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Review Article

Characteristics of the Main Constituents Used in Bone Tissue Engineering

Oryan A* and Monazzah-Harsini S

Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

***Corresponding Author**: Oryan A, Professor of Comparative Pathology, Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

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Abstract

Background: In most instances the fractured bone can regenerate itself into a normal bone; however, when the bone gap or defect is beyond 2.5 times of the bone radius, the body fails to regenerate such a critical sized bone defect spontaneously. Such a defect should be treated by bone grafting, transplantation, scaffolding and tissue engineering. Due to the disadvantage of autograft, allograft, or xenograft the orthopedic scientists directed to repair the injured bone by natural or synthetic implants and bone tissue engineering.

Results: Tissue engineering needs three essential elements including mesenchymal stem cells, differentiators and/or growth factors and scaffolds. The cellular components can be obtained from an exogenous source or endogenously from the surrounding environment and are the main key elements in regenerating the defected tissue structurally and functionally.

Conclusion: Studies are ongoing in many relevant fields, and it is hoped that bone disorders due to trauma, bone resections in surgery, ageing, and genetic or metabolic bone disorders to be successfully treated with novel bone regeneration methods in near future.

Keywords: *Bone; Tissue Engineering; Regeneration; Scaffold*

Introduction

Bone fracture is breaking or detachment in the structural unity and it is always associated with damaging in the surrounding tissues [1]. The severity of damage depends on the energy of what made the break [2]. In small and uncomplicated fractures the fractured bone can spontaneously regenerate its structure and function into a normal bone by several overlapping stages including inflammatory or exudative, fibroplasia or proliferative and remodeling or maturation phases [3]. Growth factors of different sources which are activated by the injury procedure activate the surrounding pluripotent osteoprogenitor cells [4]. These cells produce bone morphogenetic proteins, which bound to collagen fibers [3]. These proteins with hormones and cytokines result in migration of mesenchymal stem cells and make them to proliferate and differentiate to osteoblasts [5]. Hyperemia, chemotaxis, inflammatory cell infiltration, and secretion of pro-inflammatory and inflammatory mediators, cytokines, metalloproteinase, fibronectin, fibroblast growth factor and angiogenic factor are the main consequences of the inflammatory phase of fracture healing. Proliferation, differentiation and migration of fibroblasts, production of collagen and glycosaminoglycans, angiogenesis, tissue organization, chondrogenesis, endochondral ossification and intramembranous bone formation are the key steps in the proliferative and remodeling phases of fracture healing [6,7].

When a bone is broken, a gap or defect is created which is then filled with necrotic bone, blood (from broken vessels) and inflammatory cells (because of chemotaxis) [8,9]. The healing process then depends on osteoconduction by a material that acts as an acceptable scaffold to the newly formed bone, and the osteoprogenitor cells that allow osteoinduction [10]. A defect beyond 2.5 times the bone radius or critical size defect is a clinical problem and should be treated by bone grafting and transplantation (autograft, allograft, or xenograft), natural or synthetic scaffolds and tissue engineering [11,12]. However, application of autografts is limited due to high percentage of donor and recipient site complications [13]. As the allografts and xenografts undergo sterilisation and purification, they do not provide osteoinductive signals, and do not have living cells [14]. Therefore, the orthopedic surgeons have been directed to bone tissue engineering [15]. This technique takes benefits of the bone's regeneration potential and avoids the problems associated with bone grafting [16].

What is tissue engineering?

Tissue engineering is defined as combining those branches of scientific fields in which the principles of the life knowledge and engineering are applied to regenerate, maintain, restore, or increase in the quality of tissue structure and function [17]. It is based on the understanding of regeneration and tissue formation, and production of new functional tissues [14]. The tissue engineering scientists hope to reach this purpose by combining knowledge from materials science, physics, chemistry, engineering, medicine, and biology [18].

