

Review

Estrogen-mediated dental tissue regeneration

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Summary. As the key regulator of hard tissue metabolism in both men and women, estrogen regulates the processes necessary for cell growth, proliferation, and differentiation through estrogen receptor (ER). Estrogen deficiency usually causes systemic osteoporosis not only in long bones but also in jaw bones, and exogenous estrogen can enhance the osteogenic potential of mesenchymal stem cells. Dental mesenchymal stem cells (DMSCs) represent a group of stem cells isolated from different parts of the tooth, including dental pulps, apical papillae and periodontal ligaments. A number of studies have proved that estrogen plays an important role in the proliferation, differentiation and tissue regeneration of human DMSCs. Thus, this review will focus on the effects of estrogen on proliferation, apoptosis, and differentiation of dental stem cells, discuss evidence from studies in rodents that estrogen plays an important role in dental morphogenesis as well as periodontal remodeling, and suggest directions for future studies in estrogen-related tooth regeneration.

Key words: Estrogen, Osteogenic differentiation, Proliferation, Tooth regeneration, Dental mesenchymal stem cells

Introduction

Estrogen, a kind of fat-soluble hormone, is mostly produced by ovaries in female and by testicles in male. Endogenous estrogen includes estradiol, estrone and estriol, among which estradiol has the highest activity. Estrogen level is usually in a state of fluctuation throughout life. Adipose cells are the main storage unit of estrogen both in male and female. When the control center in the thalamus sends the related instructions, estrogen will be secreted into the blood, most of which is combined with albumin and sex hormone binding globulin (SHBG), and the rest will remain free, reaching the target cells to exert its unique biological actions via the diffusion effect (Kato, 2001). Various studies have demonstrated that estrogen is associated with the development, regeneration and remodeling of hard tissues, including teeth (Bernick and Ershoff, 1963; Moriya et al., 1998; Hong et al., 2006; Reginster and Burlet, 2006; Xu et al., 2014).

Teeth consist of multiple tissues, including enamel, dentin, cementum, pulp and periodontal tissues, while tooth loss is a common disease especially in old patients. Periodontitis is the leading cause of tooth loss in adults by destroying the alveolar bone and soft tissues around teeth. Osteoporosis may also lead to tooth loss (Taguchi et al., 1995). A relationship between osteoporosis and periodontitis might be predictable (Weyant et al., 1999). Moreover, dental trauma and caries are putative factors that can bring about pulp injuries and tooth loss. The mechanism and technology of tissue engineering have been introduced into the study on repairing or regenerating the injured pulps and tooth structures, in which stem cells, signal molecules and scaffold

materials are thought to be three indispensable factors for this process (Langer and Vacanti, 1993).

For the regeneration of the whole tooth or tooth components, stem cells play a paramount role during the morphogenesis of dental tissues (Ringe et al., 2002). To date, several dental mesenchymal stem cells (DMSCs) have been isolated from different parts of tooth structures, including dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP), and periodontal ligament stem cells (PDLSCs) (Gronthos et al., 2000; Miura et al., 2003; Seo et al., 2004; Sonoyama et al., 2008). These stem cells present the ability of self-renewal, high proliferation and multi-lineage differentiation potential, which may be potentially useful to regenerate tissues not only for the bone and tooth (Fig. 1), but also for other tissue types (Laino et al., 2006; Graziano et al., 2008; Saito et al., 2015; Zhu and Liang, 2015). Moreover, DMSCs represent an easily accessible alternative to bone marrow MSCs for future usage in the regeneration therapy. Many MSC-related surface markers, such as STRO-1, CD146 and CD105, are expressed in DMSCs. However, specific surface markers associated with the hierarchical commitment to differentiation pathways of DMSCs are not yet identified (Huang et al., 2009; Sedgley and Botero, 2012; Saito et al., 2015). Many research results have indicated that DPSCs play an important role in dentin-pulp tissue regeneration (Yu et al., 2006a, 2007; Yan et al., 2011;

Potdar and Jethmalani, 2015). In addition, SCAPs can generate a typical dentin-pulp-like complex and bone-like tissue (Abe et al., 2008). Transplantation of SCAPs and PDLSCs *in vivo* promotes the formation of dentin, cementum and periodontal ligament, which represents a practical approach to biological root engineering (Sonoyama et al., 2006; Otsu et al., 2014).

