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Stem Cells, Scaffolds and Signaling Molecules as a Multidisciplinary Approach for Tissue Engineering

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Abstract

Undifferentiated types of cells found in multicellular organisms are called stem cells, having characteristic to differentiate into a range of different types of adult cells. These basic cells have the ability to self-renew them without any limit and are also considered as multipotent. These two main features make them potent for tissue engineering and regenerative medicine. Differentiation of these potent cells depends upon various factors, including physical, chemical and biological cues. The fate of stem cells can be controlled by the deliberate regulation of the interaction between stem cells and their microorganisms. Cell differentiation is known to be greatly influenced by the surrounding environment, such as a composite scaffold system along with naturally occurring matrix containing a synthetic polymer, providing a microenvironment for differentiation. In this review, we present an introduction to the biomaterials used in the gene therapy, immunotherapy, stem cell therapy and tissue engineering. Biomaterials which are under clinical trials have also been discussed in this review. Tissue engineering stem cells, scaffolds, and signaling molecules are three essential elements provides an effective outcome in preclinical animal studies.

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Introduction

Friedenstien was the first scientist who opened door to the era of stem cell therapy when he discovered first stem cell lines from the stroma of bone marrow in 1970. These stem cells were non-hematopoietic, capable of forming different colonies, able to grow and having morphology like fibroblast cells [1]. Stem cells are actually unspecialized cells that transform into specialized cells [2]. Despite several drug availability, there is always vacuum present in controlling diseases. This approach provided alternative ways to cure diseases such as stem cell therapy [3]. Stem cells can treat many ailments of humans and animals. They can differentiate into more than 200 tissue or organ-specific cells under specific conditions [3]. Stem cell research is creating a lot of hope in modern therapeutics, especially in the fields of replacement of lost tissue and organ transplantation. Stem cells exist in two broad categories: embryonic stem cells and adult stem cells. Embryonic stem cells can be derived from blastocyst [5]. They are derived from those embryos, which still does not attach with a uterus. At this stage, the embryo may be at 32 cell stage [4].

On the other hand, adult stem cells are found in different adult tissues (children or adult). In these tissues, these stem cells are used for different purposes such as to regenerate damaged part or to form new cells to take place of dying cells. Stem cells are further divided into many types. One such type is totipotent stem cells. Totipotent stem cells have the ability to form any tissue of the body, including placenta. Only morula cells can be regarded as totipotent cells. The second type is pluripotent stem cells. These cells are descendent of totipotent cells. Their differentiation ability is limited than totipotent cells. They can differentiate into only those cells which are derived from three germ layers, *i.e*., ectoderm, mesoderm and endoderm. They cannot differentiate into placental tissue. They can be derived from blastocyst inside blastula before implantation within five days of fertilization. The third type is multipotent stem cells, which can produce only related family of cells, for example, hematopoietic stem cells can differentiate into blood cells only (red blood cells, white blood cells, platelets), whereas, unipotent is the fourth type of stem cells are able to produce only single type of cells [5]. The aim of this review was to describe the biomaterials used for gene therapy, immunotherapy, stem cell therapy and tissue engineering. Biomaterials which are under clinical trials have also been discussed in this review.

Different stem cells and their therapeutic effects

First successful bone marrow transplantation incident started a great era of stem cell therapy in 1968 [6]. Habitats, differentiation and therapeutics of some stem cells are given below:

Amniotic fluid-derived stem cells (AFSCs)

AFSCs have easy accessibility, the capacity of renewal and differentiate into multiple lineages. AFSCs can differentiate into all three-germ layer. AFSCs can transform into adipose, muscle, bone and neuron cells. AFSCs are considered an interesting source of stem cells in cell-based therapy. They have shorter doubling time, i.e., 36 hours as compared to other sources of stem cells and can easily differentiate into many cell lines under specific conditions. Their increased rate of proliferation and differentiation into a variety of cell lines, which includes osteogenic lineages as well, make them more attractive [7].

