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*Review Article*

# The effects of combination therapy by solid lipid nanoparticle and dental stem cells on different degenerative diseases

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**Abstract:** Stem cells have multiple therapeutic applications, as well as solid lipid nanoparticles. Solid lipid nanoparticle has appeared as a field of nano lipid technology with various potential applications in drug delivery, clinical medicine and research. Besides, the stem cells have a high proliferation rate and could differentiate into a variety of tissues. Stem cells derived from human dental pulp tissue differ from other sources of mesenchymal stem cells due to their embryonic neural crest source and neurotrophic potential. These consist of both dental pulp stem cells from dental pulp tissues of human permanent teeth and stem cells from human exfoliated deciduous teeth. With the emergence of stem cell banks, stem cells are considering for tissue engineering with respect to therapies attitude and regenerative medicine. The present study aimed to evaluate the advantages and disadvantages of the solid lipid nanoparticle and stem cells combination therapy in different therapeutic applications. The solid lipid nanoparticles have anticancer activity against tumors, induce neural differentiation in pluripotent stem cells, and regulate the mesenchymal stem cells. They also have immunomodulatory effects on human mesenchymal stem cells, the gene transfection efficiency, osteogenic differentiation and bone regeneration. But, the crucial health hazards related to stem cell transplantation such as immune rejection reactions and the interaction with other tissues and the effect of solid lipid nanoparticles must not be neglected. Overall, more experiments need to approve the synergism and antagonism effects of the stem cells and solid lipid nanoparticle combination therapy on different degenerative diseases.

**Keywords:** Stem cells, tooth, deciduous, transplantation, nanoparticles, combined modality therapy

## Introduction

Various types of stem cells interrelate together to protect homeostasis or growth, and the interconnections are intricate and broad. There are different human dental mesenchymal stem cells derived from human permanent and deciduous teeth. For example, stem cells from human exfoliated deciduous teeth (SHED) have effects on wound healing. Mesenchymal stem cells (MSCs), multi-potent adult stem cells, are existent in various tissues, such as umbilical cord, bone marrow and fat tissue. MSCs could renovate themselves with proliferation and also could differentiate into various tissues such as bone, cartilage, muscle and fat cells, and connective tissue. MSCs have been utilized for clinical application in tissue engineering and regenerative medicine. Todays, the most common source of MSCs is bone marrow. But, the bone marrow aspirate is a harmful and painful method. So, the discovery and identification of another basis of exploring MSCs are pending. This study aimed to survey SHED with dental pulp stem cells (DPSCs) and bone marrow-derived mesenchymal stem cells (BMMSCs) [1] (**Figure 1**).

Therapeutically researches of stem cells have performed for various states such as neurodegenerative situations like Parkinson's disease and Multiple Sclerosis, liver disease, diabetes, cardiovascular diseases, autoimmune disorders, and musculoskeletal disorders, and for nervous system regeneration after brain or spinal cord damage. Nowadays, the stem cells are used for treating patients who suffer bone breakage, cancer and spinal fusion surgery. Treatments of diseases using stem cells include acute and chronic leukemia, myeloproliferative,

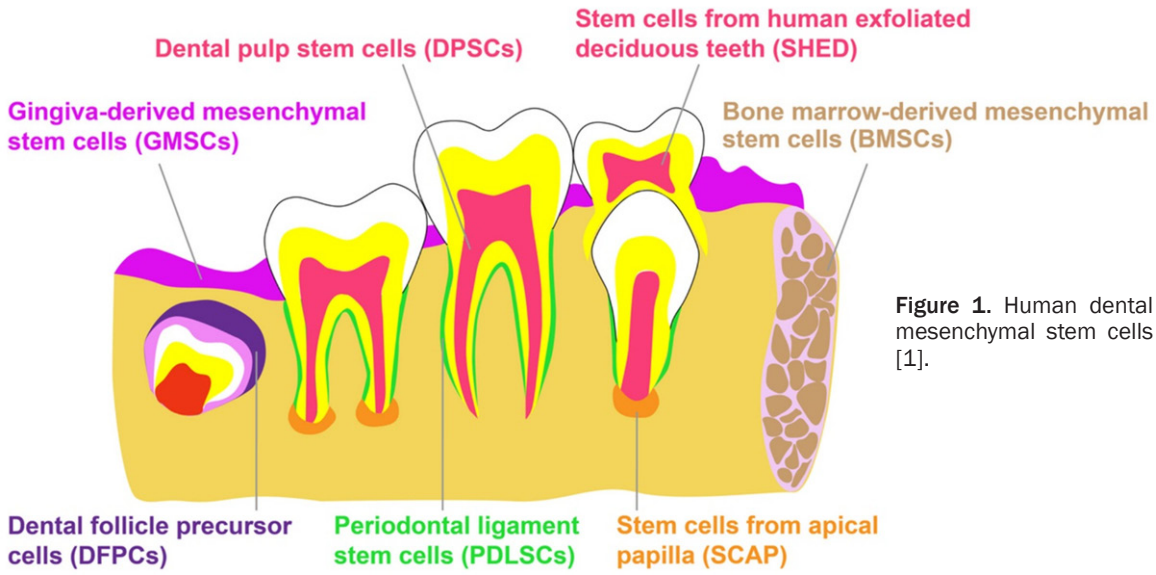


**Figure 1.** Human dental mesenchymal stem cells [1].

myelodysplasia, lymphoproliferative dysfunctions, histiocytic and phagocyte diseases, inherited erythrocyte, platelet, and immune system abnormalities, liposomal storage disorders, and also plasma cell malignancies [2].

On the other hand, solid lipid nanoparticles (SLNs) are nanoparticles consist of biodegradable lipids and act as a drug carrier. Surfactant (emulsifiers), matrix lipid, and loaded drug determine the size of SLNs that is important for penetration into the cells [3-5]. These nanoparticles are constructed to facilitate drug uptake via tissue cells, including tumor cells. Coating these nanoparticles with hydrophilic agents facilitate the uptake of SLNs. After transportation, the long-lasting of the drugs in biological circumstances is a critical problem [6]. SLNs could protect drugs from destruction and also reduce medicine side effects [7, 8]. SLNs could deliver drugs from various routes such as oral and systemic administrations [9]. After targeted SLN degrading, release of the drugs and availability of proper amount of the drugs for therapeutic aims need to be fallowed *in vitro* [5, 10].

In this article, we have reviewed the efficiencies of SLNs on stem cells beside its therapeutic capacities on different degenerative diseases *In vitro* and *In vivo*.

## SLN

SLN applications in medical nanotechnology, the preparation techniques, the advantages, biological applications, drug delivery, and also cellular uptake were discussed as follows.

