Review Article

Angiogenic and osteogenic potentials of dental stem cells in bone tissue engineering

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ABSTRACT

Manipulation of dental stem cells (DSCs) using current technologies in tissue engineering unveil promising prospect in regenerative medicine. DSCs have shown to possess angiogenic and osteogenic potential in both in vivo and in vitro. Neural crest derived DSCs can successfully be isolated from various dental tissues, exploiting their intrinsic great differentiation potential. In this article, researcher team intent to review the characteristics of DSCs, with focus on their angiogenic and osteogenic differentiation lineage. Clinical data on DSCs are still lacking to prove their restorative abilities despite extensive contemporary literature, warranting research to further validate their application for bone tissue engineering.

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1. Introduction

The end game of tissue engineering is to facilitate the healing of the damaged tissue and restore the function sustainably.¹ Deploying dental stem cells (DSCs) to clinically validate their potential as biological raw material for tissue engineering is widely studied.² The synergy between the stem cells (SCs), one of the

troika in 3D construct for tissue engineering (TE) with two other machineries (the growth factor and scaffold) is required to commence and deliver its regenerative function.³ Once transplantation is executed, the DSCs seeded in the 3D construct grow and differentiate into the preferred fate.⁴ The integration with the host system, especially the vascular system will ensure the survival and sustenance of the engineered tissue and the reparation success.⁵ Fundamentally, a matured organism originates from single pluripotent embryonic SC.⁶ After reaching adult stage, there are some mesenchymal stem cells (MSCs) populating in the terminally differentiated tissue, mainly in the perivascular niche for homeostasis and tissue injury healing.⁷ The objectives of this

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review are to deliberate the type of dental derived SCs and their properties. The focus will be emphasised on angiogenic and osteogenic features. Lastly, we outline the gaps in translating the findings in TE for therapeutic applications.

2. Dental stem cell characteristics

About more than 10 years ago, Gronthos and his team reported on their discovery of dental pulp stem cells (DPSCs) that marked the extensive pursuit for other dental derived stem cells sources.⁷ DSCs are clonogenic and self-renewal postnatal MSCs derived from dental tissues that are craniofacially located. Scientists have documented several types of SCs that have been successfully isolated and expanded *in vitro*, namely, DPSCs,⁷ stem cells from human exfoliated deciduous teeth (SHED),⁸ periodontal ligament stem cells (PDLSCs),⁹ SCs isolated from dental follicle precursor cells (DFPCs)¹⁰, stem cells isolated from apical papilla (SCAP)¹¹ and stem cells from human gingiva.¹² Though embryonic SC is of paramount importance with regard to all potency, however, DSCs have the edge over the rest since it involves non-invasive collection procedure and is ethically less hassle.¹³ DSCs originate from the embryonic source of neural crest ectomesenchyme, expressing typical MSC surface markers (Fig. 1). Until recently, the mainstream thesis believed that perivascular cells contribute to dental MSCs, however, peripheral nerve-associated glia dedifferentiated into a population of MSCs and involve in the development, self-renewal and repair of a tooth. The molecular profiling of DSCs exhibits the presence of angiogenic and osteogenic markers, revealing their plasticity which suggests their huge potential in tissue engineering.¹⁴

At the cellular level, Nestin is a neural stem cell (NSC) marker expressed by DSCs and also an intermediate filament constituting the cytoskeleton.¹⁵ The regulation of odontoblastic differentiation, tooth development and dentin reparation are associated with the nestin expression.¹⁶ The DSCs also express notch, another type of NSC marker. Notch signaling was shown to participate in maintaining the stemness and proliferative state of the cells.¹⁷ Notch signaling factors (receptors and ligands) regulate dental germ development as well as for impacted mature teeth

restoration.¹⁸ The availability of these markers in DSCs reveals the lineage of the progenitor cells and explains their potential of specializing into neuronal cells.¹⁹ Thus, DSCs can be a good SC source to be differentiated to neural cells, which can be utilised to treat neurodegenerative diseases or nerve traumatic injuries.²⁰ Moreover, the high proliferation rate of DSCs is an advantage in comparison to the other sources of SCs in terms of propagation efficiency.⁷

DSCs display angiogenic property due to the expression of endothelial differentiation markers. Angiopoietin 1 (Ang-1), cyclooxygenase-2 (Cox-2) and Ephrin B2 are among the documented markers that occur in the DSCs. Physiologically, Ang-1 controls the proliferation and survival of endothelial cells as well as vessel maturation.²¹ Cox-2 activates the signaling pathways controlling cell proliferation, migration, apoptosis, and angiogenesis,²² while Ephrin B2 controls the vascular endothelial growth factor (VEGF) receptor that promotes vasculogenesis and angiogenesis.²³ Any irregularity to this feature may cause pathological conditions as a faulty angiogenic regulation is also associated with arthritis, psoriasis and blinding retinopathy.^{22,24,25}