Adipose tissue-derived stem cells (ADSCs)

ADSCs are considered the attractive source of stem cells [8]. They are easily accessible for research and other purposes and grow abundantly. They have a high proliferation rate and don't need a long time to preserve in cell banks. They don't contain HLA-DR antigens, due to which there is no chance of transfer of contagious diseases or rejection during auto and allograft transplant of these cells.

Endothelial colony forming cells (ECFCs)

Blood (especially umbilical cord blood) contains progenitor cells, which differentiate into ECFCs. ECFCs are considered the best source of stem cells in tissue engineering and repair of blood vessels. These cells can be used as a treatment due to their regenerative capability. They possess high proliferation rate in a short time [9]. The ability of neovascularization is found in ECFCs but not in MSCs and myeloid cells. ECFCs have the remarkable ability in modulating the behavior of other cells because they can also be used as gene delivery vehicles. ECFCs can be used in the treatment of diabetes mellitus, cancer and hemophilia A. ECFCs can also be used to make new blood vessels in peripheral ischemia, retinal ischemia lung diseases, brain injury trauma, vessel damage and in stroke. In patients with hemophilia A and anemia, ECFC-based gene delivery mechanism is

considered appropriate for the administration of recombinant proteins. ECFCs produced coagulation factor VIII, when transfected with a plasmid containing cDNA can be used in the treatment of hemophilia. Their specific uses are also noticed in ischemic diseases and tissue reconstruction in the previous ten years [10].

Mesenchymal stem cells (MSCs)

MSCs have tissue regenerative features and remarkable role in tissue engineering and cellular therapy. They are present in bone marrow (adult), fetal tissues (bone marrow, liver), placenta, umbilical cord (matrix, blood), dental tissues, peripheral blood, adipose tissues, synovial membranes, deciduous teeth, liver, periosteum and muscles. MSCs can differentiate into skeletal tissue, bone, tendon, adipocytes, osteoblasts, chondrocytes, neurons, myocytes and cardiomyocytes [11]. MSCs disturb natural pathway of infection because they increase the formation of interleukin (IL-10), suppress cytokine production and shift macrophages to the anti-inflammatory site. In a study, animals show resistance against *Mycobacterium tuberculosis* when MSCs are administered to them. In an experiment, MSCs were used in rats affected by myocardial infarction (MI). These MSCs maintains structure (fibrotic tissue decreased) as well as function (left ventricular systolic increased) of heart [12]. Similarly, heart function is improved in chronic heart failure as well as in MI [13]. MSCs can treat not only different diseases of bone but also congenital skeletal diseases [14]. For example, MSCs can treat osteogenesis imperfecta (OI), which is a congenital skeletal disease, caused by a mutation in genes. Similarly, many studies have been conducted in animal models suffered from OI and treated successfully with MSCs. MSCs can also be used in the repair of long bone fractures [15]. For example, studies proved the reparative effect of MSCs in bone fractures [16]. Similar effects are noticed in rats with a femur fracture. MSCs were successfully transplanted and differentiated into the bone tissue [17]. Quarto and coworkers also mentioned MSCs as effective for large bone defects [18]. MSCs are also reported in the treatment of disc degeneration [19]. Yoshikawa and his coworkers also mentioned the use of MSCs in humans suffered from lumbar spinal canal stenosis [20]. In another study, 10 patients suffered from back pain and lumbar disc degeneration, were treated with MSCs. Remarkable progress noticed when pain and disability improved within three months and water level raised in one year. MSC injection improved degeneration of enthesis (connective tissue between tendon or ligament and bone) in rats within 45 days [21]. In racehorses suffered from digital flexor tendinopathy, MSCs intra-lesion injections reduced injury [22]. Autologous MSCs and bone marrow stem cells also treated 10 patients of degenerative disc defects and 15 patients of intertrochanteric hip fractures, respectively [23]. Everything has pros and cons. Similarly, MSCs have some disadvantages too. MSCs support proliferation of cancer cells despite their therapeutic effects [24], especially breast cancer cells (T47D, BT474, ZR-75-1). MSCs support the growth of tumor cells in a study of mice. MSCs also produce drug resistance in cancer cells and increase their metastasis.

