INTRODUCTION AND REVIEW OF LITERATURE

Periodontal disease is one of the most common diseases affecting humans ***(Dentino et al., 2013)***. In 2010, severe periodontitis was estimated to be the sixth most predominant disease worldwide, affecting about 743 million people ***(Kassebaum et al., 2014)***. In the recent 11th European Workshop on Periodontology, specialists confirmed that the high prevalence of periodontitis in Europe affects more than 50% of the adult population. The severe form of periodontitis affects 11% of the adult population ***(Tonett et al., 2015).***

Periodontitis is not a "silent" disease, periodontally affected patients have a poor awareness of their oral health and an inferior quality of life compared to healthy individuals. Proper periodontal treatment may improve the oral health-related quality of life for these patients ***(Shanbhag et al., 2012).*** The periodontium contains cementum, alveolar bone, a functionally oriented periodontal ligament and gingiva. Pathologic and/or traumatic procedures may lead to the damage or loss of the anatomical structure ***(Al-Harthi et al., 2013)***.

The term 'periodontal diseases' consists of a wide variety of chronic inflammatory conditions of the gingiva, alveolar bone, cementum and periodontal ligaments supporting the teeth. Periodontal disease starts with gingivitis (the

localized irritation of the gingiva) that is caused by bacteria in the microbial biofilm (dental plaque) attached to the teeth and gingiva. In this situation, the diagnosis of gingival inflammation is plaque induced gingivitis ***(Lopez and Baelum, 2003)***. On the other hand, chronic periodontitis occurs when untreated gingivitis causes considerable degradation of alveolar bone, cementum and periodontal ligament, which frequently leads to tooth loss ***(Gotsman et al., 2007).*** Alveolar bone loss and attachment loss are hallmarks of periodontitis. The periodontitis is generally a result of a chronic inflammatory response elicited by several infectious and non-infectious pathogenic stimuli. The aim of that inflammatory response is to limit and repair the damage ***(Darveau, 2010; Yucel-Lindberg and Båge, 2013)***.

Furthermore, periodontal disease is not only related to plaque, but also many systemic conditions may aggravate the disease ***(Jeffcoat et al., 2003)***. Periodontitis is currently being linked bidirectionally to the pathogenesis of several systemic diseases and conditions such as rheumatoid arthritis ***(de Pablo et al., 2009),*** diabetes ***(Chapple and Genco, 2013)***, pre-term low birth weights ***(Zi et al., 2015)*** and dementia ***(Abbayya, 2015)***. Also, periodontitis is linked in one direction to the pathogenesis of other systemic diseases like coronary heart disease ***(Tonetti and Van Dyke, 2013)*** and respiratory diseases ***(Bansal et al., 2013)***.

The inflammatory bone loss is a result of the upregulation (and thus hyperactivity) of osteoclasts as well as the downregulation (and thus hypo-activity) of osteoblasts, which leads to a profound net reduction in bone mass. So that, in periodontitis there is uncoupling of bone resorption with consequent bone formation leading to loss of alveolar bone ***(Lerner, 2006).***

Intrabony defects

In general, the pattern of osseous destruction in periodontitis can be classified according to clinical and radiographic criteria into horizontal or angular (vertical) defects. Angular defects are associated with infrabony (intrabony) pockets (the base of bony defect is positioned apical to the crest of alveolar bone) and horizontal defect is related to supra-bony pockets (the base of the defect is coronal to the crest of the alveolar bone) ***(Lommer and Verstraete, 2001).***

The morphology of an intrabony defect is frequently described by the number of remaining bony walls (one, two, three osseous wall). Defect morphology affects the availability of cellular and vascular componants required to regenerate the defect ***(Becker et al., 1986)***. The occurrence of vertical bone defects may be associated with lacking of root cementum ***(Lindhe et al., 1975; Blomlöf et al., 1987)*** or occlusal trauma ***(Nunn and Harrel, 2001).***

