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Adipose Derived Mesenchymal Stem Cells Origin, Characteristics and Promises

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Abstract

Under a variety of physical and experimental settings, stem cells are able to self-renew and differentiate into specialized adult cells. MSCs (mesenchymal stromal/stem cells) are multipotent stem cells present in a wide range of fetal, embryonic, and adult tissues. They are the progenitors of a variety of specialized cells and are considered a crucial tool in tissue engineering. MSCs, derived from various tissues including cord blood, placenta, bone marrow, and dental tissues, have been extensively examined in tissue repair, immune modulation, etc. Increasing the vitality of MSCs and restoring cellular mechanisms are important factors in treatment success. Oxidative stress is to get harm of cellular molecules such as DNA, proteins, and lipids as a result of overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells and tissues or insufficiency of antioxidant systems that can inactivate them. Oxidative stress has a close link with inflammation as a pathophysiological process. ROS can mediate the expression of proinflammatory genes via intracellular signaling pathways and initiate the chronic inflammatory state. At the same time, inflammatory cells secrete a large number of reactive species that cause increased oxidative stress at sites of inflammation. In inflammatory diseases, the differentiation of stem cells, the regenerative and wound healing process can be affected differently by the increase of oxidative stress. Recent studies have indicated that dental pulp stem cells (DPSCs), as a resource of adult stem cells, are an attractive option for cell therapy in diseases such as neurological diseases, diabetes, cardiological diseases, etc. as well as its treatment potential in pulp inflammation. The future of oxidative stress-inflammation cycle and/or ageing therapies involves selective elimination of senescent cells, also known as senolysis, which prevents various age-related diseases. Most pathologies are implicated on the effects of ageing without exerting undesirable side effects.

Keywords: Reactive Oxygen Species; Stem Cell-Based Therapies; Inflammation; Stem Cell Metabolism

Abbreviations: MSCs: Mesenchymal Stromal/Stem Cells; ROS: Reactive Oxygen Species; RNS: Reactive Nitrogen Species; ALP: Alkaline Phosphatase; Hh: Hedgehog; BMP: Bone Morphogenetic Protein; FGF: Fibroblast Growth Factor; TGF-β: Transforming Growth Factor β; PAMPs: Pathogen-Associated Molecular Patterns; DAMPs: Danger-Associated Molecular Patterns; PRRs: Pattern-Recognition Receptors; PRDX2: Perodoxin 2; TNF-α: Tumor Necrosis Factor-α; M1-like: Proinflammatory Conventionally Activated; M2-like: Anti-Inflammatory Alternatively Activated; IFN-γ: Interferon-γ; TLR4: Toll-Like Receptor 4; Wnt: Wingless/Patient1 Ligands; SHED: Stem Cells From Human Exfoliated Deciduous Teeth; PDLSCs: Periodontal Ligament Stem Cells; SCAP: Stem Cells From Apical Papilla; GFSCs: Gingival Fibroblastic Stem Cells; DFPCs: Dental Follicle Stem Cells; GFAP: Glial Fibrillary Acidic Protein; BMSCs: Bone Marrow

Stromal Cells; TGF-β: Tumor Growth Factor-β; FGF: Basic Fibroblast Growth Factor; PDGF: Platelet-Derived Growth Factor; EGF: Epidermal Growth Factor; IGF: Insulin-Like Growth Factors; GSTZ1: Glutathione S-Transferase Z1; VEGF: Vascular Endothelial Growth Factor; NGF: Nerve Growth Factor; BMPs: Bone Morphogenetic Proteins.

Introduction

Stem cells are undifferentiated cells that can transform into specialized mature cells under various physical and experimental conditions and have the capacity for self-renewal [1]. Based on their differentiation capacity, stem cells are characterized as totipotent, pluripotent, multipotent, or unipotent. Another classification based on the source of origin categorizes the stem cells into two major groups: embryonic and adult stem cells [2]. All three germ layers can be differentiated from embryonic stem cells produced from blastocyst cells. Even though they have a greater potential for tissue regeneration than adult stem cells, therapeutic use of such cells is not legally and ethically appropriate. However, adult stem cells reside in differentiated organs and maintain tissue homeostasis with their self-renewing, clonogenic, and multipotent properties [3]. Having many types of cell groups, adult stem cells contain hematopoietic stem cells (HSCs), MSCs, and other tissue-specific stem cells such as gut stem cells, neuronal stem cells, etc. is shown in (Figure 1). Among these cell types, MSCs have been an attractive option to develop clinical applications not only because of their multipotent properties, but also their angiogenic, neurogenic, and immunomodulatory features [4].

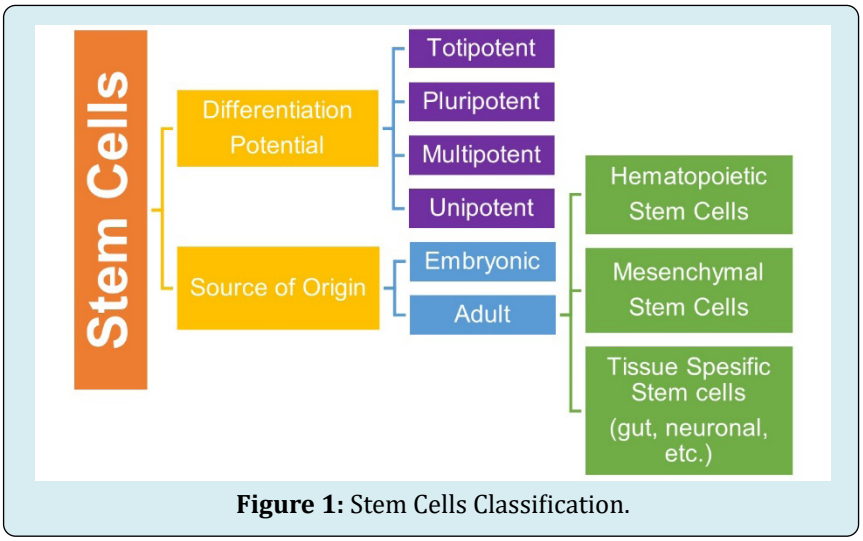


Figure 1: Stem Cells Classification.