This question is mooted that what is needed by orthopedic surgeons to properly apply tissue engineering for new bone formation? Bone tissue engineering, like any other tissue healing methodologies, needs the three following essential elements including cells, extracellular matrix, and growth factors [19]: Cellular components must be present to give rise to new structural tissue [20]. They can be obtained from an exogenous source or endogenously from the surrounding tissues [21]. Differentiators and growth factors must be present for the suitable development of the cellular elements [22]. They can be provided exogenously, produce by the transferred cells, or derived from endogenous sources [21]. A good scaffold can be instituted to supply a substrate for cellular proliferation, attachment and differentiation [23]. It may serve to prevent mobilization, provide biomechanical support, organize and align tissue hierarchy and orient growth factors or drugs to the responding cells [24]. Bone has a three dimensional configuration, and the cells don't grow in a three dimension fashion *in vitro*, therefore a scaffold as a three dimensional structure, mimicking bone structure, must be used so that the new tissue can be grown in a three dimension manner [25].

Characteristics of a good scaffold for tissue engineering

Any normal tissue consists of cells and matrix. The matrix is a three-dimensional structure for cells within a tissue [26]. Such a three-dimensional scaffold stores nutrients, water, and growth factors. In tissue engineering, this complex network has the following functions: cell attachment, participation in cellular communication pathways, structural organization and mechanical support. So far many materials have been used as scaffold in bone tissue engineering [27]. However, in overall the following characteristic of a scaffold are essential in influencing bone formation and its applicability in tissue engineering. These include topography, Porosity, three-dimensional architecture, surface chemistry, osteoinductivity, mechanical performance, immunogenicity and biodegradability [28]. Mechanical support is one of the basic functions of skeletal tissues. When a skeletal damage occurs, fixation is needed to reposition the damaged structures and provide suitable environment for functional healing. By completing the healing process, removal of the implanted scaffold is desirable clinically and biomechanically. Therefore, a degradable polymer is used in tissue engineering, because it is used as an implant and won't need a second surgery to remove it [29]. The biodegradable polymers should have seven criteria to be applied in bone tissue engineering: 1) the surface of scaffold must permit cell adhesion and growth; 2) after *in vivo* application the degradation products must not induce inflammation and toxicity; 3) the polymer must be processable into three dimensional structures; 4) it should be highly porous and has big surface area to facilitate regeneration of extracellular matrix; 5) the polymer should provide sufficient mechanical support for the injured structure; 6) the polymer must be resorbed after fulfilling its purpose; and 7) the rate of scaffold degradation must match the rate of tissue regeneration [27,30].

Optimum interaction on a cellular level and the biomechanical competence is needed to provide a better outcome in tissue formation [31]. The Specifications of a degradable polymer to be respected before implantation is divided into two main categories: biofunctionality and biocompatibility [32]. Biocompatibility means absence of toxicity, carcinogenicity, thrombogenicity, and immunogenicity or the ability of a substance to create a proper host response in a specific situation [33]. Biofunctionality indicates that an implant is physically, mechanically, chemically, biologically and thermally biocompatible, easy to handle, storable, resorbable and sterilizable [34]. In addition a scaffold must also have the following criteria: I. It must be produced from biological materials, II. It should have a relevant shape to fit a defect, anatomically, III. It should have high porosity of appropriate size, IV. It should have rough surface, V. It should have osteoinductivity, and VI. It should possess adequate mechanical properties for given load-bearing conditions [35].

Biocompatibility of a scaffold

The material used and the surrounding cells/tissues are both important factors regarding biocompatibility. However, interaction of biomaterials and cells is very complex, and just a part of it has been understood. In physiological situation, cells bind to the surrounding ECM via ligands and many proteins interact with the cells by means of evoking a myriad of responses [36]. Identification of biomaterials by a cell is mediated by proteins; pre-adsorption of small peptides has also been shown to improve cellular response [24]. Biocompatibility of a scaffold or biomaterial can be increased by changing surface features of the substrate that in turn results in elevated or reduced protein adsorption [37]. Biocompatibility plays an important role in the success of all implants. An implant or scaffold and its remnants or degradation products should be non-toxic and biocompatible [38].

