Potential of Stem Cells from Human Exfoliated Deciduous Teeth (SHED)-derived Secretome Gel in the Wound Healing Process Post Tooth Extraction

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Potential of Stem Cells from Human Exfoliated Deciduous Teeth (SHED)-derived Secretome Gel in the Wound Healing Process Post Tooth Extraction

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Abstract

Tooth extraction is a process of removing teeth from the alveolar process. The tooth extraction process always causes tissue damage, both hard tissue and soft tissue. Based on the results of Basic Health Research in 2018, the prevalence of tooth extraction in Indonesia reached 2.9%. Tooth socket wounds that are exposed to the oral environment allow the entry of pathogenic microorganisms which can cause alveolar osteitis, oroantral fistula and bacteremia. Apart from that, socket wounds after tooth extraction cause discomfort in the sufferer's oral cavity. Wounds resulting from tooth extraction often cause pain and interfere with eating activities. Spongostan is effective in healing wounds after tooth extraction and is often used in dentistry. Spongostan has several weaknesses, including the fact that it can cause hematomas, foreign body allergic reactions, extensive fibrosis, and toxic shock syndrome.

To determine the potential of stem cells from human exfoliated deciduous teeth (shed)-derived secretome gel in the wound healing process after tooth extraction.

The secretome has potential for angiogenesis, neurogenesis, tissue repair, immunomodulation, wound healing, anti-fibrotic and antimicrobial and tissue regeneration. The secretome of mesenchymal stem cells is known to contain various cytokines and growth factors. The stem cell secretome has low stability and retention in tissue so it needs to be combined with biomaterials to overcome the low tissue retention of the secretome and controlled release of bioactive materials for tissue healing. The combination of primary tooth stem cell secretome based on Hydroxypropyl methylcellulose (HPMC) gel as a carrier medium containing anti-inflammatory cytokines and growth factors is expected to act as an immunomodulator which can improve the wound healing process after tooth extraction at the inflammation, proliferation and regeneration/remodeling stages.

Stem cells from human exfoliated deciduous teeth (shed)-derived secretome gel has the potential to accelarate the wound healing process after tooth extraction.

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Introduction

Tooth extraction is a process of removing teeth from the alveolar process. The tooth extraction process always causes tissue damage, both hard tissue and soft tissue.[1] The results of Basic Health Research (Riskesdas) in 2018 conducted by the Indonesian Ministry of Health stated that the prevalence of dental and oral diseases in Indonesia reached 57.6 %. One of the treatments that can be done to treat dental and oral diseases is tooth extraction. Based on the results of Basic Health Research in 2018, the prevalence of tooth extraction in Indonesia reached 2.9 %.[2,3]

Tooth socket wounds that are exposed to the oral environment allow the entry of pathogenic microorganisms which can cause

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alveolar osteitis, oroantral fistula and bacteremia. Apart from that, socket wounds after tooth extraction cause discomfort in the sufferer's oral cavity. Wounds resulting from tooth extraction often cause pain and interfere with eating activities. These things have encouraged various research in the field of Dentistry to look for materials that can speed up the closure of socket wounds after tooth extraction.[4]

In dental practice, agents are needed to speed up the healing process after tooth extraction. Spongostan is a gelatin sponge made from natural gelatin foam or 100% porcine gelatin which has the same density. Spongostan is effective in healing wounds after tooth extraction and is often used in dentistry. Spongostan on the other hand also has several weaknesses, including causing hematomas, foreign body allergic reactions, extensive fibrosis, and toxic shock syndrome.[5,6]

The secretome of mesenchymal stem cells is known to contain various cytokines and growth factors.[7,8] Combination of primary tooth stem cell secretome based on Hydroxypropyl methylcellulose (HPMC) gel as a carrier medium which contains anti-inflammatory cytokines and growth factors is expected to act as an immunomodulator which can improve the wound healing process after tooth extraction at the inflammation, proliferation and regeneration/ remodeling stages.[9,10] This article explains the potential of stem cells from human exfoliated deciduous teeth (shed)-derived secretome gel in the wound healing process after tooth extraction.

Healing Wounds After Tooth Extraction

The healing process after tooth extraction is generally almost the same as the healing process for wounds on other bodies. When the tooth is removed, what remains in the socket is: the cortical bone (lamina dura in radiographic images) covered by the torn periodontal ligament, with the left oral (gingival) epithelial edge coronal. The socket is filled with a blood clot that protects the socket from the oral environment. This process is a secondary healing process that occurs over months to the point where the socket becomes difficult to distinguish from the surrounding bone when viewed radiographically.[11]

The phases in the healing process after tooth extraction consist of:[12]

1. Coagulation and hemostasis, which occur aberrantly after tooth extraction;
2. Inflammation, which begins soon afterward;
3. Proliferation, starting in the following days and incorporating most of the healing process;
4. Formation and remodeling, aims to restore form and function. This phase occurs over several months.

