REVIEW ARTICLE

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Cell Therapy and Tissue Engineering in Bone Defect Reconstruction; A Review

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Abstract

Background: Extensive research on bone tissue engineering as a novel therapeutic approach to design and fabricate suitable scaffolds is in progress to overcome the limitations of conventional bone repair techniques. In recent years, tissue engineering and remedial medicine have come up with the strategy of designing, fabricating, and optimizing synthetic and natural scaffolds containing cells and growth factors to facilitate the direct and indirect mechanisms of bone tissue repair in the body. Based on many studies, cellular source, cell medium condition, and biological scaffolds are critical factors in bone defect repair in the field of tissue engineering.

Aim: In this review, we focus on the combination of mesenchymal cells derived from the human adipose tissue, stem cell-to-bone differentiation medium, and biocompatible polyvinyl alcohol-graphene oxide scaffolds in bone lesion repair to gain a better understanding of each factor. This would, in turn, help us design and develop optimal therapeutic approaches for bone repair and regeneration.

Conclusion: The combination of mesenchymal cells and biocompatible scaffolds proved promising in the process of bone lesion repair.

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Introduction

Nowadays, advances in medical science have increased life expectancy. However, this increase has led to the new challenge of the high prevalence of age-related diseases thereby reducing the quality of life. Loss of skeletal tissue as a result of trauma or skeletal degenerative diseases (scoliosis, osteoporosis, osteoarthritis, infection, and tumors) has dramatically elevated the mortality rates and socio-economic costs. Along with the increasing quality of life and life expectancy in today's societies, bone lesions and their complications have also grown substantially. Nevertheless, conventional methods of repairing these lesions are not responsive to the medical needs of these patients due to their limitations.

Although various techniques have been proposed in the past few years to treat bone lesions, this is still one of the major challenges in orthopedics (1).

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Conventional bone repair approaches

Bone transplantation is the second most common type of transplant following blood transplantation in the world (2). Despite the long history of bone marrow transplantation as a standard treatment for bone lesions, the application of this treatment has been limited due to several constraints. The autograft technique (the patient's own source) which has been the gold standard method for treating bone lesions also poses challenges such as restricting the area, increasing the risk of injury at the supplier site, the risk of new fractures, the likelihood of transplant failure, and the need for surgery. Secondly, the allograft technique (human source) and the xenograft (animal source) also face the challenge of transplant rejection and pathogen transmission (3-5). The two common treatment approaches for bone lesions are metal implant strategies (titanium and steel alloys) and iron/plum graft techniques, both of which have their specific defects and may be associated with complications including implant movement, inflammation, bone resorption, long-term surgery, transplant deficiency, transplant rejection problems, and transmission of pathogens. The metal implants (joint prosthesis, bolts) are most commonly used for structural and mechanical support of arthroplasty and long bone fractures whose most important limitations are lack of biodegradability, high hardness, fatigue, fracture, failure to integrate into the host tissue, protrusion, and infection. The limitations of these therapeutic approaches along with the societies' growing need for efficient therapies have encouraged researchers to develop new therapeutic strategies to restore or replace damaged skeletal tissues.

Tissue engineering-based therapeutic approaches

In recent years, tissue engineering and remedial medicine have come up with strategies for designing, manufacturing, and optimizing synthetic and natural scaffolds containing cells and growth factors to eliminate the side effects of traditional therapies. Due to the growing need for tissue transplants and the lack of appropriate donors, tissue engineering-based therapeutic strategies are increasingly being considered as a promising approach. The key factors in the success of tissue engineering-based therapeutic strategies include the cellular source and optimal culture medium with biological and biodegradable scaffolds (6, 7).