The predictable regeneration of intrabony defects depends mainly on three criteria; the vertical depth of the intrabony defect, the deepest defects often exhibit the greatest periodontal regeneration. the angle of the defect (narrow or wide angle), intrabony defects that are narrow and mostly self-contained by two or three bony walls usually respond well to regenerative treatment (wide shallow defects responded with less bone gain compared with narrow deep intrabony defects). The number of the remaining bony walls, the greatest potential for regeneration is associated with the two or the three osseous wall intrabony defect ***(Nevins et al., 2003, 2013).*** There are no predictable regenerative methods for one wall intrabony defects ***(Kao et al., 2015)***.

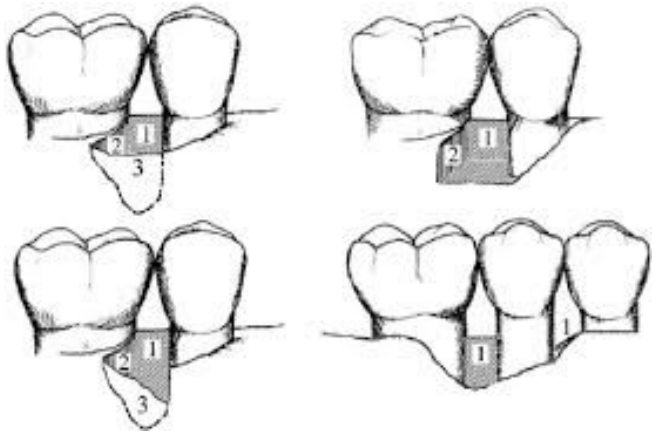

Fig. (1): Classification scheme for intrabony defects ***(Reynolds et al., 2010).***

Moreover, the presence of a vertical bony defect increases hazard of further loss of supporting bone in absence

of proper periodontal therapy. Therefore, the recognition and assessment of vertical bony defects is an important factor in prognosis and treatment plan ***(Papapanou and Wennström, 1991)***.

The choice of a regenerative approach is mainly depending on the morphology of an intrabony defect. Defect morphology directly affects the availability of cellular and vascular elements required to regenerate the defect in addition to the inherent structural support given by the surrounding alveolar bone, which can influence clot stability and space maintenance. If the intrabony defects become progressively less bounded by bone (because of decreased height of bony walls, decreased number of bony walls, and/or increased defect angle), the inherent potential for periodontal regeneration declines ***(Garrett, 1996).***

Consequently, three wall intrabony defects, mainly when being narrow and deep, seem to provide the highest inherent potential for periodontal regeneration ***(Wang and Cooke, 2005).*** On the other hand, one and two wall intrabony defects are often managed using a combination of regenerative strategies, including biologically active materials such as growth factors ***(Li, 2017).***

Challenges facing periodontal regeneration

Tissue damage associated with gingivitis is reversible if the causative agent is removed. Definitely, the gingival tissues have a remarkable capacity to regenerate to their original form and function following inflammation, as evidenced by the lack of scar tissue formation and ability to rapidly resume a functional anatomic relationship with the tooth surface and underlying structures following periodontal therapy. However, in periodontitis, once the destructive phase reaches the alveolar boneand root cementum, regeneration does not usually occur on a clinically predictable basis ***(Melcher, 1976)***.

Periodontal regeneration is a histological term which means renewal of the tooth supporting tissues, including periodontal ligament, cementum and alveolar bone over previously diseased root surface. Regeneration of lost tissues is based on the availability of the progenitor cells, appropriate signals to regulate progenitor cell differentiation, and sufficient space for tissue regeneration. This place is provided by either a physiological (blood clot) or therapeutically (bone graft and/ or membrane) placed scaffold ***(Grzesik et al., 2002)***.