Bone defects will have a bone healing process called bone healing. Bone healing consists of 3 phases, which are reparative, inflammation and remodeling phase. The remodeling phase is the final process of bone healing.[13]

Stem Cells from Human Exfoliated Deciduous Teeth (SHED)-derived Secretome

Dental pulp stem cells are mesenchymal stem cells that originate from dental pulp tissue. Primary tooth stem cells compared with permanent tooth stem cells have a higher proliferation ability, with a shorter doubling time, as well as a higher clonality and osteoblast differentiation ability.[14] Primary tooth stem cells show a morphology like fibroblast cells and have a higher proliferation rate compared to permanent tooth stem cells and bone marrow mesenchymal stem cells. This could be caused by the high expression of genes related to cell proliferation and extracellular matrix in primary tooth stem cells compared to permanent tooth stem cells.[15]

Miura, et al . (2003) found that exfoliated primary teeth contained multipotent stem cells. Exfoliated primary tooth stem cells are known to have a highly proliferative and clonogenic cell population that is capable of differentiating into various types of cells, namely cells similar to nerve cells, adipose cells and odontoblasts. After in vitro transplantation, stem cells from exfoliated primary teeth were able to induce bone formation and produce dentin.[16]

Mesenchymal stem cells that are cultured in conditioned media for several days will release natural by-products, namely the secretome containing cytokines, chemokines, immunomodulatory molecules, growth factors and extracellular vesicles (EV). Primary tooth stem cells are a source of stem cells that secrete various growth factors, cytokines and exosomes, and can be detected in stem cell culture media. These secreted growth factors function as paracrine mediators for immunoregulation and tissue regeneration. The content of the secretome is also influenced by the conditions and type of cells when cultured. Secretrom has

the potential for angiogenesis, neurogenesis, tissue repair, immunomodulation, wound healing, anti-fibrotic and antimicrobial and tissue regeneration.[17,18]

The secretome of mesenchymal stem cells is known to exhibit therapeutic properties as it is capable of inducing cell migration, proliferation, immunomodulation, and tissue regeneration. The secretome of mesenchymal stem cells is known to contain various cytokines and growth factors.[7,8] In research by Bhandi et al., (2021), it was identified that the SHED secretome had higher expression of IL-10, TGF-β, and VEGF. The results of comparative analysis of GFs responsible for cell proliferation showed that the highest expression of fibroblast growth factor -2 (FGF-2), hepatocyte growth factor (HGF), and platelet derived growth factor (PDGF) was observed in the secretome of deciduous tooth stem cells.[19]

In vitro and in vivo studies on mesenchymal stem cells are pushing clinical research forward, due to their advantages, such as plasticity, stemness, and the absence of adverse reactions or tumor formation upon transplantation. Among various sources of mesenchymal stem cells, dental pulp is expected to be a nearly ideal source of multipotent mesenchymal stem cells, which can be used in clinical research fields, including regenerative dentistry, orthopedic injury repair, and treatment of degenerative neurological disorders. Currently, there is increasing evidence that dental stem cells have many similarities with bone marrow mesenchymal stem cells. Dental stem cells have the advantage of being easily isolated compared to mesenchymal stem cells from bone marrow which require more expensive and invasive techniques. The administration of mesenchymal stem cell conditioned medium gel to burn wounds can heal wounds better than silversulfadizin cream and placebo as seen from the percentage reduction in wound diameter. Research by Nugraha et al . (2023) proved that the secretome of gingival stem cells can decrease osteoclastogenic and bone resorption related markers such as TRAP, NFATc1, and sclerostin expressed in osteoclasts.[19,20,21]

Hydroxypropyl methylcellulose (HPMC) gel

Hydroxypropyl methyl cellulose (HPMC) is a semi-synthetic cellulose derivative gelling agent that is resistant to phenol and stable at pH 3 to 11. HPMC can form a clear, neutral gel and has a stable viscosity in long-term storage. HPMC functions as a gelling agent which is a gel-forming material. Propylene glycol functions as a humectant which will maintain the stability of the preparation by absorbing moisture from the environment and reducing evaporation of water from the preparation. As the concentration of HPMC increases, the adhesive power will increase in each formula. The higher the concentration of gelling agent used, the greater the consistency of the gel and the greater the adhesive power. HPMC forms a gel base by absorbing solvent so that the liquid is retained and increases fluid resistance by forming a compact liquid mass. The more HPMC that dissolves, the more liquid will be retained and bound by the gelling agent.[22]

Discussion

Healing of tooth extraction is needed as soon as possible by dentists. Late wound recovery process post tooth extraction, may have some trouble with soft and hard tissues in the post extraction area. This process can prevent experiences to complications in alveolar bone and gingival tissues. The use of medicine post the tooth extraction can reduce the possibility of complications and it is often expected to be able to gain the process of coagulating of blood, so that it will also have the process of wound recovery soon.[23]