### *SLN application in medical nanotechnology*

Medical nanotechnology, nanotechnology application in medical science, has wide effects in various side of research including tissue engineering, cell-based biosensors, high-power microarrays, and restorative medicine [11]. It might be supposed that activity in nanometer amount could apply for wide areas of medical research, but, in molecular approach, medicine is still in initial steps [11]. Medical nanotechnology is useful in diagnosis, treatment and prevention of the diseases by engineered nanotechnologies and nanostructures, the technology that perform in a molecular scale. Different medical nanotechnology tools were used to detect disease and distribute drugs, as well as distribute hormones in chronic diseases and defects of the body system. Much more advanced tools, such as nanobots, act as small surgeons *In vivo* [12]. In nano medicines, medicines were used with confined structure of molecules. These molecules can be changed and programmed like smart machines to treat a specific disease and have yield promising results. They can be equipped by the sensors that could create a powerful machine to have resolution and be affected by the environment. These particles can keep side effects and allergic reactions. They are adaptable with the bodies, and they just act specifically when they arrive to their targets, which is called targeted drug delivery [13]. Nano medicines can prevent

large release of the medicine before activation and being toxic. Todays, numerous drug delivery systems are made of nanoparticles such as anticancer substances. They can enhance the effect of treatment and the constancy and the safety of these drugs. The materials used to release cancer drugs include various types of polymers, magnetic, and biomolecules. These materials can also equip with surface modifications including antibodies or ligands for specific diagnosis and treatment [14, 15].

*Various preparation techniques of SLNs*

A solid fat nanoparticle is usually spherical and has a diameter between 10 and 1000 nanometers. Solid fat nanoparticles have a solid fat core matrix that can dissolve lipophilic molecules. The fat core is stabilized by surface activators (emulsifiers). The emulsifier depends on the administration method and is more limited for injectable administration [16-18]. The term lipid is used in a broader sense here and includes triglycerides (e.g. tristarin), diglycerides (e.g. glycerol), monoglycerides (e.g. glycerol monostrate), fatty acids (e.g. acid), steroids (e.g. cholesterol) and waxes (e.g. acetyl palmitate). All classes of emulsifiers (depending on the charge and molecular weight) have been used to stabilize the fat dispersion. It has been shown that a combination of emulsifiers may prevent the accumulation of particles more effectively. SLN is typically spherical and consists of a solid fat nucleus stabilized by a surfactant. The main fats can be fatty acids, acyl glycerol, waxes and mixtures of these surfactants. Biological membrane lipids such as phospholipids, sphingomyelins, bile salts (sodium tauroclate) and sterols (cholesterol) are used as stabilizers. Biological lipids with minimal carrier cytotoxicity and solid fat state allow better drug control due to increased resistance to mass transfer [19].

There are several methods for producing these nanoparticles that are described as follows: There are different formulation methods such as high shear homogenization and ultrasound, emulsion/solvent evaporation or micro-emulsion. Nanoparticle 130-180 nm size range by ultra-sonication with long ultrasound time is reachable. Solvent emulsion is suitable for the scattering of size homogeneity of lipid nanoparticles by heat consumption [20].

*The advantages of SLN over other colloidal drug delivery systems*

SLNs are colloidal particles, consist of lipids being solid at both room and body temperatures that dispersed in an aqueous surfactant solution. SLNs combine benefits of other colloidal drug delivery systems such as emulsions, liposomes and polymeric nanoparticles, and also avoid or minimize some of their problems. Some advantages of SLN are the ability to immobilize hydrophilic or lipophilic drugs in the solid matrix and maintain the drug release, and the ability to prevent the premature degradation of the incorporated drug. Another advantage is physiological components and excipient of accepted status (FDA-approved constituents) in the matrix that decrease the risk of acute and chronic toxicity [21, 22].

*The biological applications of SLNs*

SLN has appeared as a field of nano lipid technology with various potential applications in drug delivery, clinical medicine and research. The unique properties of the size-dependent SLN enable drugs to integrate into nanoparticles and develop a novel drug delivery prototype in therapeutic methods. The SLN ability provides increasing bioavailability for site-specific controlled drugs strategies. SLNs are also usually biodegradable because of their composition and physiologically similar lipids. Common approaches include penetration enhancers, surface modification, pro-drug synthesis, complicated composition, and colloidal fat carrier-based for drug delivery system to intestinal lymph have been utilized. Additionally, polymer nanoparticles, self-emulsifying delivery systems, liposomes, micro-emulsions, micellar solutions, and SLN have been developed as potential carriers of intestinal lymphatic delivery [23].

*SLN drug delivery application in different tissues*

SLNs have recently appeared as a novel approach to oral and intravenous drug delivery systems. SLNs with *in vivo* stability combine the advantages of lipid-lipid emulsion nanoparticles and confront the pharmacological approaches of common nanoparticles such as polymer nanoparticles. SLN has several bene-

fits over than colloidal carriers, such as the mixture of hydrophobic and hydrophilic drugs, nonbio toxicity, prevention of organic solvents, possibility of controlled drug release and targeting, and increased drug stability and no difficulty related to large-scale production [24]. A recent research showed the use of SLN as a substrate for the oral delivery of iron, by combining the hydrophilic molecule of iron sulfate (FeSO4) in a lipid matrix composition of stearic acid. Carodilol-filled SLN was prepared as lipids and surfactants by hot homogenization method for oral delivery with Compritol and Pluxamer 188, respectively [25]. Another example of drug delivery by SLN is the oral solid suspended SLN in distilled water, designed to trap drugs in the SLN composition. After digestion, SLNs are exposed to stomach and intestinal acids that dissolve SLNs and release drugs into the body [26]. Many nanostructure systems have been utilized to deliver ocular drugs. SLNs have been emerged as a strong drug delivery system since the 1990s. SLN does not show bio-toxicity because it is produced from physiological lipids. SLN is especially beneficial in ophthalmic drug delivery because it can enhance corneal absorption of drugs and modify ophthalmic bioavailability of both hydrophilic and hydrophobic drugs. Also, SLN could be sterilized by autoclave, a required stage in ophthalmic formulation [27].

The advantages of SLN include the use of physiological lipids (reduces the risk of acute and chronic toxicity), the avoidance of organic solvents, a broad range of applications (dermal, intravenous), and high-pressure homogenization as a fixed production method. Additionally, improved bioavailability, protection of sensitive drug molecules from the environment and even controlled release properties were reported by the integration of weakly water-soluble drugs in the SLN matrix. Also, SLNs can be hydrophobic and hydrophilic drugs and are more cost-effective than polymer/surfactant-based nanoparticles. Moreover, SLNs act as nucleic acids' carrier naturally and both lipids and nucleic acids have a negative electric charge, so it means that they do not combine easily with each other. However, cationic lipids (positively charged lipids) attach lipids to nucleic acids to transfer them into the cells, but, next laboratory experiments revealed that they had adverse side effects on cell membranes [28].

