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An overview of autophagy in the differentiation of dental stem cells

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Abstract: Dental stem cells (DSCs) have attracted significant interest as autologous stem cells since they are easily accessible and give a minimal immune response. These properties and their ability to both maintain self-renewal and undergo multi-lineage differentiation establish them as key players in regenerative medicine. While many regulatory factors determine the differentiation trajectory of DSCs, prior research has predominantly been based on genetic, epigenetic, and molecular aspects. Recent evidence suggests that DSC differentiation can also be influenced by autophagy, a highly conserved cellular process responsible for maintaining cellular and tissue homeostasis under various stress conditions. This comprehensive review endeavors to elucidate the intricate regulatory mechanism and relationship between autophagy and DSC differentiation. To achieve this goal, we dissect the intricacies of autophagy and its mechanisms. Subsequently, we elucidate its pivotal roles in impacting DSC differentiation, including osteo/odontogenic, neurogenic, and angiogenic trajectories. Furthermore, we reveal the regulatory factors that govern autophagy in DSC lineage commitment, including scaffold materials, pharmaceutical cues, and the extrinsic milieu. The implications of this review are far-reaching, underpinning the potential to wield autophagy as a regulatory tool to expedite DSC-directed differentiation and thereby promote the application of DSCs within the realm of regenerative medicine.

Introduction

The extensively investigated mesenchymal stem cells (MSCs) were collected from several adult tissues, such as bone marrow, adipose tissue, dental tissue, and birth-derived tissues [1]. However, MSCs obtained from bone marrow showed certain shortcomings, including pain, morbidity, and limited cell number [2]. MSCs from adipose tissue isolated via lipoaspirates or lipectomy faced the disadvantages of techniques and the limitation of donor age [3]. Currently, there is growing evidence that dental stem cells (DSCs) have several distinct advantages that make them an ideal candidate for regenerative medicine. Specifically, these cells are accessible and reliable, conventionally isolated from discarded teeth or periodontal tissue, obviating invasive procedures. More importantly, DSCs exhibit multi-lineage differentiation, low immunogenicity, circumventing ethical concerns, and heightening the viability of autologous transplants.

It is now understood that DSCs can be readily obtained from tissues originating from the oral region [4]. To date, numerous different types of human DSCs have been identified and named according to their origin, including dental pulp stem cells (DPSCs) [5], stem cells from human exfoliated deciduous teeth (SHED) [6], stem cells from apical papilla (SCAPs) [7], periodontal ligament stem cells (PDLSCs) [8], dental follicle cells (DFCs) [9], and gingival mesenchymal stem cells (GMSCs) [10]. The main characteristics of DSCs are shown in Table 1. However, DPSCs exhibit a higher proliferation potential, superior neuroprotective property and neurogenetic ability as compared with MSCs from bone marrow, adipose tissue, and umbilical cord [11].

DSCs belong to the MSC category, mirroring the characteristics of MSCs, including self-renewal ability and multi-lineage differentiation potential [12,13]. It has been demonstrated that DSCs possess the abilities of dentinogenesis, cementogenesis, osteogenesis, adipogenesis, chondrogenesis, myogenesis, angiogenesis, neurogenesis, and hepatogenesis when provided with the appropriate inductive

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signals [14]. In addition, DSCs exert potent immunomodulatory functions [14,15]. Their easy availability and advantages position them as promising entities in various biomedical fields, including regenerative dentistry, osseous reconstruction, and neural tissue regeneration [16]. Therefore, a comprehensive exploration of the regulatory mechanisms underlying DSC differentiation into specific cellular and tissue types is imperative.

The differentiation process of DSCs is significantly influenced by a range of regulatory factors, with previous studies primarily focusing on genetic, epigenetic, and molecular aspects [17–19]. Recently, the role of autophagy in DSC differentiation has emerged as a research hotspot. Autophagy typically orchestrates cellular homeostasis by degrading redundant or detrimental cytoplasmic constituents, particularly during stress conditions. Dysregulated autophagy has been linked to various clinical diseases, including cancer, cardiovascular diseases, inflammation, metabolic syndromes, neurodegeneration, and musculoskeletal disorders [20]. In dental medicine, autophagy participates in the physiology and pathophysiology of teeth, including tooth development, pulp aging, periapical lesions, and stress adaptation [21,22]. It also reportedly participates in suppressing inflammation within the dentin-pulp complex, dental pulp, and periapical tissues [22,23]. Additionally, autophagy is intricately connected to stem cell developmental processes encompassing self-renewal, lineage commitment, and cell aging [24,25].

TABLE 1

Characteristics of six types of DSCs

Cell types	Tissue origin	Negative markers	Positive markers	Multilineage differentiation	Applications in tissue repair and regeneration
DPSCs	Permanent tooth pulp	CD14, CD34, and CD45 [5,26]	CD29, CD44, CD73, CD90, CD105, CD146, and STRO-1 [27]	Osteoblasts Odontoblasts Adipocytes Chondrocytes Myocytes Neuronal cells Endothelial cells Hepatocytes Melanocytes [28]	Dentin/pulp-like complex [29] Regenerative endodontics [30] Periodontal regeneration [31] Corneal epithelium regeneration [32] Central nervous system injuries [33,34] Craniofacial bone defects [35] Mandible bone defects [36] Brain ischaemia (stroke) [37] Myocardial infarction [38] Parkinson's disease [39]
SHED	Exfoliated decidous tooth pulp	CD14, CD18, CD19, CD24, CD34, and CD45 [40]	CD13, CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166, and STRO-1 [40]	Osteoblasts Adipocytes Chondrocytes Neuronal cells [41] Hepatic cells [42,43]	Bio-root regeneration [44] Regenerative endodontics [45] Corneal epithelium regeneration [46] Central nervous system injuries [47] Mandibular defects [48] Liver fibrosis [49] Parkinson's disease [50]
SCAPs	Apical papilia tissue	CD14, CD34, CD45, and CD150 [40]	CD13, CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166, and STRO-1 [40]	Osteoblasts Odontoblasts Lipoblasts Neuroblasts [51]	Periodontal regeneration [52] Regenerative endodontics [53] Nerve tissue regeneration [54] Bone and dentin regeneration [55]
PDLSCs	Peridontal Ligament tissue	CD11b, CD14, CD19, CD31, CD34, CD40, CD45, CD79a, CD80, CD86, and HLA-DR [56,57]	CD10, CD13, CD26, CD29, CD44, CD73, CD90, CD105, CD166, STRO-3/4, Neuron-glial antigen 2 (NG2), and neural crest derived cell markers (Nestin, β-tubulin III CD271/ p75NTR, SLUG, and SOX10) [56,57]	Cementoblasts Osteoblasts Chondrocytes Adipocytes Neurons Cardiomyocytes Pancreatic islet cells Corneal keratocytes [56,57]	Periodontal regeneration [58] Bone regeneration [59] Neural regeneration [60] Jaw bone regeneration [61]

(Continued)

Table 1 (continued)