Mesenchymal Stem Cells

MSCs are the progenitors of connective tissues that have increasingly been used in regenerative medicine by reconstructing damaged mesenchymal tissues due to their potential to develop into diverse cell types like osteoblasts, adipocytes, chondrocytes, and neuroblasts [4,5]. Mesenchymal stem cells are spindle shaped, and fibroblast-like adherent cells that proliferate into undifferentiated cells [6]. Although bone marrow stem cells are considered a significant source for mesenchymal stem cells, MSCs have also been derived from different tissues including placenta, umbilical cord blood, adipose tissue, dermis, and orofacial regions tissues and extensively evaluated in various research fields such as tissue repair, immune modulation [7,8]. Numerous developmental and environmental signals mediate the balance of division of mesenchymal stem cells to generate the suitable number of stem and differentiated cells [9]. The proliferation capacity of differentiated cells is limited due to resulting in a shorter telomere after each division. However, since MSCs express telomerase enzyme, they have longer telomere than other somatic cells, and therefore their proliferation capacity is quite high [10].

According to the literature, there is a close relationship between stem cell differentiation's direction and mitochondrial functions and dynamics. The level of mitochondria activity in MSCs is relatively low, following stimulation, mitochondrial biogenesis, such as protein levels of respiratory enzymes, oxygen consumption rate, mtDNA gene number, mRNA levels, and intracellular ATP content, increases. To benefit from regenerative medicine, it is important to have knowledge of the roles of mitochondrial dynamics such as energy metabolism and redox state related to MSC differentiation [7,11]. In addition, when the metabolic activity conditions in MSCs are examined, while the energy requirement in MSCs is provided by glycolysis, differentiated cells are more dependent on oxidative metabolism [12,13]. In the osteogenic differentiation process, with the activation of mitochondria in MSCs, ATP production is provided by

oxidative phosphorylation [7,14]. In both adipogenesis and osteogenic differentiation, oxygen consumption rate and respiratory enzyme activities goes up and mitochondrial membrane potential decreases significantly [7,15]. In mammalian cells, oxygen is used in aerobic energy synthesis reactions as a substrate for cytochrome oxidase, the mitochondrial respiratory chain's terminal enzyme [16]. Mitochondria, one of the main sources of O_2 production, affect redox potential, ion balance and energy output of the cells. With the reduction of mitochondrial membrane potential, electron leaking from the electron transport chain increases the mitochondrial ROS production, which can generate super-radicals and cause tissue breakdown. Due to the oxidation of intracellular components, high amounts of ROS are considered to produce cellular dysfunction and destruction [17,18].

Oxidative Stress

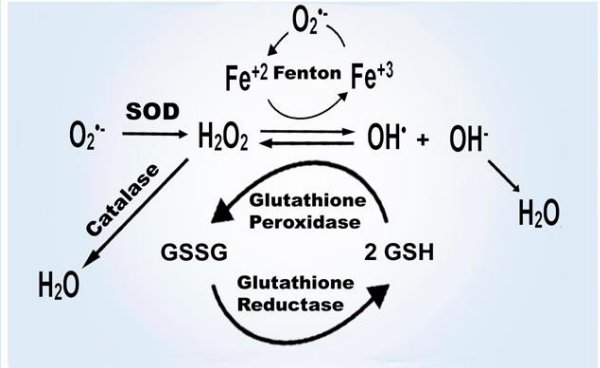
With the contribution of metabolic activities and environmental stressors, the body produces a variety of reactive species as free radicals or non-radicals. These compounds called pro-oxidants, which may be oxygen-derived or nitrogen-derived, are highly reactive and lead to cell and tissue damage by attacking macromolecules such as protein, lipids, and DNA [19]. In a healthy state, the human body has sufficient mechanisms to neutralize the effects of oxidant molecules through antioxidant systems including endogenous or exogenous antioxidant molecules and enzyme systems. However, if the balance between pro-oxidants and antioxidants shifts in favor of pro-oxidants, it causes the phenomenon called oxidative stress. This imbalance can develop due to either deficiency of antioxidants or accumulation of ROS [20]. Excessive ROS production in the body may change DNA structure, lead to protein modification and lipid peroxidation, as well as activate several transcription factors and produce pro/anti-inflammatory cytokines [21]. On that account, oxidative stress causes the initiation and progression of many diseases and pathological processes, including chronic inflammation, aging, carcinogenesis, cardiovascular diseases, diabetes, and neurological disorders [22]. Therefore, understanding the mechanisms involved in the regulation of oxidative stress is so critical.

Free Radicals and Their Neutralization

Free radicals, with an uneven number of electrons, are highly reactive species as they tend to bind with another electron to stabilize themselves. Although having a very short half-life, overproduction of these oxygen, nitrogen, or sulfur-derived species can result in damage to biomolecules and cells through a large chain of chemical reactions called oxidation [23].