Stem cells from apical papilla (SCAP)

SCAP was first discovered by Sonoyama and coworkers from the apical papilla (tissue present at the apex of incompletely developed permanent teeth). SCAP can differentiate into bone, teeth, adipose and neuron under specific conditions [25]. Stem cells from human exfoliated deciduous teeth (SHED) were initially discovered by Miura and coworkers from remnants of the vital pulp of exfoliated deciduous teeth [26]. SHED is having a faster proliferation rate, self-renewal capacity and multilineage differentiation. SHED can differentiate into bone, adipose, teeth and blood vessels. In another experiment, stem cells were used to treat glioblastoma (brain tumor), which was incurable before [27]. Stem cells can treat different injuries of the spinal cord, bone and cartilage [28]. Stem cells are also capable to rebuild cartilage [29]. Stem cells isolated from dental pulp [30], muscle cells, bone marrow, periosteum and adult bone [31] are used in bone tissue engineering. Stem cells obtained from healthy pulp tissue, apical papilla, SHED and SCAP are used in the regeneration of dentin or pulp. Using some transcription factors, induced pluripotent stem cells (iPSCs) can be obtained from adult somatic cells or terminally differentiated cells by the reverse process. But instead of adult somatic cells, other stem cells (MSCs, DPSCs, SCAP, SHED) can also be changed into iPSCs more easily [32]. iPSCs can transform into nearly all cell types and do not initiate rejection by immunity. iPSCs can also be changed into a neural crest-like cells *in vitro* and odontoblasts.

Combined effect of stem cells with factors

Some stem cells can also combine with other stem cells and/or factors to treat diseases. There are many

examples available related to this combination. The craniofacial defect-osseous defect of the jaw was regenerated after treatment with a combination of tissue repair cells (TRC) and guided bone regeneration (GBR), which was further confirmed by radiological and tomographic measures. Combination of ECFCs with decalcified bone, skin sheets or pancreatic islets helps in tissue repair. Combination of ECFCs with pancreatic islets is also used in diabetic rats, which results in a reduced inflammatory response, better glucose level regulation in blood and graft function. Different cell types can be co-implanted with ECFCs and showed remarkable results in tissue or organ function, regeneration and improved new vessel formation. These cell types include adipose tissue-derived stem cells (ADSCs), mesenchymal stem cells (MSCs), smooth muscle cells (SMCs) and myeloid cells. Combination of ASCs and ECFCs with pancreatic islets (pig) or mature adipocytes (human) induced neovascularization at the subcutaneous flank region of mice. These studies confirmed the use of ECFCs in the treatment of type 1 diabetes [10]. Combination of ECFCs with MSC-derived smooth muscle cells treated cranial defects in mouse [33]. ECFCs (alone or combined) loaded spheroids improved bone formation [10]. ECFCs have the ability to make new blood vessels. Their ability was increased when ECFCs were mixed with MSCs or ADSCs. Neural crest-like cells (NCLC) can transform into odontoblasts when co-culture with mouse dental epithelium. Pluripotent stem cells along with epithelium and MSCs, induced the formation of dentin-like and dental pulp like structures in the mouse. Lee and coworkers repaired human knee cartilage by MSCs mixed with hyaluronic acid in 70 patients [34]. Ueda and coworkers treated 14 patients of mandibular defects with a mixture of plasma rich protein (PRP), MSCs and thrombin/calcium chloride [35].

Use of scaffolds in tissue engineering

The scaffold provides a synthetic extracellular matrix (ECM) environment to cells for tissue regeneration and plays an important role in tissue engineering. On the basis of different scaffolds, tissue engineering has been developed for restoration and repair of damaged skeletal systems. Scaffolds were made from several composite materials, but only a few were capable to induce osteogenesis. An ideal scaffold should have good osteoinductive bioactivity, biocompatibility, proper pore size, high porosity and desired hierarchical structure. An ideal scaffold should have a 3D porous structure. Porosity should be 70% and pore size should be from 50-900 µm. Because of orderly structure, scaffolds were fabricated by low-temperature deposition manufacturing (LDM) techniques. Some techniques formed hierarchical porosity after washing with deionized water, fabricating and drying at more than 90^{o}C [36].

