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Review Article

Tissue regeneration therapy by Nano composite scaffolds based on PLGA hydrogel embedded with human dental pulp stem cells: a systematic review

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Abstract: Tissue regeneration is the procedure of renewal, restoration and growth of injured tissues and defective organs including nerve, bone, tooth, cartilage and blood vessels. Repair process of damaged tissues needs non-invasive methods; so, the scientists have recently focused on alternative treatment pathways. Nano gels based on Poly Lactic-co-Glycolic Acid have been designed for different purposes in medicine. It is a biodegradable and biocompatible polymer composite. Also, human dental pulp stem cells embedded in the Poly Lactic-co-Glycolic Acid scaffold have proliferation ability and differentiation potential. They can differentiate into different cell lineages, including bone, cartilage, nerve, tooth and other tissues. So, this treatment technology can be used for tissue engineering in regenerative medicine. On the other hand, this structure is a promising application for targeted cancer therapy. Therefore, this review studied tissue, especially tooth regeneration based on the new designed Nano composite scaffolds embedded with Poly Lactic-co-Glycolic Acid hydrogel and dental pulp stem cells.

Keywords: Stem cells, dental pulp, polylactic acid-polyglycolic acid copolymer, regeneration, Nano gels

Nano composite scaffolds and PLGA hydrogel

Tissue regeneration is the procedure of renewal and growth to repair or replace tissues which are injured or suffered from a disease. Poly Lactic-co-Glycolic Acid (PLGA) polymers frequently used as scaffolds for drug delivery systems in tissue regeneration. Also, PLGA is broadly used for bone-tissue-engineering, because of its good biocompatibility and biodegradability. Tissue engineering needs three common ingredients: (1) reparative cells which could produce a practical matrix; (2) a useful scaffold for transplant as a bed; and (3) biochemical reactive molecules, including cytokines and growth factors which can form the favorable tissues [1].

PLGA is a copolymer of lactic acid and glycolic acid approved by the Food and Drug Administration. It typically synthesizes as a ring-opening polymerization of lactic and glycolic acids. Also, these biomaterials are derived from natural sources and can generate biodegradable and biocompatible composites such as Nano gels for therapeutic applications. Besides, PLGA could be a controlled-release drug delivery system because of its mechanical strength. Moreover, PLGA is a thermoplastic linear polymer, aliphatic polyester. Therefore, it can be modified to create a targeted polymeric nanoparticle for drug delivery. So, PLGA scaffold could be formulated for tissue engineering and intracellular interactions [2].

Cancer treatment pathways include invasive methods such as surgery, radiotherapy, chemotherapy, immunotherapy, and hormone therapy designed to treat and shrink a cancer or stop the progression of a cancer. Stem cell or bone marrow transplant and other alternative treatments are the new methods of cancer treatment. These methods are usually used in leukemia, lymphoma, multiple myeloma, and myelodysplastic syndromes to treat blood cells. They could also be utilized for neuroblastoma, Ewing sarcoma, brain tumors in children, germ cell tumors, and testicular cancer [3].

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Human dental pulp stem cells

Human dental pulp stem cells (hDPSCs) derived from the neural crest have proliferation and differentiation abilities. Proliferation and differentiation of hDPSCs have no adverse effects such as immune responses and cytotoxicity. Also, long term preservation is the other benefit of hDPSCs. These advantages of hDPSCs make them more appropriate for tissue engineering. Thereby, hDPSCs can be used in the treatment of tissue degeneration, especially neural, and bone defects [4, 5].

At the present time, treatment attitudes based on PLGA Nano gels embedded with hDPSCs have been developed regarding the design of a drug delivery system for a differentiation of diseases. In the current study, the novel designed Nano composite PLGA Scaffolds for differentiated hDPSCs in regenerative medicine were studied.

The designed Nano composite scaffolds based on PLGA embedded with hDPSC are explained below.

