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Exosomes Based Therapies In Periodontitis: A New Frontier

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ABSTRACT:

INTRODUCTION: Periodontitis is defined as an inflammatory disease, initiated by bacteria, that results in the destruction of the supporting tissues of the teeth. Among the periodontal diseases, periodontitis remains one of the leading cause which is characterized by chronic inflammation and progressive destruction of the supporting structures of the teeth. Traditional treatment therapy to eradicate the cause are supra-gingival scaling, sub-gingival curettage and root planning. These therapies focus on mechanical debridement and anti-infective strategies. Exosomes play a vital role in tissue regeneration. Exosomes are small extracellular vesicles which are secreted by various cell types. Exosomes are of two types natural exosomes and engineered exosomes. Exosomes are secreted by almost all cells, including mesenchymal stem cells (MSCs), periodontal ligament stem cells (PDLSCs), dendritic cells (DCs), B cells, T cells and mast cells. Exosomes derived from mesenchymal stem cells show higher potential ability in the regeneration of alveolar bone, periodontal ligament and cementum. Mesenchymal stem cells have healing and immunomodulatory properties which allows them to be widely used in the field of medicine. The common source of MSCs are bone marrow (BM-MSCs), dental pulp (DPSCs), periodontal ligament (PDLSCs) and gingiva (GMSCs). The use of exosome-based strategies in medicine and dentistry is associated due to their high potential.

KEYWORDS: Exosome, stem cells, periodontitis, periodontal regeneration, mesenchymal stem cells.

INTRODUCTION:

Periodontics is a branch of dentistry that deals with the diagnosis and conditions of supporting and surrounding tissue of the teeth. Periodontal tissues are a complex and dynamic system comprised of both hard and soft tissues that envelop and uphold teeth. Hard tissues comprise of dental cementum, alveolar bone and soft tissues comprises of periodontal ligament and gingiva which distribute the forces generated during mastication [1]. Periodontitis is a chronic inflammatory disease that occurs in the periodontal tissues and is caused by the improper balance in the microbiota surrounding the periodontal tissues. Periodontitis is irreversible. Supra-gingival scaling, sub-gingival curettage and root planning are the commonly used

treatment plans to treat periodontitis. Newer treatment approaches such as Guided Tissue Regeneration (GTR), autogenous bone grafting, allograft bone filling.

Tissue engineering is a multidisciplinary field that provides periodontal tissue regeneration and repair, helps to form biological substitutes replacing the function of damaged or diseases tissues. In tissue regeneration, stem cells scaffolds are currently used. By using biomaterial scaffolds to load exosomes can provide higher therapeutic effects.

Exosomes were originally found in the supernatant of sheep erythrocytes cultured in vitro. Exosomes are secreted by all cells including Mesenchymal Stem Cells (MSCs), dendritic cells (DCs), B cells, T cells and mast cells and found in many body fluids such as plasma, urine, breastmilk, semen, amniotic fluid and saliva [1]. Exosomes are extracellular vesicles (EVs) which are in the size between 50 & 150nm [2]. Based on the particle size, EVs can be classified into 1) Apoptotic bodies (ApoBDs), 2) microvesicles (MVs) and 3) exosomes (EXOs) [3]. Exosomes are rich in proteins, DNA, mRNA, and miRNA.

Based on their origin and their source, EXOs are divided into natural exosomes and engineered exosomes. Natural exosomes are divided into animal derived exosomes and plant-derived exosomes [4]. They are natural drug delivery vehicles [5]. Exosomes are released into the extracellular space through fusion of plasma membrane with certain endosomes such as multivesicular bodies (MVB) [6]. Exosomes are useful in monitoring the progression of the disease and early diagnosis detection [7]. In periodontal regeneration, periodontal ligament stem cells (PDLSCs) play an important role [8]. They secrete more EXOs. PDLSCs that seen within the periodontal ligament helps in the repair of alveolar bone and their regeneration [9,10,11]. Many studies are reported that PDLSCs in regenerative medicine is done by secreted bioactive compounds such as EXOs, cytokines, chemokines and growth factors [12].