- **Cellular Source**

The cellular source is one of the challenges in tissue engineering which requires specific optimization. Today, the potential of stem cell-based therapies in the repair and restoration of living tissues has been considered as a novel therapeutic solution and an alternative to traditional methods of treatment. The use of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSC) has many advantages; however, due to the lack of precise regulation of the regulatory cycle, as well as technical and ethical problems in genetic isolation and manipulation, its application is currently limited (8). The mesenchymal stem cells (non-blood cells derived from embryonic mesoderm) are another source of stem cells that have valuable stem cell characteristics such as self-renewal and in vitro replication, differentiation into specialized cells (bone, cartilage, muscle, tendon, etc.), easy accessibility and separability from various autologous and allogeneic sources (bone, cartilage, muscle, tendon, ligament, embryonic structures, etc.) which could be a suitable cell candidate in reconstructive medicine (9-16). Although mesenchymal cells isolated from various sources have many advantages, limitations in the number of available cells and inadequate access to the amount of tissue required for cellular extraction have limited their therapeutic processes. Adipose-derived stem

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cells, however, do not impose these limitations. Adipose tissue and its mesenchymal cells are readily accessible without the risk of patient injury or death and can be used as an autologous cell source in research and clinical phases (8). Based on studies of adipose tissue-derived mesenchymal cells (ADSCs) in humans (17, 18) and mice (19), they have been able to acquire the characteristics of differentiated (morphological and functional) metastatic cells such as in vitro extracellular matrix calcium phosphate, expression of osteoblast-specific proteins (osteocalcin and alkaline phosphatase) and response to mechanical stress (increased expression of alkaline phosphatase, osteocalcin, collagen type Ia1 and COX2) and shear stress of fluids. These results suggest that adipose tissue-derived mesenchymal cells have the potential to differentiate into mechanical stress-sensitive bone cells and are considered as valuable cellular candidates in bone tissue engineering (17, 18, 20, 21). Transcription factors affecting the differentiation of ADSCs into bone marrow cells have not been fully identified, but the effective roles of Menin, Shh, Notch-1, BMP2, RunX2, TBX3, and valproic acid have been reported (22-27). FGF2 has also been confirmed as an inhibitor of the differentiation of ADSCs into bone marrow cells (28). Another prominent feature of these cells is the secretion of growth factors required for the indirect repair of bone lesions such as VEGF, HGF, IGF-1, and TNFα (8, 17). No specific markers have been reported for these cells so far, and their differentiation from fibroblast cells is very challenging for researchers. Generally, mesenchymal cells have no or low level of expression of hematopoietic markers including CD11b, CD14, and CD19, CD34, CD45, CD79a, CD117, HLA-DR, CD73, CD90, and CD105. In addition to the importance of specific differentiation factors of ADSCs to bone cells, synthetic extracellular matrix and three-dimensional cell culture medium are other essentials of the cell differentiation process (5, 7). Note that, in the field of bone tissue engineering, animal cells have been mostly used in research and further studies using human cells are required as well. The application of tissue engineering scaffolds with human cells also needs to be further validated in pre-clinical studies to step into the next phases of clinical research.

- **Culture medium optimization**

Culture conditions and optimum culture medium are other influential parameters in living tissue engineering, which is currently undergoing extensive research to improve the culture conditions of these cells and to provide suitable differentiation environments. Culture media containing dexamethasone, ascorbic acid, beta-glycerophosphate, valproic acid, vitamin D3, and BMP-2 are commonly used to differentiate adipose tissue mesenchymal stem cells (8). The transcription factors, specific genes, and functional proteins that are involved in the process of stem cell differentiation to specific bone cells can be examined to confirm cellular differentiation. The most important molecular properties of an osteoblast-differentiated cell include the ability of mineralization, cell membrane-dependent alkaline phosphatase activity, bone matrix molecules such as type I collagen (COLL I), osteocalcin, bone cysteoprotein, and bone sialoprotein (BSP), and BSP parathyroid-dependent growth factors which are all influenced by the key transcription factor Runx2, regulating the morphology of osteoblasts. In addition to the Runx2 transcription factor, the molecules that are involved in various signaling pathways such as TGF-1, BMP, Wnt, HH, and NELL-1 are also required to complete the differentiation stage of these cells (8, 17, 28).

