Regenerative medicine in dental and oral tissues: dental pulp mesenchymal stem cell

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ABSTRACT

Background. Regenerative medicine is a new therapeutic modality using cell, stem cell and tissue engineering technologies. Purpose. To describe the regenerative capacity of dental pulp mesenchymal stem cell. Review. In dentistry, stem cell and tissue engineering technologies develop incredibly and attract great interest, due to the capacity to facilitate innovation in dental material and regeneration of dental and oral tissues. Mesenchymal stem cells derived from dental pulp, periodontal ligament and dental follicle, can be isolated, cultured and differentiated into various cells, so that can be useful for regeneration of dental, nerves, periodontal and bone tissues. Tissue engineering is a technology in reconstructive biology, which utilizes mechanical, cellular, or biological mediators to facilitate regeneration or reconstruction of a particular tissue. The multipotency, high proliferation rates and accessibility, make dental pulp as an attractive source of mesenchymal stem cells for tissue regeneration. Revitalized dental pulp and continued root development is the focus of regenerative endodontic while biological techniques that can restore lost alveolar bone, periodontal ligament, and root cementum is the focus of regenerative periodontic. Conclucion. Dentin-derived morphogens such as BMP are known to be involved in the regulation of odontogenesis. The multipotency and angiogenic capacity of DPSCs as the regenerative capacity of human dentin / pulp complex indicated that dental pulp may contain progenitors that are responsible for dentin repair. The human periodontal ligament is a viable alternative source for possible primitive precursors to be used in stem cell therapy.

Keywords: mesenchymal stem cells, dental pulp, tissue engineering, regenerative medicine

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ABSTRAK

Latar belakang. Pengobatan regeneratif merupakan suatu metode baru dengan menggunakan sel stem dan teknologi rekayasa jaringan. Tujuan. Menguraikan kemampuan regenerasi sel stem mesenkim pulpa gigi. Telaah Pustaka. Di bidang kedokteran gigi, sel stem dan teknologi rekayasa jaringan berkembang pesat dan menarik banyak perhatian karena kemampuannya dalam memfasilitasi inovasi bahan gigi serta regenerasi jaringan gigi dan mulut. Sel stem mesenkim yang berasal dari pulpa gigi, ligamen periodontal dan folikel gigi dapat diisolasi, dikultur dan berdiferensiasi menjadi pelbagai jenis sel sehingga bermanfaat untuk regenerasi jaringan gigi, saraf, periodontium dan tulang. Rekayasa jaringan merupakan suatu teknologi rekonstruksi biologik yang menggunakan prinsip mekanik, sel, mediator biologic, untuk memfasilitasi regenerasi atau rekonstruksi jaringan tertentu. Kemampuan multipoten, kecepatan proliferasi yang tinggi, dan tersedianya sumber yang cukup, membuat pulpa gigi merupakan suatu sumber sel stem mesenkim yang menarik bagi regenerasi jaringan. Revitalisasi pulpa gigi dan kelanjutan pembentukan akar merupakan fokus endodontik regeneratif sedangkan teknik biologik untuk memulihkan tulang alveolar yang hilang, ligamen periodontal dan sementum akar merupakan fokus dari periodontik regeneratif. Kesimpulan. Dentin-derived morphogens seperti bone morphogenic protein (BMP) diketahui berperan dalam regulasi odontogenesis. Adanya kemampuan angiogenik dan multipoten sel stem pulpa gigi (DPSc) sebagai kapasitas regeneratif komplek pulpa dentin manusia mengindikasikan bahwa pulpa gigi mengandung progenitor yang bertanggung jawab terhadap reparasi dentin. Ligamen periodontal manusia merupakan sumber alternatif kehidupan, sebagai prekursor dalam terapi menggunakan sel stem.