PDLSCs derived EXOs transport genetic materials and plays a vital role in communication and cellular activities [13]. They exhibit biological roles such as stimulation of cell proliferation, inhibition of apoptosis, advancement of angiogenesis and enhance tissue repair capacity [14]. EXOs carry signalling chemicals that are involved in biological process and cell signalling. They cross the blood-brain barrier (BBB) and control inflammation [15]. EXOs derived from bone marrow (MSCs) [16,17], adipose tissue MSCs [18], dental follicle cells [19] have been used in the regeneration of periodontium. Mesenchymal stem cells MSCs are spindle shaped cells that disintegrate into various types of cells that aid in regeneration of damaged tissues. Electron microscopy imaging is the gold standard for characterizing exosomes.

ORIGIN AND FORMATION OF EXOSOMES:

The formation of early endosomes is by the invagination of the plasma membrane forming the early endosomes. The early formed endosomes internalize the surface proteins, lipids and extracellular materials. The early endosomes mature into late endosomes or multivesicular bodies (MVB) through the inward budding of endosomal membrane. Intraluminal vesicles (ILVs) are formed inside the MVBs which later become exosomes upon release. Through ESCRT-dependent pathway, ESCRT-independent pathway, the molecular CARGO such as proteins, lipids, RNA which are loaded into the intraluminal vesicles (ILVs) as exosomes. These exosomes react with the cells through fusion and ligand receptor binding. Some MVBs combine with lysosomes, whereas other MVBs combine with the plasma membrane [4].

MATERIALS AND METHODS:

Databases searched for literature review included Scopus, Web of Science and PubMed. The search was accomplished using the keywords “exosomes”, “periodontitis”, “stem cells”, “tissue regeneration”, “bone loss”. Only articles published in English were included.

SEARCH STRATEGY:

A literature search was conducted in Scopus, Web of Science and PubMed to identify articles that investigated the exosomes-based therapies in periodontitis. The following search lines were used for each database: (exosomes) OR (periodontitis) OR (stem cells) OR (tissue engineering) OR (tissue regeneration) OR (mesenchymal stem cells) OR (periodontal ligament stem cells) OR (periodontal tissue regeneration) OR (exosomes-based therapies).

CLASSIFICATION:

Exosomes are divided into natural exosomes and engineered exosomes. Natural exosomes are further subdivided into animal derived exosomes and plant derived exosomes. All types of cells produce EXOs like Mesenchymal stem cells (MSCs), T cells, B cells, macrophages, dendritic cells (DC), Periodontal ligament stem cells (PDLSCs) and widely seen in many body fluids such as plasma, urine, breastmilk, semen, amniotic fluid and saliva. Extracellular vesicles (EVs) originate from extracellular space and can be classified into exosomes (EXOs) derived from endosomes, microvesicles (MVs) and apoptotic bodies (ApoEVs).

MESENCHYMAL STEM CELLS (MSCs):

Mesenchymal stem cells (MSCs) was first isolated and described by Friedenstein and his colleagues [20]. They are isolated from bone marrow [21], umbilical cord [22], adipose tissue [23], cord blood [22], placenta [24], dental pulp [25], endometrium [26], amniotic fluid [27], skeletal muscle tissue and dermal tissue [28]. The best source and standard for MSCs from other sources is the bone marrow derived MSCs. MSCs are heterogeneous and they are cultured and can differentiate into bone cartilage, fat to mesodermal tissues. MSCs paracrine factors play a vital role in immunomodulation [29], tissue healing [30,31], anti-fibrosis [32,33], anti-apoptosis [34] and angiogenesis [35]. MSCs paracrine factors reach the injured tissues to help repair the tissue. MSCs prevent fibrosis through the paracrine factors and suppresses and reduces the chronic inflammation. By amplifying autophagy, MSCs derived exosomes help in tissue repair and preventing apoptosis [36].