The main members of the ROS family are superoxide anion ($0_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), hypochlorous acid (HOCl), peroxyl radical ($ROO^{\bullet}$), and hydroperoxyl radical ($HOO^{\bullet}$) which are produced over the normal oxygen metabolism in the body, mainly in the mitochondria, both depending on enzymatic and nonenzymatic reactions [24]. Respiratory chain, phagocytosis, prostaglandin synthesis, and cytochrome P450 system are the main enzymatic processes resulting in ROS production [25]. In addition to mitochondrial respiration, nonenzymatic production occurs due to ionizing radiation, heavy metals, xenobiotics, and when oxygen reacts with organic compounds [26].

Including enzymatic and nonenzymatic antioxidant molecules, multiple mechanisms in the human body compose the antioxidant system to counteract the disruptive effects of ROS and oxidative stress [24,27,28]. Major compounds of the antioxidant system are shown in (Figure 2).



Enzymatic Antioxidants	Main Localization
Superoxide dismutase	Cytosol, Mitochondria, Extracellular fluid
Catalase	Peroxisomes, Cytosol
Glutathione Peroxidase	Mitochondria, Cytosol, Membrane-bound, Nucleus, Endoplasmic Reticulum, Extracellular fluids
Glutathione Reductase	Mitochondria, Cytosol
Non-enzymatic Antioxidants	**Main Localization**
Vitamin E (α-tocopherol)	All Membranes
Carotenoids	All Membranes
Vitamin C (Ascorbic Acid)	Both Extracellular and Intracellular including Mitochondria, Cytosol, Peroxisomes
Glutathione (GSH)	In all compartments of cells including Cytosol, Membranes, Peroxisomes, Endoplasmic Reticulum

Figure 2: Neutralization of free radicals by major enzymatic and non-enzymatic antioxidants.

ROS and MSCs Differentiation

Due to the increasing potential applications of MSC in regenerative medicine, studies on MSC differentiation have shown that ROS can play an influential role in determining the direction of differentiation. In fact, undifferentiated cells are less resistant to exogenous ROS than differentiated cells [29]. While uncontrolled ROS can be harmful, a regulated basal level of ROS can perform as a signaling molecule to keep cell processes including proliferation, differentiation, and survival [17,30]. According to Rodrigues et al., increased ROS stimulates the stress-induced MAPK pathways JNK, p38MAPK, and ERK, as well as apoptotic proteins and ant apoptotic pathways [31]. Up regulation of ROS has been reported to suppress osteogenic differentiation by reducing the activity of alkaline phosphatase (ALP) and Hedgehog (Hh) signaling pathway, which is fundamental for bone growth and maintenance [29,32]. In fact, for osteogenic differentiation, the interaction of hormones (glucocorticoid and parathyroid hormones) and various extracellular signals such as wingless/patient1 ligands (Wnt), bone morphogenetic protein (BMP), fibroblast growth factor (FGF), transforming growth factor β (TGF-β), and Hh signaling pathway is constitutive [33,34]. Chen, et al. reported a substantial drop in intracellular H_2O_2 and O_2 levels on the second day following osteogenic induction, as well as a notable overexpression of the antioxidant enzymes SOD2 and Catalase after 14 days of induction. Furthermore, throughout osteogenic differentiation of hMSCs, the number of copies of mtDNA, protein levels of respiratory enzymes, oxygen consumption rate, mRNA levels of mitochondrial biogenesis genes, and intracellular ATP content increased, while intracellular ROS levels decreased dramatically, and antioxidant enzymes were up regulated. These findings imply that in osteogenic induction, mitochondrial respiratory activities increase in response to the growing energy demand [35]. As a result, it is suggested that Excess ROS suppresses osteoblastic differentiation and antioxidants possibly restore this process. Moreover, considering age-related bone loss and bone strength, it should be noted that elevation of intracellular ROS in the MSC of the elderly patient may result in decreased osteoblastic differentiation [36].

The generation of ROS in MSCs was found to be higher during adipogenic development. Differentiation of human MSCs into adipocytes begins with an elevation in mitochondrial oxygen consumption in addition to increasing of intracellular ROS generated from mitochondrial complex III due to mTORC1 signaling. The addition of exogenous H_2O_2 has been observed to stimulate adipogenesis in human adipose-derived MSCs depending on applied concentration. In addition, in a study examining the stimulating role of endogenous nitric oxide (NO) in adipogenesis, there was a 50% elevation in basal NO levels in the first two days after adipogenic differentiation [37]. These findings showed that enhanced ROS generation is not just a result of adipocyte development [38,39]. In another study, differentiation of human bone marrow-derived MSCs into adipocytes resulted in increases in SOD2, catalase, SOD3 mRNA and protein expression [40,41]. Moreover, Yasunari et al. revealed that N-acetyl-cysteine (NAC) treatment reduced increased ROS formation, which in turn blocked adipogenic differentiation and mitochondrial targeted antioxidants can inhibit adipogenic differentiation [38]. Similar to adipogenic differentiation of MSCs, in chondrogenic differentiation, the level of ROS is elevated with an advanced decline in catalase activity [42]. ROS produced by NADPH oxidase 2 and 4 is required for differentiation of murine primary chondrocytes and the ATDC5 cell line. It was observed that Superoxide dismutase (SOD3) contributed for the reduction ROS in the extracellular matrix, whereas NAC inhibited chondrogenic differentiation. Consistent with this, SOD3 enzyme was diminished upon chondrogenesis [41,43]. Furthermore, the addition of H_2O_2 increased chondrogenic differentiation, while administration of NAC inhibited it, implying that ROS is important in chondrogenesis [7,29].