Cell types	Tissue origin	Negative markers	Positive markers	Multilineage differentiation	Applications in tissue repair and regeneration
DFCs	Dental follicle tissue	CD11b, CD34, and CD45 [62,63]	CD44, CD73, CD90, CD105, STRO-1, Notch-1, Oct-4, Sox-2, Nanog, and Nestin [62,63]	Osteoblasts Adipocytes Chondrocytes Cementoblasts Periodontal ligament cells Neuronal cells [63]	Central nervous system injuries [34,64] Bone regeneration [65] Periodontal regeneration [66] Bio-root regeneration [67]
GMSCs	Gingival tissue	CD14, CD34, and CD45 [68]	CD31, CD44, CD73, CD90, CD105, CD117, CD146, CD166, SSEA, and STRO-1 [68,69], Oct-4, Nanog, Sox2, SSEA4, TRA 160/181, Nestin , and β-tubulin III [70]	Osteoblasts Adipocytes Chondrocytes [68]	Periodontal regeneration [69,71] Mandibular and calvarial defects [72,73] Facial nerve defects [74]

Note: Abbreviations: DFCs, dental follicle cells; DPSCs, dental pulp stem cells; DSCs, dental stem cells; GMSCs, gingival mesenchymal stem cells; PDLSCs, periodontal ligament stem cells; SCAPs, stem cells from apical papilla; SHED, stem cells from human exfoliated deciduous teeth.

Mammalian autophagic pathways can be classified into three types based on morphological and mechanistic features: macroautophagy [75], microautophagy [76], and chaperone-medicated autophagy (CMA) [77]. Among these, macroautophagy is the predominant system and has been extensively investigated. Macroautophagy involves membrane elongation, resulting in the formation of the autophagosome that has a double-membraned structure [78]. Microautophagy involves membrane internalization, generating intraluminal vesicles [79,80]. The above two pathways undergo selective or non-selective processes that transport cytoplasmic components into lysosomes to be degraded. However, CMA could directly and selectively transport cytosolic materials into lysosomes without membrane deformation [77,81].

Recent research has corroborated that autophagy activation is involved during cell differentiation [82,83]. Autophagy inhibition could turn young MSCs into a relatively aged state that exhibits degenerative changes [84]. In an *in vivo* study, autophagy activation could restore bone loss in aged mice, suggesting autophagy might be a future target for clinical treatment of age-related bone loss [84]. Thus, it is highly conceivable that autophagy plays a pivotal role during stem cell differentiation compared to their undifferentiated state. However, excessive autophagic activity may lead to cell function dysregulation and cell death [85–88], accentuating the challenge of appropriately harnessing autophagy in directing stem cell differentiation. Recent studies have substantiated autophagy modulation as pivotal for DSC lineage commitment and potentially pivotal in maxillofacial tissue regeneration, yet no comprehensive review has been reported.

This review aims to document recent advances in understanding the intricate regulatory mechanisms and relationships between autophagy and DSC differentiation. We conducted a comprehensive search for relevant articles based on up-to-date databases, including PubMed, Google Scholar, and Web of Science. The keywords used in our search included “DSCs”, “DPSCs”, “SHED”, “SCAPs”, “DFCs”, “GMSCs”, “DPLSCs”, “autophagy”, “mitophagy”, “cell differentiation”, and related terms. We applied no publication date limits and incorporated articles that published in English. We screened the titles, abstracts, and full texts of the identified articles to select articles related to the topic of autophagy in DSC differentiation. Studies with insufficient or poor-quality data and duplicates were excluded. Our final selection included articles that contributed valuable insights into the role of autophagy in the differentiation of dental stem cells. This review delineates the autophagy mechanism, emphasizes its function and regulation in DSC differentiation, and provides insights into the regulatory mechanisms that can be harnessed as targets in DSC-driven regenerative medicine. Furthermore, this review outlines a potential approach to the precise regulation of autophagy in stem cell differentiation, serving as a guide for DSC-based research and promoting their application in tissue reconstruction and regeneration.

The Process and Mechanism of Autophagy

Macroautophagy, henceforth referred to as autophagy, represents a self-degradation and recycling mechanism, serving as a “housekeeping” function by eliminating dysfunctional organelles, pathogenic proteins, and intracellular pathogens [89,90]. It is a dynamic process that can be briefly subdivided into five phases: initiation, nucleation, phagophore expansion, sealing and cargo sequestration, and autolysosome formation (see Fig. 1) [75,91].

The process of autophagosome biogenesis is intricate and is typically initiated by a wide range of cellular events, including oxidative stress, infection, nutrient deficiency, and hypoxia, among others [92]. These events lead to the recruitment of