Various growth factors, hormones and biological signals can improve the regenerative capacity of DMSCs. Estrogen is the key regulator of bone metabolism in both men and women. Both osteoporosis and periodontal diseases are related to estrogen deficiency which causes impaired osteogenic differentiation of PDLSCs (Zhang et al., 2011). It has been revealed that there is a close relationship between estrogen therapy and osteoporosis that occurs in postmenopausal women (Tella and Gallagher, 2014). Estrogen provides several functions to regulate cell growth, cell survival, osteo/odontogenic differentiation and inhibits apoptosis and osteoclast activity via estrogen receptor (ER) in mesenchymal stem cells (MSCs) (Zhou et al., 2001). In addition to promoting the committed differentiation of stem cells, estrogen can also regulate the functions of stem cells by promoting the secretion of growth factors and inhibiting the release of inflammatory factors (Jilka et al., 1992; Kodama et al., 2004; Ryan et al., 2005).

To date, the relationship between estrogen and tooth regeneration has not been fully elucidated. The current

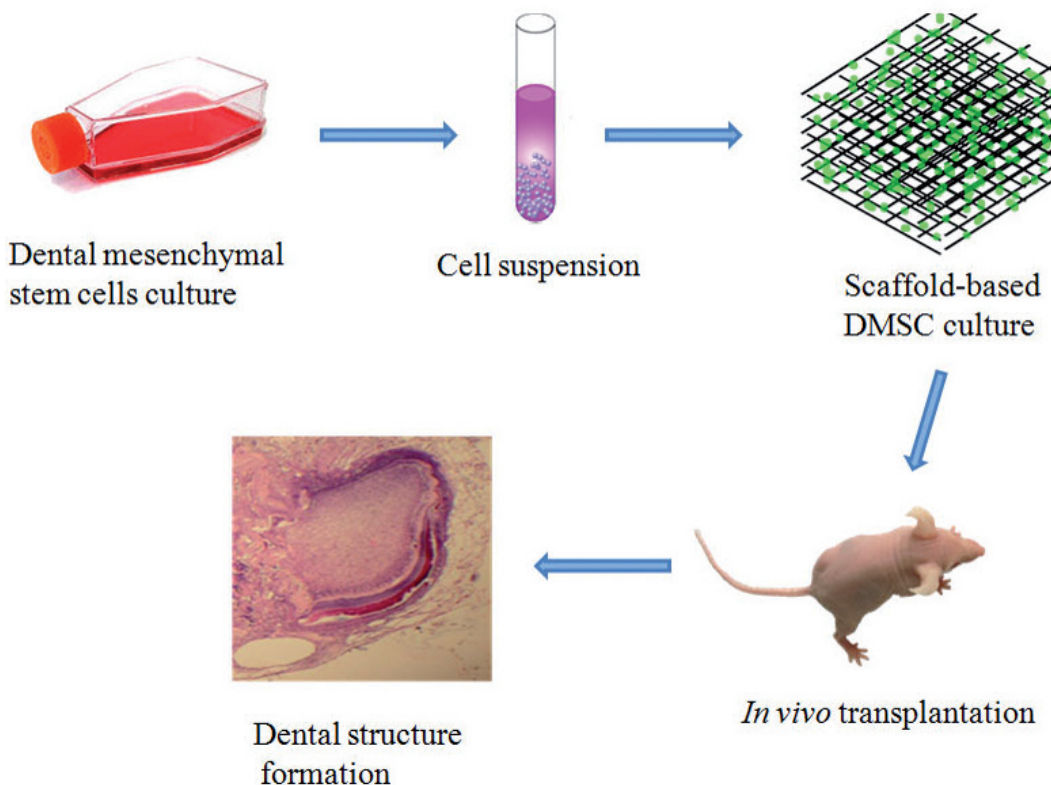


Fig. 1. DMSCs have the capability to form dental structures. After digestion and resuspension, dental mesenchymal stem cells (DMSCs) are seeded and recombined with scaffolds. Then, DMSCs/scaffold recombinants are transplanted *in vivo* to form tooth structures.

review will discuss the influence of estrogen on the proliferation, committed differentiation and morphogenesis of dental mesenchymal stem cells, in order to provide useful cues for their future applications in dental tissue regeneration.