Because free radicals modulate the activity of transcription factors, cell proliferation, and certain levels of differentiation, studies on neurogenic differentiation have shown associations between low amounts of ROS and certain signaling pathways [44,45]. The reduced level of ROS suppressed the Notch1 pathway and Hes1 expression, caused progressive elevation in the expression of Nestin, a neural stem cell-specific protein, and the antioxidant b-ME induced the differentiation of BMSCs in neural cells. Considering future MSC-based therapeutic approaches, these results suggest that ROS-based regulation of the Notch1 signaling pathway may be associated with neurogenic differentiation of MScs [46]. The overall regulatory role of ROS on stem cells is shown in Figure 3.

However, it has been shown that in addition to acting as second messengers in intracellular signaling that regulates cell functions including cell proliferation, differentiation, survival, and apoptosis, ROS can act as a antimicrobial agent when overproduced. There is strong evidence that excessive ROS production can contribute to cell and tissue damage as well as chronic inflammation, as they are involved in inflammatory events such as increased vascular permeability, leukocyte extravasation, phagocytosis, respiratory burst, and angiogenesis [47].

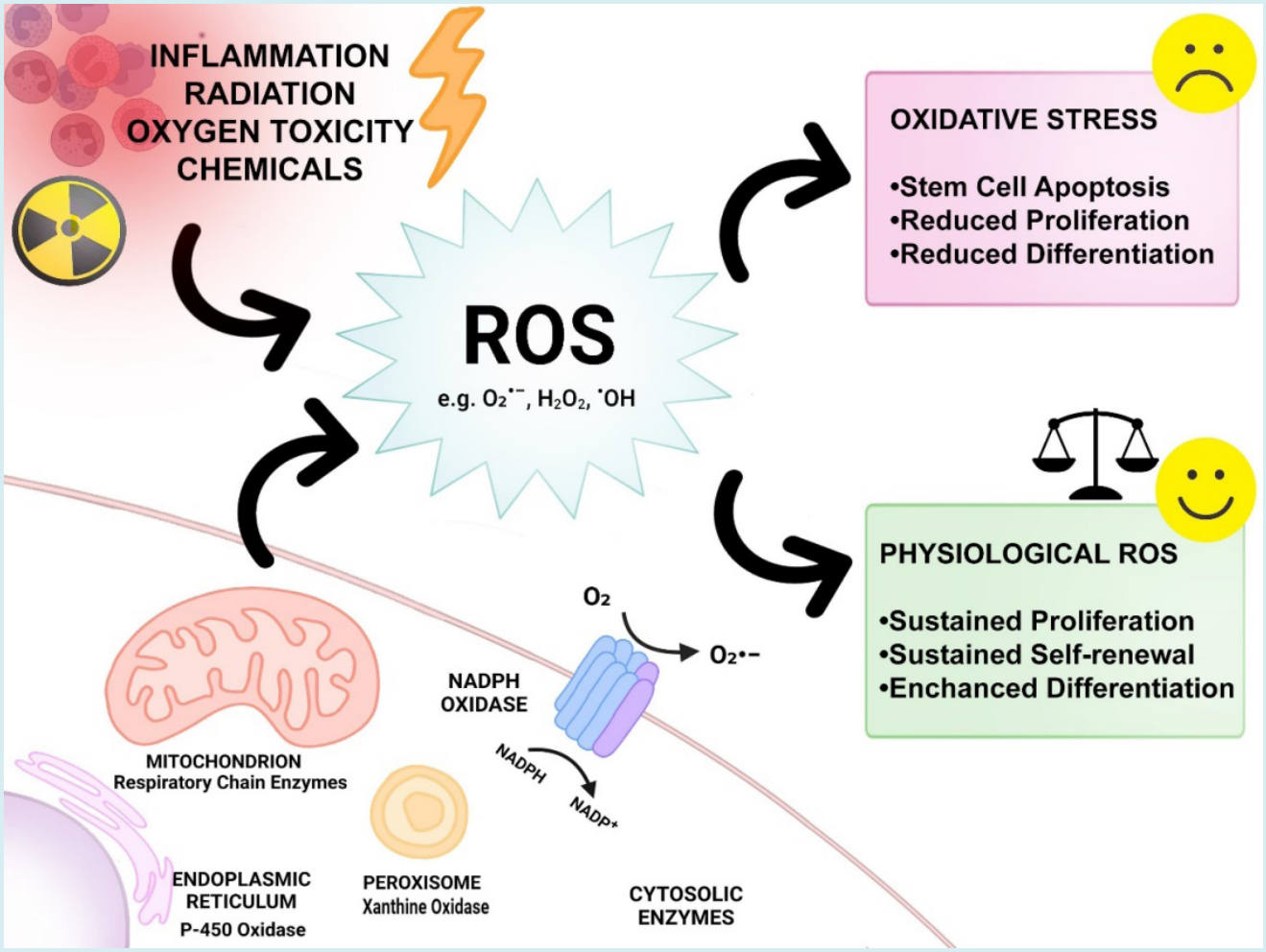


Figure 3: Sources of ROS and their effect on stem cell metabolism.

Inflammation

Inflammation is a protective immune response to invading pathogens, and it is operated by both the innate and adaptive immune systems. The acute inflammatory response with the events of systemic vasodilation, vascular permeability, and leukocyte emigration takes place when the innate immune system confronts the tissue damage by pathogens or stress [48]. Pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), which are released by injured cells due to trauma, ROS, bacterial inflammation, etc., can be recognized by the family of pattern-recognition receptors (PRRs). Macrophages, monocytes, dendritic cells, and neutrophils are among the immune cells that express PRRs [49]. As the NF-κB transcription factor is activated by stresses including oxidative stress, several cytokines and chemokines are released. Further, immune cells such as neutrophils recruit and migrate through the vascular wall into the infection site (Figure 4). Although this process is considered advantageous for the organism at the beginning, if it is not controlled, it can lead to excessive inflammation and many related inflammatory diseases [48]. The excessive generation of ROS and RNS can directly induce the secretion of inflammatory mediators and the stimulation of inflammation [49].