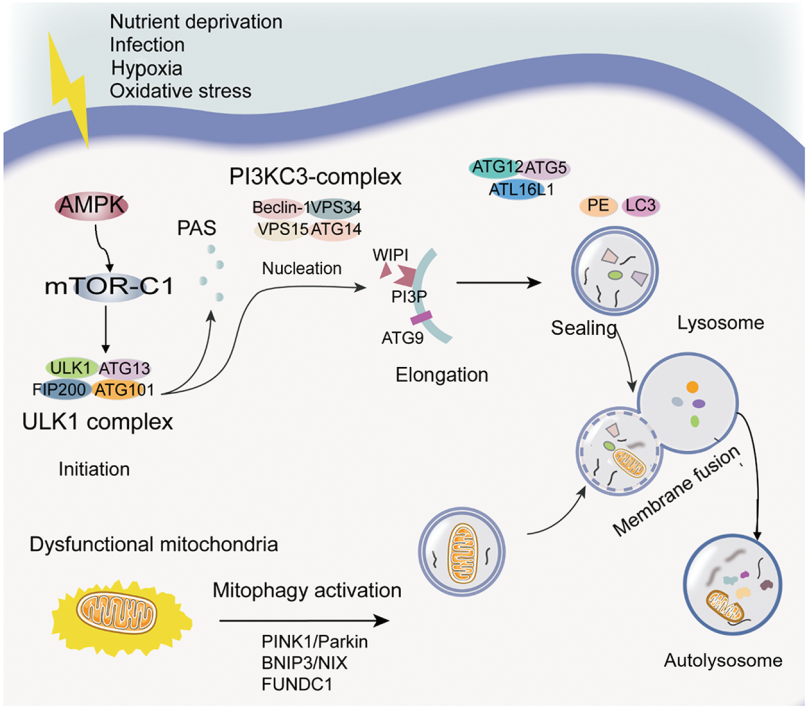

FIGURE 1. Diagram of the autophagy process. Initiation: The initiation of the autophagic process can be induced by various stress conditions, which result in AMPK activation, mTOR-C1 inactivation, and ULK1 complex phosphorylation. Nucleation: PI3KC3-complex phosphorylation and PI3P generation contribute to phagophore nucleation. Elongation: WIPI binding to PI3P and the transmembrane protein ATG9-containing vesicles contribute to the elongation of the phagophore membrane. Sealing: Two ubiquitin-like protein conjugation systems (ATG12 conjugates with ATG5 and ATL16L1, LC3 conjugates with PE) are required for elongation and closure of the phagophore membrane, and its cargo sequestration can be nonselective or selective. The selective autophagy is related to LC3 and LC3 homologs. Autolysosome formation: The autophagosome outer membrane fuses with the lysosomal membrane to form a single-membrane autophagic body termed the autolysosome. Mitophagy: Mitophagy, targeted dysfunctional mitochondria, is a particular type of autophagy. PINK1/Parkin, BNIP3/NIX, and FUNDC1 signaling pathways are mainly mitophagy regulatory pathways. Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; ATG, autophagy-related proteins; BNIP3/NIX, BCL2 interacting protein 3/NIP3-like protein X signaling; FUNDC1, FUN14 domain containing 1; LC3, microtubule-associated protein 1 light chain 3; mTOR-C1, the mechanistic target of rapamycin complex 1; PAS, phagophore assembly site; PE, phosphatidylethanolamine; PI3KC3, the class III PI3K; PI3P, phosphatidylinositol-3-phosphate; PINK1/Parkin, the phosphatase and tensin homolog-induced putative kinase 1/E3 ubiquitin ligases Parkin signaling; ULK1, Unc-51-like kinase 1; WIPI, WD-repeat domain phosphoinositide-interacting.

approximately 16–20 core autophagy-related (ATG) proteins to the phagophore assembly site (PAS), acting at specific stages of autophagosome initiation or formation [93,94]. Current evidence suggests that adenosine monophosphate-activated protein kinase (AMPK) activation is triggered in response to energy insufficiency-induced stress, leading to the inhibitory phosphorylation of the mechanistic target of rapamycin complex 1 (mTOR-C1). AMPK-induced autophagy activation can also circumvent mTOR and directly phosphorylate key proteins in initiation, such as Unc-51-like kinase 1 (ULK1) and ATG13. Notably, the ULK1 complex, comprising ULK1, ATG13, FIP200, and ATG101, holds pivotal importance in autophagy initiation, prompting phagophore nucleation through the phosphorylation of components within the class III PI3K (PI3KC3) complex I (PI3KC3-C1 consists of Beclin 1, VPS34, VPS15, and ATG14) [92,93]. Beclin 1, a critical component in the PI3KC3 complex, plays a vital role in initiating the autophagic process [95]. Besides, it has been reported that PI3KC3-C1 leads to phosphatidylinositol-3-phosphate (PI3P) generation to act in autophagosome nucleation [93].

The phagophore is a specific structure that arises from the membrane generated during nucleation. It has been established that the involvement of WD-repeat domain phosphoinositide-interacting (WIPI) proteins and related entities occurs during the early phases of membrane elongation via PI3P binding [91]. Furthermore, the transmembrane protein ATG9-containing vesicles contribute to the elongation of the phagophore membrane by delivering various cellular membranes such as the plasma membrane, mitochondrial membrane, recycling endosomes, and the Golgi complex membrane [92]. Over time, the phagophore membrane elongates, enclosing a portion of the cytosol, leading to membrane expansion and sealing into a characteristic double-membraned spherical vesicle known as the autophagosome [92]. Studies have indicated that two ubiquitin-like protein conjugation systems are integral to phagophore membrane elongation and closure: ATG12 conjugates with ATG5 and ATL16L1, and members of the ubiquitin-like protein LC3 (microtubule-associated protein 1 light chain 3) subfamily, part of the ATG8 protein family, conjugate with membrane-resident phosphatidylethanolamine (PE) [89,93].

This autophagosome entraps substances, excess or potentially hazardous cytoplasmic entities, and intracellular pathogens for subsequent degradation as autophagic cargo [93]. While autophagy has been considered primarily as a

non-selective degradation pathway, it plays a critical role in the digestion of specific cargoes like proteins and organelles. LC3 and its homologs are pivotal in sequestering specifically labeled autophagic substrates into autophagosomes through cargo receptors containing the LC3-interacting region during selective autophagy. Conversely, nutrient scarcity initiates bulk autophagy, which is relatively nonselective, engulfing a range of cytoplasmic substrates [75]. The closure of the autophagosomal membrane marks the completion of autophagosome formation.

Subsequently, the outer membrane of the autophagosome fuses with the lysosomal membrane, resulting in the formation of a single-membraned autophagic body termed the autolysosome. Within the lysosomal lumen, acidic hydrolases facilitate the degradation of the autolysosome, releasing salvaged nutrients back into the cytoplasm for cellular reuse [92].

According to the selectivity towards degradation substrates, autophagy can be divided into mitophagy, reticulophagy, ribophagy, pexophagy, etc. [96]. Recently, mitophagy, an autophagic process that targets mitochondria, has attracted significant interest as a specialized form of autophagy. It maintains mitochondrial quality and homeostasis by selectively eliminating dysfunctional or surplus mitochondria [97]. Mitochondrial dysfunction can be assessed in several ways, such as: mitochondrial membrane potentia ($\Delta\Psi m$) measured using fluorescent probes like JC-1 or TMRM, the elevated ROS levels detected by DHE (dihydroethidium) or MitoSOX, abnormal mitochondrial morphology and mass assessed by electron microscopy and staining with fluorescent dyes like MitoTracker, and functional assay including those measuring ATP production, oxygen consumption, and mitochondrial respiratory chain activity, which can provide insight into mitochondrial function and dysfunction [98,99]. In addition, laser-induced autofluorescence could be used to detect mitochondrial dysfunction *in vitro* [100].

Mitochondrial dysfunction plays a crucial role in the autophagy cascade. Membrane depolarization of mitochondria is a trigger of autophagy [101]. The cellular mitochondria of ROS production and oxidative modification may be primary targets of autophagy [102]. Mitophagy is initiated when mitochondrion becomes damaged or impaired. Mitophagy, a specialized form of autophagy, is the selective targeting and elimination of damaged mitochondria. Hence, mitochondria contribute to prosurvival functions of autophagy. Currently, regulatory pathways of mitophagy primarily revolve around the phosphatase and tensin homolog-induced putative kinase 1 (PINK1)/E3 ubiquitin ligases Parkin signaling, BCL2 interacting protein 3 (BNIP3)/NIP3-like protein X (NIX) signaling, and FUN14 domain containing 1 (FUNDC1) signaling [25,103].

Briefly, autophagy/mitophagy is a self-digestive process that is conserved in eukaryotic cells and responsible for maintaining cellular homeostasis through degrading of harmful components in cells. Previous research has found pharmacological inhibition of autophagy via chloroquine (late-stage autophagy inhibitor, already in clinical use) may be a promising strategy for alleviating pathologic bone loss induced by glucocorticoids and by ovariectomy in murine models [104], suggesting late autophagy regulation may play a protective role in a hostile environment.

Effects of Autophagy on DSC Differentiation

Stem cell-based tissue engineering holds promise for more robust tissue repair and regeneration. A mounting body of evidence underscores the correlation between autophagy and DSC differentiation (see Table 2), especially osteo/odontogenic differentiation, neuronal differentiation, and vasculogenic differentiation.