Apart from neural and angiogenic properties, osteogenic markers also present in DSCs include alkaline phosphatase (ALP), collagen type I (Col I), osteocalcin (OCL), and osteopontin (OPN).^{26,27} The differentiation of DSCs into odontoblasts is regulated by ALP. ALP is also a key marker of pluripotent embryonic SC identification as well as in MSCs.²⁷ At the tissue level, Col I is the major structural protein found in bone, skin,²⁸ tendons and ligaments.^{29,30} The defect in Col I gene may result in various disorders such as Caffey disease and osteogenesis imperfecta.^{30,31}

The presence of three different markers, neural, angiogenic and osteogenic in the DSCs show its differentiation potentials. These entice interest from the pre and clinical communities to exploit their capability to produce engineered tissue such as blood vessel or bone.^{32,33} DSCs are known as MSCs, and thus, they are capable of giving rise to chondrogenic, osteogenic and adipogenic lineage of cells.³⁴ In addition, DSCs also show neurogenic and angiogenic potentials. Therefore, these multipotent cells attract fundamental and clinical communities to exploit their capability to produce engineered tissues such as blood vessel or bone.^{32,33}

Fig. 1. The angiogenic and osteogenic potential of dental stem cells for bone tissue engineering.

3. Angiogenic potential of dental stem cells

The formation of new blood vessels or angiogenesis is the focal point in tissue engineering that happens to be a great challenge.³⁵ The failure to do so will interrupt the supply of basic needs of newly transplanted tissue and may compromise the survival and success rate.³⁶ Physiologically, angiogenesis allows the process of growth and development and wound healing to take place.^{37,38} However, angiogenesis is also associated with serious pathological conditions.²⁴ Defining angiogenesis regulation is the major milestone as this will be of great benefit in therapeutic applications in patients in inducing neovascularization. Selection of DSCs as one of the components of engineered construct provides an advantage due to its angiogenic behavior.³⁹ DSCs displayed their pro-angiogenic effects via two manners; firstly, paracrine effects by secreting angiogenic factors and secondly, by differentiating into endothelial cells and directly integrating with host.^{40,41}

DPSCs induced angiogenesis by releasing angiogenic factor in the DPSCs conditioned medium (CM).³⁹ Profiling of the media revealed the presence of VEGF and monocyte chemotactic protein-1 (MCP-1), classic components that promote angiogenesis.⁴² DPSCs were able to significantly promote the formation of blood vessels in the chicken chorioallantoic membrane assay.⁴² Another study investigated the paracrine effects of DPSCs in murine model, which found that wound healing is enhanced by the injected DPSCs.⁴³

Besides DPSCs, SHED have been previously reported to differentiate into vascular endothelial cells both *in vivo* and *in vitro*.³² SHED were seeded in tooth slice as scaffold and implanted subcutaneously into immunodeficient mice.⁴⁴ Consequently, they noted that SHED differentiated into new functional blood vessels and attached to the host circulatory system.⁴⁴ The presence of cellular elements in the newly formed vessels suggested that these capillaries anastomosed with the host vasculature. The scaffold utilized was also believed to induce endothelial differentiation of SHED due to the angiogenic factors residing in the stromal cells⁴⁵ or dentin matrix,⁴⁶ while, in the *in vitro* study, SHED turned into endothelial cells³² after VEGF administration, an effective growth factor in inducing angiogenesis.⁴⁷ The endothelial differentiation markers, namely, vascular endothelial growth factor receptor 2 (VEGFR2) and CD31 were positively expressed.³² Treatment with VEGF was also responsible for the higher number of capillaries sprouting without having an effect on the proliferation rate of SHED.⁴⁸ Primary DPSCs also displayed endothelial differentiation triggered by VEGF.³² The differentiated cells expressed the common endothelial markers, VEGFR2 and CD31.³² Based on these evidences, it can be concluded that DSCs are able to differentiate into endothelial cells.

The other type of SCs previously described on their involvement in angiogenesis, both *via* co-culture and differentiation pathways which include embryonic SCs (ESCs),^{49,50} adipose derived SCs^{51,52} and cardiac SCs⁵³ and endothelial progenitor cells.^{54,55} However, among the SCs, the ESCs possess a major concern with regards to its tumorigenic potential.^{56,57} Hence, this might incur additional studies to ensure that the end products are fit and safe for consumers.