- **Biological and biodegradable scaffold**

Another requirement in the field of tissue engineering is the design and manufacture of scaffolds. Most scaffolds used for bone tissue

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engineering are made of polymers, bioactive ceramics, composites (hybrids), depending on their application type (solid scaffold or injectable scaffold). Three-dimensional (3D) scaffolds are used in various in vitro and in vivo studies which are usually made of natural polymers such as those in extracellular matrix proteins including collagen, fibrin, elastin, hyaluronic acid. Alternatively, they could be synthetic polymers such as various macroalcohols including polyethylene glycol, polycaprolactone, polyvinyl alcohol, or a combination of two groups. In the field of bone tissue engineering, three-dimensional micro- or nanostructured scaffolds with a pore size between 100 μm and 350 μm and permeability of over 90% have been typically used for various materials and fabrication methods (29).

These scaffolds typically support the attachment of extracellular matrix cells and proteins to the surface and provide a suitable biological environment for the proliferation, differentiation, cell migration, and secretion of extracellular matrix proteins as well as the targeted release of biomolecules, along with active and controlled nutrients at the right time and in the desired space. The toxicity, immunogenicity, biocompatibility, biodegradability, and mechanical properties of 3D scaffolds are important factors. Thanks to the desired mechanical and physicochemical properties of the polyvinyl alcohol hydrogel polymer and its wide application in bone tissue engineering research, this compound has been recently used as the main basis of scaffold construction; in this regard, polyvinyl alcohol-graphene oxide hydrogel nanocomposites can also be used for scaffold fabrication (30). Polyvinyl alcohol (PVA) is a synthetic, non-toxic, non-carcinogenic, water-soluble, biocompatible and bioadhesive hydrogel polymer with favorable physicochemical properties which provides extracellular matrix topography very well. This FDA-approved polymer has been widely used in orthopedic implants and other medical applications such as contact lenses, artificial pancreas, hemodialysis, and so on.

PVA is a linear carbon polymer with a hydroxyl group in the side chain which is well capable of forming fibrous forms and combining with other polymers. This synthetic polymer is formed from partial hydrolysis to complete polyvinyl acetate and removal of acetate groups (31, 32).

The degree of PVA hydroxylation determines its physical, chemical, and mechanical properties. This polymer dissolves in water well, but it is resistant to most organic solvents. The higher the degree of hydroxylation of this polymer, the lower its solubility in water and the more crystalline it becomes. As such, they are crosslinked in the form of cross hydrogels to be used for different purposes. The cross-linking of this polymer with chemical or physical agents enhances its structural strength when absorbing water or biological fluids. The degree of crosslinking of PVA hydrogels determines the rate of water uptake followed by its physical, chemical, diffusion, and biological properties. In addition to the cross-linking degree of PVA hydrogel, its molecular weight and concentration also affect its final properties (32, 32). The PVA polymer is crosslinked with various agents such as Genipin, citric acid, glyoxal, glutaraldehyde, UV, borax vanadate (Na tetraborate, Na2B4O7, etc.). The structural and physical properties of polyvinyl alcohol-borax aqueous composition have been extensively studied. The cross-linking reaction occurs between PVA and borate ion, which is called the di-diol compound in which two PVA hydroxyl ions are combined with one borate ion. The cross-linking degree of PVA with borax can be easily adjusted by altering the concentration and molecular weight of PVA and borax, where a reversible gel is produced with favorable physicochemical properties (33-37). 3D scaffolds made of chitosan, fibrin, and

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tricalcium phosphate, and PVA have been reported as a suitable scaffold for differentiating ADSCs into bone cells (5, 7).

Graphene oxide-polyvinyl alcohol composite along with other natural and synthetic polymers have been widely used in bone tissue engineering. Recent studies have suggested that graphene oxide not only optimizes the physicochemical properties of the composites but also reduces the diameter of nanofibers and creates a suitable surface for cell attachment and proliferation. Further, it stimulates the osteogenesis in mouse bone marrow mesenchymal cells.