Tissue regeneration therapy

In a study, Salehi et al designed a novel bio absorbable copolymer scaffold. It contains PLGA combined with Poly N-Isopropyl Acrylamide Block (PNIPAAm-b-PLGA) to grow hDPSCs, *in vitro*. PNIPAAm is a temperature-responsive polymer fabricated with free-radical polymerization. It may be used in macroscopic gels, microgels, membranes, sensors, and biosensors. The tendency of hydrophilic solutions with PNIPAAm for enhancing viscosity and also pH-sensitivity make it a good candidate for drug delivery systems. The fabrication process of this scaffold was performed by emulsion freeze-drying and salt leaching methods. hDPSCs are grown on this scaffold for fourteen days. SEM revealed the shape of this scaffold; cell viability and contact of hDPSCs and the scaffold. The results demonstrated that, PNIPAAm-PLGA scaffolds are biocompatible and could be used in tissue regeneration [6, 7].

Treatment for cartilage damage

In a study, Ghandforoushan et al designed a new Nano gel scaffold consisting of gelatin (natural compound) and PLGA-PEG (poly ethylene glycol)-PLGA (synthetic compound) combined with transforming growth factor-β1 (TGF-β1) and hDPSC. This scaffold was analyzed with hydrogen-1 nuclear magnetic resonance (1HNMR), and the attenuated total reflectance Fourier transform infrared spectroscopy (FTIR), and also scanning electron microscopies (SEM) which approved the nanoparticle size and shape of the synthesized scaffold. The scaffold with pore size of about 202.05 μm and its morphology have shown interconnection structure and permeability all over the scaffold. Phalloidin/DAPI staining confirmed the extended cytoskeleton of the chondrocytes. The degradation, mechanical properties, density, and porosity were also investigated. In this study, they use Alcian blue staining to define sulfated glycosaminoglycan (sGAG) synthesis. Also, the expression of cartilage-specific genes, type-II collagen, SOX 9, and aggrecan, were measured by real-time RT-PCR due to survey the effect of Nano gel on hDPSCs, on day 21. The results of cell encapsulation showed improved adhesion, viability, and differentiation of the stem cells in the designed scaffold. So, PLGA-PEG-PLGA-TGF-β1 Nano gel would be a promising feature of chondrocyte damage recovery [8].

Intra-articular treatment

In another study, Ghandforoushan et al designed a PLGA-collagen/PLGA-PEG-PLGA Nano gel scaffold combined with TGF-β1. Several physicochemical factors were evaluated with 1HNMR, FTIR, SEM, Brunauer-Emmett-Teller (BET), and Dynamic light scattering (DLS) techniques. In this study, the degradation profiles, porosity, density, swelling ratios, mechanical features, shapes, and cytotoxicity of the synthesized scaffold have been measured. SEM images have been shown with proper cell adhesion and appropriate distribution of hDPSCs all over the scaffold. Besides, the MTT assay has revealed the cell viability. The expression of Sox-9, collagen type II, and aggrecan genes were approved by real-time RT-PCR assay. So, hDPSCs could differentiate into chondroblasts. Based on the results, h-DPSCs had high adhesion, proliferation, and good differentiation on PLGA-collagen/PLGA-PEG-PLGA-TGF-β1 Nano gel scaffold. Therefore, this designed Nano gel scaffold is an appropriate candidate

for intra and extra articular reconstruction [9] (**Figure 1**).

Bone regeneration and osteogenesis

Cementum is a calcified tissue covering the surface of the root of the tooth. Also, the cementum protein 1 (CEMP1) is a protein coding gene associated with diseases including Type C Thymoma and Cryptosporidiosis [10].

In a study, Colorado et al designed a recombinant cementum protein 1 (rCEMP-1) in combination with hDPSC and PLGA/hydroxyapatite (PLGA/HA) scaffold in order to investigate the healing reaction of major damage in an animal model. The proliferation and differentiation of hDPSCs grown on a monolayer PLGA/HA scaffold were investigated by qPCR. Also, bone formation was measured by histology, histomorphometry, SEM and radiographic assessment. The data showed that PLGA/HA scaffold combined with hrCEMP-1 have been promoted the proliferation and differentiation of hDPSCs via increasing mRNA expression of alkaline phosphatase (ALP), *osx*, *runx2*, op, and *col-I* genes. Besides, the rCEMP-1/hDPSCs/PLGA/HA scaffold showed connective tissue with large central radiolucency, and few peripheral densities, after 10 weeks. So, it wa concluded that, rCEMP-1/PLGA/HA scaffold induces hDPSCs, *in vitro*, but it does not make a large effect on bone size, *in vivo* [11].