Nacre

Nacre is biomineralization product of a living organism, which makes pearls and shells. In this case, nacre is defined as ideal to make scaffold due to its properties such as the genetic relationship with bone, good biocompatibility and osteoinductive bioactivity. Instead, nacre has also been proved to be as osteoinductive *in vivo* [37]. Nacre implant has excellent biodegradable property and can be dissolved and absorbed *in vivo*. When shell nacre and pearl nacre were compared, later one was found better as osteogenetic agent and contains more organic substances. Efficacy of scaffolds can also be increased by adding inorganic materials to them. However, excess pearl powder distorted slurry jet process and interrupted succession of polyester matrix due to which structural quality and mechanical properties of scaffolds were affected. Although pearl is perfect natural compound for osteogenesis, its applicability is limited due to damageable osteoinductive bioactivity, difficulty in molding and low porosity [38].

Poly (lactic-co-glycolic acid) (PLGA) and others

Polylactone-type polymers, PLGA, has also been approved for human clinical use as material for scaffolds by US Food and FDA [39]. PLGA is considered as excellent biomaterial with many good features. The combination of PLGA and pearl can be used to make suitable scaffolds. The ratio of PLGA/pearl 70:30 was better-ordered structure than the ratio of PLGA/pearl 50:50. PLGA/pearl scaffolds are used for the growth of cells and replaced by autologous bones. In a comparison of PLGA/pearl with PLGA/TCP scaffolds, stem cell proliferation and adhesion were higher in PLGA/pearl scaffolds [39]. PLGA can be used with RP techniques over the long run.

Tri-calcium phosphate (TCP)

Another material is TCP that can also be used with RP techniques. Other biomaterials used in bone tissue engineering are natural or recombinant bone morphogenetic proteins, demineralized bone matrix, natural or synthetic hydroxyapatite (HA) and ceramic-based scaffolds. Although ceramic-based

scaffolds are not suitable for cells due to brittleness, poor cell infiltration and low cell-adhesion property [40]. Hydrogels and polyesters can also be used as scaffolds in bone tissue engineering.

Use of different stem cells on scaffolds

Amniotic fluid stem cells (AFSCs)

Human AFSCs also differentiated into osteoblasts when grown on scaffolds. In many papers, AFSCs were discussed that they were grown on scaffolds and used for different purposes in tissue engineering [41].

Adipose-derived stem cells (ADSCs)

Alginate and collagen scaffolds can be used to grow ADSCs. In a study, ADSCs were grown on HA-TCP and transformed into osteoblasts to repair defects in tibia bone in an animal model. Cell-free HA-TCP was used as a control. The result showed that formal group was more beneficial and increased mineral matrix, more strength and suitable environment were seen [42].

Endothelial colony-forming cells (ECFCs)

ECFCs have been grown on scaffolds efficiently as evidenced by some researchers [10].

Mesenchymal stem cells (MSCs)

Use of MSCs on scaffolds has also been reported in works by different scientists. Hydroxyapatite (HA) scaffold is considered as similar to natural bone and suitable for bone tissue engineering. In another study, MSCs were grown on PLGA/pearl scaffolds prepared by LDM technology [39]. Similar studies were also reported by different scientists [43]. Other scaffolds used for MSCs were PCL, type I collagen, silk, PLA/DBP, PLLA-COL1, PLCL/gelatin, PLGA/HA and PCL/nHAp. In another study, MCM coated with hydroxyapatite used to grow MSCs and treated four patients of a thoracic-lumbar spine fracture. The damaged tendon was also repaired by MSCs grown on scaffold [44]. Three patients with anterior maxillary cleft defects were also treated with MSCs grown on biphasic scaffolds. In another study, biomimetic composite nanofibrous scaffolds made of hydroxyapatite/ chitosan (HAp/CTS) were used to grow murine mesenchymal stem cells (mMSCs) for osteogenesis [45]. MSCs also transformed into cardiac progenitor cells grown on collagen scaffolds.

