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Characteristics and properties of gingival mesenchymal stem cells

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ABSTRACT : The gingiva is a unique oral tissue overlaying the alveolar ridges. It is recognized as a biological mucosal barrier and a distinct component of the oral mucosal immunity. This tissue can easily be obtained in the clinic as a discarded biological sample. Recently, gingival mesenchymal stem cells (G-MSCs) have been isolated and characterized as abundant and easily accessible, as compared to other mesenchymal stem cell sources.

Many reports described G-MSCs as having much potential for multilineage differentiation and show remarkable tissue regenerative properties. Primary experimental therapeutic applications of G-MSCs are potentially superior to conventional clinical treatment modalities. Several studies have recently applied G-MSCs to stem cell-based therapy and revealed that G-MSCs have many advantages as a candidate cell source for bone regeneration and periodontal tissue regeneration. This review summarizes the scientific evidence on G-MSCs' isolation, their characterization, and their regenerative properties.

Key Words : mesenchymal stem cell, gingiva, regeneration, multilineage differentiation

Introduction

The gingiva is one component of the periodontium which supports the tooth according to the function and histologically composed of epithelium and connective tissue. Furthermore, the gingiva has regenerating capability during wound healing with regard to the reconstitution of tissue architecture[1].

This tissue is easily accessible and is often resected during standard surgical procedures, such as periodontal surgeries, with little disturbance to the patient[2-4].

In recent years, gingival mesenchymal stem cells (G-MSCs) have been confirmed to possess stem cell properties, including the formation of clonogenic colonies, the expression of a typical MSCs surface marker profile and the capacity of multilineage differentiation in vitro[3, 5-10]. Also clinically, as a candidate cell source for stem cell therapy, G-MSCs are considered to be superior to other stem cells based on the fact that G-MSCs showed stable morphology, uniform homogeneity and fast proliferation[6-8, 11, 12]. Thus, G-MSCs have several advantages over other stem cells for cell therapy in regenerative medicine[6].

Herein, we review the scientific aspects of the current investigations on G-MSCs' isolation, their characterization, and their regenerative properties.

1. G-MSCs isolation

In many reports describing the methods for isolation of G-MSCs, gingival tissue samples from human subjects or animals via gingivectomy techniques surgically obtained. The tissue biopsies were minced and digested to obtain single-cell suspensions[6, 8, 11, 13] or kept intact and the tissue explants culture method was used to grow out the adherent connective tissue cells[2, 3, 14, 15]. The obtained cells were subsequently cultured and expanded in vitro for further experiments.

Several protocols for isolation and expansion of G-MSCs were proposed. Some of the protocols did not attempt to select stem cells' population from the heterogeneous gingival connective tissue cells via single-cell cloning[8, 13, 16] or magnetic activated cell sorting (MACS) techniques[2, 17]. A recent

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study relying on a STRO-1/MACS scheme for G-MSCs' isolation demonstrated that the STRO-1/MACS+-cell population, in contrast to the STRO-1/MACS- one, harbored the cells with stem cells' characteristics and distinctive osteogenic marker expression[2].

2. G-MSCs' associated markersa

In the field of mesenchymal stromal cells' (MSCs) isolation, characterization, and research[18], most studies referenced the minimal criteria proposed by the International Society for Cellular Therapy (ISCT) for MSCs' characterization[19]. According to the criteria, MSCs should show self-renewal capabilities and plastic adherence under standard culture conditions. More than 95% of the MSCs' population should express the surface markers CD73, CD90, and CD105, and these cells must lack the expression (less than 2%) of the surface markers CD11b, CD14, CD19, CD34, CD45, CD79α, and HLA-DR. Many studies for G-MSCs' identification further augmented the ISCT's list by additional markers, including CD13, CD38, CD44, CD54, CD117, CD144, CD146, CD166, Sca-1, STRO-1, SSEA-4, Oct-3/4, Oct-4A, Nanog, nestin, integrin β1, and vimentin[2, 14, 20-23]. Most commonly explored markers listed in Table 1 and STRO-1 positive rat G-MSCs are shown in Fig. 1.