*The cellular uptake mechanism of SLNs*

Nanoparticles located in the extracellular environment can attach to the plasma membrane, which may lead to the uptake of the nanoparticles through endocytosis process. If the nanoparticles cannot be internalized, the drug release from the nanoparticles and enter to the cells, but it can disperse to the surrounding normal tissues rather than be delivered mostly to the target cells. But, *in vitro* and *in vivo* studies revealed that the intracellular concentration of the drug is much higher when it is released from nanoparticles into the cytoplasm after internalization. The form of endocytosis in nanoparticles uptake can affect the nanoparticle's intracellular localization and trafficking. Understanding endocytic mechanisms is necessary for the development of nanoparticles in clinical therapeutics. Besides, most nanoparticles have been shown to exploit more than one pathway to gain cellular entry. The endocytosis of nanoparticles also depends on the cell type treated. Also, cell type could be crucial in defining the nanoparticle entry and final destination in the cells. Endocytosis is known as a general entry mechanism for different extracellular materials, and can be divided into two main categories: phagocytosis (uptake of large particles) and pinocytosis (uptake of fluids and solutes). Phagocytosis is followed by specialized professional phagocytes, such as macrophages, monocytes, or dendritic cells. The phagocytic pathway of cellular entry consists of recognizing the particles, followed by the adhesion of the opsonized particles onto the cell membrane and ingestion of the particle by the cells [29].

Pharmacologic inhibitors can clarify the specific endocytosis mechanism of nanoparticles internalization. Endocytosis as a usual entry mechanism for different extracellular materials, and it is an energy-dependent uptake. So, due to clathrin role for SLN internalization, incubation under hypertonic environments for disruption of the clathrin coated vesicles' formation on the cell membrane would be done. SLN cellular uptake was evaluated by the caveolae or lipid-rafts pathway, so the cells could be pretreated by the drug filipin that disrupts the cholesterol distribution within the cell membrane. If the cellular entry occurs by macropinocytosis (gliomas) or phagocytosis (macrophages) the cells

might be pretreated with cytochalasin B, a potent inhibitor of macropinocytosis/phagocytosis that depolymerizes the actin filaments inhibiting the formation of the structures essential to enclose particulates. Finally, SLNs could enter the cells and increase the concentration of the drugs [30].

## Stem cell

The isolation and tracking of stem cells and also the comparison between different stem cells are mentioned as follows:

### *The isolation and tracking procedure of stem cells*

Stem cells have to be successfully and carefully extracted and identified. For example, SHEDs were usually obtained from discarded exfoliated deciduous teeth from a child and transplanted with a single infusion by the tail vein *in vivo*. SHEDs were marked by the PKH26 red fluorescent cell linker mini kit for tract distribution. SHEDs have to be verified using flow cytometry [31].

### *The comparison between SHEDs, DPSCs, BMMSCs and cord blood stem cells*

SHED is a novel source of stem cells, recognized as a population of postnatal stem cells. MSC specifications like fibroblastic morphology and the expression of MSC markers exhibit in DPSCs, SHED, and BMMSCs isolated cells. The dividing rate of SHED was significantly more than that of DPSCs and BMMSCs ($P < 0.05$). There are 2.0-fold or more expression rates of 4386 genes by an altered expression among DPSCs and SHED cells that has been revealed by gene expression profiles using DNA microarray analyzer. The genes participated in cell proliferation and extracellular matrix, such as various cytokines like fibroblast growth factor and tumor growth factor beta, had more expression in SHED. It is concluded that SHEDs have superior proliferation ability, plentiful cell supply, and painless stem cell collection with the least invasion. So it could be a favorable choice as a cell source for potent treatment applications [32]. SHED and DPSCs were evaluated for their cell surface antigens and proliferation by measuring the cell cycles, growth rates, Ki67-positive efficiencies, and colony-forming units (CFUs). The multi-differentiation was demonstrated by alizarin red and oil red O and real-time PCR *in vitro*. The mineralization ability of the cells was investigated by implanting with ceramic bovine bone (CBB) into subcutaneous of immune-compromised mice for 8 weeks *in vivo*. A three-dimensional pellet culture system was used to recreate the biological microenvironment like a regenerative milieu in SHED and DPSCs. The mRNA expression levels of inflammatory cytokines, including matrix metalloproteinase-1 (MMP1), tissue inhibitors of metalloproteinase-1 (TIMP1), matrix metalloproteinase-2 (MMP2), tissue inhibitors of metalloproteinase-2 (TIMP2) and interleukin-6 (IL-6) were assessed. So, SHED is a feasible, available and potential other source of regenerative medicine for therapeutic applications [33]. Besides, SHEDs are complementary for cord blood stem cells. SHEDs are capable of renewing solid tissue types while cord blood stem cells could not repair the connective tissues, dental tissues, neuronal tissue and bone. However, cord blood stem cells are invaluable for the refinement of blood cells.

## The effects of stem cells on different degenerative diseases

The effects of stem cells on different degenerative diseases such as kidney injury, liver injury, nerve impairment, systemic lupus erythematous, brain injury, inflammation, hair regeneration, dentistry, bone problems, and diabetes mellitus and also the effect of laser therapy on stem cells are discussed as follows.

### *The applications of stem cells in kidney injury treatment*

Stem cell therapy is useful for the ischemic kidney damage. *In vivo* assay demonstrated the effects of stem cells therapy in comparison with the control group. Also, *in vitro* study showed that stem cells could significantly reduce MCP-1 secretion in tubular epithelial cells (TEC) stimulated with $H_2O_2$. And, stem cells enhance HGF expression and could improve wound in the scratch experiment, that it was down regulated with anti-antibodies. So, stem cells could be utilized for acute kidney injury [34].

### *The effects of stem cells on wound healing*

Also, wound healing could improve with basic fibroblast growth factor (b-FGF) in addition to

human deciduous teeth dental pulp cells (hDPCs), which is better than using one of them. The immune-histologic experiments showed that human type I collagen, surround PKH26-positive cells at day 14 *in vivo* (a nude mouse full-thickness skin defect model) and collagen fibril zones significantly enhanced at days 7 and 14 in wound induced in hDPC/b-FGF groups compared with the normal one [35].

*The therapeutically effects of stem cells on liver impairment*

Liver transplantation is an end treatment for incurable liver involvement. Stem cells are considered as a suitable cell source for liver renewal. The therapeutic effects of stem cells on hepatogenesis were investigated in carbon tetrachloride (CCl4)-induced liver fibrosis model mice. The stem cells transplanted into CCl4-induced liver fibrosis model mice by the spleen homed to recipient livers, and expressed human leukocyte antigen-ABC (HLA-ABC), human hepatocyte specific antigen hepatocyte paraffin 1 and human albumin. Stem cells transplantation significantly improved liver disorders and caused anti-fibrotic and anti-inflammatory influences on the recipient livers. Stem cells-derived HLA-ABC-positive cells that were sorted from the primary recipient liver tissues with CCl4 damage did not mix with the host mouse liver cells. Sorted HLA-positive cells expressed human hepatocyte-specific genes including albumin, cytochrome P450 1A1, fumarylacetoacetase, tyrosine aminotransferase, uridine 5'-diphospho-glucuronosyltransferase, transferrin and transthyretin, and also secreted human albumin, urea and blood urea nitrogen. Besides, stem cells-derived HLA-ABC-positive cells were transplanted into CCl4-treated mice, again. The donor cells homed into secondary recipient livers, and expressed hepatocyte paraffin 1 and human albumin, as well as HLA-ABC. The secondary transplantation recovered a liver impairment in secondary recipients. Thereby, stem cells could promote hepatic disorders and directly transform into hepatocytes without cell fusion in CCl4-treated mice, and are suitable for liver renewal [36]. Stem cells have few oncogenesis, proliferative, multi-potency, and immune-suppressive ability. They have an anti-fibrotic influence on liver fibrosis *in vivo*. Stem cell administration and the bio 3D printer that can provide scaffold-free 3-D images of the liver and diaphragm are a novel treatment for uncontrolled pediatric surgery including biliary atresia and diaphragmatic hernia in regenerative medicine [2].