The mouth area, maxilla and mandible jaws are appropriate environment for tissue engineering, because of their high regenerating ability [12]. In a study, Sohrabi et al designed a PLGA-silk fibroin (SF) Nano fibrous scaffold combined with inorganic polyphosphate (polyP) by Electro spinning [13]. Electro spinning is a nanometer ultrafine fiber production method that first produces a polymer or solution via a spinneret with high potential, then firm it to become a filament [14]. The data showed better viability of hDPSCs because of the growth on the PLGA/SF/polyP Nano fibers in comparison with PLGA/SF. Besides, hDPSCs growth on the PLGA/SF/polyP Nano fibers show strong osteogenic differentiation ability rather than PLGA/SF Nano fiber and normal culture. So, the PLGA/SF/polyP Nano fibrous scaffold combined with hDPSCs had functional ability for bone regeneration [13, 15].

Adipose-derived stem cells (ADSCs) are mesenchymal stem cells (MSCs) which are isolated from abundant adipose tissue with minimal invasive process, in contrast with bone marrow-derived MSCs. They can adhere to plastic culture flask and expand, *in vitro*. Also, they have an ability to differentiate into various cell lineages. So, ADSCs could be used for injured organs in tissues engineering. Also, because of differentiation of ADSCs into osteogenic cells, they could be used for bone regeneration [16].

In a study, Park et al assessed the bone regeneration abilities of ADSCs by genetic engineering. They cultured and transduced ADSCs with recombinant adenovirus expressing bone morphogenetic protein-2 (rAd/BMP-2). They induced two bone injuries on the parietal bones of twenty-four rats. The injuries were left alone in twelve rats, and recovered with a scaffold in another twelve rats that were implanted by ADSCs in osteogenic media, or implanted by rAd/BMP-2-transduced ADSCs (n = 12). They performed histological examinations, bone histomorphometry, and microcomputed tomography (micro-CT) imaging at four and eight weeks after implanting. Bone regeneration was detected in the rAd/BMP-2-transduced ADSC group, in comparison with others. Micro-CT revealed significant difference in bone volume to tissue volume ratio between rAd/BMP-2-transduced ADSCs groups and the other group ($P<0.05$). The data indicated that transmission of BMP-2 could induce the osteogenic differentiation of ADSCs and also increased bone regeneration. Overall, genetic engineering of ADSCs by BMP-2 is functional in regenerative medicine [17].

Tooth regeneration

In a study, Zhang et al cultured hDPSCs on a PLGA scaffold in a Rotary Cell Culture System (RCCS) to simulate microgravity (SMG) (**Figure 2**). Then, they evaluated the influence on the reproduction, adherence, migration, and cytoskeletal structure of the hDPSC. They performed MTT cell proliferation assay, Bromodeoxyuridine (BrdU) incorporation assay, and flow cytometry, and also western blot tests to recognize the proliferation rate of hDPSCs in simulated microgravity condition. Besides, immunofluorescence assay, SEM analysis, and cell migration, and adherence assay were per-