Marker expression of G-MSCs was shown to be altered by culturing conditions or adding biomolecules. For example, G-MSCs cultured as 3D spheroids demonstrated elevated expression Stro-1, Oct-4, and Nanog, important transcriptional factors relevant to stem cell properties, and decreased expression of other MSCs-associated markers, including CD29, CD90, and CD105[24]. On the other hand, ascorbic acid (vitamin C) significantly elevated the expression of SSEA-3, Sox-2, Oct-3/4, and Nanog[15]. Oct-3/4, Nanog, and Sox-2 expression are vital for maintaining a progenitor status with an unlimited stem cells' division, without affecting their self-renewal or differentiation capacity[25, 26]. In recent some studies, Nanog is suggested as a key gene for maintaining the cellular pluripotency[25, 27].

These varied expressions of characteristic markers of G-MSCs under different culturing conditions indicate that the potential of G-MSCs has been elucidated not yet completely. Further extensive research is needed in this area to precisely define the potential of G-MSC and their cellular characteristics.

3. Multilineage differentiation potential of G-MSCs

Similar to previous investigations on MSCs from other sources, several studies reported on a multilineage differentiation ability of G-MSCs into osteoblastic, adipocytic, chondrocytic, endothelial, and neural directions, when incubated in in vitro inductive culture conditions[3, 5, 7, 11-13, 28] (Table 1, Fig. 1).

Osteogenic differentiation was demonstrated by the expression of alkaline phosphatase (ALP) activity and the formation of calcified Alizarin-Red positive deposits[2, 3, 5-7, 11, 13, 29] and through transmission electron microscopic (TEM) ultrastructural examinations, showing cellular structure of mature osteoblasts[30]. Osteogenic differentiation was further demonstrated on the mRNA level through the expression of bone specific markers, including Runx2, collagen I, collagen III, ALP, osteonectin (ON), osteopontin (OP), and osterix[2, 13, 16, 31].

Adipogenic differentiation was demonstrated by Oil-Red-O staining and the expression of the adipogenic markers peroxisome proliferator-activated receptor gamma (PPARγ), fatty acid synthase, and lipoprotein lipase (LPL)[2, 6, 13, 16].

Chondrogenic differentiation was evident by Toluidine-Blue staining and the expression of Sox-9, aggrecan, and Col-II or by Alcian-blue staining and aggrecan expression[2, 32].

Recently a further study reported on the ability of G-MSCs for endothelial and neuronal differentiation[13, 21, 33-35].

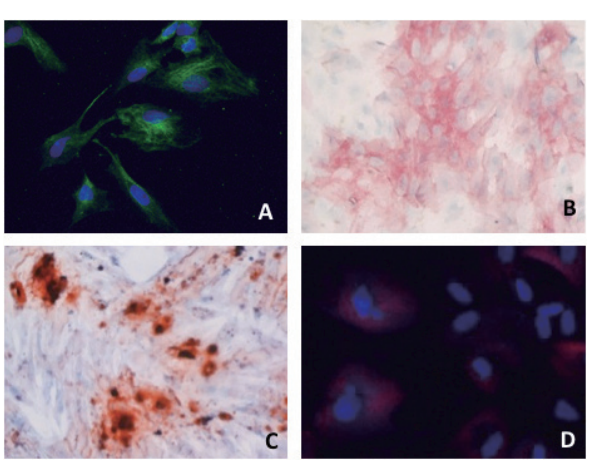

Fig. 1

- G-MSCs were isolated from gingiva in 6 week-old Wistar rats. A : G-MSCs were positive for STRO-1.
- Osteogenic differentiation of G-MSCs.
  B : Histochemical staining for ALP C : Alizarin Red staining
  D : Immunostaining for osteopontin