With the above-mentioned cellular processes which are interconnected especially with tissue regeneration, both ROS and inflammation take a significant regulatory role in signaling mechanisms and the metabolism of the stem cells.

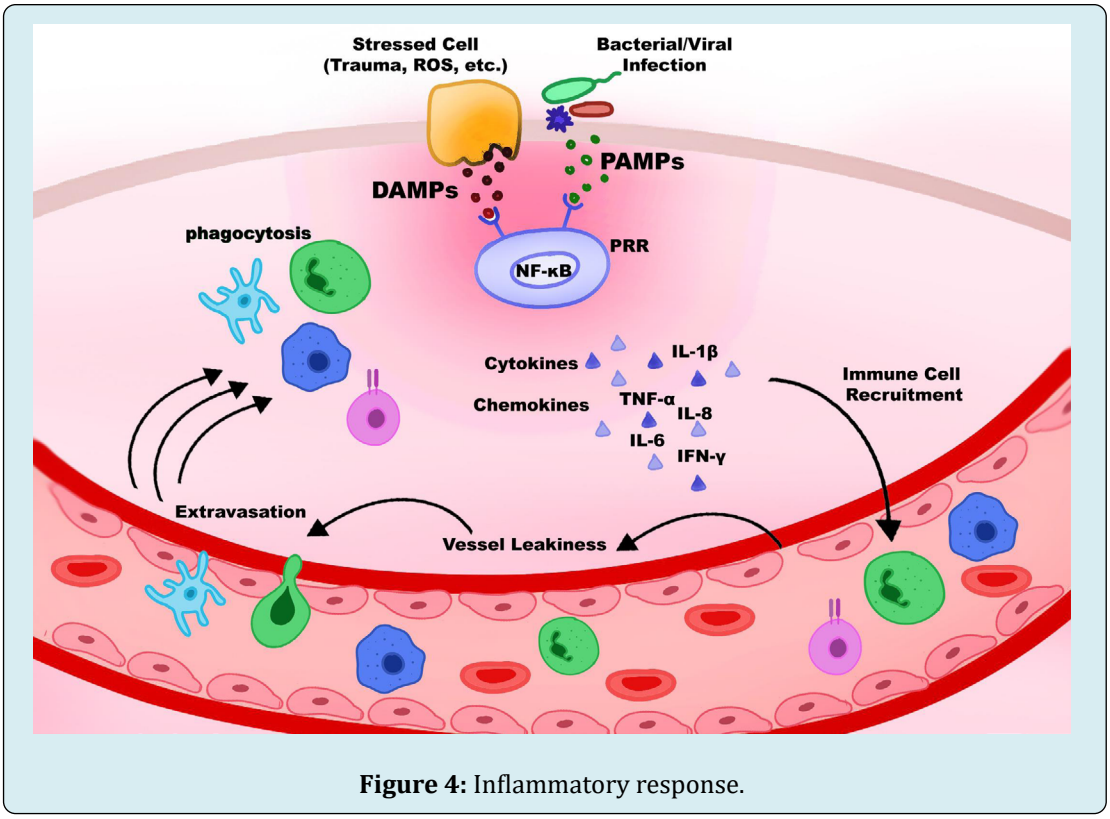


Figure 4: Inflammatory response.

Inflammation and ROS Connection

Many authors have recognized the correlation between oxidative stress and inflammation. ROS can initiate intracellular signal transductions and mediate the stimulation of various transcription factors e.g., NF-κB [47]. These transcription factors in turn increase the expression of pro-inflammatory genes and induce the chronic inflammatory state [50]. At the same time, inflammatory cells promote oxidative stress by releasing multiple reactive species at sites of inflammation [51]. Most of the chronic disorders associated with high levels of ROS cause oxidative stress, which results in protein oxidation, glycosylated products, DNA damage, and lipid peroxidation [52]. It was reported that protein oxidations activate the release of signal molecules such as the pyridoxine 2 (PRDX2) molecules, which is considered an inflammatory signal. Once released, it functions as a redox-dependent inflammatory mediator that causes macrophages to secrete tumor necrosis factor-α (TNF-α) [53]. Macrophages play a key role in the regulation of inflammatory. In the inflammatory process, monocytes are recruited by neutrophils and move to the site of injury, where they transform into macrophages [54]. The link between macrophage population shift, also known as polarization, and tissue remodeling has been studied extensively. Based on their physiological properties, macrophages are classified as either pro-inflammatory conventionally activated (M1-like) or anti-inflammatory alternatively activated (M2-like). Once recruited and differentiated, macrophages are polarized to the M1-like state when stimulated by interferon-y (IFN-y), TNF-α, and Toll-like receptor-4 (TLR4) activation. Production of IL-10 and IL-4 causes the population to shift towards M2-polarization that signals tissue remodeling and repair [55-57].