Biofunctionality of a scaffold

Additional support is needed to hold the mechanical function of a defected bone; for example, in spinal problems which cause instability, degeneration, and severe deformations, spinal fusion in these segments may be required [39]. The devices which are used for spinal fusion must restore and maintain the spinal anatomy and create a proper mechanical environment for spinal fusion. The device used to bear load, in case of spinal fusion, is called cage that is provided with a load-transducing filler part. Compared to implants and bones that should resist noticeable loads, metals are popular materials used for cages [40]. Although the metals or alloys have some beneficial effects on healing but disadvantages such as wear, late foreign body reaction, and infection limits their application in fracture healing [41,42].

Porosity of a scaffold

Scaffolds should have generously porous structure and open pores, with wide surface area to volume ratios, to allow cell growth and cell distribution and promote neovascularization from the surrounding tissues inside the porous structure [43]. In addition, the scaffolds must exhibit sufficient microporosity, to allow capillary growth. Interconnectivity and porosity are also important for sufficient diffusion of oxygen and nutrients and for the removal of dioxide carbonic and metabolic waste resulting from the living cells which have grown into the scaffold [44]. The rank of porosity influences other properties of the scaffolds such as its mechanical stability; therefore, the value of porosity must be balanced with the mechanical requirement of the particular tissue that is going to be replaced [45]. The pore size is an important issue because, if the pores are too small, they would be occluded by the cells and this phenomenon prevents cellular penetration, ECM production, and neovascularization throughout the scaffold. The pore size must be within the 200 - 900 μm range, in bone tissue engineering [46].

Surface Properties of a scaffold

Surface properties and topography of a scaffold affect cellular adhesion and proliferation [47]. Topographical properties of a scaffold are important in osteoconduction. Osteoconduction is a process that the osteoprogenitors migrate on the scaffold surface trough a fibrin clot that is established after the material application. Migration of the osteoprogenitor cells trough the fibrin clot would cause retraction of the temporary fibrin mesh. So, it is of great importance to properly secure the fibrin to the implant or scaffold. In addition when the osteoprogenitor cells start to migrate, the fibrin would be removed from the scaffolds during wound contraction. A rougher surface would be able to confine the fibrin, and then promote migration of the osteoprogenitor cells into the materials texture [48].

Osteoinductivity of a scaffold

In the fractured bone it is needed that the stem cells differentiate to the periosteoblasts; the materials that have this role are called osteoinductive materials [49]. In addition, when a bone defect is large, natural osteoinductivity combined with a biocompatible scaffold may not be enough and a osteoinductive scaffold is needed to effectively promote bone healing [50].

Mechanical property and biodegradability of a scaffold

A scaffold must have adequate mechanical strength to endure the hydrostatic pressures and to maintain the spaces needed for cell growth and ECM production [51]. As bone is under continuous pressure, the mechanical properties of the scaffold must match the living bone, so that mobilization or even normal physical activity of the fractured bone can be made possible [52]. In addition, the rate of degradation of scaffolds should be coincident with the growth rate of the new tissue; on the other hand when the fractured bone totally healed the scaffold should totally been degraded [53].

Scaffold fabrication

Numerous processing technologies have been introduced to fabricate the porous three dimensional polymeric scaffolds for bone tissue engineering. These techniques include emulsion freeze-drying, solvent casting and particulate leaching, rapid prototyping, electrospinning, gas foaming, and thermally induced phase separation [54].