Table 1 Characteristics of G-MSCs

Surface Markers	In vitro Multipotency	In vivo Tissue Formation	Experimental Therapeutic Applications
CD29	adipocyte	connective tissue	wound healing
CD44	chondrocyte	bone	bone regeneration
CD73	osteoblast	cartilage	periodontal tissue regeneration
CD90	neural cell		immunomodulatory and anti-inflammatory
CD105	endothelial cell		
CD146			
STRO-1			
SSEA-4			
Nanog			

When cultured on fibronectin-coated slides in endothelial cell growth medium, G-MSCs expressed the endothelial cell marker CD31[13]. Recently, human gingival fibroblasts including G-MSCs possessed capacity for being induced and differentiated into vessel endothelial-like cells with typical and specific morphological, ultrastructural, and immunological characters of endothelial-like cells by induction with VEGF[33].

Under neural differentiation conditions, G-MSCs are positive for GFAP (glial fibrillary acidic protein), neurofilament 160/200 (NF-M), MAP2, nestin, and βIII-tubulin[13]. When subjected to a glial differentiation regimen, G-MSCs induce neurogenesis and support survival of PC12 cells in serum-free medium[4]. Furthermore, recent study demonstrated that G-MSCs incubated in neural cell culture medium could differentiate into neuronal cells which expressed Nestin, Sox-1 and Pax-6 as markers of neural stem cell-related genes and promoted peripheral nerve regeneration in a crush-injury model of rat sciatic nerve[35].

Thus, many studies revealed that, as same as MSCs from other sources, G-MSCs potentially have a multilineage differentiation ability according to the inductive stimuli and specific cellular conditions.

4. Immunomodulatory properties of G-MSCs

G-MSCs, similar to other MSCs sources, exhibit outstanding immunomodulatory properties, besides the self-renewal, multipotent differentiation, and tissue regeneration capabilities. Generally, MSCs are nonimmunogenic and hold immunomodulatory capability, allowing for their allogenic transplantation without immunosuppression.

In regard to immunomodulatory and anti-inflammatory capabilities of G-MSCs, several studies have reported that these cells are capable of eliciting a potent inhibitory effect on T-cell proliferation in response to mitogen stimulation[6, 13]. Mechanistically, G-MSCs express their anti-inflammatory effect partly via the IFN-γ-induced expression of indoleamine 2, 3-dioxygenase (IDO), IL-10, cyclooxygenase 2 (COX-2), and inducible nitric oxide synthase (iNOS), which are well known as immunosuppressive factors[13, 36]. In addition, toll-like receptors (TLRs) are germ line-encoded pattern-recognition receptors (PRRs), detecting specific pathogen-associated molecular patterns (PAMPs) and thereby promoting immune cells' activation[37, 38]. G-MSCs may interact with their inflammatory environment via TLRs. In a recent study, G-MSCs displayed a characteristic expression profile of TLRs 1, 2, 3, 4, 5, 6, 7 and 10 in basic culture condition and with inflammation up-regulated the expression of TLRs 1, 2, 4, 5, 7, and 10, while diminished TLR6 expression[39].

These immunomodulatory properties of G-MSCs are currently a matter of intense research to ameliorate inflammatory diseases, representing as a potentially promising tool in cellular therapy[3].

5. Experimental therapeutic applications of G-MSCs (Table 1)

In concerned with actual applications of G-MSCs, G-MSCs with suitable carriers implanted subcutaneously into immunocompromised mice generated connective tissue-like structures[11, 13], bone matrix[5, 8, 40], or mineralized tissues that exhibited certain similarities to cementum and bone, positively staining for collagen (Col), Ca, cementum attachment protein (CAP), cementum protein 1 (CP-1), bone sialoprotein (BSP), ALP, and

osteocalcin (OC)[41].

