Implant mucositis, Peri-Implantitis, Peri-Implant soft and hard tissue deficiencies.
Periodontal health and gingival health are further divided into Clinical gingival health on an intact periodontium and Clinical gingival health on a reduced periodontium which can be on stable periodontitis patient or in Non-periodontitis patient
Gingivitis: Dental biofilm-induced is further divided into Associated with dental biofilm alone, mediated by systemic or local risk factors and Drun-influenced gingival enlargement.
Gingivitis: Non-dental biofilm-induced is further divided into Genetic/ developmental disorders, Specific infections, Inflammatory and immune conditions, Reactive processes, Neoplasms, Endocrine, nutritional and metabolic diseases, Traumatic lesions and Gingival pigmentation.
Necrotizing periodontal diseases are further classified into Necrotizing gingivitis, Necrotizing periodontitis and Necrotizing stomatitis.
Periodontitis is further classified depending upon severity and complexity of management into Stage I: Initial periodontitis, Stage II: Moderate periodontitis, Stage III: Severe periodontitis with potential for additional tooth loss and Stage IV: Severe periodontitis with potential for loss of the dentition. Depending upon extent of distribution into Localized, Generalized and Molar-incisor distribution. Depending upon risk of rapid progression, and anticipated treatment response into Grade A: Slow rate of progression, Grade B: Moderate rate of progression and Grade C: Rapid rate of progression.
Other periodontal conditions are further classified into periodontal abscesses and Endodontic-periodontal lesions.
Mucogingival deformities and conditions around teeth are further classified into Gingival phenotype, Gingival/Soft tissue recession, Lack of gingiva, Decreased vestibular depth, Aberrant frenum/Muscle position, Gingival excess, Abnormal color, Condition of exposed root surface.
Traumatic occlusal forces are further classified into Primary occlusal trauma, Secondary occlusal trauma and Orthodontic forces.
Tooth and prosthesis related factors are further divided into Localized tooth related factors and Localized dental prosthesis related factors.

CONCLUSION

As the progress is being made in the field of technology and science there is an advancement that is also observed in the understanding of the etiology and various the factor responsible for periodontal disease. This broader understanding is helping in the development of various newer treatment modalities in the field of Periodontology.
However, there is much yet to be discovered to remove all the present obstacles and to provide with even better technology and materials for the future.

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