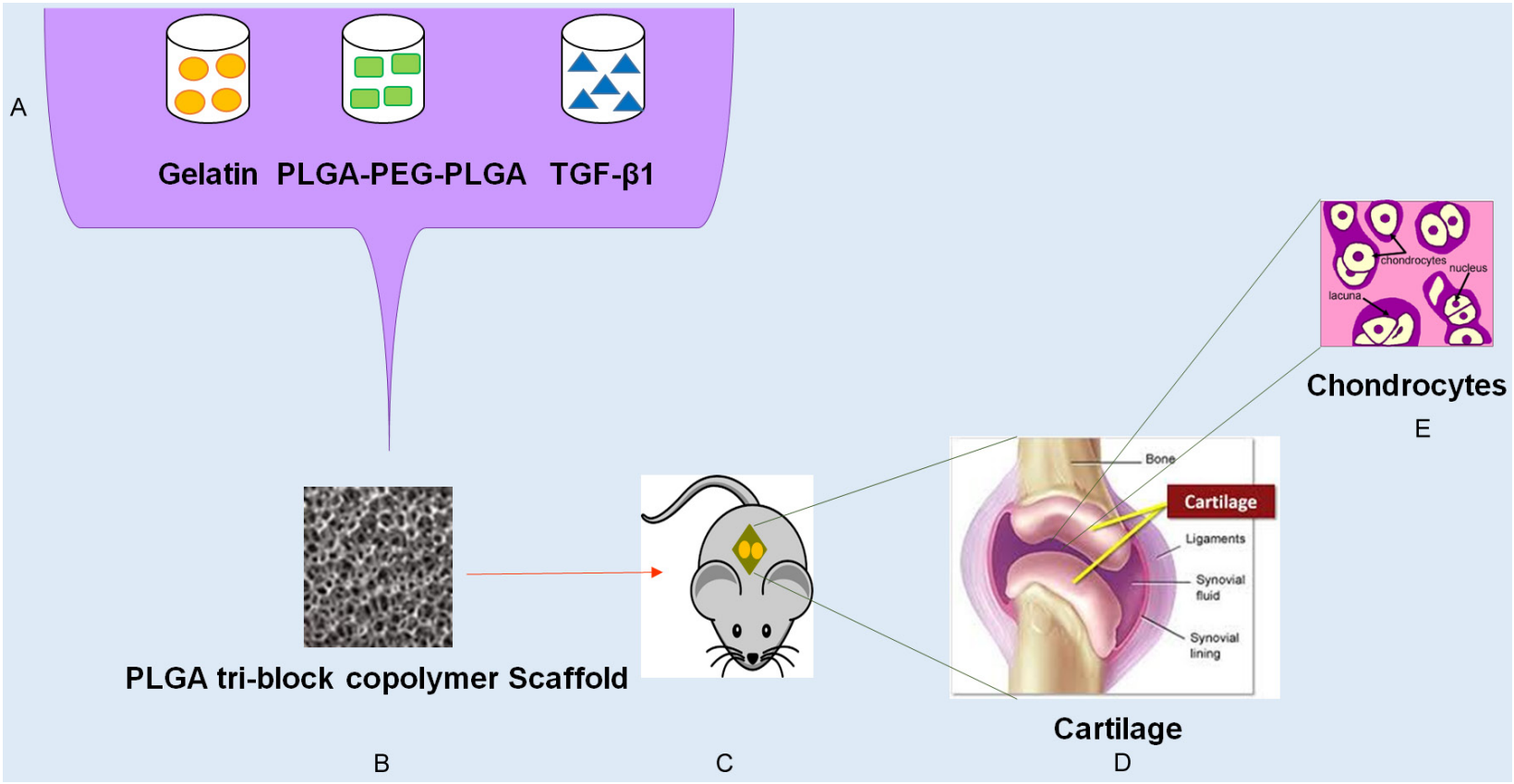

Figure 1. Schematic illustration of PLGA-PEG-PLGA-TGF-β1 hydrogel scaffolds for cartilage regeneration. A: Synthesis of PLGA-PEG-PLGA-TGF-β1 hydrogel nanocomposite. B: Schematic illustration of PLGA-PEG-PLGA-TGF-β1 hydrogel Scaffold. C: Uptake of the PLGA-PEG-PLGA-TGF-β1 in an animal model. D, E: The inserted PLGA-PEG-PLGA-TGF-β1 differentiated hDPSCs into chondrocytes due to cartilage regeneration in cartilage defect animal models.