*The effects of stem cells on nerve impairment*

Stem cells and their medium could have influence on neuron disorders via several mechanisms, such as cell replacement, paracrine effects, angiogenesis, synaptogenesis, immunomodulation, and apoptosis inhibition. Stem cell-Exos is an appropriate regenerative agent in neuron related dysfunctions [37].

Stem cells have a great immunomodulatory and neuroprotective potential. An experiment showed that stem cell transplantation regulates peripheral c-Jun in the trigeminal ganglia (TG) in a rat model of trigeminal neuralgia, and it had analgesic effects. In a study, chronic constriction damage to the infra-orbital nerve (CCI-ION) was induced. The mechanical threshold was evaluated by von Frey filaments, and the mRNA levels of c-Jun in the ipsilateral TG were tested. The phosphorylation of c-Jun in the ipsilateral TG was tracked using immunohistochemistry and Western blotting. PKH26-labelled stem cells were distributed to any parts of TG, lung, liver and spleen. Systemic stem cells transplantation significantly elevated the mechanical thresholds in CCI-ION rats and down-regulated the c-Jun mRNA levels in the TG created by nerve ligation. Stem cell administration obstacle the activation of c-Jun in the TG. So, systemic stem cell transplantation down-regulates the c-Jun in the TG, returns trigeminal neuralgia and removes pain. The phosphorylation of c-Jun leads to the hyperalgesia and allodynia improvement, and stem cell transplantation might improve trigeminal neuralgia [38].

Mechanical allodynia is a painful sensation that happens with innocuous stimuli like light touch. Although inflammatory hyperalgesia plays a maintenance role, allodynia has no biological effect. Stem cells could reduce mechanical allodynia *in vivo* by the siglec-9/MCP-1-mediated tissue-improving mechanism. The effects of stem cells on mechanical allodynia were evaluated in an animal model. Systemic transplantation of stem cells and conditioned medium from stem cells (Stem cell-CM) significantly inverted the mechanical allodynia

caused by spinal nerve transection at day 6. Stem cell or stem cell-CM significantly reduced the activated transcription factor 3-positive neurons and macrophages in the ipsilateral side of the dorsal root ganglion (DRG) at day 20. Stem cell or stem cell-CM also down-regulated the activation of microglia and astrocytes in the ipsilateral side of the dorsal spinal cord. Systemic administration of secreted ectodomain of sialic acid-binding Ig-like lectin-9 and monocyte chemoattractant protein-1 had no influence on the mechanical allodynia, but systemic transplantation stem cell-CM with 30-50 kDa proteins inverted the pain. So, the 30-50 kDa molecular weight proteins secreted by stem cells could maintain and repair DRG neurons injury and also promote mechanical allodynia [39].

Trigeminal neuralgia is an immedicable advanced nervous disease that is remained for several months or years. Stem cells have neuroprotective and immunomodulatory effects to attenuate trigeminal neuralgia. The analgesic effects of stem cells in chronic constriction damage to the infra-orbital nerve (CCI-ION) were investigated in rats. The systemic or local administration of stem cells reduced the sensitivity of rats to mechanical stimuli after nerve damage for 8 weeks. PKH26-labeled stem cells spread to the ipsilateral trigeminal ganglions 24 and 72 hours after local administration. Stem cell administration at the lesion site decreased inflammatory cell infiltration and pro-inflammatory cytokine levels in the damaged nerve, and suppressed CCI-ION- of transient receptor potential vanilloid type 1 expression in the trigeminal nerve and ganglion in the early phase. So, stem cell ED could treat the trigeminal neuralgia and potentially other chronic neuropathic pain [40].

*The effects of stem cells on systemic lupus erythematous*

The MSC properties of SHED in comparison with BMMSCs were done. In vitro stem cell analysis, like flow cytometry, inductive differentiation, telomerase activity, and Western blot analysis to access multipotent differentiation of stem cells and *in vivo* implantation to find tissue regeneration of stem cells were performed. Besides, systemic stem cells transplantations were done to treat systemic lupus erythematosus (SLE)-like MRL/lpr mice. The results show that, stem cells are able to differentiate into osteogenic and odontogenic cells, adipogenic cells, and neural cells. They could express mesenchymal surface molecules such as STRO-1, CD146, SSEA4, CD73, CD105, and CD166, and also activate multiple signaling pathways, like TGFβ, ERK, Akt, Wnt, and PDGF. However, BMMSCs have an immunomodulatory activity that leads to successful treatment of immune diseases. But, the immunomodulatory properties of SHED compared with BMMSCs have significant influences on inhibiting T helper 17 (Th17) cells in vitro. Besides, SHED transplantation is able to effectively reverse SLE-associated disorders in MRL/lpr mice. Cellular characterizations revealed that SHED transplantation increased the ratio of regulatory T cells (Tregs) by Th17 cells. So, SHEDs are an accessible and feasible MSC source for recovering immune disorders like SLE [41].

*The effects of stem cells on brain injury*

Traumatic brain injury (TBI) is a main problem of mortality and disability for all ages all over the world. For this reason, BV-2 microglia cells with stem cells were cultured by a trans-well assay. The secretion levels of neuro-inflammatory agents and nitrite were examined using enzyme-linked immunosorbent assay (ELISA) and Griess test. Then, purified stem cell-Exos were cultured by activated BV-2. ELISA, Griess assay, flow cytometry, immunofluorescence, and qRT-PCR were done for inflammatory agents. At last, stem cells and stem cell-Exos were administrated by local injection into TBI animal model. The motor functional improvement was assessed by Basso, Beattie, and Bresnahan (BBB) scores. The lesion volume and neuro-inflammation were measured by histopathology and immune-fluorescence. Stem cell-Exos could decrease neuro-inflammation with replacing microglia polarization. The transplantation of stem cell-Exos significantly promotes animal motor functional improvement and decreases cortical lesion in comparison with the control group at 14 day. Stem cell-Exos contributed to functional improvement after traumatic brain damage with changing microglia M1/M2 polarization in animal model. So, the stem cell-Exos could decrease the neuro-inflammation. The beneficial effects of odontogenic stem cells and their exosomes on TBI or other neurological diseases must be explored [42].