The fact that macrophage blockade regulates Wnt recipient stem cell differentiation in intestinal crypts indicates a direct relationship between macrophages and stem cells [58]. MSCs activate macrophages toward an immunosuppressive M2 phenotype. As a result, MSCs create a more anti-inflammatory state by reducing TNF-α, IFN-y secretion, and NK killer proliferation and increasing IL-10 and IL-4 secretion [56,57,59,60]. Furthermore, MSCs secrete low levels of MHC class I and co-stimulatory molecules such as CD40, CD80, and CD86 to prevent all reactive antibody production and degradation [61,62]. Because of their multimodal properties, MSCs are thought to have potential uses in different therapy modes such as new tissue formation, healing of tissue damage, grafting of other cells and tissues, and treatment of immune-based pathologies [63,64].

The significance of ROS in macrophage-mediated immunity unquestionable. ROS has direct antibacterial effect against bacteria and parasites, in addition to redox control of immunological signaling and inflammatory activation [65]. Macrophages are frequently the first immune cells that come

into contact with invading pathogens. Recognition of bacteria by macrophages results in the generation of ROS in many cellular compartments, which perform several antibacterial roles, including destruction of bacteria phagocytized by the oxidative burst produced by Nox2 [65,66]. The most important point is at what stage ROS production tends to repair or destruction in tissue damage.

Dental Pulp Stem Cells

Among the MSCs, the ability of orofacial MSCs to self-renew and differentiate into many cell types has made them an ideal option for tissue regeneration. Since dental stem cells are ecto-mesenchymal origin, they can have both mesoderm and ectoderm properties and are easily accessible sources for multipotent postnatal stem cells [67]. Mesenchymal stem cells obtained from orofacial tissues are not only recommended for remodeling of hard tissue on account of their superior properties compared to bone marrow MSC cells but also suitable for regeneration of craniofacial tissues originating from the neural crest [10,67]. Among the rich MSCs source identified in oral tissues are DPSCs, stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAP), gingival fibroblastic stem cells (GFSCs), and dental follicle stem cells (DFPCs). These stem cell populations are obtained from various oral and maxillofacial tissues at different stages of development. DPSCs, for example, are isolated from the pulp tissues of permanent teeth, whereas SHED is isolated from the pulp of deciduous teeth. However, it has been noted that depending on the environmental conditions, there are multiple stem cell populations in the pulp tissue [68,69].

Dental pulp, which includes both mesenchymal and ectodermal tissue and neural crest cells, is composed of blood vessels, nerve fibers, and connective tissue [70]. DPSCs originating from migrating neural crest cells are ectoderm-derived stem cells. Numerous *In vitro* and *In vivo* studies on cell growth, differentiation capacity, and potential to lead stem cell functions have revealed that DPSCs have impressive application potential for regenerative medicine [11,71]. The multilineage differentiation potential of DPSCs is shown in Figure 5. Similar to that of MSCs, DSPCs exhibit a fibroblast-like morphology, adhesive quality to plastic surfaces, surface marker expression, proliferation, and colony forming behavior. In addition to mesenchymal stem cell markers such as CD73, CD90, and CD105, The immunophenotype of DPSCs has been found to include another mesenchymal stem cell marker, STRO-1, which is co-expressed with CD146 and pericyte antigen, [68,72,73] DPSCs also express neural lineage markers such nestin, B III tubulin, and glial fibrillary acidic protein (GFAP), which are seen on neural stem cells [74,75].

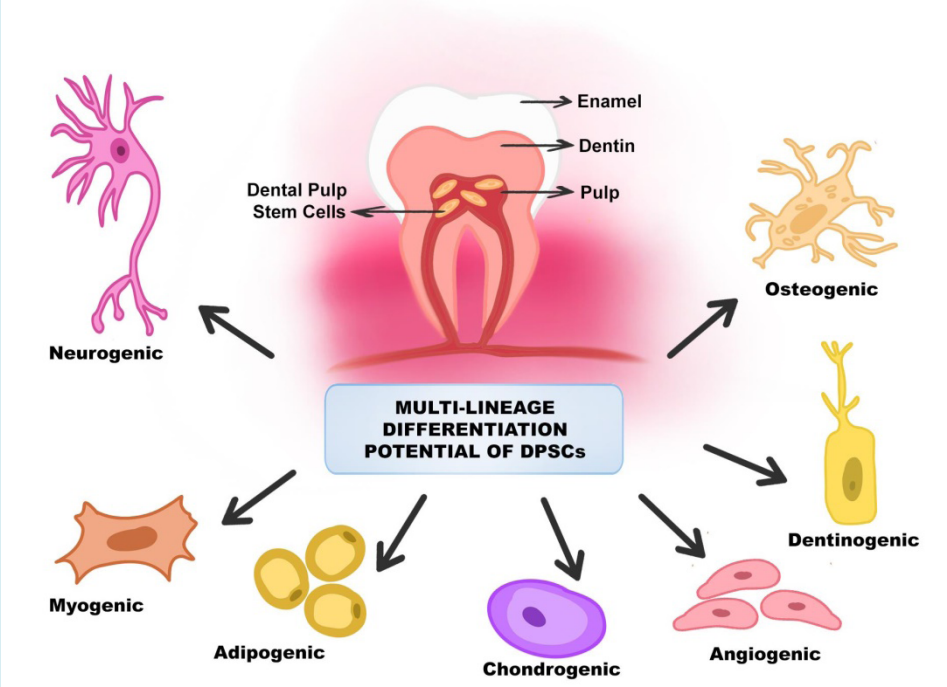


Figure 5: Multi-lineage differentiation potential of DPSCs.

Gronthos, et al. published the first study on DPSCs, demonstrating that their stem cell capabilities are comparable to those of bone marrow stromal cells (BMSCs), with similar immunophenotype and calcified nodule formation under differentiation medium treatment [71]. DPSCs have shown a high differentiation ability, such as neurogenesis, adipogenesis, osteogenesis, chondrogenesis, angiogenesis, and dentinogenesis, and are tightly controlled by a variety of growth factors, including tumor growth factor-β (TGF-β), basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), TNF-, and insulin-like growth factors (IGF) I and II [76].