Kata kunci: *sel stem mesenkim, pulpa gigi, rekayasa jaringan, pengobatan regeneratif.*

INTRODUCTION

In recent years, stem cell research has grown fast indicated that stem cell-based therapies have potency to improve the quality of life patients with conditions that range from Alzheimer's disease to cardiac ischemia as well as regenerative medicine, like bone or tooth loss.[1,2] Stem cells play an important role in development and tissue regeneration.[3]

Certain tissues contain more stem cells such as the dental tissues that considered a rich source of mesenchymal stem cells, in proper to tissue engineering applications. Dental mesenchymal stem cells were discovered from dental pulp, periodontal ligament, dental papilla, and dental follicle that have potency to differentiate into several cell types, including odontoblasts, neural progenitors, osteoblasts, chondrocytes, and adipocytes. In dentistry, stem cell biology and tissue engineering are of great interest that may provide an innovative for generation of clinical material and/or tissue regeneration. The multipotency, high proliferative rates, and accessibility make the dental stem cell an attractive source of mesenchymal stem cells for tissue regeneration.[1,2]

The discovery of stem cells in dental pulp has led to a new paradigm especially in field of understanding mechanisms involved in maintenance of dental pulp homeostasis both in health condition and pulp response to injury. Stem cells are involved with the dental pulp tissue physiology within lifespan of the tooth. It has also been accepted that stem cells are involved in pulp angiogenesis control during response to cariogenic process.[4]

Regenerative endodontics is a new treatment concept that focuses on pulp vitality reestablishment and root development continuing[4]. Stem cell that have been utilized to periodontal regeneration, including bone marrow-derived mesenchymal stem cells (BMSCs) and the main dental-derived mesenchymal stem cell such as periodontal ligament stem cells (PDLSCs), dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), stem cells from apical papilla (SCAPs) and dental follicle precursor cells.[5,6]

LITERATURE REVIEW

Stem cells and tissue engineering

Stem cells are characterized by the ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. The highly proliferative capacity and plasticity make these cells become a new source of seed cells in tissue engineering in a wide range of applications.

Tissue engineering purposes to stimulate either to regenerate or to grow tissue outside the body, which can then be implanted as natural tissue. The research exploration of tooth tissue engineering mainly focuses on three parts: seeding cells, scaffolds and growth factors.[7,8]

Sources of dental-derived mesenchymal stem cells are PDLSCs, DPSCs, SHEDs, SCAPs and dental follicle precursor cells[9]. Gronthos, *et al.* first identified adult DPSCs in human dental pulp and revealed that DPSCs could regenerate a dentin-pulp-like complex composed of mineralized matrix with tubules lined by odontoblasts and fibrous tissue containing blood vessels in an arrangement similar to the dentin-pulp complex found in normal human teeth.[10] Dental follicle is a mesenchymal tissue that surrounds the developing tooth germ during tooth root formation. Dental follicle progenitors created periodontal components, such as cementum, periodontal ligament, and alveolar bone.[11] Alternative source of primitive progenitors in stem-cell therapies is the human periodontal ligament [12] while SHEDs might be an ideal resource of stem cells to repair damaged tooth structures and induce bone regeneration.[13]

Regenerative endodontics stem cells and caries-induced dentinogenesis

Dental pulp contains a small sub-population of stem cells that are involved in pulp response to caries progression. Specifically, stem cells replace odontoblasts that have undergone cell death as a consequence of cariogenic risk. Stem cells also secrete factors that have potency to enhance pulp vascularisation, provide oxygen and nutrients required for dentinogenic response that is typically observed in teeth with deep caries.[3]

Dentinogenesis is a unique process which involves interaction between odontoblasts, endothelial cells, and nerves. The odontoblasts, ecto-mesenchymal derived cells are the first cells that respond to the injury caused by bacterial invasion during caries progression. The responses to bacterial stimuli are possible because of odontoblasts expressed Toll-like receptors (TLR). TLR2 primarily involved in Gram-positive and TLR4 for Gram-negative bacteria. Expression of TLR4[14] and nociceptive neurons in dental pulp cells increase during pulpitis.[15] TLR2 and TLR4 play critical role in regulation of dental pulp angiogenesis within response to bacterial stimuli.[16,17] DPSCs express TLR-4 while exposure DPSCs to bacterial LPS enhances vascular endothelial growth factor (VEGF) expression.[18]