These studies highlighted the angiogenic potential of DSCs that can be useful for in applications. Further development and trials need to be carried out to assess the safety and efficacy of DSCs and translate their potential to the patients.

4. Osteogenic potential of dental stem cells

The exploration of DSCs osteogenic potential has been extensively investigated in the last decade since the discovery of DSCs.⁷ Physiologically, the formation of bone involves MSC

accumulation for mesenchymal condensations.⁵⁸ This process partially resembles the formation of tooth except that there is no epithelial invagination.⁵⁹ The nearby cells naturally restore the impacted bone tissue when there is minor fracture including chondroblasts, osteoblasts, endotheliocytes, and fibroblasts.⁶⁰ However, when the injuries are beyond repair by the local cells (such as large bone defects created by trauma, infection, tumor resection, and skeletal abnormalities), stem cell therapy can be an option (such as bone marrow SCs) for bone regeneration.⁶⁰

DSCs may be one of the possible SC sources for bone regeneration as they are able to differentiate into osteocytes and osteoblasts under special conditions *in vitro*.^{23,27,61–63} DFPCs were shown to have osteogenic differentiation potential, although it was cultured in the absence of dexamethasone, a pro-osteogenic supplement.⁶² Osteo/odontogenic differentiation by SCAPs was observed and addition of KH_2PO_4 promoted proliferation and differentiation of SCAP.⁶³ Interestingly, the mineralized tissue formation ability by DPSCs can be enhanced by genetic modification study as well as during *in vitro* study.³³

Various *in vivo* studies that employed DSCs from different sources such as human, murine, rabbit, canine and minipig showed that DSCs also differentiated into bone-like structure.^{33,64–67} A defected mandibular was treated with cryopreserved SHED, and it was found that osteogenesis took place without immune response from the host.⁶⁸ Another study was done in periodontal fenestration defected rats and after treatment with allogeneic PDLSCs, periodontal defects were repaired.⁶⁹

Recently, a clinical trial verified the efficiency of autologous PDLSCs to regenerate periodontal tissue in periodontitis patients with deep intraosseous defects (>5 mm) and the safety of PDLSCs in clinical periodontal regenerative medicine.²

In comparison with bone marrow SCs, DSCs were more competitive as they have immunosuppressive properties, are highly proliferative and can endure cryopreservation without harming the SCs.^{70,71} Despite these positive features reported, Asatrian et al. suggested that the limited quantity of pulp in a tooth may hinder DSCs' potential as an alternative source for therapeutic application.⁷²

All of the studies discussed earlier provide preclinical and clinical evidences of DSCs' ability to differentiate into bone tissue. This feature can be linked to the embryonic origin of DSCs which is from the neural crest.^{73,74} Hence, it can be concluded that DSCs have the osteogenic differentiation ability.

5. Angiogenesis and osteogenesis: bone tissue engineering duo

More than 200 years ago, the intimate relationship between angiogenesis and osteogenesis had been acknowledged.^{75,76} Angiogenesis along with efficient supply of blood are prerequisites to support and sustain the bone development and maintenance.⁷⁷ The blood vessels also serve as communication network for the skeletal and neighboring tissue.⁷⁸ In the field of reconstructive orthopaedic, autograft is the first option should orthopedic surgeons are required to perform bone graft.⁷⁹ Autogenous bone graft is the most preferable, as it is osteoconductive, osteoinductive and osteogenic.⁸⁰ Autogenous cancellous bone extracted from the iliac crest is considered a popular option due to the fact that it fulfills the three features.⁸¹ Nonetheless, there are few complications associated with the invasive autograph extraction procedure which include wound infection, persistent pain, swelling and altered sensation.⁸²

Previously, some approaches employed in bone tissue engineering co-transplanted osteoprogenitor cells with either hematopoietic or endothelial cells aiming to establish a supportive blood supply to the transplanted cells.^{83–86} Besides using co-culturing

Table 1

The differentiation potential of dental derived stem cells.

	Stem Cells	Source	Scaffold	Host	Gene Markers	Result	Ref.
Angiogenic differentiation	SCAP	Human	synthetic scaffolds consisting of poly-D,L-lactide/glycolide	Mouse	DSP, BSP, ALP, CD105	Regeneration of pulp-like tissue with well-established vascularity	104
	DPSC	Human	N/A	Mouse	CD31	Promoting tubular network formation of endothelial cells and the proliferation of dermal fibroblasts	105
	SHED	Human	human tooth slice	Mouse	EGFR2 and CD31	Differentiation of primary SHED into endothelial cells	32
Osteogenic differentiation	SHED	Human	Tooth slice	Mouse	VEGFR2, CD31, and VE-Cadherin	Differentiated into functional odontoblasts and vascular endothelial cells	48
	DPSC	Human	N/A		ALP, Coll I, OSC, OPN, IGFBP-5, JunB, and NURR1	osteoblastic differentiation process, IGFBP-5, JunB, and NURR1 gene expression is significantly increased	27
	Gingiva	Human	Biografts containing hydroxyapatite/tricalcium phosphate	Mouse	Cbfa 1, ALP, osterix, collagen type I, OSC	Tissue mineralisation	106
	SHED	Human	Hydroxyapatite/tricalcium phosphate	Mouse	BSP, OSC	calvarial defects were reversible with substantial bone formation	94