PERIODONTAL LIGAMENT STEM CELLS (PDLSCs):

Periodontal ligament stem cells are also known as Mesenchymal stem cells. The main characteristic feature of PDLSCs is self-renewal, multipotency and immunomodulation [37]. They are heterogenous, multipotent cells and competent to differentiate into osteoblasts, cementoblasts, chondrocytes and adipocytes [38,39,40]. They endorse neoformation of periodontal tissues. Osteoblasts are responsible for tissue homeostasis of alveolar bone, cementoblasts in cementum and fibroblasts in periodontal ligament. PDLSCs travel to the injured site, activated and differentiate into osteoblasts- bone formation, cementoblasts-cementum regeneration, fibroblasts- ligament fibre repair. These cells secrete anti-inflammatory cytokines (IL-10, TGF-β). They are suppreses pro-inflammatory cytokines (IL-1β, TNF-α). They regulate the immune cells such as Tcells, macrophages and release growth factor to elevate blood vessel formation and help in tissue repair. The tissue repair produces extracellular matrix proteins and collagen which helps to rebuild the periodontal structure [41].

ISOLATION TECHNIQUES:

Isolation techniques are as follows: differential ultracentrifugation, ultrafiltration, precipitation-based methods, size exclusion chromatography, immunoaffinity capture and microfluidic based isolation [42.

1. DIFFERENTIAL ULTRACENTRIFUGATION: It is a common and gold standard isolation technique. The isolation is done based on the size and density of the cells with sequential centrifugation steps. It is a bit time consuming.
2. ULTRAFILTRATION: This isolation method uses membrane fillers to separate particles by size. The main disadvantage is the risk of vesicle damage.
3. PRECIPITATION BASED METHODS: This method uses polyethylene glycol (PEG) to precipitate exosomes. It is simple and suitable for small samples. It is less pure.
4. SIZE EXCLUSION CHROMATOGRAPHY: The main principle of this method is, it separates particles based on size through porous gel matrix. It has high purity. It requires specialized column.
5. IMMUNOAFFINITY CAPTURE: It uses antibodies targeting exosomes surface markers. It is expensive and shows low yield.
6. MICROFLUIDIC-BASED ISOLATION: It is a newer technology. It relies on lab-on-a-chip systems to isolate exosomes using size, charge or markers. This method is rapid and requires small sample volume. This technique is still under development and very expensive.

PRESERVATION TECHNIQUE:

Preservation technique for exosomes such as cryopreservation with cryoprotectants, freeze drying, spray drying and storage at -80°C [43.

1. Storage at -80°C: It is a gold standard technique. It helps to prevent degradation of membrane integrity. This method requires ultra-low freezer.
2. Cryopreservation: This is a successful method. Cryopreservation is associated with cryoinjury. Cryoinjury is caused by osmotic imbalance and intracellular ice formation [44. This method causes cell death which can be prevented by the use of cryoprotectants. Cryoprotectants are of two types namely intracellular agents (penetrating cryoprotectants) and extracellular compounds (nonpenetrating cryoprotectants).
3. Drying method preservation: in this method, the water is removed using two methods such as freeze drying and spray drying.
   I. Freeze-drying: the initial step of this method is sublimation (primary drying) which followed by desorption (secondary drying). The basic principle in which freeze-drying takes place is that the water changes from solid state to vapour state without changing into liquid state and it takes place under pressure (4.579mmHg) and temperature below 0.0099°C [45.
   II. Spray drying: This is suitable to produce therapeutic agents such as vaccines, proteins and peptides [46. It is faster, undergoes single step and more economical. This method undergoes five steps such as concentration, atomization, droplet-air contact, droplet-drying and separation.