*The effects of stem cells on inflammation*

Temporomandibular joint osteoarthritis (TMJ-OA) is an inflammatory joint disorder. Exosomes secreted by stem cells (stem cell-Exos) have an anti-inflammatory effect on temporomandibular joint chondrocytes via miR-100-5p/mTOR. They were confirmed using western blot, nanoparticle tracking analysis, and transmission electron microscopy. The anti-inflammatory effects of stem cell-Exos were verified by western blotting and RT-qPCR. The miRNA expression profiles of stem cell-Exos were determined by MicroRNA (miRNA) array analysis. Chondrocytes were treated with a miR-100-5p mimic or rapamycin. The molecular effect of the exosomal miR-100 target mTOR was detected by a luciferase reporter assay. The results revealed that MiR-100-5p was in the stem cell-Exos, abundantly. Stem cell-Exos down-regulated the expression of interleukin-6 (IL-6), IL-8, matrix metalloproteinase 1 (MMP1), MMP3, MMP9, MMP13, and dis-integrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5). Chondrocytes treated with the miR-100 mimic revealed low expression of MMP1, MMP9, MMP13, ADAMTS5, and mTOR. But, the expression of the MMPs and mTOR were increased. Rapamycin treatment enhanced miR-100 and suppressed MMPs and ADAMTS5. So, the luciferase reporter assay showed that miR-100-5p directly targeted the mTOR 3' untranslated region and that stem cell-Exos miR-100-5p down-regulated mTOR expression. Consequently, stem cell-Exos repress inflammation in TMJ chondrocytes and might be useful for TMJ inflammation treatment. Therefore, stem cell-Exos have a therapeutic effect on TMJ inflammation with basic mechanisms [31].

*The effects of stem cells on hair regeneration*

The evidence revealed that mesenchymal-epithelial interactions in early morphogenesis steps of tooth and hair follicles have numerous homologies. Stem cells extracted from 8 to 12 years old children accelerate the hair regeneration cycle and enhance and aggregate the skin cells *in vitro*. Stem cells and dermal cells of C57 mice were subcutaneously administrated to nude mice *in vivo*. So, hair appeared from dermal cells without stem cells. To further explore the molecular mechanism, epidermal and dermal cells were freshly extracted and co-cultured with stem cells. Then many signaling molecules in regenerated hair follicle were indicated. The expression of Sonic Hedgehog (Shh) and Glioma-associated oncogene 1 (Gli1) was increased. So, stem cells might promote the generation of hairs by increasing Shh/Gli1 pathway that is a revolution in tissue engineering and damaged tissue improving [43].

*Medical use of stem cells in dentistry*

The capacities of stem cells were investigated by tissue engineering and regenerative medicine. Some recognized main principles of these investigations are in dental revascularization of the necrotic root canal using disinfection following establishment of bleeding into the canal system with many instruments. The application of intra-canal irritants (NaOCl and chlorhexidine) with the antibiotics (a mixture of ciprofloxacin, metronidazole, and minocycline pulp), for many weeks, is a major stage, to disinfect the root canal and enhance revascularization of the necrotic tissue. So, there are not any immune rejections and pathogen transmission in revascularization because of insider blood cells resulted in this regeneration [33].

For pulp implantation, when the apex is open, post-natal stem cells derivative from skin, buccal mucosa, fat, and bone are transplanted into the dis-infected root canal. The harvesting and transfer of autogenous stem cells, using syringe, is simple, and it is a benefit of this procedure. The other preference is the capacity of these cells to stimulation of pulp retreatment. However, this procedure has some unfavorable results, such as the less survival rate of the cells and the migration of the cells to another sites throughout the body. To improve the chance for success and the usage of the maximal capacity of stem cells in pulp regeneration, all the 3 parameters including cells, growth factors, and scaffold must be investigated [33].

Post-natal stem cell treatment to turn 2 dimensional pellet culture system into 3 dimensional cells, pulp cells could be cultured on biodegradable membrane filters. The major benefit of this transfer process is the easiness of the growth of these cells on filters in the laboratory for evaluating the cytotoxicity of the cells. But the difficulty of the generated pulp sheets implantation is the need for specialized process for appropriate attachment to the root canal walls. As coronal canal filled by scaffolds that able to

provide cellular proliferation, just the apical part of the canal will reach these cellular constructs because the sheets of cells have no any vascularity [44].

A scaffold has to consist of many growth factors such as fibroblast and vascular endothelial growth factors, and Bone Morphogenetic Protein (BMP) that need for proliferation and differentiation of the stem cells. Besides, for scaffold implantation and transfer success, it must consist of nutrients which will amplify cell survival and growth and also antibiotics to avoid any growth of the bacteria in the dental canal. The scaffold substances might be natural or synthetic, biodegradable or permanent. The synthetic substances such as poly-lactic acid, poly-glycolic acid, and poly-caprolactone degrade throughout the body and could be utilized for tissue engineering targets. The limitations of this process are related to achievement of too much porosity and regular pore size problems [45].

By 3-dimensional printing, cells could be located in the way that just the cells that generate tissue will simulate the native tooth pulp tissue construction. The major principle for this method is precise trend of the pulp tissue manufacture when place it into the arranged and formed root canal in ordination of its apical and coronal symmetry [46].

Gene therapy is a novel technique that inoculates a gene encoding a treatment protein into the cells, which could later express the purpose protein [47]. In a study, Rutherford transferred the ferret pulps containing cDNA (complementary DNA) into mouse BMP-7, and no reparative reply was resulted, but proposed that more investigations are required for potential of pulp gene treatment. Despite the successful application of viral delivery systems in many tissues, there were crucial health risks such as mutagenesis, carcinogenesis, and immune system responses to virus and its proteins [48].

In another study, Huang et al. found that stem cells derived from apical papilla and dental pulp could generate cells resemble to odontoblasts that produce dentin like tissue on the present dentinal walls by stem progenitor cell attitude and tissue engineering methods. Also, pulp-like tissue could produce renewal tissue in an emptied root canal space in animal models [49].

*The effects of stem cells on bone problems*

More researches for stem cells have to be performed in rhesus monkeys, because alveolar bone microenvironments are more resemble to humans. More studies are required to provide a balance between the osteogenesis and osteoclast genesis procedures. By the progress in the stem cell biology, dental stem cells will fortunately be capable to repair cleft palate, rescue damaged teeth and jaw bones, refine periodontal disorders, and also regenerate the whole tooth construction [50].

*Therapeutic application of stem cells on type 2 diabetes patients*

MSCs are able to treat diabetes, but the beneficial effects are not clear. In particular, the medical factors that control MSC treatment in this manner are unclear. In a follow-up study, twenty-four type 2 diabetes mellitus (T2DM) patients treated with insulin were isolated for receiving three systemic injections of stem cells from 42 days to 12 months. Glycosylated serum albumin and hemoglobin level reduced significantly after stem cell administration with total effective rate at 86.36% and 68.18%, respectively in the end of therapy and follow-up duration. Three patients desisted from insulin injection after stem cell administration. A steamed bread meal test revealed that the serum levels of postprandial C-peptide at 2 hours were significantly higher than those at the baseline. The research revealed that patients with a high level of blood cholesterol and a low baseline level of C-peptide had low reply to stem cell administration. But some patients have an unstable fever (11.11%), fatigue (4.17%), or rash (1.39%) after stem cell administration. Totally, stem cell administration is an assured and efficient treatment to recover glucose metabolism and islet activity in T2DM patients. Besides, serum lipid levels and baseline islet activity might be major factors related to treatment result of MSC administration in T2DM patients [51].