ROS and Dental Pulp Stem Cell Differentiation

According to the literature, as in DPSCs, mitochondrial functions and dynamics determine the pathway of stem cell development [11]. In cardiomyocyte coculture, DPSCs showed greater mitochondrial respiratory capacities and lower mtROS than BM-MSCs and AD-MSCs. In addition, depletion of mitofilin has induced osteogenic/dentinogenic differentiation in DPSCs. Mitofilin regulates osteogenesis and is a transmembrane protein found in the inner mitochondrial membrane [77]. It was demonstrated that odontogenic differentiation in DSPCs, initiated by mitochondrial elongation with developed crista, increasing mitochondrial oxygen consumption rate, ATP production, and upregulation of mitochondrial glycolytic enzyme activity [78]. Long-term H_2O_2 treatment of DPSCs increased ROS production, which reduced cell survival and overexpression of antioxidant molecules Cu/Zn and Mn SOD as well as odontogenic/osteogenic markers like dentin sialophosphoprotein, dentin matrix protein-1, osteopontin, bone sialoprotein, Runx-2, and bone morphogenetic protein 2 and 7. However, PPAR- overexpressed cells enhanced dentin mineralization despite being exposed to oxidative stress [79]. In a recent study, a high O_2 environment compared to physiological oxygen levels activated p38 / p21 phosphorylation and /NRF-2 signaling pathway in DPSCS and decreased DPSC proliferation due to increased oxidative stress [80]. Moreover, SHED differentiated into neuronal cells with higher mitochondrial membrane potential and mt DNA in addition to longer mitochondria [81].

However, it was revealed that DPSCs are heterogeneously located in different niches in dental pulp tissues including subodontoblast layer, pulpal vasculature, and central pulp. Therefore, their proliferative capacity may be different [82]. Alaidaroos, et al. showed that when DPSC has a high proliferative ability, they resist H_2O_2-induced senescence and increase SOD2/glutathione S-transferase Z1 (GSTZ1) expression and SOD activities [83]. Whereas, low proliferative subpopulations exhibited accelerated senescence with low SOD, catalase and glutathione-associated antioxidant activities. Thus, this increases the complexity of individual DPSC subpopulations regarding their origin and regenerative properties and may cause significant limitations in mesenchymal stem cell (MSC)-based therapies.

Inflammation, Regeneration and Dental Pulp

DPSCs have been proven in numerous studies to develop into odontoblast-like cells and create a dentin/pulp-like complex [84-86]. To form new pulp-like tissue, two tissue engineering approach can be applied in the root canal, the first is cell transplantation and the second is cell homing. Based on cell transplantation technique, stem cells together with growth factors such as FGF, PDGF, TGF-β, IGF, vascular endothelial growth factor (VEGF), nerve growth factor (NGF) and bone morphogenetic proteins (BMPs) are placed on a proper scaffold and applied directly to the root canal. In the cell homing approach, recombinant signaling molecules or endogenous, dentin-derived growth factors attract the local stem cells to migrate from periapical tissues into the root canal. This approach can also be performed to repair damaged dentin-pulp complexes caused by local inflammatory disorders [87-89].

Dental inflammation has been recognized as an adverse factor leading to pulp destruction, mostly through necrosis or apoptosis. If dental pulp inflammation is in a reversible phase, to generate a reparative dentine, stem precursors and pericytes can be mobilized for odontoblastic differentiation [90-92]. Wnt receiving stem cell activation that can differentiate into odontoblast-like cells requires the presence of macrophages in the dental pulp. It was reported that elevation of Wnt cells significantly stimulated the up regulation of TGF-β1 in the dental pulp and produced acceleration in the polarization of the Wnt/β-catenin pathway from the pro-inflammatory phase to the anti-inflammatory phase (M1-M2 polarization) via GSK-3 antagonist small molecules, thus enhanced the reparative capacity [93-95]. Moreover, Neves, et al. showed that when macrophages are depleted, they significantly impair the reparative dentin-forming capacity, causing an accumulation of neutrophils at the injury site that leads to excessive inflammation. On the other hand, depletion of neutrophils suppressed inflammation and increased reparative dentin secretion in the pulp [96]. Regulation of inflammation with macrophages in dental pulp stem cells is shown in (Figure 6).