Primary odontoblasts are induced to secrete a dentin matrix that mineralizes as reactionary dentin in response to shallow caries. This type of tertiary dentin protects dental pulp from irritants and maintains dental pulp integrity. Meanwhile, when caries advances more deeply towards the pulp, odontoblasts die and cells originated from DPSCs pool typically substitute dying odontoblasts.[3,18] This process depends on a cascade of events that involve stem cell proliferation, migration, and differentiation into odontoblasts. Stem cell-derived odontoblasts can also contribute to the generation of a dentin bridge in cases of pulp exposure.

Stem cells and pulp angiogenesis

Signaling molecules underlying active interchange during dentinogenesis is exist upon the spatial proximity between odontoblasts and blood. Stem cells secrete factors that have potency to enhance pulp vascularisation and provide the oxygen and nutrients required for the dentinogenic response.[3,18] One of these factors is vascular endothelial growth factor (VEGF), a potent inducer of endothelial cell differentiation and survival, and the most effective angiogenic factor. VEGF also plays a critical role on vascular permeability changes during physiological and pathological events.[19]

VEGF is strongly expressed by odontoblasts and in the sub-odontoblastic layer *in vivo*.[17,18,20] Indeed, VEGF is potently expressed in dental pulp tissues of teeth undergoing caries-induced pulpitis as demonstrated by immunohistochemical studies.[21] Among its receptors, VEGFR2 is quite associated with the angiogenic potential of

endothelial cells[22]. VEGFR2 is expressed in dental pulp of permanent and primary teeth in accordance with pulp cells ability to respond to VEGF-induced signaling.[23] VEGF expression enhanced in dental pulp cells exposed to Lipotheicoic acid (LTA) or lipopolysaccharides (LPS).[20,24] LTA and LPS are important toxins associated with Gram-positive and Gram-negative bacteria, respectively.

Growth factor

Dentin is an important bioactive molecules reservoir that involved in the regulation of dental pulp responses to stimuli.[25] Dentin-derived morphogens play an important role in the odontoblastic differentiation of SHED[1]. Bone morphogenetic proteins (BMP) are involved in the regulation of odontogenesis and dentin regeneration.[26,27] Dentin-derived BMP-2 is required for SHED odontoblastic differentiation.[1] VEGF plays a key role in the promotion of dentinogenesis. VEGF induces vascularization required to sustain the high metabolic needs of odontoblastic cells in active process of dentin matrix secretion.[19]

Stem cells and regenerative periodontics

The periodontal ligament is a specialized connective tissue, derived from dental follicle and originated from neural crest cells. Mesenchymal stem cells obtained from PDLSCs are multipotent cells with similar features of the BMSCs and DPSCs, capable of developing different types of tissues such as bone and tooth associated-tissues.[5]

Stem cell populations that have been utilized to periodontal regeneration, including BMSCs and the main dental-derived mesenchymal stem cell populations such as PDLSCs, DPSCs, SHEDs, SCAPs and dental follicle precursor cells.[5,6]

DISCUSSION

Tissue engineering has been defined as "understanding and applying the principles of tissue growth to produce functional replacement tissue for clinical use". The ability of DPSCs to differentiate into odontoblasts and regenerate the dental pulp has raised the interest towards regenerative endodontics that use these cells for the revitalization of the necrotic teeth.[1,13,28-30]

Regenerative endodontics focuses on reestablishment of pulp vitality and continued root development. This clinical procedure relies on the intracanal delivery of a blood clot (scaffold), growth factors (possibly from platelets and dentin), and stem cells.[4]