*The effects of low-level laser therapy on stem cells*

The low-level laser (LLL) releasing fractionated total energy with single or multiple irradiations could induce stem cells renewal. Cell viability with MTT and trypan blue exclusion (TBE), and cell proliferation with crystal violet (CV) and sul-

forhodamine B (SRB) tests were determined at 24, 48, and 72 h after the first irradiation. The use of laser releasing fractionated total energy (2 or 3 times of 2.5 J/cm$^2$) stimulated cell viability at 48 h, but the single irradiation with 2.5 J/cm$^2$ did not induce metabolic function and the proliferation. The 5.0 and 7.5 J/cm$^2$ single doses and the 3 time uses of 2.5 J/cm$^2$ protected cell viability and induced proliferation of stem cells at 72 h [52].

### The beneficial effects of stem cells

It provides storage of cells as a donor for life, before any injury happens. It could be used for any age and its collection and isolation are simple and safe. Not only for the donor, but it can also be used for close relatives of donor including grandparents, parents, uncles and siblings. It is not expensive and its price is less than 1/3 of cord blood storage. Embryonic stem cells and stem cells are considered without ethical concerns.

By fantastic progress in the prevention, detection and therapeutic effects on human diseases, stem cell study provides the hope of receiving main clinical advances. By tissues generated from stem cells, researches are attempting to develop therapeutic applications to rebuilding and replacing injured cells and give hope to patients involving with various illnesses. Nowadays, for the in vitro culture of stem cells, several methods have been introduced which will support unexpected chances to study and finding human embryology. Besides, a set of standard surgery process and safe measurement methods have to be determined to prove the possibility of the medical use of stem cells.

### The therapeutic effects of SLN on tumors, stem cells and systemic prescription and also bone regeneration

*The anticancer activity of drugs coated by SLNs against different tumors*

Perfect cancer therapies with chemical drugs target tumor cells especially and have better tendency to remove tumor stem cell populations. As CD44 would be expressed on more types of cancer stem cells, and it attaches particularly to hyaluronic acid, SLNs coated by hyaluronic acid (HA-SLNs) could deliver paclitaxel (PTX) to CD44 expressed on targeted melanoma cells specially. Shen et al. established a model system made of melanoma stem-like cells for *in vitro* and in mouse xenografts tests. It demonstrated that the cells which express high level of CD44 have a potent cancer stem cell feature than cells with low level of CD44. This feature was determined by sphere and colony establishment, more amount of side population cells, present of cancer stem cells markers such as ALDH, CD133, Oct-4 and also tumor formation, *in vivo*. It concluded that PTX-loaded HA-SLNs deliver PTX sufficiently into the cells and induce apoptosis considerably in CD44 expressed cells, *in vitro*. PTX-loaded HA-SLNs with B16F10-CD44 over expressing attacked the cancer respiratory cells effectively in a lung metastasis model. Consequently, HA-SLNs increases anticancer potential against Melanoma Stem-like Cells shown in **Figure 2**. It could exhibit enough anticancer outcomes with low dose of PTX, and without side effects. It defines the concept of viability that prepares remarkable profits. So, the HA-SLNs targeting system is a promising treatment for cancer [53].

MicroRNA-200c (miR-200c) delivery system with SLNs could increase the effect of PTX on breast cancer stem cell. Since breast cancer stem cells are resistant to chemical therapeutics, it has made treatment difficult. It has been shown that MiR-200c could sensitize breast cancer stem cells to microtubule-targeting chemical therapeutics with reduction of the presentation of class III β-tubulin. Liu et al. combined miR-200c and PTX with SLNs to eliminate breast cancer stem cells. SLNs in this method were made of a cationic lipid named 1, 2-dioleoyl-3-trimethylammonium-propane loaded miR-200c. Nanostructured lipid carriers (NLC) with 20 weight percent oleic acid were provided to deliver PTX. Mammospheres with breast cancer stem cells features were utilized to evaluate the efficiency of this therapeutic model. The cationic SLN could compress anionic miR-200c to create SLN/miR-200c complexes by charge interactions and could maintain miR-200c from destruction with ribonuclease enzymes. SLN/miR-200c complexes could achieve 11.6-fold presentation of miR-200c after incubation for 24 hr, significantly better in comparison with Lipofectamine™ 2000/miR-200c complexes. Twelve hours after intracellular uptake, miR-200c release occurred from

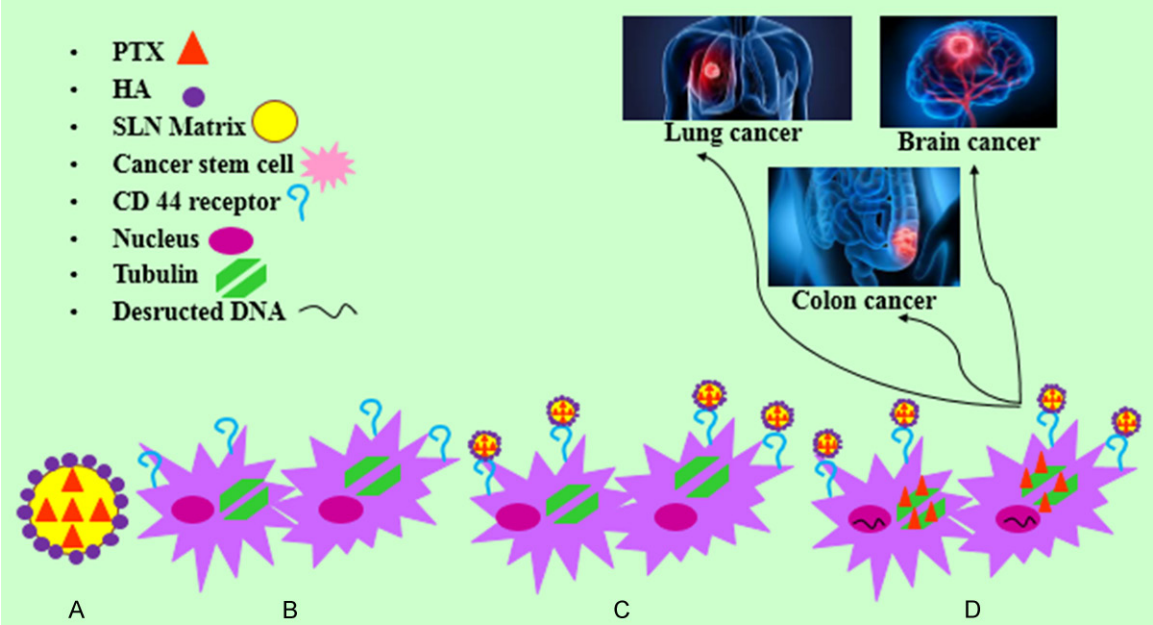


**Figure 2.** Schematic illustration of PTX-loaded HA-SLN effect on cancer stem cell deliver CD44. A: Schematic illustration of PTX-loaded HA-SLNs. B: Cancer stem cell deliver CD44. C: Attachment of PTX-loaded HA-SLNs to CD44 receptor on the surface of cancer stem cells. D: PTX-loaded HA-SLNs deliver PTX sufficiently into the cells and induce apoptosis in different cancers such as lung, brain and colon cancer.