Stem cell from human exfoliated deciduous teeth (SHED)

SHED transformed into a dental pulp tissue when loaded on scaffolds/tooth slices and transplanted into immunocompromised mice [46].

Pluripotent stem cells (PSCs)

PSCs were grown on a 3D cellulosic scaffold and they transform into hepatocyte-like cells. These cells showed close similarity to primary human hepatocytes as compared to conventional 2D systems [47].

Human embryonic stem cells (HESCs)

HESCs and bovine osteoblasts were grown in the porcine bladder submucosa matrix–poly (D, L-lactide-co-glycolide) (BSM-PLGA) composite scaffolds. These scaffolds are degradable and biocompatible [48].

Bone marrow stem cells (BMSCs)

Human BMSCs are also considered the most common source of osteoprogenitors for bone tissue engineering. But to induce osteogenesis by BMSCs, certain points to be considered, such as there should be high calcium content, the presence of HA, appropriate stiffness of scaffold [48] and proper supply of specific hormones and growth factors is necessary.

Conclusions and future prospects

Stem cells derived from all sources hold immense medical promises. Stem cell therapies have virtually unlimited medical and dental applications. This review discusses a number of already established new stem cell approaches and strategies. Further studies are necessary to develop optimized growth and differentiation protocols and reliable safety assays to evaluate the potential of stem cells and their derived specialized cells for the broader application in regenerative medicine and drug development. The use of scaffolds for tissue regeneration has also been discussed. Besides that, the materials used to develop scaffolds in tissue engineering are reviewed. This review is intended to illustrate the important roles of the materials science of scaffolds and engineering in the field of tissue engineering. Despite these recent improvements to the mechanical properties, porosity, and bioactivity of scaffolds, future research is needed to overcome any remaining limitations in the fabricating process. We believe no one material will satisfy all design parameters in all applications, but a wide range of materials will find uses in various tissue engineering applications.

Conflict of interest

None of the authors have any potential conflict of interest.