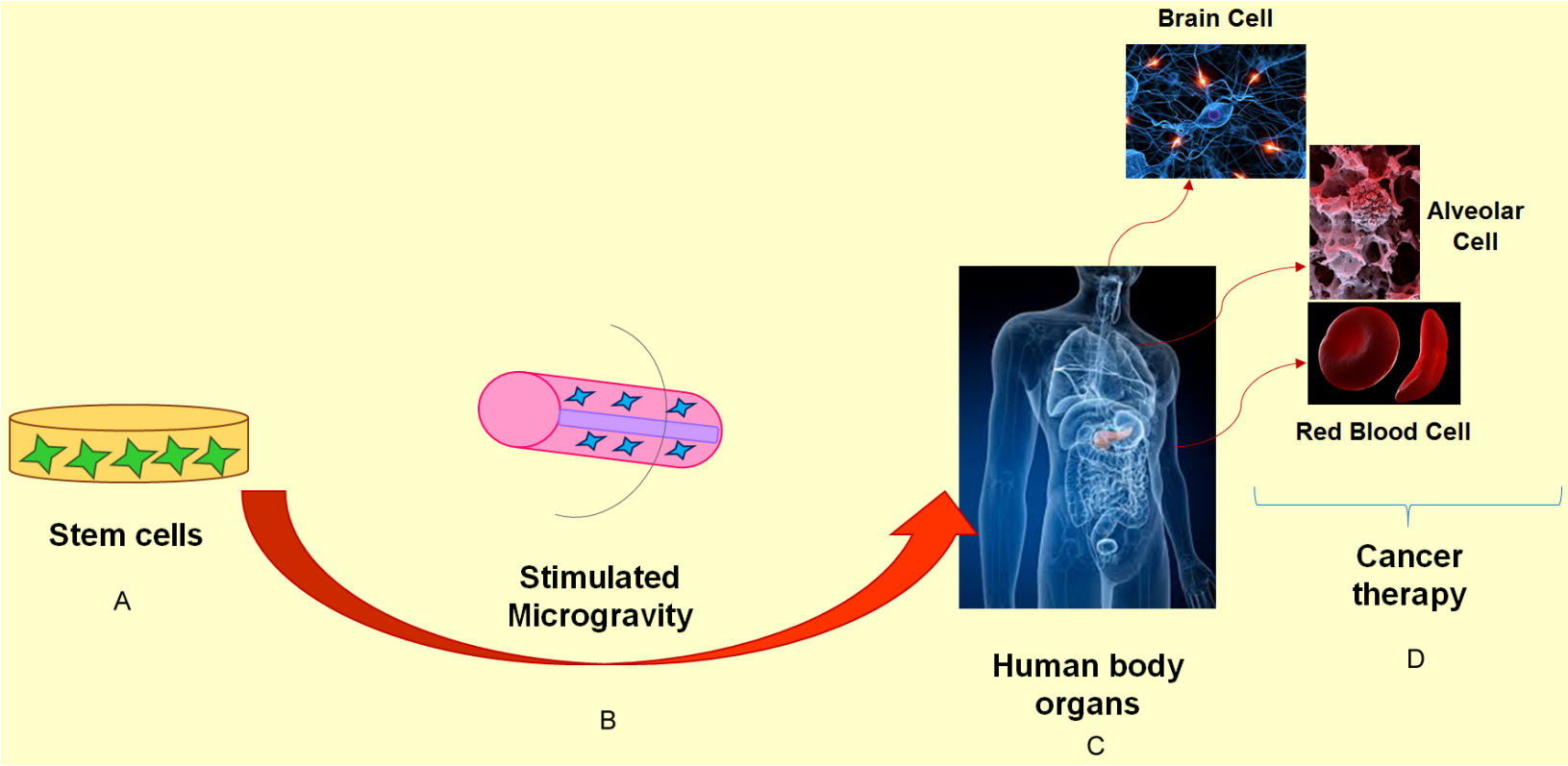

Figure 2. Schematic illustration of the effects of microgravity on differentiation and cell growth of the stem cells and cancer stem cells. A: Stem cells cultured in vitro. B: Stimulated Microgravity system used to investigate the influence of this system. C: Different cancer cells derived from human organs. D: Brain, Alveolar, and Red Blood Cells.

formed in comparison with normal gravity (NG) condition. This study showed that, SMG could accelerate the proliferation and adherence of hDPSCs, but it reduces migration and organization of the cytoskeletal structure of hDPSCs compared with NG condition. SMG could up regulate *integrin alpha-VI, integrin alpha-5, integrin beta-I, laminin beta-I* and *tenascin-C* genes, significantly. Hence, SMG can control the hDPSC growth in PLGA scaffold via integrin-mediated pathway. It might contribute to the enhancement of the expandability and adherence of the scaffold, so, this scaffold can be used in dental tissue engineering [18].