DSP = dentin sialoprotein, BSP = bone sialoprotein, ALP = alkaline phosphatase, CBF α 1 (RUNX2) = core binding factor subunit alpha 1, OSC = osteocalcin, Collagen type I = coll I, Osteopontin = OPN, IGFBP-5 = insulin-like growth factor binding protein 5, NURR1 = nuclear receptor related 1 protein, Ref. = reference.

technique, there is another strategy to promote vascularization for the engineered tissue using VEGF⁸⁷ and prefabricated tissue engineered construct.⁸⁸ However, both methodologies come with few hiccups that require more validation in the *in vivo* models and major clinical setback that patients will need to go surgery at least twice, double the expenditures and complexity of which could overshadow its future clinical applications.

With regard to these factors and gaps mentioned, a fresh approach for bone regeneration require a new strategy by employing single cell source of MSCs that are not only osteogenic but also have the ability to differentiate into endothelial cells and integrate with host vasculature network to support cell viability and to fully restore the tissue function.

Bone marrow SCs were harvested and expanded into bone repair cells (BRCs) which was proposed as a new single source having osteogenic and angiogenic potentials.⁸⁹ Though the study noted that the BRCs phenotypically showed vascular-like profile and molecularly expressed markers for both endothelial and pericytic cells, this study was unable to determine the source of cells that contributed to tissue regeneration.⁸⁹ Another SC source, the stromal vascular fraction (SVF) of adipose tissue containing progenitor cells, has the ability to differentiate into endothelial cells and various musculoskeletal lineages.⁹⁰ A study showed that SVF was able to stimulate neovascularogenesis in the mouse ischemic hindlimb.⁹¹ Nevertheless, the mechanism underlies the vascularisation of this model yet to be elucidated and the more challenging pre-clinical trials design should be tested.

Alternatively, DSCs have the potential to differentiate into osteogenic and endothelial cell types that develop into mature bone (Table 1). Human DPSCs,⁹² PDLSCs⁹³ and SHED⁹⁴ have demonstrated the ability to form engineered bone grafts, and also differentiate into vascular cells.^{95,96} To date, investigation on thorough potential of DSCs as a single SC source for bone tissue engineering is lacking; from the upstream of the fundamental research, the pre-clinical and clinical trials that have been approved by the regulatory bodies and to formulate the most efficient biomanufacturing of clinical grade SC.

6. Dental stem cells in tissue engineering: angiogenesis and osteogenesis

The prospective of DSCs in tissue engineering is vast not only for dentistry field but also for various types of regenerative medicine that include neural and bone.^{8,27,97–100} The angiogenic and

osteogenic potentials of DSCs entice the research and clinical community to get more understanding on this subject matter for future clinical practice.^{27,35,101} However, these experimental findings are not conclusive and need to be validated in certified facilities abiding international standards. An integrated roadmap is needed to develop a sustainable tissue engineering value chain. An effort has been done by Hiroshima University setting up a commercial teeth bank in 2004 and later followed by a lot of bio-based companies globally to offer clinical grade cell storage service.¹⁰² The double-blind randomized controlled trials need to be conducted to clinically prove the ability of DSCs for therapeutic functions.¹⁰²

Ideally, for stem cell based therapy for therapeutic approval, according to Ministry of Health, Malaysia, SCs must be reproducible, proliferate extensively and generate sufficient quantities of tissue. Besides that, they should differentiate into the desired cell types and survive in the recipient after transplantation and integrate into the surrounding tissue afterwards. Then, they should be functional throughout the recipient's life without harming the recipient in any way.¹⁰³

7. Conclusion

The multipotent DSCs which can be isolated from various sites of dental tissues demonstrated promising prospects for future therapeutic applications. The angiogenic and osteogenic potential possessed by DSCs can be further investigated and validated as a common stem cell source for bone tissue engineering application. Addressing the gaps highlighted previously may provide concrete evidences that can offer better treatment strategy and benefit the patients as a whole. Innovation for a better healthcare solution will be an exciting endeavour for DSCs and bone tissue engineering research community.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

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