Osteo/odontogenic differentiation

In recent years, significant emphasis has been placed on the osteogenic and odontogenic differentiation abilities of DSCs. Given the shared components and properties of bone and dentin, we attempt in this section to describe the potential impact of autophagy on osteogenic/odontogenic differentiation and mineralization of DSCs, offering a tantalizing prospect for tissue regeneration.

Recent *in vitro* investigations have predominantly focused on autophagy's ultrastructure and the expression levels of autophagy-related genes in stem cells cultured in osteo/odontogenic induction conditions. In this regard, human dental pulp cells (DPCs) have become a research hotspot. During odontoblastic differentiation, the expression of dentin matrix protein-1 (DMP-1) and dentin sialoprotein (DSP) is steadily increased, paralleled by autophagosome and autophagolysosome formation. Furthermore, there is a notable shift from cytosolic LC3-I to the autophagosome marker LC3-II, albeit with diminished levels of the selective autophagy cargo receptor p62 [105]. A similar trend was observed in the osteoblast differentiation of DPSCs. Core ATG proteins (ATG3 and ATG7) are upregulated, while the autophagy-inhibiting factor mTOR is significantly downregulated [106]. These findings collectively indicate autophagy activation during the osteo/odontogenic differentiation process.

The prevailing consensus suggests that autophagy activation could potentially potentiate osteoblast and odontoblast mineralization, whereas its inhibition might yield opposite effects [107–111]. It has been corroborated that autophagy facilitates type I collagen synthesis, thereby modulating the osteoblastic differentiation of periodontal ligament (PDL) cells [112]. Moreover, Nollet et al. [113] reported that autophagy bodies of living cells can be used as carriers to secrete apatite crystals into the extracellular matrix. Additionally, autophagy interfaces with signaling pathways to amplify cell differentiation and mineralization. In this respect, research has shown that autophagy fosters odontoblastic differentiation via the phosphatidylinositol 3-kinase (PI3K)/Akt signaling while simultaneously attenuating stemness in DPCs under odontogenic induction conditions [105]. AMPK activation has been reported to promote osteogenic differentiation of DPSCs through different approaches: early mTOR inhibition-mediated autophagy and later activation of the Akt/mTOR signaling axis [114] suggesting that the differentiation ability of DSCs is closely associated with the regulatory effects of autophagy.

The emphasis should be placed on the distinct autophagic shifts occurring at different osteo/odontogenic differentiation stages. Activation or inhibition of autophagy barely affected early differentiation markers. During extended cultures with an osteogenic differentiation medium, autophagy markers experience significant downregulation. Under these circumstances, autophagy activation significantly mitigates biomineralization ability of DFCs, while autophagy inhibition yields a divergent effect [115]. However, another report proves that initiating autophagy could enhance early osteogenic activity via AMPK/mTOR signaling pathway [116]. This temporal regulation and stem cell type might account for the discrepancies between the inhibition and stimulation effects of autophagy in response to mineralization induction. Essentially, by accelerating the hard tissue formation potential of DSCs, the precise modulation of autophagy may emerge as a crucial factor in promoting dentinogenesis and osteogenesis.

Neuronal differentiation

Although most studies have focused on the abilities of DSCs to differentiate into odontoblasts or osteoblasts, it should be borne in mind that DSCs also possess a powerful neurogenic differentiation capacity [11,60]. Originating from the neural crest, DSCs express distinct neural markers such as nestin, glial fibrillary acidic protein (GFAP), β-tubulin III, synaptophysin, and S100 protein [7,117]. Furthermore, it has been reported that human DPSCs exhibit higher neurotrophin levels and more extensive innervation than human bone marrow-derived MSCs [118], positioning DSCs as a promising candidate for neural tissue regeneration and repair [119].

Upon 7 days of DPSC cultivation in neural differentiation-specific media, differentiated cells adopt a neural-like pyramidal morphology characterized by short dendrite projections and elongated axon-like extensions, accompanied by a significant elevation in neural-specific markers [120]. Prateeksha et al. [120] revealed significant upregulation of autophagy and mitophagy-associated markers during neural differentiation (ND) of DPSCs. Enhancing autophagy and mitophagy levels notably enhanced ND, underscoring the pivotal role of autophagy and mitophagy in directing DPSCs toward neural commitment. Another research has demonstrated that the autophagy inducer, 5-Azacytidine, enhances neuronal differentiation of MSCs [121], there remains a paucity of studies investigating the role of autophagy in stem cell neuronal differentiation, warranting further exploration of the underlying mechanisms.

It is now understood that mitochondria, which are the targets of autophagy, play a pivotal role in providing energy during cell differentiation. Previous studies have observed heightened mitochondrial membrane potential, increased mitochondrial DNA, and elongated mitochondria during the neuronal differentiation of SHED. Inhibition of mitochondrial activity has been shown to impede neuronal differentiation, emphasizing the significance of mitochondrial function [122]. Additionally, increased mitochondrial respiration and spare respiratory capacity have been observed in neuron-like differentiated cells [120]. Upon depletion of mitochondrial fission factor (MFF) in SHED differentiating into dopaminergic neurons, the marker protein of mitochondria biogenesis was downregulated, and neurons showed impaired neurite outgrowth and excessive reactive oxygen species (ROS) [123]. It has been confirmed that autophagy is an important regulator in fighting against excessive ROS-induced impairment [124,125]. These findings suggest the crucial function of mitochondria in the neuronal differentiation of stem cells.

Therefore, it is unsurprising that mitochondrial dysfunction has been implicated in various human diseases, including neurodevelopmental and neurodegenerative disorders. Impaired neurite development, linked to mitochondrial dysfunction, has been observed in dopaminergic neurons differentiated from stem cells obtained from children with neurodegenerative diseases like Rett syndrome [126], autism spectrum disorder (ASD) [127], attention deficit hyperactivity disorder (ADHD) [128], and Down syndrome [129], as well as DPSCs obtained from a patient with metabotropic dysplasia and multiple neuropsychiatric symptoms [130], suggesting mitochondria might be a potential therapeutic target of neuro-related diseases. Nevertheless, further investigations are warranted to fully elucidate the role and mechanism of mitophagy in neuro-related disorders.

Vasculogenic differentiation

First of all, we should make clear the difference between vasculogenesis and angiogenesis, which are two subsequent processes of the development of blood vessels system. Angiogenesis refers to the establishment of new blood vessels from the preexisting vasculatures, occurs throughout life, and consists of four stages. Vasculogenesis is the formation and development of the vascular system, including the formation of blood vessels from endothelial cells during embryogenesis (see Fig. 2) [131,132].

The induction of angiogenesis remains a pivotal challenge in tissue engineering and regeneration. DSCs have demonstrated the ability to differentiate into vasculogenic endothelial cells capable of generating functional blood vessels [133]. DSCs have been increasingly recognized for their therapeutic potential in dental pulp and periodontal tissue engineering.