Classification of estrogen

There are two main kinds of estrogen according to its origin, i.e., endogenous estrogen and phytoestrogen. Endogenous estrogen, a kind of steroid hormone from animals with extensive biological activities, is mainly secreted by the ovaries and a little by testicles and adrenal glands. Serum estrogen is mainly derived from the adrenal cortex at a very low level before puberty. Endogenous estrogen is mainly composed of estradiol, estrone and estrinol, among which estradiol has the strongest activity and estrinol the weakest. Estrogen level is gradually decreased with age and a series of symptoms may appear both in male and female, i.e., menopause symptoms, arteriosclerosis, cerebral vascular obstruction and osteoporosis. This natural estrogen, usually extracted from the urine of pregnant mares, is mainly used for clinical HRT (Hormone Replacement therapy, HRT) to relieve the clinical symptoms. Unfortunately, it also results in some side-effects (such as endometrium hemorrhage and increased risk of breast cancer) (Laforest and Taurelle, 1994; Al-Azzawi and Wahab, 2012).

Phytoestrogens are plant-derived xenoestrogens (also called "dietary estrogens") that consist of heterocyclic phenols of estrogenic activity formed by enzymatic metabolic conversions in the gut after consumption of phytoestrogenic plants. They are a group of nonsteroidal plant compounds (e.g., isoflavones, lignans and coumestans), and are particularly abundant in soybeans and flaxseed (Dalais et al., 1998; Murkies et al., 1998). These compounds are structurally similar to estradiol and have the capacity to bring about estrogenic or antiestrogenic effects by sitting in or blocking relevant receptor sites. Many studies have demonstrated that high intake of phytoestrogens can reduce the risk of menopausal symptoms, cardiovascular disease, breast cancer and osteoporosis with few undesirable side-effects (Dittfeld et al., 2015; Kyro et al., 2015; Luo et al., 2015; Sobenin et al., 2015).

Phytoestrogens might be considered as another alternative to HRT. However, the clinical value of phytoestrogens should be extensively investigated by long-term clinical trials.

General functions of estrogen

Estrogens play important roles in almost all female systems, i.e., the reproductive system, the nervous system, cardiovascular system, skeletal, and so on (Maggi et al., 2004; Mikosha et al., 2015; Menazza and Murphy, 2016). In the physiological state, ovarian functions begin to gradually decline at age of 35 along

with some fluctuations of hormone levels. However, the estrogen level is dramatically decreased in the menopause and then menstruation and reproductive functions will vanish.

Recently, the effects of estrogens on male have attracted more and more attention. Two thirds of estrogens in the male are from the conversion of androgens by aromatases and others from the testis (Hess et al., 2001). Several studies have proved that estrogen is a main regulator of the gonadal-pituitary feedback for the gonadotropin axis, and the deficiency of aromatase or estrogen can result in a significant reduction in round and elongated spermatids and abnormalities of the reproductive tracts in male rodents (Robertson et al., 1999; Mauras et al., 2000; McKinnell et al., 2001; Carreau et al., 2007).

In addition to their effects on male reproduction, estrogens are also necessary for the maintenance of bone mass in males (Pentikainen et al., 2000). Because estrogen receptors and aromatase exist in male osteoblasts, male bone is considered to be an important target tissue of estrogens. It has been demonstrated that the deficiency of aromatase or mutation of estrogen receptor genes can lead to osteoporosis in males (Vanderschueren et al., 1997; Vidal et al., 2000). A recent study further reveals that estrogens can enhance mnemonic retention without improving organization abilities in male mice (Al Abed et al., 2016).

Effects of estrogen on the proliferation and apoptosis of dental mesenchymal stem cells

Cell proliferation is often used as an important indicator during cell development and reproduction. During cell development, proliferation means the enrichment or increase in cytoplasm, organelles, and hereditary substances. However, during cell reproduction, it means the growth of cell populations, in which one mother cell gives birth to two daughter cells. In general, cell proliferation is affected by many factors, i.e., growth factors, extracellular matrix and other stimuli (e.g., estrogen) (Nurse, 2000; Strom et al., 2004).