EXOSOMES IN PERIODONTITIS:

Periodontal disease is a chronic inflammatory disease that caused by bacteria called Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis, destructing the supporting structures of the tooth. Thus, it eventually leads to the destruction of supporting tissues of the periodontium. It can affect 90% of the global population [47. Many researchers have reported that exosomes found in all body fluids can be isolated from saliva are useful for diagnosis and investigation of various diseases [48. The cells produce ATP (adenosine triphosphate) in periodontitis. Exosomes reduced ATP to ADP (adenosine diphosphate) and at the very last into adenosine releasing CD39 and CD73 and finally apoptosis is inhibited [49. Periodontal regeneration is a multifactorial which is achieved by treatments such as scaling and root planning and several agents to prevent periodontal pocket. Long junctional epithelium is responsible for the healing of periodontal pocket. On treatment basis, GTR is the best option for periodontal pocket. Periodontal regeneration is achieved by two methods such as scaffold and controlled delivery of drugs. There is not much literature about scaffold application. The mesenchymal cells (MSCs) derived exosomes are suitable option for periodontal regeneration. In periodontal regeneration, use of scaffolding is the best option with minimal destruction [50. Many studies are reported that, collagen sponges are used as scaffold for loading exosomes in regeneration of the periodontium. Exosomes are nanocarriers which help in the delivery of therapeutics to the infected tissue. Exosomes are applied to the periodontal defect sites as scaffold, membranes and biomaterials such as drugs and hydrogels.

Engineered exosomes are used to diagnose periodontitis. Mant studies have shown a decreased levels of CD9 and CD81 levels of salivary exosomes in patients with periodontitis when compared to healthy individuals [51. Exosomes amplify the periodontal regeneration through osteogenesis-angiogenesis coupling [52. The mesenchymal stem cells (MSCs) derived exosomes boost angiogenesis by enhancing the migration and proliferation of endothelial cells. This causes stimulation of osteoblast and fibroblast regeneration [53. Exosomes intensify the bone matrix synthesis and matrix mineralization by raise the growth factors such as IGF-1 and TGF-1β [54. Periostin is a protein that is responsible for adhesion and migration of fibroblasts and osteoblasts that lead to periodontal regeneration [55. The advantages of exosome-based therapies are cell-free, ready-to-use, easier to obtain and easier to explore various routes of drug administration [56. Many studies have reported that stem cell derived exosomes increase the proliferation of osteoblast/osteocyte and thus preventing apoptosis [57. The use of exosomes among clinicians have shown effect on increased bone formation and reduced bone resorption are expected to raise.

EXOSOMES IN PERIODONTAL REGENERATION:

Exosomes play an important role in periodontal tissue regeneration. By enhancing the process of angiogenesis, exosomes help in repair of periodontal regeneration [58. Bone marrow MSCs contain growth factors such as IGF-1, VEGF, and TGF-β1 to promote cell migration, proliferation and vascularization enhancing early bone formation [59. EXOs from periodontal stem cells enhance angiogenesis by delivery growth factors such as VEGF, FGF-2, Ang-1 and pro-angiogenic miRNAs to the endothelial cells resulting in new blood vessel formation to improvise periodontal healing.

Periodontal tissue engineering mainly includes three aspects such as cell therapy, biomaterial scaffold fabrication technology and bioactive factors [60. Biomaterial scaffold plays an important role in tissue engineering. These scaffolds tend to mimic the structural and functional components of natural extracellular matrices [61. The main characteristics feature which makes tissue engineering ideal would be good biocompatibility and biodegradation; controllable degradation and absorption rates; three-dimensional porous structures which support the growth of cells and help in transportation of nutrients and metabolic waste; good strength; optimal mechanical properties [62.

Recent advancements in tissue engineering utilizing mesenchymal stem cells provide new therapeutic way for the regeneration of periodontal tissues. Stem cells used for periodontal regeneration can be divided into dental and non-dental stem cells. There are many varieties of stem cells such as dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAPs) and dental follicle cells (DFCs) tend to provide a significant potential in tissue regeneration [63. Embryonic stem cells, induced pluripotent stem cells, bone marrow-derived mesenchymal stem cells and adipose-derived stem cells also demonstrated to enhance the process of regeneration of periodontal tissues.