In primary DPSCs and SHED, a specific sub-population characterized by high vascular endothelial growth factor receptor 1 (VEGFR1) expression has been identified, enabling these cells to respond to vasculogenic stimuli [134]. However, they lack VEGFR2, CD31, and VE-cadherin expression [135,136]. When subjected to endothelial differentiation medium supplemented with VEGF, DPSCs progressively express endothelial cell-related markers (e.g., VEGFR2, VE-cadherin, CD31) [137]. Upon transplantation into murine hosts, DPSCs differentiate into functional CD31-positive mature blood vessels [135,136], underscoring the angiogenic potential of DPSCs for tissue regeneration. Despite these promising findings, the precise mechanisms governing vascular differentiation induction remain largely unclear.

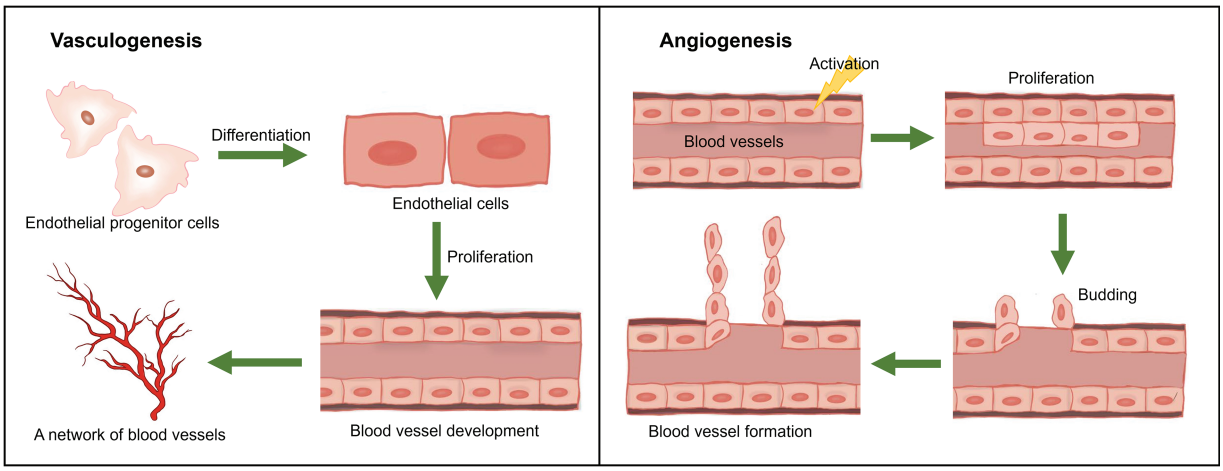

FIGURE 2. Hypothetical figure showing the processes of vasculogenesis and angiogenesis.

Emerging evidence has linked autophagy to the process of angiogenesis. For instance, colocalization of endothelial marker CD31 and autophagic marker LC3 has been observed in regenerated pulp-like tissue, suggesting the potential involvement of autophagy in pulp revascularization [138]. This hypothesis is corroborated by findings indicating that autophagy regulates endothelial cell processing and maturation [139]. In another study, the angiogenic effect of erythropoietin on PDLSCs was linked to autophagy activation under an inflammatory microenvironment modulated through the Akt/Erk1/2/BAD signaling pathway [140]. This novel approach presents a promising strategy for angiogenic tissue engineering.

It has been reported that PDLSCs can contribute to vasculogenesis not only through direct differentiation into endotheliocytes but also by secreting pro-angiogenic factors. In an inflammatory milieu, increased autophagy levels in PDLSCs correlated with heightened secretion of pro-angiogenic cytokines, including angiopoietin and basic fibroblast growth factor (bFGF), ultimately leading to enhanced tube formation [141]. Autophagy's role in bolstering the paracrine function of PDLSCs increases their pro-angiogenic potential, offering a pathway to expedite blood circulation establishment within DSC-based tissue-engineered constructs. In summary, an autophagy-centered approach to angiogenic differentiation holds considerable therapeutic potential for dental pulp and periodontal tissue regeneration.

Adipogenic differentiation

Currently, published data are conflicting about the potential of DSC adipogenic differentiation *in vitro*. DSCs could differentiate into adipocytes under suitable conditions, which was confirmed by oil red O staining of the accumulated lipid droplets [142]. However, a recent study disclosed that DPSCs cultured in the adipogenic induction medium had a limited capacity to differentiate into adipocytes, which is supported by their gene expression profile [143]. There is no doubt that DSCs still possess adipogenic differentiation potential. Adipocytes are fat-storing cells with limited clinical application, but they could be used for further molecular research. However, no literature related to autophagy in adipogenic differentiation of DSCs has been retrieved. The impact of autophagy on the adipogenic differentiation ability is worth exploring.

Chondrogenic differentiation

Several studies have investigated the chondrogenic potential of DSCs. After 21 days of chondrogenic induction, toluidine blue staining could be used to assess glycosaminoglycans production, and alcian blue and picrosirius red staining could evaluate collagen deposition. Gene expression analysis has confirmed the presence of chondrogenic markers, including *Sox9*, *Comp*, and *Acan*, during chondrogenic differentiation of DPSCs [144]. It has been reported that the chondrogenic differentiation potential of SCAPs enhanced by distal-less homeobox 5 (DLX5) and homeobox C8 (HOXC8) [145]. Fas cell surface death receptor ligand (FasL) stimulation is implicated in supporting the chondrogenic differentiation of DPSCs [146]. Chondrogenic differentiation is integral to cartilage repair and regeneration. It is a pity that how autophagy regulates chondrocyte differentiation remains still unclear.

Other differentiation routes

DSCs hold significant potential for myogenic differentiation [147,148]. Furthermore, DPSCs can differentiate into insulin-producing cells [149] and hepatic-like cells [150]. The effects of autophagy on the multi-lineage differentiation ability of DSCs are worth exploring, and reports on the function and regulation of autophagy in the DSC differentiation into the above tissue-like cells are still lacking.

Furthermore, it is worth noting the concept of hormesis, which has gained prominence in the study of stem cell biology. Hormesis is a biphasic dose/concentration response. It displays a low-dose/concentration stimulation and a high-dose/concentration inhibition [151–153]. The hormetic response is typically demonstrated during preclinical investigations, markedly enhancing therapeutic applications through selecting optimal hormetic doses in clinical trials. Recently, hormesis and its potential relevance to dental stem cells have extensively been studied, especially in cell proliferation and differentiation [154–156]. Additional researches are still needed to investigate the implications of

hormesis on dental stem cell behavior and its potential applications in regenerative dentistry.

Regulation of Autophagy in DSC Differentiation

The differentiation of DSCs is influenced by various factors involving autophagy pathways. In the field of tissue engineering, scaffold materials play a pivotal role in impacting DSC differentiation. Additionally, drug stimuli function as signaling agents, orchestrating DSC differentiation. The external environment surrounding DSCs must also be considered. This comprehensive array of factors impacting autophagy-mediated DSC differentiation is elaborated upon in the subsequent sections (see Fig. 3).