In addition, the predictability of periodontal regeneration is influenced by several factors including; features of the defect site (e.g., gingival biotype, root topography, and bone morphology) ***(Stavropoulos et al., 2004; Wang and Cooke, 2005)***, surgical technique (e.g., conventional flab design or

minimal invasive technics ***(Cortellini and Tonetti, 2009),*** related to the patient (e.g., age, smoking and other systemic conditions) ***(Patel et al., 2012),*** an esthetic consideration**,** and finally, early periodontal intervention ***(Reynolds et al., 2015)***.

At least four principles must be considered in order to have a successful regeneration: 1) A functional epithelial closure must be re-established at the coronal portion of the tissues 2) New Sharpey's fibers must be inserted into the previously exposed root surface to reproduce both the dentogingival fiber complex and the periodontal ligament. 3) New acellular, extrinsic fiber cementum must be re-established on the previously exposed root surface. 4) Alveolar bone height must be returned to within 2mm apical to the cementoenamel junction (CEJ) ***(Bartold et al., 2000).***

When periodontal wounds are closed and sutured, one of the wound margins is the avascular and rigid periodontitis-affected root surface ***(Kina et al., 2008)***. The fibrin clot provides a weak initial attachment to that root surface, to prevent epithelial apical growth and to form a scaffold for development of a cell and collagen fiber attachment mechanism. The fibrin clot must form and adhere to the altered root surface for adequate time to allow a proper wound maturation. If these series of events are disrupted, or if the initial attachment of fibrin or/and immature connective tissue is ruptured, then healing by a long junctional epithelium extend to

the base of the original periodontal pocket is expected ***(Kina et al., 2013).***

Periodontal tissues are unique due to the various criteria need to periodontal regeneration. These include; 1) the coordinated formation of the three tissues of the periodontal ligament; the potential role of bacterial contamination during healing; 2) the specific requirement for dental cementum formation, a tissue that is not seen in other parts of the body; 3) the requirement for coronal regeneration of tissues towards the overlying superficial tissues. In addition, the regeneration and organization of periodontal ligament appears to be significantly dependent on the continued functional loading (and therefore micromovement) of the tooth. The function load is coming from occlusal forces and the tension stress from mucosal membranes and gingiva, that adds a further challenge in regeneration ***(Hughes et al., 2010; Li, 2017).***

Recently, many randomized controlled clinical studies approved the ability to achieve periodontal regeneration in intrabony defects using a diversity of regenerative therapies: (1) the bone replacement grafts BRGs serves as a scaffold for new bone formation;(2) guided tissue regeneration (GTR) which creates spaces using barrier membranes that may be filled with new bone;(3) growth factors (recombinant human platelet-derived growth factor-BB [RHPDGF-BB] and enamel matrix derivative [EMD] and/or cell therapy (stem/ osteoprogenitor cells) to

encourage new bone formation, as well as combination therapies of all of the above ***(Pellegrini et al., 2013; Kao et al., 2015).***

In periodontal regeneration, the expression of different phenotypes and generation of cementum or alveolar bone may be dependent on whether a common lineage of progenitor cells attach on residual cemental or exposed dentinal substrata or stay in the alveolar bone side of the periodontal ligament space. The presence of exposed dentine may preferentially modulate the expression of the cementogenic phenotype on readily available cementoblasts and their progenitors attached to dentine or residing within the periodontal ligament space ***(Ripamonti et al., 1994)***. The regeneration of the periodontium is promising but challenging due to complex structure of periodontium, tissue integration required for functional restoration, limited blood supply due to avascular root surface and limited number of cells in periodontal defect ***(Hughes et al., 2010).***

Periodontal tissue engineering

The field of regenerative medicine incorporates diverse areas of technology, such as stem cells and tissue engineering ***(Hasetine. 1999)***. Tissue engineering; one of the main components of regenerative medicine, uses different sciences for the purpose of improving the biological behavior that can restore and preserve normal function. Tissue engineering is defined as "an interdisciplinary field which applies the

principles of life sciences and engineering towards the development of biological substitutes that goal to maintain, restore or improve tissue function" ***(Atala and Lanza, 2001)***.