In a study, Zheng et al used four bio compatible types of three-dimensional (3D) scaffolds for dental tissue regeneration. These scaffolds contain a net of PLGA (70/30, mol/mol) and three types of calcium phosphate. So, they combined 50 wt% PLGA and 50 wt% hydroxyapatite, tricalcium phosphate or calcium carbonate hydroxyapatite. These scaffolds were produced by the particulate leaching and phase separation techniques. Surface alterations of these scaffolds were performed with ammonia plasma treatment and collagen anchorage techniques. They investigated the influences of these scaffolds on proliferation of hDPSCs with MTT test, *in vitro* and BrdU labeling, *in vivo*. Besides, hDPSCs differentiation abilities were evaluated with calcification assessment, and ALP activity. The implanted 4-dpn rat tooth bud could produce dentin- and pulp tissues. These outcomes indicated that calcium phosphate can produce tooth tissue successfully. However, the PLGA scaffold combined with tri calcium phosphate is better for the proliferation and differentiation of hDPSCs and also dentin regeneration, rather than other three scaffolds [19].

Scaffold substances make a functional structure that can recover injured tissues by emulating the extracellular matrix (ECM). In a study, it was supposed that ECM of periodontium and dental pulp/dentin compounds could produce the tooth root. For this reason, aligned PLGA/Gelatin electrospun sheet (APES), treated dentin matrix (TDM) and native dental pulp extracellular matrix (DPEM) were synthesized and incorporated into APES/TDM for periodontium and DPEM/TDM for dental pulp regeneration. Then, the physicochemical properties and biocompatibility of APES and DPEM were investigated, *in vitro*. After that, the degradation of APES and revascularization of DPEM were evaluated, *in vivo*. Also, the ability of APES/TDM and DPEM/TDM in odontogenic stimulation was investigated with dental stem cells. Ultimately, they confirmed periodontium and dental pulp/dentin regeneration in the swine jaw. The data indicated that APES attracted aligned fiber direction and conducted cell proliferation by DPEM and also protected the intrinsic fiber structure and ECM proteins. Besides, both APES/TDM and DPEM/TDM could differentiate into dental stem cells, *in vitro*. Implanted stem cells and APES/TDM/DPEM compounds could produce tooth root, 12 weeks after transplantation. In pulp-dentin tissues, a column of odontoblasts layers organized throughout the junction of the new predentin matrix and dental pulp tissue, while revascularization was observed. In periodontium tissue, cellular cementum and periodontal ligament tissues were produced on the TDM surface. Consequently, these findings propose that APES and DPEM have suitable physicochemical properties and good bio compatibility with TDM. So, it can arrange an ECM environment for tooth root regeneration [20].

Li et al evaluated the influence of dynamic 3D SMG culture system on the proliferation and differentiation potential of hDPSCs on PLGA scaffold in nude mice. The hDPSCs cultured on PLGA scaffold were protected individually in the 3D SMG culture and static 3D culture with osteogenic media for seven days, *in vitro*. Consequently, the hDPSCs-PLGA compounds have been seeded on the back of nude mice in subcutaneous injection pathway, for four weeks. The histological and immune histochemical results of Ki-67, type I collagen, dentin sialoprotein and DMP-1 showed the proliferation and differentiation potential of hDPSCs provided in the 3D SMG culture system. The data demonstrated that dynamic 3D SMG culture could recover the proliferation and differentiation potential of hDPSCs, *in vivo* [21].