It seems contradictory to the influence of estrogen on the growth features of mesenchymal stem cells. 10^{-8} M 17β -estradiol (E2) can significantly reduce growth rates in cultured UMR106 cells (a clonal osteoblastic cell line), whereas E2 had no effect on the growth kinetics of S90E (a human fibroblastic cell line) (Gray et al., 1987). Likewise, E2 can decrease the cell proliferation of hFOB/ER9 in a dose-dependent manner and cause a significant decrease at a concentration as low as 10^{-11} M (Robinson et al., 1997). In contrast, 10^{-7} M E2-treated MSCs display a significant increase in proliferation rate and a decrease in apoptosis (Zhou et al., 2001). Yu et al. have proved that the higher concentration of E2 (10^{-5} M) can significantly inhibit the proliferation of SCAPs and DPSCs, while 10^{-7} - 10^{-9} M E2 had no influence on cell growth, indicating that there may exist a dose-dependent manner of E2 in regulating

the growth of SCAPs (Wang et al., 2013a; Li et al., 2014). *In vivo* research has demonstrated that DPSCs respectively from OVX and Sham-operated rat incisors show no significant difference in cell proliferation (Wang et al., 2013b).

However, the effects of E2 on cell proliferation in these cells may be affected not only by the concentration of E2 but also by the levels of ER. The latter can activate some ER-mediated signaling pathways (e.g., Wnt/ β -catenin, mTOR and PI3K/Akt/STAT3) required for the expression of proliferation-related genes (Yin et al., 2015; Zhang et al., 2015; Zhu et al., 2016). Zhang et al. have revealed that PDLSCs from ovariectomized (OVX) rats (estrogen deficiency animal model) present a significant increase in cell growth in comparison with Sham group (Zhang et al., 2011). When periodontal ligament (PDL) cells are transfected by the short interfering RNA (siRNA) technique to inhibit ER β expression, an enhanced proliferation is detected in non-transfected hPDL cells after estradiol stimulation, as compared with transfected cells, implying that estrogens/ERs axis may exert an effective action on PDL cell proliferation (Mamalis et al., 2011).

On the other hand, E2-ER α and E2-ER β complexes can also regulate the expression of key genes in the G1 phase of the cell cycle to alter cell proliferation, such as *c-Myc*, *cyclin D1*, *cyclin E*, *Cdc25A*, *p45Skip2*, and *p27Kip1*, which are involved in activation of Cdk2, a crucial step in moving the cell into S phase (Doisneau-Sixou et al., 2003; Strom et al., 2004).

Effects of estrogen on the differentiation of dental mesenchymal stem cells

Cell differentiation is a common process of a cell changing from one type to another with some modifications in cell size, shape, and response to signals, by which adult stem cells can generate fully differentiated daughter cells during tissue repair and regeneration. This process is usually relatively stable and results in a selective gene expression in time and space, in which different genes are switched on or off, eventually producing a specific protein (Luo et al., 2002). Many investigations have confirmed that diverse factors (e.g., hormones, regulating factors, transcription factors and biomechanical factors) can induce DMSCs to differentiate into osteo/odontogenic lineages (Birmingham et al., 2012; Uddin and Qin, 2013).

Estrogen deficiency can reduce the dentinogenic capacity and calcium deposition in rat incisors, and inhibit the odonto/osteogenic differentiation of DPSCs, while 17 β -estradiol (E2) can upregulate the osteo/odontogenic capacity of DPSCs via activating the NF- κ B pathway. In general, osteo/odontogenic differentiation of MSCs is a complicated process characterized by the expression of the main transcription factor *Runx2* and other osteo/odontogenic marker genes, such as alkaline phosphatase (*ALP*), type I collagen (*COL I*), osteocalcin (*OC*) and dentin sialoprotein (*DSP*),

followed by extracellular matrix mineralization (Karsenty, 2001). It has been widely accepted that exogenous estrogens can prevent postmenopausal bone loss by enhancing osteoblastic activity and formation of bone tissues (Lindsay et al., 1976; Talmage et al., 1986). Other studies have revealed the molecular mechanisms by which E2 increases the alkaline phosphatase activity of UMR106 cells and stimulates the sequential osteoblastic differentiation by regulating extracellular matrix expression (Gray et al., 1987; Robinson et al., 1997; Qu et al., 1998).

In physiological conditions, the E2-dependent increase in bone formation requires the proliferation and differentiation of osteoblast precursors. E2 can regulate the osteogenic activity of MSCs in bone marrow (Zhou et al., 2001). Meanwhile, estrogen is thought to be a functional molecule in the osteo/odontogenic differentiation of dental MSCs. Indeed, estrogen has been shown to stimulate the bone formation capacity of cultured periodontal ligament cells (PDLs) by increasing ALP activity, osteocalcin distribution and the formation of mineralized nodules (Morishita et al., 1998, 1999), in which ER α and ER β may play a crucial role in the biological changes in estrogen-treated PDLs (Jonsson et al., 2004; Cao et al., 2007).