Ethylene polytetrafluoroethylene (PTFE) also provides a porous structure and a suitable surface for connecting and regenerating bone cells. Carbon polytetrafluoroethylene or Teflon is a thermoplastic polymer consisting of an ethylene framework with four fluorine groups (two fluorine groups per carbon atom). The presence of fluorine groups and the strong bonding of carbon and fluorine with this polymer is very important. Accordingly, PTFE is a stable polymer that is biologically neutral and chemically inactive. PTFE is a tough, flexible material with moderate tensile strength, heat resistance, and chemical properties. This polymer has the low abrasion resistance and excellent thermal stability up to 300° C, but it decomposes at over 350° C. PTFE is a neutral biomaterial with a low coefficient of friction**.** In 2008, Jeongand colleagues investigated the repair of scaffold hybrid chondrocytic lesions composed of polydioxanone-polyvinyl alcohol by melt-molding particulate-leaching method containing human bone marrow mesenchymal cells. Based on the successful results of the in vitro study, scaffolds containing rabbit bone marrow mesenchymal cells were also studied in vivo (27). The team reported that MSC / PDO / PVA hybrid scaffold created a new cartilage tissue in vitro and improved osteochondral lesion repair in vivo (27).

In 2009, Asran and colleagues prepared an electrophoretic nanofiber scaffold made of polyvinyl alcohol-collagen type I-nano-hydroxyapatite and proposed it for bone tissue engineering due to the physicochemical properties of the scaffolds (38). Qu and colleagues (2010) prepared a bilayer osteochondral scaffold using polyvinyl alcohol-gelatin-nanohydroxyapatite-polyamide 6 to repair cartilage and bone lesions and post-implantation of bone marrow-derived mesenchymal stem cells. They were implanted in an ectopic area in rabbits. This group suggested scaffolds for bone and cartilage tissue engineering (39).

In 2010, Nitzscheand colleagues developed a composite scaffold containing collagen I-chitosan-hydroxyapatite-polyvinyl alcohol in the freeze-drying method and applied it in bone tissue engineering (40). Zhang and colleagues in 2011 developed a polyvinyl alcohol-nano graphene oxide composite by melting-freezing. They observed a 132% increase in tensile strength and 32% in compressive strength of PVA compared to pure PVA hydrogel in response to adding 0.8% wt of graphene oxide. Note that the addition of this amount of GO had no effect on PVA toxicity on osteoblast cells (41).

In 2011, Qi and his colleagues developed a nanofiber composite using the electrospinning method composed of polyvinyl alcohol-graphene oxide. They observed a decrease in the fiber diameter, an increase in tensile strength (less than 1% wt GO), and binding and growth of murine osteoblast cells (MC3T3-E1) on the scaffold without affecting the viability (42). Depan and colleagues reported that the presence of graphene oxide in chitosan nanocomposite structure enhances the proliferation and growth of osteoblasts and improves cell-scaffold and cell-cell interactions. It also improves the mechanical and physicochemical properties of the scaffold (43). The graphene and graphene oxide demonstrated to affect the growth and

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differentiation of mesenchymal cells in terms of the type of bonding interaction (covalent, electrostatic, or hydrogen) with the surface of mesenchymal cells. Also, graphene and graphene oxide has been identified as effective inducers of bone marrow mesenchymal cells to the osteocyte cell line. According to this study, graphene inhibits chondrocyte differentiation pathway due to its specific interaction with insulin, while graphene oxide does not interfere with this process (44).

In 2012, Wang and his colleagues fabricated polyvinyl alcohol-graphene oxide nanofibers by electrospinning. Elsewhere, Chahal and colleagues utilized cellulose nanofibers and polyvinyl alcohol to fabricate suitable bone tissue engineering scaffolds by electrospinning. They found that the spinning conditions of cellulose improved and their mechanical properties increased after the addition of PVA (45,46).

In 2013, Pandele and his colleagues developed a casting method for chitosan-polyvinyl alcohol-graphene oxide nanocomposites (47).

Conclusion:

The combination of mesenchymal cells and biocompatible scaffolds proved promising in the process of bone lesion repair.

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Conflicts of Interest

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