The wound healing process after tooth extraction begins with the healing process in the soft tissue, then continues with the healing process in the hard tissue, namely the alveolar bone. Post-extraction wound healing involves growth factors that determine the wound healing process, namely the inflammatory phase, proliferation phase, and remodeling phase.[24]

The acute inflammatory response peaks in the first 24 hours and is complete after 7 days. The first phase of the inflammatory phase of bone healing is the formation of a hematoma from peripheral and intramedullary blood cells, as well as bone marrow cells. This response causes the hematoma to coagulate between and around the fracture tips, and within the medulla to form a template for callus formation. The inflammatory phase is important as the main factor in hemostasis and requires the innate immune

system, neutrophils and monocytes as the body's main defense against pathogens and assists in phagocytosis of damaged tissue. If tissue damage occurs, there will be a signal called "danger signals" which is characterized by two categories, namely Damage-Associate Molecular Patterns (DAMPs) and Pathogen-Associated Molecular Patterns (PAMPs).[25,26]

DAMPs are intrinsic "danger alarms" and PAMPs are extrinsic factors that are connected to interactions with tissue damage and are part of the inflammatory mediator High Mobility Group Box 1 (HMGB-1). Activation of DAMPs makes HMGB-1 bind to the Receptor Advanced Glycation End Products (RAGE) and also Toll-like receptors TLR2/TLR4 via the MyD88 pathway which induces transcription of the Nuclear Factor-Kappa Beta (NF-κB) pathway. Activation of the NF-κB pathway can increase the activation of pro-inflammatory cytokines such as IL-1, IL-6 and TNFα. Activation of several pro-inflammatory cytokines can increase the activity of Bone Morphogenetic Protein -2 (BMP-2) and VEGF, which are bio markers that play a role in the proliferation stage process.[27,28]

The proliferative phase occurs after the inflammatory process until the 3rd week. The proliferation phase begins with the process of necrotic bone resorption in the damaged bone area using osteoclasts. This is then continued with the process of callus formation, vascular repair and osteoid secretion. This phase involves the gradual replacement of soft callus by immature woven bone. The masenchymal tissue will differentiate into chondrocytes in the soft callus area and stabilize the fracture zone.[29,30]

Remodeling phase begins when osteoclast precursor cells receive signals from osteoblasts to differentiate into osteoclasts. Mature osteoclasts then synthesize proteolytic enzymes that digest the collagen matrix. This bone resorption is the initial stage of the remodeling cycle which is regulated by osteoclast apoptosis. The next phase of the remodeling cycle preosteoblasts are drawn from mesenchymal stem cells in the bone marrow. Mature osteoblasts synthesize bone proteins, especially osteocalcin and regulate bone mineralization and become osteocytes. So osteocalcin acts as a marker for active osteoblasts in the proliferation phase.[31]

The regeneration / remodeling phase is the last and longest phase of bone healing. This phase is a complex process involving bone resorption followed by new bone formation. During bone remodeling, there is communication between osteoblasts and osteoclasts at the cellular level. Osteoblasts play a major role in the process of bone formation, while osteoclasts play a role in the process of bone resorption. The sequence of bone regeneration phases is activation, resorption, reversal and formation. The activation step depends on osteoblast-derived cells, either on the bone surface or in the marrow, acting on blood cell precursors (hematopoietic cells) to form bone-resorbing cells or osteoclasts. The resorption process can occur below the cell layer. After the reversal phase, osteoblasts begin to produce new bone. Some osteoblasts remain in the bone and are converted into osteocytes, which connect with each other to the osteoblast surface. The three phases, namely activation, resorption (absorption), and reversal are relatively fast, perhaps only lasting 3 weeks in humans. The final phase of bone formation takes longer, up to 3 or 4 months. In this phase, BMP-2 plays a role in controlling the complex reaction between osteoclast and osteoblast cells in carrying out the remodeling stage.[21,32] In conclusion, primary tooth stem cell secretome gel has the potential to accelerate the wound healing process after tooth extraction at the hemostasis, inflammation, proliferation, and regeneration/ remodeling stages by increasing osteogenesis activity and suppressing osteoclastic activity. Further research to determine the exact levels and passage of primary tooth stem cell secretomes is needed.

Conclusions

From this review, it can be concluded that SHED-derived secretome gel has the potential to accelerate the wound healing process after tooth extraction. Application of SHED-derived secretome gel can play a role in the hemostasis, inflammation, proliferation, and regeneration/remodeling stages by increasing osteogenesis activity and suppressing osteoclastic activity, thus accelerating the wound healing process after tooth extraction.

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Declaration of Interest

The authors report no conflict of interest.

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