Recently, increasing evidence supports the notion that estrogen is closely associated with the committed differentiation of human dental mesenchymal cells. PDLSCs isolated from the OVX rats generate fewer calcium deposits and present lower levels of estrogen receptors (ER α and ER β) than those from Sham group. Moreover, E2 treatment significantly enhances the osteogenic differentiation of PDLSCs *in vitro* (Pan et al., 2011; Zhang et al., 2011), and promotes the odonto/osteogenic differentiation of stem cells from apical papilla via the mitogen-activated protein kinase pathway (Li et al., 2014). The expression of osteogenic proteins and genes (e.g., alkaline phosphatase, runt-related transcription factor 2, osterix, dentin matrix protein 1, dentin sialoprotein, dentin sialophosphoprotein and osteocalcin) are significantly upregulated in E2-treated SCAPs (Li et al., 2014). Recent studies have demonstrated that E2 can also trigger the odonto/osteogenic potency of human dental pulp stem cells (DPSCs) through NF- κ B and c-Src/MAPK signaling pathways (Wang et al., 2013a,b; Woo et al., 2015).

It is commonly believed that estrogens can bind directly to ERs to modulate bone metabolism and the activity of target cells (Robinson et al., 1997). As the classic steroid receptors, ERs including ER α and ER β generally mediate a variety of physiological signals. During E2-mediated odontoblastic differentiation, fulvestrant, as a selective ER antagonist, can significantly downregulate the gene expression of dentin sialophosphoprotein (*DSPP*), dentin sialoprotein (*DSP*) and dentin matrix protein 1 (*DMPI*) by reducing the phosphorylation of c-Src and MAPK signaling pathways (Woo et al., 2015). Human dental pulp cells expressing

ER mRNAs can be stimulated by E2 to express the genes related to odontogenesis and odontoblast differentiation (e.g., *BMP2* and *LEF1*) (Inaba et al., 2013). In addition, both ER α and ER β were involved in the process of osteogenic differentiation of PDLSCs, in which ER β is more effective during the osteogenic process (Tang et al., 2008; Pan et al., 2011). Previous studies have demonstrated that ER α functions as an activator and ER β acts as a repressor in the osteogenic differentiation of MSCs (Maruyama et al., 2001; Lindberg et al., 2003). Estrogen regulation of cell functions is determined by the stages of differentiation and the isoforms or concentrations of ERs (Waters et al., 2001; Heldring et al., 2007). However, there are still many discrepancies in the study of the mechanism of ERs in the formation of tooth/bone tissues.

Together, estrogens/ERs axis can stimulate osteo/odontogenic differentiation in dental stem cells and contribute to their capacity in dental tissue regeneration.

Effects of estrogen on the morphogenesis of dental tissues

Morphogenesis of dental tissues is a continuous process that causes a tooth germ to develop its shape. It is one of three basic aspects of odontogenesis along with the regulation of cell proliferation and differentiation. The process determines the spatial distribution of dental cells (e.g., ameloblasts and odontoblasts) in the tooth germ. Tooth morphogenesis can take place also in a

mature tooth, in cell culture or inside cell pellets (Dassule and McMahon, 1998; Yu et al., 2006a,b, 2008). Morphogenetic responses may be triggered in teeth by hormones (e.g., estrogen), growth factors, transcription factors, receptor proteins, environmental elements or mechanical stresses, which form a signal network to precisely regulate the morphology of dental structures (Zhang et al., 2005).