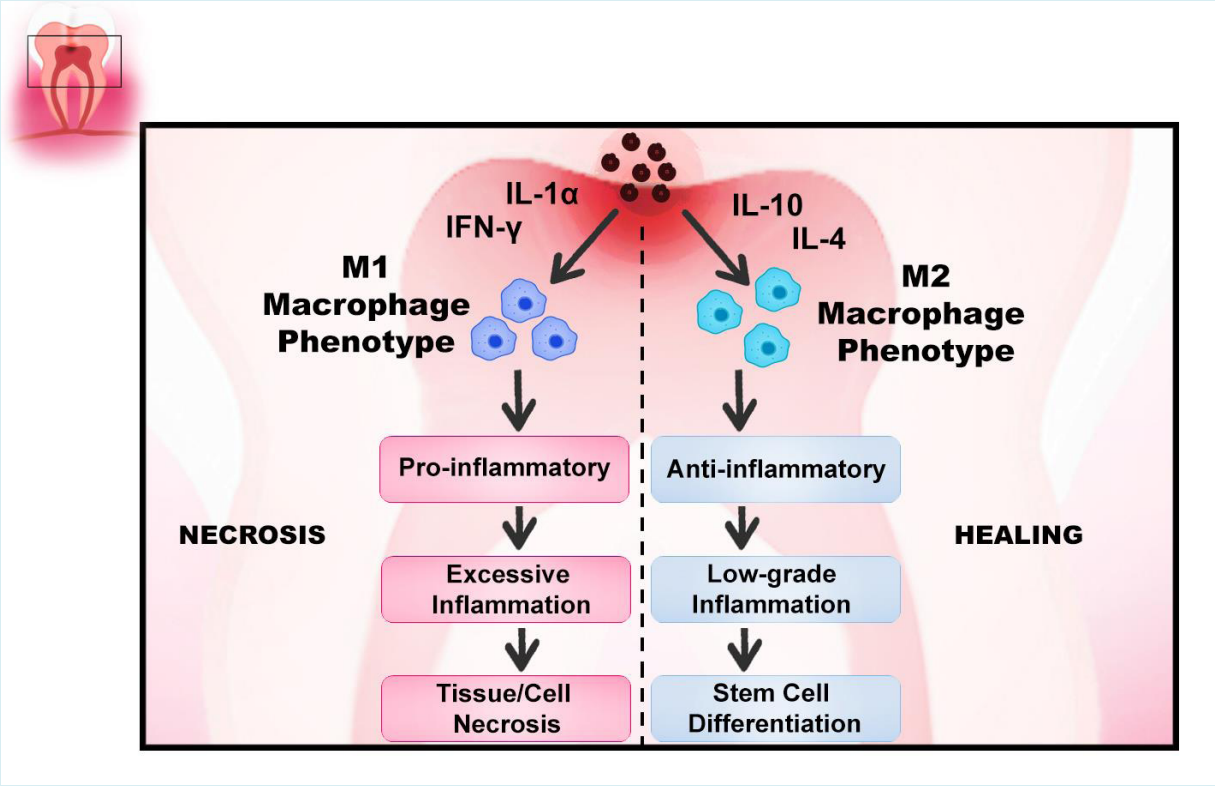


Figure 6: M1-M2 macrophage polarization is one of the key steps in tissue repair as they regulate dental pulp stem cell activation and inflammation.

However, the development of reparative dentin is a complex mechanism that needs a mild inflammatory reaction within the dental pulp [92,96,97]. Odontoblasts, immune-competent cells (monocytes, macrophages, and stem/progenitor cells), and subsequently pulp fibroblasts generate a large number of signaling molecules to regulate the immune response following recognition of the pathogen. Cells slide along the root and migrate to the wound side, towards the crown. Inflammatory processes cause the proliferation of pulp progenitor stem cells and increase in number and size. Their phenotype changes and they become odontoblast-like cells that produce collagen, ALPase, and SPARC/osteonectin [90].

Considering pulp inflammation caused by deep caries, it is well recognized that bacterial lipid byproducts increase intercellular ROS, as well as inhibition of cell growth, cell cycle kinetics, and protein synthesis [90]. The control of the cytotoxicity of generated intercellular ROS, in turn, can stimulate the recruitment of certain pulp cells such as progenitor stem cells, involved in reparative and regenerative processes [98,99]. Since glutathione effectively removes the excessive ROS, in a recent study, MTA agent was used with soluble N-acetyl cysteine antioxidant molecule and enhanced the differentiation capacity of DPSCs [100]. Therefore, in the design of reparative dentin formation and treatment models in regenerative medicine, it is important to know the inflammatory processes that occur in the tooth pulp as a result of dentin injury, as well as their impact on local stem cell activation [90,96].

On the other hand, it has been shown that ROS accumulation leads to premature senescence of dental pulp cells. In addition, the vitality of senescent cells can be damaged by exogenous ROS caused by infection, trauma, and chemicals. Therefore, such inflammatory process contributes to the impairment of pulp homeostasis and reduces the regenerative ability of dental stem cells [101]. Considering the interactions mentioned above, the future of dental pulp therapies involves the selective elimination of senescent cells, also known as senolysis, which prevents various age-related diseases. Most oral pathologies are implicated on the effects of ageing without exerting undesirable side effects. Senolytic drugs are increasing the resistance to ageing. They may delay or treat the geriatric syndromes and extend the life of the organisms. Studies have shown that several senolytics, including dasatinib plus quercetin, 17-DMAG, and navitoclax, are effective in reducing senescent cell burden in mice with decreases in senescent cell indicators such as cellular senescence-associated β-galactosidase activity. Therefore, 1st generation senolytics such as dasatinib, quercetin, fisetin

and curcumin, and 2nd generation senolytic therapies such as immunomodulators are considered as future therapeutic drugs of senolysis [102-106].

Conclusion

ROS and inflammation play a significant regulatory role in cell signaling mechanism which controls the balance between regeneration and tissue damage, and these are essential processes for stem cell metabolism and stem cell-based therapies. Indeed, there is interplay between both phenomena as the accumulated ROS can directly induce the secretion of inflammatory mediators and stimulation of inflammation, the other way around, also inflammatory cells promote oxidative stress by releasing multiple reactive species at sites of inflammation. Key molecules in transcription, protein modulation, and protein stability can be modified for regulation of oxidative stress and inflammation which is fundamental for future studies to improve stem cell proliferation, differentiation, and survival as well as to prevent cell senescence and ageing process. Since dental pulp stem cell-based therapies have gained attention in this field lately, future therapies are also necessary to focus on eliminating senescent cells and improvement of senolytic drugs in terms of resisting ageing.

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