The development of bioengineered organ replacement strategies and the appropriate seeding cells, plus biodegradable polymer scaffolds and proper microenvironment, ensure a substantial advance in tooth engineering. The increase time of culture will increase the number of cells. However the over initial number seeding cells will make cells apoptotic.[31]

The obvious advantages of SHEDs based on higher proliferation rate compared with stem cells from permanent teeth; easy to be expanded *in vitro*; high plasticity since they can differentiate into neurons, adipocytes, osteoblasts and odontoblasts; readily accessible in young patient[32]; especially suitable for young patients with mix dentition.[33] The long-term storage for 2 years of DPSCs is still capable of differentiating into pre-osteoblasts and produced woven bone tissues. In addition, DPSCs still expressed certain surface antigens, confirming cellular integrity.[34,35] Dental follicle stem cells (DFSCs) lines were heterogeneous.[36] DFSCs able to differentiate into osteoblasts/cementoblasts, adipocytes, and neurons.[12,37,38]

PDLSCs-based therapy will congregate the inflammation and infection control to stem cells capable in regenerate new periodontal tissues therefore problem that residual PDLSCs are limited in patients with periodontitis due to the long-term inflammation will be overcome.[39] PDLSCs could differentiate into cells that can colonize and grow on biocompatible scaffold, suggesting an easy and efficient autologous source of stem cells for bone tissue engineering in regenerative dentistry.[40] PDLSCs are able to differentiate into osteogenic and cementoblastic lineages. Developing apical tooth germ cells able to provide a cementogenic micro-environment and induce differentiation of PDLSCs along the cementoblastic lineage.

The ability of odontoblasts and stem cells to sense LPS through TLR signaling contributes to the overall response of the pulp to bacterial infection that is characterized by an increase in vascular density and influx of immune cells. The endothelial cells and nerve cells located in close to the carious lesion modulate the odontoblastic response.[18,20]

The multipotency and angiogenic capacity of DPSCs could be exploited therapeutically in dental pulp tissue engineering. The dentin healing depends at least in part on the regenerative potential of stem cells from the pulp core that actively migrate towards the carious site, differentiate into odontoblasts, and secrete mineralizable matrices. DPSCs are potential therapeutic targets in cases of reversible pulpitis. Importantly, these cells may become the primary strategy for the revitalization of necrotic immature permanent teeth.

The immature tooth treated by apexification as common procedures demonstrates healing of apical periodontitis, but does not achieve the goals of continued root development or restoration of functional pulp tissue.[8] Mesenchymal stem cells obtained from periodontal ligament as well as BMSCs and the main dental-derived mesenchymal stem cells are multipotent cells that have been utilized to periodontal regeneration. Growth factors in general act to stimulate pre-osteoblastic cells upregulating differentiation into new bone as well as stimulating the inflammatory cascade, which promotes wound healing. Growth factor technology is commercially available today, such as platelet-derived growth factor.

CONCLUSION

Dental mesenchymal stem cells are candidates for regenerative medicine, therefore the knowledge of the cell differentiation mechanisms is very important for the development of tooth engineering. The potential mesenchymal stem cells for tooth regeneration mainly include SHEDs, DPSCs, SCAPs, DFSCs, PDLSCs and BMSCs.

SHEDs are distinctive with the osteoinductive ability and high plasticity. The three main lineages of DFSCs were highly undifferentiated state of periodontal ligament-type lineage and cementoblastic or alveolar bone osteoblastic lineage. Dentin-derived morphogens such as BMP are known to be involved in the regulation of odontogenesis. The multipotency and angiogenic capacity of DPSCs as the regenerative capacity of human dentin / pulp complex indicated that dental pulp may contain progenitors that are responsible for dentin repair. The human periodontal ligament is a viable alternative source for possible primitive precursors to be used in stem cell therapy.

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