As we all know, estrogen deficiency has received universal attention because of bone fracture in older women due to the loss of calcium contents in the vertebrae and long bones. In the field of dentistry, a large number of missing teeth and low mineral density in the mandible have been reported to have a positive correlation with systemic osteoporosis by a series of epidemiological studies (Do Lee and White, 2005; Inagaki et al., 2005; Takaishi et al., 2005; Yoshihara et al., 2005). Meanwhile, severe bone loss and structural damage were detected after ovariectomy in the jaw bones and alveolar bones (Ejiri et al., 2008). In addition, clinical research has proved that there is more prevalence of periodontitis and periodontal attachment loss in postmenopausal women (DeBaz et al., 2015; Al Habashneh et al., 2016). Our work has demonstrated that the clinical crown length, compressive strength, radiodensity, and calcium content in ovariectomized rat incisors are significantly decreased in comparison with Sham incisors (Xu et al., 2014). Additionally, the predentin structures in Sham incisors are thicker than those in OVX incisors (Fig. 2), and the odonto/osteoblast specific proteins (e.g., dentin sialoprotein, runt-related

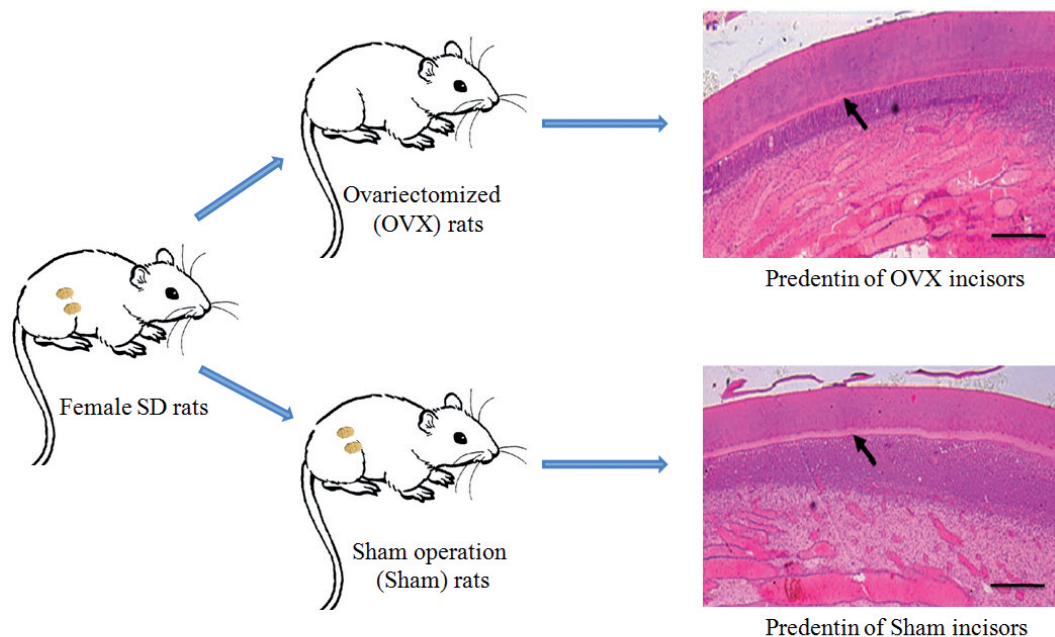


Fig. 2. Estrogen deficiency diminishes the predentin thickness of rat incisors. The predentin structures in Sham incisors are thicker than those in OVX incisors after ovariectomy. Scale bars: 50 μ m.

transcription factor 2, osterix, and osteocalcin) in the dentin–pulp complex of OVX incisors are also significantly down-regulated, suggesting that estrogen can affect the dentinogenesis, protein expression and calcium deposition of tooth structures *in vivo* (Xu et al., 2014; Kim et al., 2015; Yamamoto et al., 2015). Thus, estrogen deficiency can bring about the injuries not only to periodontal tissues (including alveolar and jaw bones) but also to tooth development (including dentinogenesis and mineralization), and ultimately affect the regenerative ability of dental mesenchymal stem cells, as well as dentin–pulp complex. Together, DMSCs-mediated tissue engineering seems to be promising in the not too distant future, during which estrogen may act as an important regulator for the regular and typical morphogenesis of tooth structures (Wang et al., 2013b).

Conclusion/Prospects

In summary, estrogens can regulate the proliferation in a dose-dependent manner and promote the committed differentiation of human dental mesenchymal cells via the ER-mediated signaling pathway. The estrogens/ERs axis can enhance osteogenesis or dentinogenesis of DMSCs both *in vitro* and *in vivo*, indicating that estrogens/ERs may have clinical implications for dental tissue regeneration as well as alveolar bone reconstruction. More extensive studies are required to investigate the potential mechanisms associated with estrogen-mediated osteo/odontogenic differentiation of DMSCs and regeneration of dental tissues.

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