DISCUSSION:

Periodontitis is a chronic inflammatory disease that occurs in periodontal tissues which is irreversible. Common periodontal treatment modalities are supra-gingival scaling, sub-gingival curettage and root planning. Tissue engineering helps to repair damaged periodontal tissues. Extracellular vesicles are divided into apoptotic bodies (ApoBDs), microvesicles (MVs) and exosomes (EXOs) [58. Exosomes are secreted by almost all types of cells and is a natural drug delivery vehicle. Natural exosomes and engineered exosomes are their types. EXOs carry cell signalling chemicals and have the ability to enter the Blood Brain Barrier (BBB) and suppress the inflammation due to their low immunogenicity and high transport efficiency. EXOs help to monitor disease progression, early diagnosis and detection.

In the formation of EXOs, early endosomes mature into multivesicular body (MVB). Cargo sorting into intraluminal vesicles. MVB fuses with lysosomes, some MVB move to plasma membrane. The intraluminal vesicles are released outside as exosomes. EXOs are secreted by all types of cells such as mesenchymal stem cells (MSCs), periodontal ligament stem cells (PDLSCs), dendritic cells (DCs), B cells, T cells and mast cells. Immune cells (Tcells and macrophages) release growth factors that produces blood vessel in the injured site which helps in tissue regeneration. MSC derived EXOs help in tissue repair by promoting autophagy and stopping apoptosis. Due to their high immunomodulation ability, MSCs are best resource for cell-based therapy. PDLSCs also known as mesenchymal cells. Characteristic features of PDLSCs are they self-renewal, multipotent, immunomodulation. PDLSCs move to the injured site and differentiate into osteoblasts for bone formation, cementoblast for cementum regeneration and fibroblasts for ligament fibre repairs.

Ultracentrifugation, ultrafiltration, precipitation-based methods, size exclusion chromatography, immunoaffinity capture and microfluidic are the isolation techniques used to isolate exosomes. Ultracentrifugation is the gold standard and commonly used isolation technique. Cryopreservation with cryoprotectants, freeze-drying, spray-drying, room temperature with stabilizers and storage at 80C are the preservation techniques used to preserve exosomes. Storage at -80C is the god standard method. Cryopreservation is a successful method with a main drawback called cryoinjury which is prevented by use of cryoprotectants.

In periodontal defect sites, EXOs are applied as scaffolds, membranes and biomaterial such as drugs and hydrogels. Collagen sponges are used as scaffold for loading EXOs in periodontal regeneration. EXOs accelerate periodontal regeneration through osteogenesis-angiogenesis coupling. Studies report that stem cell derived EXOs increase osteoblast/osteocyte proliferation to prevent apoptosis. Periodontal tissue engineering mainly includes three aspects such as cell therapy, biomaterial scaffold fabrication technology, and bioactive factors [60. Tissue engineering should possess certain characteristics making it ideal are good compatibility and biodegradation, controllable degradation and absorption rates, cell growth and transportation of nutrients, good strength and good mechanical strength [62. The traditional diagnostic criteria for periodontitis tend to rely on clinical symptoms such as periodontal pocket depth and pathological loss of alveolar bone.

CONCLUSION:

Exosome show explicit use as a therapeutic tool for periodontal regeneration. The use of stem cell derived exosomes shows positive outcomes in the repair of periodontal tissue defects. Exosome loaded scaffolds have been used in repair of tissues such as bone, cartilage and skin. They have higher therapeutic potential. The scaffolds used for loading exosomes in periodontal regeneration include hydrogels, collagen sponges, and β-tricalcium phosphate (β-TCP) scaffolds. In most studies, the use of exosomes have been used in animal models and have limited access in clinical field. Exosomes have been emerged as a new research frontier in the field of periodontics. Future studies should focus on the application in clinical practice to make it more effective therapy. Exosomes have been in spotlight recently for the diagnosis and treatment for many diseases.

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