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References

[1] Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 1970; 3(4):393-403.
[2] Arias AM. Drosophila melanogaster and the development of biology in the 20th century. Methods Mol Biol 2008; 420:1-25.
[3] Avasthi SR, Srivastava, Singh A, Srivastava M. Stem cell: past, present and future--a review article. IJMU 2008; 3(1):22-31.
[4] Bach FH, Albertini RJ, Joo P, Anderson JL, Bortin MM., Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. Lancet 1968; 2(7583):1364-6.
[5] Niwa H, Miyazaki Ji, Smith AJ. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nature Genet 2000; 24(4):372.
[6] Hipp J, Atala A. Sources of stem cells for regenerative medicine. Stem Cell Rev 2008; 4(1):3-11.
[7] Hashemiben, Razavi BS, Esfandiari E, Karbasi S, Mardani M, and Nadali F. Designing of human cartilage tissue, by differentiation of adipose-derived stem cells with BMP-6 in alginate scaffold. JIAS 2010; 8(31):117-27.
[8] Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. Arterioscler Thromb Vasc Biol 2008; 28(9):1584-95.
[9] Tasev, Koolwijk DP, Van Hinsbergh VW. Therapeutic Potential of Human-Derived Endothelial Colony-Forming Cells in Animal Models. Tissue Eng Part B Rev 2016; 22(5):371-382.
[10] Karadas, Yucel OD, Kenar H, Torun Kose G, Hasirci V, Collagen scaffolds with in situ-grown calcium phosphate for osteogenic differentiation of Wharton's jelly and menstrual blood stem cells. J Tissue Eng Regen M 2014; 8(7):534-545.
[11] Hsiao, Tsai CP, Chu P. Transplanta-tion of Wharton's jelly mesenchymal cells to improve cardiac function in myocardial infarction rats. J Biomedical Sci 2016; 5:1.
[12] Segers VF, Lee RT. Biomaterials to enhance stem cell function in the heart. Circ Res 2011;. 109(8):910-22.
[13] Saeed, Ahsan HM, Saleem Z, Iqtedar M, Islam M, Danish Z et al. Mesenchymal stem cells (MSCs) as skeletal therapeutics - an update. J Biomed Sci 2016; 23:41.
[14] Watson L, Elliman SJ, Coleman CM. From isolation to implantation: a concise review of mesenchymal stem cell therapy in bone fracture repair. Stem Cell Res Ther 2014; 5(2):51.
[15] Kumar S. Bone defect repair in mice by mesenchymal stem cells. Methods Mol Biol 2014; 1213:193-207.
[16] Xu L, Zhang W, Lv K, Yu W, Jiang X, Zhang F. Peri-Implant Bone Regeneration Using rhPDGF-BB, BMSCs, and beta-TCP in a Canine Model. Clin Implant Dent Relat Res 2016; 18(2):241-52.
[17] Quarto, Mastrogiacomo RM, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A et al. Repair of large bone defects with the use of autologous bone marrow stromal cells. N Engl J Med 2001; 344(5):385-6.
[18] Bach FC, Willems N, Penning LC, Ito K, Meij BP, Tryfonidou MA. Potential regenerative treatment strategies for intervertebral disc degeneration in dogs. BMC Vet Res 2014; 10:3.
[19] Yoshikawa, Ueda TY, Miyazaki K, Koizumi M, Takakura Y. Disc regeneration therapy using marrow mesenchymal cell transplantation: a report of two case studies. Spine (Phila Pa 1976) 2010; 35(11):E475-80.
[20] Nourissat, Diop GA, Maurel N, Salvat C, Dumont S, A. Pigenet, et al. Mesenchymal stem cell therapy regenerates the native bone-tendon junction after surgical repair in a degenerative rat model. PloS one 2010; 5(8):e12248.
[21] Godwin, Young EN, Dudhia J, Beamish I, and Smith R. Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. Equine Vet J 2012; 44(1):25-32.
[22] Torres J, Gutierres M, Lopes MA, Santos JD, Cabral A, Pinto R, et al. Bone marrow stem cells added to a hydroxyapatite scaffold result in better outcomes after surgical treatment of intertrochanteric hip fractures. Biomed Res Int 2014; 451781.
[23] Liu S, Ginestier C, Ou SJ, Clouthier SG, Patel SH, Monville F, et al. Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. Cancer Res 2011; 71(2):614-24.
[24] Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. Journal of endodontics 2008; 34(2):166-171.
[25] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003; 100(10): 5807-12.
[26] Bagó JR, Pegna GJ, Okolie O, Mohiti-Asli M, Loboa EG. Hingtgen SD. Electrospun nanofibrous scaffolds increase the efficacy of stem cell-mediated therapy of surgically resected glioblastoma. Biomaterials 2016; 90:116-125.
[27] Li L, He ZY, Wei XW, Wei YQ. Recent advances of biomaterials in biotherapy. Regen Biomater 2016; 3(2):99-105.
[28] Magne D, Vinatier C, Julien M, Weiss P, Guicheux J. Mesenchymal stem cell therapy to rebuild cartilage. Trends Mol Med 2005; 11(11):519-26.
[29] Wang S, Mu J, Fan Z, Yu Y, Yan M, Lei G, et al. Insulin-like growth factor 1 can promote the osteogenic differentiation and osteogenesis of stem cells from apical papilla. Stem Cell Res 2012; 8(3):346-56.
[30] Marolt D, Knezevic M, Novakovic GV. Bone tissue engineering with human stem cells. Stem Cell Res Ther 2010; 1(2):10.
[31] Yan X, Qin H, Qu C, Tuan RS, Shi S, Huang GT. iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev 2010; 19(4):469-80.