An in-depth exploration of natural and synthetic biomaterials suitable for scaffold construction in oral tissue regeneration has been outlined in prior reviews [16,157]. Experiments indicated that the nanopore structure and the pore size were found to be important for regulating the expression of autophagy pathway components (LC3A/B, Beclin-1, Atg3, Atg7, and P62) and the osteogenic pathways [158]. The nano-textured surface of biomaterials induced a stronger autophagic response, resulting in stronger osteogenesis [159]. In addition, numerous studies have corroborated the potential of specific materials to facilitate DSC differentiation through autophagy, although the precise underlying mechanisms remain partly obscured.

Tissue-derived scaffold: Platelet-rich plasma (PRP) has been effectively utilized as a tissue scaffold during clinical practice in recent years to regenerate pulp-like tissue and stimulate root development during regenerative endodontic treatment for immature teeth [160]. Notably, PRP exhibits potential for bone regeneration, with PRP-induced autophagy playing a crucial role in driving substantial osteogenic differentiation and mineralization in DPCs [109]. Furthermore, thrombin-activated PRP has been shown to augment autophagy-mediated osteogenic differentiation and viability of PDLSCs [110]. These results suggest that autophagy might be a mechanism underlying PRP-mediated osteogenic differentiation and mineralization of DSCs.

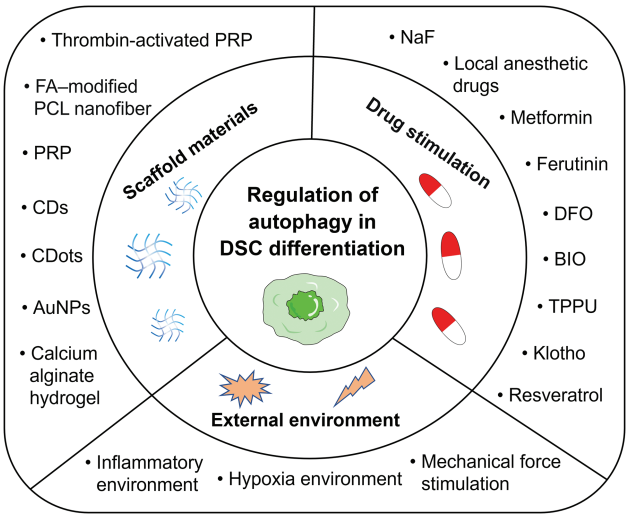

FIGURE 3. An overview of the major factors that regulate autophagy in the differentiation of DSCs. These factors can be mainly divided into three categories: scaffold materials, drug stimulation, and external environment. Abbreviations: AuNPs, gold nanoparticles; BIO, 6-bromoindirubin-3'-oxime; CDs, carbon nanodots; CDots, carbon dots; DFO, deferoxamine; DSCs, dental stem cells; FA-modified PCL nanofiber, fluorapatite-modified polycaprolactone nanofiber; NaF, sodium fluoride; PRP, platelet-rich plasma; TPPU, a soluble epoxide hydrolase inhibitor.

Nanomaterials scaffold: Recently, bioactive nanomaterials have been widely proposed to regulate DSC differentiation in tissue regeneration. Carbon nanodots (CDs) have been recognized for enhancing DSC differentiation by activating autophagy, exemplified by metformin-based CDs promoting efficient odontogenic differentiation of DPSCs [161]. Carbon dots (CDots) have been found to enhance osteo/odontogenic differentiation of DPSCs through reasonable autophagy activation via the PI3K/Akt/mTOR signaling pathway, contributing to the effective regeneration of dentin-pulp complex and blood vessels *in vivo* [162]. Graphene oxide quantum dots (GOQDs) bolster DPSC mineralization by modulating autophagy induced by ROS [163]. Moreover, fluorapatite (FA)-modified polycaprolactone (PCL) nanofibers induce DPSC differentiation and mineralization, potentially influenced by insulin, hedgehog, and Wnt signal pathways interconnected with and/or mediated by cell autophagy [164]. Gold nanoparticles (AuNPs) have been implicated in affecting PDL cells osteogenic differentiation via autophagy [165]. *In vivo* experiments have also shown that treatment with PDLSC sheets and 45 nm AuNPs increased bone structure and collagen formation, mediated by autophagy pathway activation [111]. AuNPs treatment has also been observed to rescue PDLSC osteogenic potential under inflammatory conditions by restoring the inflammation-compromised autophagy-lysosome system [166]. Hence, the development of autophagy-regulating materials holds promise for promoting tissue repair and regeneration through DSC differentiation.

Hydrogel scaffold: The combination of biomaterials with signaling factors and DSCs in a hydrogel scaffold holds significant promise for tissue regeneration. Each component yields distinct effects; when combined, they may work synergistically to enhance treatment outcomes. For instance, calcium alginate hydrogel, DPSCs, and fibroblast growth factor 21 (FGF21) have shown remarkable effectiveness in promoting recovery from spinal cord injuries. This occurs through multiple mechanism, where DPSCs differentiate into neurons, and FGF21 inhibits autophagy via the AMPK-mTOR-LC3 pathway, particularly during the chronic recovery phase. Additionally, the calcium ions within this hydrogel facilitate self-adhesion through cadherin, while alginic acid helps prevent calcium overload injuries by absorbing excess calcium ions [167]. Indeed, biomaterials loaded with signaling molecules represent a potent strategy for balancing autophagy and DSC differentiation in tissue injury repair.