Besides PLGA, nanofibers have recently achieved major encouragement for application in tissue regeneration. In research, a technique used for determining genes expressed among osteogenic differentiation of ADSCs, MSCs and Pulp cells (PCs) was incorporated into PLGA/Hydroxyapatite nanofibrous scaffold. The con-

sequences revealed that osteogenic differentiation and mineralization of stem cells implanted into the PLGA/HAp nanofiber happened and ALP activity and calcium analysis occurred. Besides, it was discovered that osteogenic differentiation of stem cells could influence the expression of osteogenic genes (osteocalcin, collagen type I). The mRNA and protein expression of these genes was approved by RT-PCR and western blot tests. This study demonstrated a steady differentiation of stem cells into osteogenic cells on PLGA/HAp nanofiber scaffolds. Differentiated ADSCs revealed better bone regeneration over others. Furthermore, PLGA/HAp nanofiber could promote the differentiation of ADSCs into osteogenic cells for bone and tooth regeneration [22].

Spinal cord defect regeneration

Stem cell implanting improves spinal cord damage; although, it indicates undesirable efficiency on improving wide level lesions in an organ. It is supposed that dental follicle cells (DFCs) have the ability and could regenerate spinal cord defects (SCD). In Li et al's study, mesenchymal and neurogenic lineage features of human DFCs (hDFCs) have been recognized. First, they were synthesized and aligned on electrospun materials (AEM) of PCL/PLGA. Then, hDFCs were expanded via the directed fiber and proliferated successfully on AEM. Consequently, hDFCs were implanted in the AEM and restored damage in the rat spinal cord. They performed Substantial examinations, although the outcomes are not statistically significant. The histological investigations revealed that nerve fiber permitted AEM to pass. Also, seeded hDFCs could produce oligodendrogelia linage maker 2, *in vivo,* that were able to induce myelination. So, it is concluded that hDFCs could be an appropriate choice for nerve regeneration. Besides, aligned electrospun fiber can maintain spinal cord organization and promote cell polarization. Therefore, it could be considered for the SCD regeneration [23].

Challenges and future directions

Choosing the most appropriate scaffold for further clinical application is a challenge.

Tooth regeneration via stem cell treatment strategy is an encouraging therapeutic approach for decomposed and damaged teeth. Although hDPSCs have been broadly recognized by the major ability for tooth regeneration, because of slow proliferation, and poor differentiation, *in vitro*, the culture of hDPSCs is still a problem.

Dynamic 3D SMG produced by a rotational cell culture system would be a functional process for different cells activities. RCCS is a device designed to grow 3D cell cultures in microgravity. So, this system is able to grow tissues, cancer cells, and viral cultures out of body. Tissues cultured in the RCCS are bigger and 3D than cells cultured in the laboratory, but the structural and chemical features resemble common tissues. Also, the RCCS reduces any force which may harm the sensitive cell cultures. The RCCS system is a potential procedure to test chemotherapy drugs on a patient's cancer cells out of body [24, 25]. Cellular proliferation, invasive, apoptosis, metastasis, and survival behaviors, and gene expression are the significant changes in cancer cells. The harmful effects of chemotherapeutic drugs might also occur in the stem cells or cancer stem cells. Both culture and SMG affect the biological behavior of the cancer cells. Therefore, the microgravity environment is a novel system that controls the conditions in cancer treatment strategy [26].

Conclusion

PLGA is used for medicine treatment approaches, because of its biodegradability and biocompatibly and mechanical strength. This fabricated copolymer can create an impermanent scaffold for cell adherence and development, *in vitro* and *in vivo*. It is the best polymer for controlled release drug delivery systems. Furthermore, this scaffold conducts tissue regeneration at different levels. At the present time, surgical methods for bone regeneration would be widely recovered with a greater concept, stem cells. This study analyzed different scaffolds based on PLGA. For example: PLGA-PEG-PLGA-TGF-β1 scaffold could differentiate hDPSCs with better adhesion, and viability into the chondrocyte [8]. Besides, the expression of chondrogenic genes revealed in hDPSCs cultured on the PLGA-collagen/PLGA-PEG-PLGA-TGF-β1 scaffold [9]. Although, rCEMP-1/PLGA/HA scaffold could induce hDPSCs, *in*