[32] Goerke SM, Obermeyer J, Plaha J, Stark GB, Finkenzeller G. Endothelial progenitor cells from peripheral blood support bone regeneration by provoking an angiogenic response. Microvasc Res 2015; 98:40-47.
[33] Lee KB, Wang VT, Chan YH, Hui JH. A novel, minimally-invasive technique of cartilage repair in the human knee using arthroscopic microfracture and injections of mesenchymal stem cells and hyaluronic acid—a prospective comparative study on safety and short-term efficacy. Ann Acad Med Singapore 2012; 41(11):511-7.
[34] Ueda M, Yamada Y, Kagami Y, Hibi H. Injectable bone applied for ridge augmentation and dental implant placement: human progress study. Implant Dent 2008; 17(1):82-90.
[35] Wang H, Li Y, Zuo Y, Li J, Ma S, Cheng L. Biocompatibility and osteogenesis of biomimetic nano-hydroxyapatite/polyamide composite scaffolds for bone tissue engineering. Biomaterials 2007; 28(22):3338-3348.
[36] Lamghari M, Almeida MJ, Berland S, Huet H, Laurent A, Milet C, et al. Stimulation of bone marrow cells and bone formation by nacre: in vivo and in vitro studies. Bone 1999; 25(2 Suppl):91S-94S.
[37] Wiria FK, Leong, Chua C, Liu Y. Poly-ε-caprolactone/hydroxyapatite for tissue engineering scaffold fabrication via selective laser sintering. Acta Biomater 2007; 3(1):1-12.
[38] Xu M, Li Y, Suo H, Yan Y, Liu L, Wang Q, et al. Fabricating a pearl/PLGA composite scaffold by the low-temperature deposition manufacturing technique for bone tissue engineering. Biofabrication 2010; 2(2):025002.
[39] Kim J, Jeong SY, Ju YM, Yoo JJ, Smith TL, Khang G, et al. In vitro osteogenic differentiation of human amniotic fluid-derived stem cells on a poly (lactide-co-glycolide)(PLGA)–bladder submucosa matrix (BSM) composite scaffold for bone tissue engineering. Biomed Mater 2013; 8(1):014107.
[40] Sun H, Feng K, Hu J, Soker S, Atala A, Ma PX. Osteogenic differentiation of human amniotic fluid-derived stem cells induced by bone morphogenetic protein-7 and enhanced by nanofibrous scaffolds. Biomaterials 2010; 31(6):1133-1139.
[41] Hashemiben B, Dehghani L, Sadeghi F, Esfandiari E, Gorbani M, Akhavan A, et al. Bone repair with differentiated osteoblasts from adipose-derived stem cells in hydroxyapatite/tricalcium phosphate in vivo. Int J Prev Med 2016; 7(1):62.
[42] El Backly RM, Zaky SH, Canciani B, Saad MM, Eweida AM, Brun F, et al. Platelet rich plasma enhances osteoconductive properties of a hydroxyapatite-β-tricalcium phosphate scaffold (Skelite™) for late healing of critical size rabbit calvarial defects. J Cranio Maxill Surg 2014; 42(5):e70-e79.
[43] Pacini S, Spinabella S, Trombi L, Fazzi R, Galimberti S, Dini F, et al. Suspension of bone marrow–derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. Tissue Eng 2007; 13(12):2949-2955.
[44] Peng H, Yin Z, Liu H, Chen X, Feng B, Yuan H, et al. Electrospun biomimetic scaffold of hydroxyapatite/chitosan supports enhanced osteogenic differentiation of mMSCs. Nanotechnology 2012; 23(48):485102.
[45] Kerkis I, AI. Caplan, Stem cells in dental pulp of deciduous teeth. Tissue Eng Part B Rev 2012; 18(2):129-38.
[46] Tasnim F, Toh YC, Qu Y, Li H, Phan D, Narmada BC, et al. Functionally enhanced human stem cell derived hepatocytes in galactosylated cellulosic sponges for hepatotoxicity testing. Mol Pharm2016; 13(6):1947-1957.
[47] Lee SJ, Lim GJ, Lee JW, Atala A, Yoo JJ. In vitro evaluation of a poly(lactide-co-glycolide)-collagen composite scaffold for bone regeneration. Biomaterials 2006; 27(18):3466-72.
[48] Ranganathan SI, Ferrari M, Decuzzi P. Design maps for scaffold constructs in bone regeneration. Biomed Microdevices 2013; 15(6):1005-13.