SLN/miR-200c complexes. Also, after transfection of SLN/miR-200c to breast cancer stem cells, the presentation of class III β-tubulin was declined efficiently and the effect of PTX-loaded NLC against breast cancer stem cells was significantly increased ($P < 0.01$). These findings showed that the cationic SLN could deliver miR-200c as a promising therapeutic for cancer therapy. Besides, the combination treatment of miR-200c and PTX is a new medication for the breast cancer stem cell therapy [54].

*Effects of SLN on integrin affinity and neural differentiation of induced pluripotent stem cells*

Kuo et al. provided an amphiphilic SLNs coated with PPFLMLLKGSTR peptide (Ln5-P4) (Ln5-P4/ASLNs) to load nerve growth factor (NGF) and retinoic acid (RA) to differentiate pluripotent stem cells to neuron cells. Beeswax (BW) and lecithin lave a dominant role in emulsification and define diameter, zeta potential, encapsulation efficiency of NGF and RA and release kinetics of NGF- and RA-loaded Ln5-P4/ASLNs. An enhancing BW weight percentage from 0% to 75% declines the particle size and zeta potential and refines encapsulation efficiency of RA and NGF via over expression of β-tubulin III to 93.72% in cultured cells. Potent tendency of these SLNs to α3β1 integrin expressed on induced pluripotent stem cells makes the SLNs entrance easier. The potential of Ln5-P4/NGF-RA-ASLNs to differentiate stem cells to neuron cells was more than free NGF-ASLNs and RA-ASLNs that has been proved via immunochemistry method. Flow cytometry analysis demonstrated that Ln5-P4 on NGF-RA-ASLNs could promote the uptake of NGF and RA with stem cells and accelerate neuronal differentiation. Ln5-P4/NGF-RA-ASLNs as a colloidal delivery system to create mature neuron cells from stem cells and could be promising for neurodegenerative disease treatment and neural damage in regenerative medicine [55].

Kuo et al. designed a NGF loaded heparinized cationic SLNs (NGF-HCSLNs) that made of heparin-stearic acid conjugate, cacao butter, cholesterol, stearylamine (SA), and esterquat1 (EQ1) with the average diameter of 90-240 nm. The particle size of HCSLNs with EQ1 was smaller than that with SA. The zeta potential and electrophoresis analysis demonstrated that HCSLNs-SA had a positive and HCSLNs-EQ1 had a negative charge potential at pH 7.4. The transmission electron microscope showed the NGF loaded on the surface of HCSLNs. This

study confirmed that NGF-loaded HCSLNs-EQ 1 could differentiate stem cells better than NGF-loaded HCSLNs-SA based on electrophoretic mobility and zeta potential. The immunochemistry method indicated that NGF-loaded HCSLNs could differentiate stem cells to neuron cells. NGF-loaded HCSLNs-EQ1 had better viability than NGF-loaded HCSLNs-SA for stem cells. NGF-loaded HCSLNs-EQ1 could regulate membrane charge of stem cells and might be promising for the differentiation of the nerves [56].

*SLNs functionally regulate mesenchymal stem cells*

An accelerated decrease in self regeneration, survival and activity of isolated stem cells is a main obstacle in cellular development based therapeutic drugs. It is critical to identify a supportive substance to protect the properties of the stem cells. The experiments indicated various functional effects of SLNs on mouse mesenchymal stem cells activity. SLNs are a platform for the stem cell attachment, protection of stemness, and an inducer for stem cell differentiation. Scanning electron microscopy along with expression confirms these evidences. Chabra et al. showed that SLNs are well supportive media for adhesive targets and a molecular model to differentiate the adipocytes. Also, SLNs regulate stem cell properties via harmonizing the structural adjustment along with SLN preparation [57].

*The immunomodulatory effects of SLN on human mesenchymal stem cells*

Payne et al. produced SLNs made of all Trans' retinoic acid (atRA) by the emulsification-ultrasonication method. The immune regulation effects of atRA-SLNs on A549 cells (Epithelial Cell Line Model) was investigated *in vitro* by ELISA. For this reason, human mesenchymal stem cells (hMSCs) were suspended into a methylcellulose, collagen and beta-glycerophosphate hydrogel to observe the immune regulation effects *in vitro*. So, it was demonstrated that SLNs encapsulated atRA significantly and then released it after 72 hr. A549 cells were viable with atRA-SLNs and a decrease in IL-6 and IL-8 levels happened after administration. A549 cells also were viable after adding the hMSC/hydrogel formulation, although IL-6 and IL-8 levels have been increased. It is concluded that atRA SLNs and hMSCs had pro-inflammatory effects, and they could regulate inflammatory diseases via several mechanisms that lead to various results. However, more research is needed to explore the mechanism of them in inflammatory diseases such as COPD [58].

*The gene transfection efficiency of SLNs for iPSCs generation*

Gene transfer mostly is based on viruses, but it has some disadvantages. In nuclear reprogramming, 4 transcription factors consist of Oct4, Sox2, Klf4, and c-Myc (OSKM) have been transduced into somatic cells for iPSCs generation. In a study, Alkan et al. used a Cationic Stearamide SLN (CSLN) as a yamanaka factor delivery. So, non-viral vector system is used to transfect the reprogramming factors. CSLN was provided by the solvent diffusion method. The CSLNs transfer the plasmid DNA encoding Oct3/4, Sox2, Klf4, and GFP to fibroblast cell lines. High cell viability of CSLN was achieved at 36.5 ± 0.06 mV zeta potential and 173.6 ± 13.91 nm size for the gene delivery to the fibroblast cells. The transfection efficiency was evaluated based on GFP expression that was 70% ± 0.11. Also, the expressions of the Oct4, Sox2, and Klf4 were evaluated using RT-qPCR; it showed an increment after the 12th cycle in Klf4 (Ct averages: 13, 41), Sox2 (Ct averages; 12, 4), Oct4 (Ct average; 13, 77) and also the propensity to colon formation was reported. Besides, the overexpression of Oct4 and SSEA-1 with CSLN was investigated by flow cytometry test [59].

*Curcumin encapsulated by PEGylated SLN used for systemic prescription*

Curcumin (CUR) with pharmacological effects such as anti-inflammatory and antioxidant activities could be used for the central nervous system therapy. But it has some limitations, such as lipophilic property. SLNs as a nano vector could carry and represent lipophilic composites, although they have systemic limited use because of the short half-life. The stealth SLNs (pSLNs) could control this limitation. So, Santonocito et al. designed a Curcumin-loaded-pSLNs using the solvent evaporation method. Drug antioxidant effect was investigated with Oxygen Radical Absorbance Capacity method.