Ex vivo-expanded G-MSCs were seeded onto HA/TCP grafts, incubated in osteogenic medium, mixed with collagen gel, and transplanted subcutaneously into the dorsal surface of immunocompromised mice. This study revealed that high expression levels of osteocalcin, OPN, and Col I were observed, indicating the potential of G-MSCs for in vivo bone regeneration[13]. Some studies were investigated that the regenerative capacities of G-MSCs implanted in bone tissue in vivo. Newly formed bone with a well-mineralized trabecular structure located at the inner site was also demonstrated for G-MSCs transplanted into the mandible and calvarial defect model[7, 13]. All of these results combined confirm a clear bone regenerative capacity by G-MSCs and possibility of applications for clinical regenerative medicine.

In a field of dental therapy, G-MSCs are also considered a promising and readily available cell source for periodontal tissue regeneration, including the reestablishment of cementum, periodontal ligament, and alveolar bone as a functional tooth[9, 42, 43]. In an animal study[9], G-MSCs were found to promote periodontal regeneration with significant improvement in periodontal clinical parameters. In another study for periodontal regeneration, G-MSCs' cell sheets were employed for periodontal regeneration in a class III furcation defects dog model. In this study, the transplanted G-MSCs significantly enhanced the regeneration of the damaged periodontal tissues, including the alveolar bone, cementum, and periodontal ligament[42]. Recently, periodontal regenerative potential of G-MSCs combined with hyaluronic acid based hydrogel synthetic extracellular matrix demonstrated a remarkable periodontal regenerative potential in a porcine experimental periodontitis model in vivo, with newly formed bone, cementum, and periodontal ligament[43]. Thus, G-MSCs are considered as one of potential candidate cell sources for stem cell therapy in periodontal tissue regeneration. However, to gain clinical successful achievement through stem cell-based regenerative periodontal therapy on the patients, more studies are required to explore the ability of G-MSCs to regenerate periodontal tissue and the underlying precise cellular mechanisms.

With regard to immunomodulatory properties of G-MSCs, cell-based therapy using a systemic infusion of G-MSCs significantly ameliorated the severity of inflammation-related colonic injuries in experimental colitis by suppressing inflammatory cell infiltration and increasing regulatory T-cells (Tregs) accumulation[13]. In addition, G-MSCs can elicit M2 macrophages characterized by the secretory cytokines IL-10 and IL-6 and decreased induction of Th-17 cell expansion, which resulted in a marked acceleration of wound healing[44]. Experimental administration of G-MSCs dramatically relieved the sensitization and elicitation of contact hypersensitivity (CHS) by modulating the function of immune cells through the COXs/PGE2 pathway[45]. Recently, the promising therapeutic effect of G-MSCs was demonstrated in an experimental collagen-induced arthritis (CIA) model. The role of G-MSCs in controlling the development and severity of CIA mostly depended on CD39/CD73 signals[46]. Thus, these findings suggest that G-MSCs have immunomodulatory and anti-inflammatory capacity through in vivo immune system and render a promising and easily accessible cell source for MSC-based therapies of inflammatory and allergic diseases.

Conclusion and perspective

The gingival tissue provides a readily accessible as well as easily obtainable and renewable source of multipotent postnatal stem cells for cellular approaches in different tissue repair or regeneration performances. The attributes of G-MSCs make them preferable cellular sources in the field of tissue engineering. G-MSCs show remarkable tissue regenerative potential, and primary experimental therapeutic applications of G-MSCs are potentially superior to conventional clinical treatment.

However, numerous biological and technical challenges need to be addressed prior to considering transplantation approaches of G-MSCs in humans. The further optimization of techniques for cellular integration and the improvement of their properties for clinical handling are important to apply clinical stem cell-based therapy. Presently, the inductive factors and cellular processes activated during self-renewal and differentiation are not sufficiently elucidated. Finally, in view of current knowledge of tissue development processes, to achieve reliable biologically based regenerative clinical therapies, more precise understanding of the biological functions of G-MSCs is required.

Declaration of interest

The authors declare no conflicts of interest.

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