TABLE 2

Autophagy in the differentiation of DSCs

Cell types	Function of autophagy in cell differentiation	Involved proteins and pathways in cell differentiation	Regulation of autophagy	References
DPCs	Promoted odontoblastic differentiation	LC3-I/II, p62, and PI3K/Akt signaling	3-MA	[105]
		FUNDC1	Hypoxia	[168]
	Promoted dentin repair and regeneration	LAMP-1, LC3-II, p62, and mTOR	Local anesthetics	[169]
	Promoted osteogenic differentiation and mineralization	LC3B and Beclin 1	PRP	[109]
DPSCs	Promoted osteogenic differentiation	BECN1, MAPLC3B, ATG3, ATG5, ATG7, LC3B, and mTOR	KLF2	[106]
		Akt/mTOR signaling axis AMPK activation	Autophagy inhibitors	[114]
		Insulin, hedgehog, and Wnt signal pathways	FA-modified PCL nanofiber	[164]
		ATG7 and BECN 1	Ferutinin, KLF2	[170]
	Increased neuronal differentiation	ATG5, ATG7, BECN 1, LC3B, PINK1, and Parkin	KLF2	[120]
		AMPK-mTOR-LC3 pathway	Calcium alginate hydrogel, FGF21	[167]
	Promoted odontogenic differentiation	p62 and LC3-II	Metformin-based CDs	[161]
		HIF-1α/BNIP3 pathway	DFO	[171]
	Promoted osteo/odontogenic differentiation	PI3K/Akt/mTOR signaling pathway	CDots	[162]
		Maybe related to autophagy	BIO	[172]
	Inhibited osteogenic differentiation	p62/SQSTM1 and LC3	LPS stimulation	[173]
SCAPs	Promoted osteo/odontogenic differentiation	LC3, ATG5, and Beclin 1	NaF	[174]
	Downregulated osteo/odontogenic differentiation capacity	LC3, ATG5, and Beclin 1	LPS stimulation	[175]
PDL cells	Inhibited osteoblastic differentiation	LC3, ATG5, and p62	Autophagy inhibitors	[112]
PDLSCs	Had a significant angiogenic effect	Akt/Erk1/2/BAD signaling pathway	Erythropoietin	[140]
	Increased pro-angiogenic ability	p62 and LC3-II	Inflammatory environment	[141]
	Accelerated osteogenic differentiation	LC3, Beclin 1, and SIRT1	Thrombin-activated PRP	[110]
	Increased osteogenic ability	AMPK/mTOR signaling pathway	Metformin	[176]
	Promoted odontoblasts differentiation	LC3, Atg5, Beclin 1, TFE3, and NF-κB	Inflammatory environment	[177]
	Promoted osteogenic differentiation	LC3B and ATG7	Mechanical tension force	[178]
	Promoted periodontal tissue remodeling	LC3-II/I, Beclin 1, and AKT/FOXO3 pathway	Mechanical stimuli combined with lncRNA FER1L4	[179]
	Inhibited osteoclast differentiation	LC3B, Beclin 1, p62, and ATG5	A20, hypoxia	[180]
	Promoted osteoclast differentiation	mTOR, LC3B, Beclin 1, and ATG5	circCDK8, hypoxia	[181]
	Promoted bone regeneration	LC3B, Beclin 1, and ATG5	Antiaging protein Klotho	[182]
	Promoted osteogenic differentiation	PINK1-Parkin-related mitophagy pathway	Nanoparticles that control the release of MitoQ	[183]
PDLSC sheets	Promoted bone structures and collagen formation	LC3 and p62	AuNPs	[111]
DFCs	Increased biomineralization capability	LC3A/B, ATG12, and AMPK	Autophagy inhibitors	[115]
GMSCs	Accelerated the osteogenic differentiation	AMPK-Beclin 1 pathway	Resveratrol	[184]

Note: Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; ATG, autophagy-related proteins; AuNPs, gold nanoparticles; BIO, 6-bromoindirubin-3′-oxime; BNIP3/NIX, BCL2 interacting protein 3/NIP3-like protein X signaling; CDs, carbon nanodots; CDots, carbon dots; DFCs, dental follicle cells; DFO, deferoxamine; FA-modified PCL nanofiber, fluorapatite-modified polycaprolactone nanofiber; FGF21, fibroblast growth factor 21; FUNDC1, FUN14 domain containing 1; GMSCs, gingival mesenchymal stem cells; LC3, microtubule-associated protein 1 light chain 3; mTOR-C1, the mechanistic target of rapamycin complex 1; NaF, sodium fluoride; PE, phosphatidylethanolamine; PI3KC3, the class III PI3K; PI3P, phosphatidylinositol-3-phosphate.

Drug stimulation

Autophagy's vital role in DSC differentiation provides the basis for exploring autophagy-regulating agents beneficial for dental pulp and periodontal tissue reconstruction. Rapamycin is a commonly used autophagy enhancer, while 3-methyladenine (3-MA) and the antimalarial drug chloroquine serve as autophagy inhibitors [91]. Additionally, numerous drugs have been shown to impact cell differentiation by modulating autophagy pathways.

Chemical drugs: Sodium fluoride (NaF), a key component of fluoride toothpaste and mouthwash, has been reported to participate in tooth formation and mineralization processes. Low concentrations of NaF modulate autophagy in SCAPs, promoting osteo/odontogenic differentiation and suggesting NaF-induced autophagy as a regulator of SCAP differentiation [174].

Interestingly, local anesthetic drugs frequently used in dental treatment, like Ubistesin, Ubistesin Forte, Septanest, Scandonest, and Xylocaine, have potential impacts on dentin repair and regeneration by inducing autophagy in DPCs [169].

Metformin, a widely used hypoglycemic drug, enhances the biocompatibility of polydopamine-templated hydroxyapatite (tHA). Combined with tHA, metformin increases the viability and osteogenic ability of PDLSCs through the AMPK/mTOR signaling pathway, regulating autophagy [176].

Deferoxamine (DFO) is an iron chelator clinically used in treating iron overload. It has been reported that during differentiation and mineralization of DPSCs, DFO-induced autophagy could significantly contribute to the repair ability of DPSCs through activation of the hypoxia-inducible factor-1α (HIF-1α)/BNIP3 pathway in a ROS-dependent manner [171].

Natural drugs: Ferutinin, a natural non-steroidal phytoestrogen, catalyzes osteoblast differentiation of DPSCs via KLF2-mediated autophagy/mitophagy, suggesting its potential for regenerative bone therapy [170].

Resveratrol, a naturally occurring polyphenol, has been reported to facilitate the osteogenic differentiation process of GMSCs cultured in the induction differentiation medium through the AMPK-Beclin-1 pro-autophagic pathway. This effect appeared to be intricately linked to resveratrol's role as both an autophagy inducer and a promoter of MSC osteogenic differentiation [184].

Molecule drugs: 6-bromoindirubin-3'-oxime (BIO), a promising small molecule candidate with the ability to influence Wnt signaling, has been reported to effectively promote the odonto/osteogenic differentiation of DPSCs while simultaneously reducing their adipogenic differentiation potential. Analysis of RNA sequencing data from DPSCs treated with BIO revealed differential expression of genes associated with autophagy-modulated pathways [172].

Klotho, an anti-aging protein, has been found to protect PDLSC viability in harsh environments. Moreover, it facilitated bone regeneration following transplantation *in vivo* using a rat model of cranial bone defect by maintaining a low level of autophagic activity in PDLSCs. In contrast, abnormally elevated autophagy activity was observed in locally transplanted PDLSCs that did not undergo Klotho pretreatment [182].

Despite their great potential for autophagy regulation, these drugs have not been developed for this purpose and lacked specificity. Thus, it is important to design and develop clinically targeted autophagy modulators based on the unique autophagic features associated with each specific disease.

External environment

The critical importance of an optimal environment for differentiated transplanted cells is well recognized, with harsh microenvironments compromising their stemness and impeding their therapeutic efficacy in tissue regeneration engineering. Satisfactory therapeutic outcomes in patients with conditions such as pulpitis, periodontitis, and orthodontic treatment remain elusive, potentially limited by the inflammatory milieu and insufficient oxygen supply within the cell transplant site. Consequently, a comprehensive exploration of the role and regulatory factors of autophagy in the intricate and variable transplant setting becomes imperative.