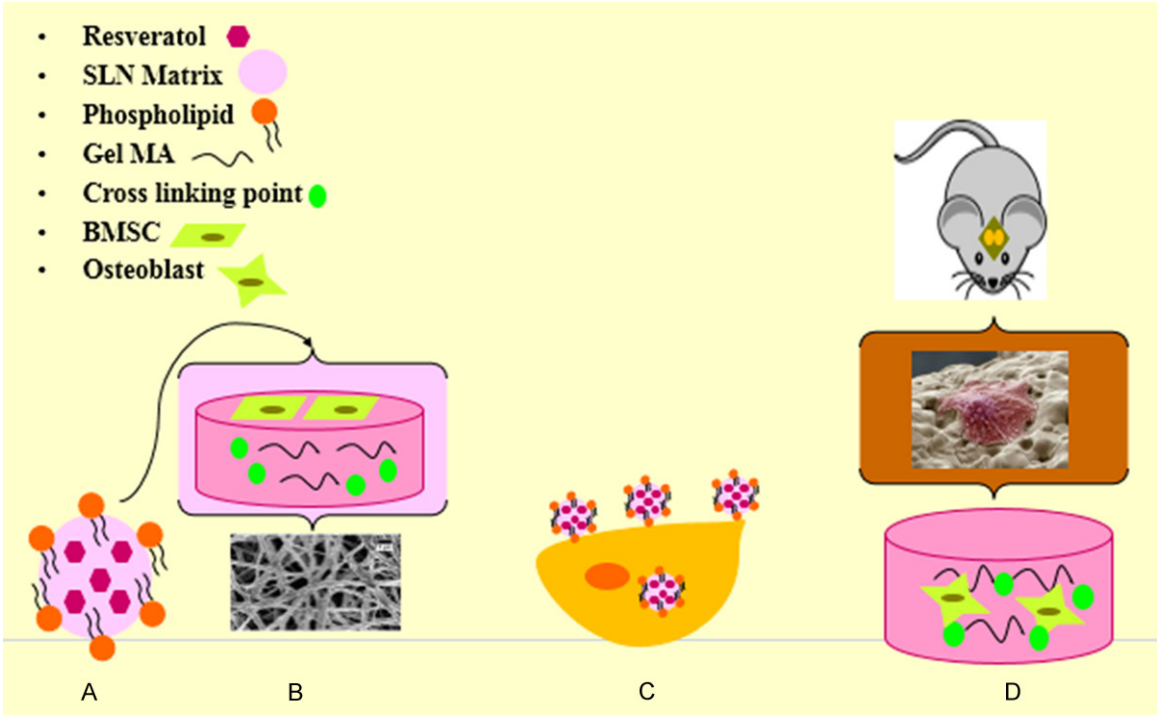


**Figure 3.** Schematic illustration of the SLN deliver Res efficiency on GelMA hydrogel scaffold for bone regeneration. A: Schematic illustration of Res loaded SLN. B: Adding Res/SLN to GelMA hydrogel scaffold. C: BMSCs uptake the Res/SLN. D: The inserted Res induce differentiation of BMSCs cultured on the scaffold to create osteoblasts and develop osteogenesis that contributes to bone renewal in bone defect animal model.

Optimized Curcumin-loaded-pSLNs had < 200 nm particle size substantiated with TEM images, a zeta potential value about -30 mV, and well long-term viability. Differential Scanning Calorimetry method substantiated the PEG micelles encapsulated with SLN. Altogether, this study proposed that this prepared compound can be considered as a promising vector for the systemic prescription [60].

*The effect of SLN on osteogenic differentiation and bone renewal*

Traumatical bone damages, tumor removal, and hereditary deformity cannot automatically resolve. Bone tissue engineering is an encouraging plan to develop *in vitro* substitutions for bone transplantation and control the limit of bone grafts. Wei et al. designed a gelatin methacrylate (GelMA) hydrogel scaffold that carries SLN encapsulated resveratrol to enhance osteogenic differentiation of BMSCs and bone regeneration. Also, an osteogenic drug that enhances bone differentiation was used in this scaffold. Different concentrations (0.01%, 0.02%, 0.04% and 0.08%) were used in GelMA scaffold. The efficiency of these scaffolds on osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) and bone renewal in rat cranial bone defect model were investigated by different characterization tests. It was demonstrated that these Res-SLNs/GelMA scaffolds could improve the osteogenic differentiation of BMSCs by the stable release of Res *in vitro* and *in vivo*, as shown in **Figure 3**. The optimum concentration of the scaffold was reported to be 0.02 Res-SLNs/GelMA. So, as a favorite release system of Res, it has good biocompatibility, osteoconduction, and osteoinduction. Therefore, it is promising for bone tissue engineering [61].

## Conclusion

This review is a summary of the effects of SLN and stem cell combination therapy on different degenerative diseases, like tumors, DM, and neural diseases. SLNs as a lipid nanoparticles are useful for drug delivery in medicine. Besides, the stem cells have a high proliferation rate and could differentiate into a variety of tissues. Stem cell banks support more usage

aspects for tissue engineering and regenerative medicine. The stem cells have good proliferation property, low invasive procurement, neuronal differentiation and neurotrophic ability, and unremarkable ethical aspects. Dental stem cells are promising novel therapy for pulp implantation, postnatal stem cell therapy, three-dimensional printing, and gene therapy. Many common therapeutic applications of stem cells were observed for neurodegenerative defects, but they concentrate on neuroprotection rather than neuroregeneration. SLN based stem cells therapies emerge as a potential therapy option for neurodegenerative disorders because they home, engraft, differentiate and generate factors for CNS improvement. SLNs have anticancer activity against tumors, induce neural differentiation in pluripotent stem cells, and regulate the mesenchymal stem cells. They also have immunomodulatory effects on human mesenchymal stem cells, the gene transfection efficiency, osteogenic differentiation and bone regeneration. They are an alternative source for regenerative medicine, disease modeling, and drug screening with their unique properties. Despite these potentials, there are still some limitations of treatment, including the effectiveness of permanent preservation of stem cells, the expansion of a vigorous GMP-grade construction protocol, regulation of the source of transplantation, and assessment of the efficacy and safety in humans. In addition, the optimization for zeta potential, particle size, size distribution, the morphology and also long-term stability of the SLN formulation performed to achieve high cell stability are hard. Overall, these outcomes contribute to preclinical documents to approve the usage of SLN and stem cells in the treatment. These evidences of the previous studies in the literature were prepared to demonstrate that the combination stem cell therapy with SLN could be compromising for different defects. But the crucial health hazards related to stem cell transplantation such as immune rejection reactions, the interaction with other tissues, and the effect of solid lipid nanoparticles must not be neglected. On the whole, more experiments are required to approve the synergism and antagonism effects of the stem cells and solid lipid nanoparticle combination therapy on different degenerative diseases and also reconnoiter if stem cells derived from one tissue could contact with other tissue cells.

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## Disclosure of conflict of interest

None.

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