vitro, it cannot make crucial bone size effect, *in vivo* [11]. On the other hand, SMG can control the hDPSC growth cultured on the PLGA scaffold via integrin-mediated pathway and enhance the expandability and adherence of the scaffold [18]. The dynamic 3D SMG could recover the proliferation and differentiation potential of hDPSCs cultured on the PLGA scaffold, *in vivo* [21]. The PLGA/SF/polyP nanofibrous scaffold had well viability, and great bone differentiation for DPSCs [15, 17]. The PNIPAAm-PLGA scaffold had good compatibility for drug delivery [6, 7]. The Tricalcium phosphate PLGA scaffold is suitable for the proliferation and differentiation of DPSCs in tooth regeneration. Implanted 4-dpn rat tooth bud could produce dentin- and pulp tissues [19]. APES/TDM and DPEM/TDM have well physicochemical properties and biocompatibility with TDM. In pulp-dentin tissues, a column of odontoblasts layers organized throughout the junction of the new predentin matrix and dental pulp tissue, while revascularization might be observed. In periodontium tissue, cellular cementum and periodontal ligament tissues produced on the TDM surface could provide ECM environment for tooth root regeneration [20]. The hDFCs incorporated into the AEM could produce oligodendrogelia linage maker 2, *in vivo*; thus it can induce myelination. So, hDFCs could be used for SCD regeneration while, aligned electrospun fiber could maintain the spinal cord organization and promote cell polarization [23]. The transmission of BMP-2 can encourage the osteogenic differentiation of ADSCs and increase osteogenesis [17]. Ultimately, increased ALP activity, and calcification, and the expression of osteogenic genes of the ADSCs cultured on the PLGA/HAp nanofiber revealed osteogenic differentiation and mineralization in bone and tooth regenerations [22].

Moreover, the efficiency of stem cells in the neuroprotection of induced neurotoxicity in rats, and the protective effects of stem cell in Alzheimer's disease, and T-Cell cancer therapy by stem cells are being investigated. On the other hand, co-culture of mesenchymal stem cell spheres with hematopoietic stem cells under hypoxia to maintain self-regeneration and homing marker expression are being evaluated [27-30].

Although PLGA is a bio compatible scaffold, choosing the most appropriate scaffold for further clinical application is a challenge. H-DPSCs had high adhesion, proliferation, and good differentiation on Nano gel scaffolds. Also, the enhancement of the expandability and adherence of the scaffold are obvious. Although, a Colorado study defined that rCEMP-1/PLGA/HA scaffold could induce hDPSCs, *in vitro*; it could not make crucial effect on bone size, *in vivo* [11]. On the one hand, Zhang's study showed that, SMG could accelerate the proliferation and adherence of hDPSCs, but it reduces migration and organization of the cytoskeletal structure of hDPSCs compared with NG condition [18]. Additionally, some supplementary material such as Tri calcium phosphate are better for the proliferation and differentiation of hDPSCs and dentin regeneration [19].

Therefore, tissue engineering for articular cartilage, osteogenic, dental and neural defects is promising. PLGA is a biodegradable and biocompatible composite used for tissue engineering and intracellular interactions. Also, it is appropriate for drug delivery, because of its mechanical strength. Among different stem cells, hDPSCs are broadly used for regenerative medicine, however, *in vivo* and *in vitro* proliferation potential, differentiation ability, and preservation condition of hDPSCs are contrariwise. The major shortcoming of the reviewed studies is the absence of *in vivo* applications in all of the studies, which is expected to happen for future research.

Overall, good regeneration potential of injured bone tissue in the face and jaw by dental stem cells make them an ideal goal for tissue regeneration. Besides, the appearance of PLGA scaffolds with good bio-compatibility and biodegradability make them the best choice for tissue regenerating. Nano gel PLGA copolymer has been provided and designed for this purpose. Tooth and bone regeneration via stem cell treatment strategy is an encouraging treatment pathway for decomposed and damaged teeth. Also, hDPSCs have been broadly recognized as a good avenue for tooth tissue regeneration, although, *in vitro* culture of hDPSCs for tissue regenerating is still a challenge. The proliferation of these cells is slow, and the differentiation is poor, *in vivo*. The PLGA compounds exhibit an ability to differentiate cultured hDPSCs into the articular cartilage, bone, tooth and neural cell/tissue. As respect to cell viability, PLGA scaffold is the best carrier for hDPSCs.

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Disclosure of conflict of interest

None.

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