Inflammatory environment: Research substantiates that an inflammatory microenvironment triggers mitochondrial damage, activating mitophagy via the PINK1-Parkin pathway to degrade impaired mitochondria [185]. The role of autophagy in DSC differentiation is multifaceted, as evidenced by reports that autophagy prompted by lipopolysaccharide (LPS) stimulation contributes to the attenuation of SCAP osteo/odontogenic differentiation capacity [175]. Dang et al. [173] revealed that LPS-induced inflammatory conditions trigger excessive autophagy in DPSCs, suppressing osteogenic differentiation, an effect reversed by the soluble epoxide hydrolase inhibitor TPPU. Intriguingly, low concentrations of tumor necrosis factor-α (TNF-α) fostered osteogenic differentiation of PDLSCs through autophagy activation via AKT/mTOR signaling inhibition [186]. Hence, it is highly conceivable that DSC differentiation is intricately connected to the activation level of autophagy driven by inflammation. Nonetheless, contrasting studies have shown that autophagy enhances odontoblast differentiation by suppressing NF-κB activation within an inflammatory milieu [177]. Additionally, the anti-inflammatory factor interleukin-37 (IL-37) augmented osteo/odontogenic differentiation of DPSCs via activated autophagy [187]. These discrepancies most likely stem from variations in the cell lines used and the level of inflammatory stimulation. Taken together, these findings unveil fresh perspectives into the mechanisms safeguarding the dentin-pulp complex during inflammatory intrusion, offering potential therapeutic implications for caries, pulpitis, and dental pulp regeneration within inflammatory contexts.

Mechanical force environment: During the course of mechanical force-induced tooth movement, the periodontal tissues undergo the development of a sterile inflammatory microenvironment. This environment is marked by heightened levels of inflammatory cytokines and chemokines, as well as increased activity of immune cells associated with inflammation [188,189]. PDLSC autophagy

induced by mechanical compressive force has been reported to facilitate M1-like macrophage polarization by inhibiting the AKT signaling pathway in periodontal tissues, thereby fostering inflammatory bone remodeling and promoting orthodontic tooth movement [190]. However, observations by Chen et al. [191] suggested that orthodontic force-triggered autophagy primarily occurs on the pressured side of PDL tissues, reducing the speed of orthodontic tooth movement by suppressing osteoclastogenesis. This discrepancy likely arises from the multifaceted roles of autophagy under different compressive force levels. Zheng et al. [178] observed heightened autophagy levels due to mechanical tension force, effectively promoting the osteogenic differentiation of PDLSCs. Recent research has unveiled the ability of the combination of mechanical stimuli and biochemical signals to influence cellular fate through modulation of the autophagy pathway. Notably, the induction of PDLSC autophagy under orthodontic compressive force was found to be orchestrated by long non-coding RNA (lncRNA) FER1L4 via the AKT/FOXO3 pathway [179]. These findings highlight autophagy modulation as a prospective strategy to bolster periodontal tissue reconstruction while also offering a promising avenue to expedite orthodontic tooth movement.

Hypoxia environment: Hypoxia, often triggered by inflammation stemming from bacterial infection or trauma, is a common occurrence in injured tissues. Certain researchers have uncovered the protective role of autophagy under hypoxic conditions. A previous report highlighted FUNDC1's role as a receptor that mediated mitophagy in response to hypoxia [103]. Diminishing FUNDC1 expression undermined hypoxia-induced proliferation, migration, and odontoblastic differentiation of DPCs, suggesting that FUNDC1-mediated autophagy potentially governs the biological behavior of DPCs [168]. Hypoxia stimulates lactate production, a glycolysis end product. Lactate has been reported to inhibit autophagy and subsequently hinder the osteogenic differentiation of PDLSCs via the MCT1-mTOR signaling pathway [192]. A20, also recognized as TNF-α-inducible protein 3 (TNFAIP3), functions as a potent anti-inflammatory enzyme. In a hypoxic microenvironment, A20 was found to repress osteoclast differentiation of PDLSCs by downregulating autophagy [180]. Importantly, circCDK8 was found to exhibit an inhibitory effect of osteogenic differentiation via autophagy induction [181]. These findings indicate the close relationship between osteogenic/osteoclast differentiation and autophagy levels, highlighting autophagy's potential as a novel therapeutic target for bone loss in periodontitis.

High glucose environment: In addition, the osteogenic ability of PDLSCs is impaired by high glucose environments [193]. Elevation of autophagy partly reversed the detrimental impact of high glucose conditions on PDLSCs, highlighting the protective role of autophagy in preserving cellular function. In a diabetic rat periodontal trauma model, the periodontium tissue exhibited partial recovery in the autophagy-enhanced cell injection group, offering stronger evidence for the regulatory role of autophagy *in vivo* [194]. This finding sheds light on the mechanistic understanding of tissue regeneration in periodontitis associated with diabetes, potentially offering a fresh perspective for treating and researching periodontitis in diabetic patients.

Other factors

LncRNAs are an abundant class of RNAs that do not encode proteins but play an important regulatory role in gene expression. A recent study uncovered the regulatory role of lncRNA insulin-like growth factor binding protein 7-antisense 1 (IGFBP7-AS1) in odontogenic differentiation of SHED through autophagy, indicating its potential as a gene target in the regeneration of dental hard tissue and the dental-pulp complex [195]. Although the gene expression levels regulated by lncRNAs can be broadly categorized into epigenetic, transcriptional, and post-transcriptional regulation, their specific role in autophagy regulation remains unclear. Additionally, cellular communication network factor 1 (CCN1), a pivotal matricellular protein, facilitated osteogenesis in PDLSCs through autophagy and the MAPK/ERK pathway [196]. CCN1 exhibits promising regulatory potential in tissue regeneration, playing multifaceted roles in cell differentiation, adhesion, and migration and serving as a key participant in bone development, osteoblast differentiation, and the remodeling of the extracellular matrix.

Conclusion and Perspectives

DSCs hold immense potential for stem cell-based medical therapies, necessitating an in-depth comprehension of their differentiation mechanisms. Based on the available literature, a viable avenue exists for enhancing DSC regenerative potential via autophagy modulation. Serving as a self-degrading and recycling system, autophagy actively participates in DSC differentiation, including osteo/odontogenic, neurogenic, and angiogenic differentiation, influenced by factors considered in tissue regeneration, such as scaffold materials, drug stimulation, and the external environment. A comprehensive synthesis of current knowledge concerning autophagy's role and regulation in DSC differentiation could significantly advance research into DSC-based regeneration. However, despite escalating interest and investigation into autophagy's contribution to DSC differentiation, there is still a considerable gap in our overall comprehension of autophagy's influence and the underlying regulatory mechanisms regulating its implications within this context.

The differentiation ability of DSCs is significantly affected by the transplantation environment mediated by the autophagy pathway. Furthermore, most autophagy modulators have limitations such as poor target specificity, low stability, low concentration, and limited drug action duration. Addressing these challenges could be achieved by a drug delivery system that leverages the controlled drug release properties of scaffold materials through intelligent response to the microenvironment. This system, which incorporates autophagy modulators and DSCs for *in-situ* implantation, offers an innovative and effective approach for repair and reconstruction [183]. This method introduces a

novel perspective, demonstrating that autophagy modulators can be safely and efficiently applied in harsh environments through scaffold-based delivery. This innovative approach paves a fresh path toward precise and rigorous control of autophagy during stem cell differentiation. Nevertheless, given the complexity and significance of autophagy in DSC differentiation for tooth and bone tissue regeneration, further research is needed to explore strategies for the precise and rigorous regulation of autophagy.

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Ethics Approval: Not applicable.

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