Tissue engineering approaches generally fall into two classes: the use of acellular matrices and cellular matrices, the acellular matrices mainly depend on the body's natural ability to regenerate for proper orientation of new tissue growth, while the cellular matrices contain the cells and used when the original tissues have limited power of regeneration or limited number of cells. Acellular tissue matrices are frequently prepared by manufacturing artificial scaffolds, or by eliminating cellular components from tissues via chemical and mechanical management to produce collagen rich matrices ***(Correia et al., 2013; Farzaneh et al., 2014).***

The matrices degrade slowly upon implantation and are usually replaced by the extracellular matrix proteins which are secreted by the ingrowing cells. Cell therapy can also be used via injection, with or without carriers such as hydrogels, collagen and bone graft ***(Amiel and Atala, 1999)***. In other hand, the cellular matrices are used for tissue engineering, a small portion of donor tissue is separated and individual cells are isolated. These cells are either implanted into the host, or are expanded in culture, attached to a matrix, and then re-implanted into the host after expansion ***(Amiel et al., 2006)***.

The three essential components required for tissue engineering include a population of multipotential progenitor cells, the presence of signaling molecules / inductive morphogenic signals ***(Nakashima and Reddi, 2003; Nakashima, 2005)*** and a conductive extracellular matrix scaffold with adequate blood supply ***(Srisuwan et al., 2006).***

The extracellular matrix scaffold not only acts as a delivery vehicle for cells to the site of regeneration but it also plays a major role in cell attachment, space retention, determination of morphological features and recruitment of oxygen and nutrients ***(Nakashima and Reddi, 2003; Nakashima, 2005)***. Several properties are of great importance in the selection of a suitable scaffold material which include porosity, tissue conductivity, biocompatibility and resorption rate ***(Murphy and Mooney, 1999; Duailibi et al., 2006).***

Cellular elements

Stem cells are undifferentiated cells which have the capability at the solitary cell level to both self-renewal and differentiation to produce more specialized and mature cells. These are biological cells found in nearly all multicellular organisms ***(Cai et al., 2004; Wagers and Weissman, 2004).*** Stem cell potency has the ability to differentiate into specialized cell types and to give rise to any mature cell type. Stem cells have been classified according to their potency to

totipotent, pluripotent, multipotent, Oligopotent and unipotent ***(Takahashi and Yamanaka, 2006; Macfarlan et al., 2012).***

Unipotent cells can give rise only to one cell type, their own, nevertheless have the property of self-renewal, which differentiates them from non-stem cells. Such as muscle and most epithelial tissues have self-renew character due to the presence of unipotent progenitor cells ***(Blanpain et al., 2007).*** While, oligopotent stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells ***(Majo et al., 2008)***. Multipotency referred to the ability to differentiate into a limited number of cells, like a mesenchymal stem cell (MSC). In contrasts with pluripotency, which has the capability to give all three germ layers but not to the extraembryonic lineages ***(Cahan and Daley. 2013).*** Finally, totipotency is the incredible capacity of the early embryo to produce all cells of an organism ***(Ishiuchi and Torres-Padilla, 2013).***

According to stem cell sources, there are three major classes of stem cells, the embryonic stem cells, the adult stem cells and recently through genetic manipulation induced pluripotent stem cells (IPSCs). Adult stem cells are undifferentiated cell found in specialized tissue and organs of adult. In comparison to the embryonic stem cells, adult stem cells are less debatable in ethical terms and easily accessible due to their availability in various organs of the body ***(Wagers and Weissman, 2004; (Sonoyama et al., 2006).***

Mesenchymal stem cells (MSCs) are self-renewable, highly proliferative, non-hematopoietic progenitor cells that have the ability to differentiate into distinct mesenchymal cell types, including osteoblastic and cementoblastic lineages (multipotency). Accordingly, MSCs are a promising tool in the regenerative treatment of periodontal defects. MSCs found in the perivascular space and other special niches in adult tissues including the PDL, gingival tissue and stromal compartment of bone marrow, unfortunately, these cells are limited in number to allow self-regeneration of periodontal wound ***(Seo et al., 2004).*** For that, many efforts to improve periodontal regeneration, by decortication, make intramarrow penetration to encourage cellular movement from bone marrow into the defect site or transplanting different stem cells in the periodontal defect ***(Huang et al., 2009; Estrela et al., 2011).***

One of the most important features of MSCs that worths considering is their unique and unexplained immune-modulatory properties. It has been reported that the adult MSCs express intermediate levels of class I major histocompatibility complex (MHC) proteins but no class II MHC proteins. Therefore, these cells are non-immunogenic, so that, no immunosuppression is needed after their transplantation into an allogenic host ***(Le and Ringden, 2005)***. Further studies have shown that MSCs have immunosuppressive properties by modulating specific T-cell functions *in vitro* ***(Beyth et al., 2005; Dazzi and Marelli-Berg, 2008).***

Stem cell-based periodontal regeneration is developing rapidly, and non-dental stem cells, such as embryonic stem cells, bone marrow-derived mesenchymal stem cells (BMMSCs) ***(Shi, 2012)***, alveolar periosteal cells (APCs) ***(Nagata et al., 2012)*** and adipose-derived stem cells show the potential for multilineage differentiation to make replacement tissue ***(Higashimoto et al., 2013)***.

Dental stem cells, such as dental pulp stem cells (DPSCs) ***(Kerkis et al., 2006)***, dental follicle cells (DFCs) ***(Guo et al., 2009),*** periodontal ligament stem cells (PDLSCs) ***(Vaquette et al., 2012)*** and gingival mesenchymal stem cell (GMSCs) are increasingly being investigated as easily available undifferentiated cells ***(Jin et al., 2015)***.

Seo et al. (2004) reported that PDLSCs expressed the mesenchymal stem-cell markers STRO-1 and CD146. Under specific culture conditions, PDLSCs differentiated into cementoblast-like cells, adipocytes, and collagen-forming cells. PDLSCs showed the capacity to generate cementum/PDL-like structure and contribute to periodontal tissue repair ***(Seo et al., 2004).***

In 2006, the autologous bone marrow mesenchymal stem cells (BM-MSCs) was used to treat the supra-bony periodontal defect in adult mongrel dogs. BM-MSCs carried on Calcium alginate scaffold were used and there was a true regeneration of

periodontium on histologic level with formation of bone, cementum and periodontal ligaments ***(Weng et al., 2006)***.

Li et al., assessed the regenerative power of BM-MSC. They used a collagen membrane scaffold for autogenous BM-MSC, and they used this to augment fenestration defects around mandibular molar in beagle dogs which lead to new periodontal ligament, bone and cementum formation ***(Li et al., 2009***).

Duan et al., evaluated new population of stem cells allogenous induced pluripotent stem cell (iPSC) was carried on apatite coated silk and enamel matrix derivative (EMD), then all that were used to regenerate the periodontal fenestration in nude mice. New bone, PDL and cementum were detected around the tooth ***(Duan et al., 2011).***

Park et al., compared the regenerative potential of autogenous periodontal mesenchymal stem cells (PDLSC), dental pulp mesenchymal stem cells (DPSC) and periapical follicular mesenchymal stem cells (PFSC) in 3 mm wide circumferential apical defects in beagle dogs. This study confirmed that PDL-MSC were the most promising stem cell for the clinical application among the three dental stem cells and can be used for treatment of advanced periodontitis ***(Park et al., 2011).***

BM-MSC, PDLSC and alveolar periosteal cells (APC) were used to treat one wall intrabony defect in dogs. Three-