Biomaterials used in scaffolds applied in bone tissue engineering

Selecting the most appropriate material is a very important stage in constructing the bone tissue engineering scaffolds [55]. Today various materials such as ceramics, metals and polymers from synthetic or natural origins have been suggested. However, most of the ceramics and metals are not degradable, but some ceramics and polymers are biodegradable [56]. Ceramics are greatly applied in the bone tissue engineering and bone substitution fields [57]. Some ceramics such as coralline hydroxyapatite have natural origin and some others such as hydroxyapatite or β-tricalcium phosphate are synthetic in nature. However, most ceramics are osteoinductive and osteoconductive, so they have been considered ideal materials in bone tissue engineering [58]. Several studies have reported acceptable bone healing results following application of ceramics either with or without bone marrow, however, low mechanical stability, difficulty in predicting the dissolution/degradation rates have been stated as the major drawback of ceramic materials [59]. Therefore the biodegradable polymers have been found as ideal products for bone tissue engineering [60]. Polymers are divided to synthetic and natural classes. Natural polymers are obtained from natural origins i.e. from animal, vegetal, bacterial or fungal origins. Chitosan, hyaluronic acid, fibrinogen, collagen, gelatin, alginate, elastin, cellulose, coral and starch are examples of these types of polymers [61] and low immunogenicity, capability to interact with the host's cells, and chemical and bioactive behavior are the main advantages of such natural polymers. Synthetic poly-

mers such as ceramics (hydroxyapatite), calcium phosphate cements (mono-calcium phosphate, di-calcium phosphate, and tri-calcium phosphates), calcium sulphates, bioactive glasses, poly-carbonates, poly-e-caprolactone, poly-propylene fumarates, poly-a-hydroxy acids, poly-phosphazenes, poly-anhydrides, and other composites are also used in the biomedical tissue engineering [62].

Cells applied in tissue engineering

The second step after preparing an appropriate scaffold is selection of a dependable source of cells which allows their proliferation, migration and differentiation to particular cell lineage. Actually, an ideal cell source must be easily expandable to higher passages, have a protein expression pattern comparable to the host tissue to be regenerated and do not initiate a severe immunological reaction [24]. From more than three and half decade ago, the scientists recognized which mesenchymal stem cells can be applied in tissue engineering so that the researchers supplied the right carrier and the appropriate set of cells which, once re-transplanted, would ensure bone repair [63]. Bone marrow has been said to be the most abounding source of mesenchymal stem cells that have a high proliferative ability and high capacity to differentiate to different cell lineages. Also, bone marrow is an accessible origin of osteogenic cells which can be collected, using a simple aspiration method. This procedure is less invasive than collecting the osteogenic cells by biopsy from the periosteum, trabecular bone, or calvarium [64].

Sources of stem cells in bone tissue engineering

Osteoblasts

Osteoblasts have strong osteogenic potential and could be used as the seed cells in bone tissue engineering, as they are non-immunogene and are also able to synthesize and secrete ECM, and promote mineralization and bone formation [65]. Long incubation time, less proliferative capacity *in vitro*, and less available donor tissue are the main disadvantages in application of osteoblasts in comparison to stem cells [66]. Isolation from biopsies which are taken from the patients, followed by restricted *in vitro* expansion is the most obvious choice in application of the osteoblasts. However, in certain bone diseases as the protein expression profile of osteoblasts is under the expected values they may not be good enough to be transplanted in the recipient [67]. In such circumstances an alternative procedure is the use of non-human cells or xenogeneic cells to solve the problem of low cell number harvest. However, the immunogenicity of xenogeneic cells, the ethical problems and transmission of infectious agents related with this subject have refrained the excitement for this method [68].

Stem cells

Stem cells are undifferentiated cells with the capacity to self-renew, produce more stem cells, and differentiate to different cell lineages under appropriate conditions. Stem cells have different degrees of differentiation potential. Stem cells are categorized as embryonic and adult stem cells base upon their sources [69]. When the stem cells are derived from the fertilized oocyte they are totipotent, and can form the embryo and the placenta. These cells can specialize and form an empty ball of cells, the blastocyst, and a cluster of cells derived to embryo, called Inner Cell Mass [70].

Embryonic stem cells

The embryonic stem cells are totipotent cells separated from the inner cell of blastocysts and can differentiate to any cell type in body [71]. Study on human embryonic stem cells has emerged major controversies with regards to immunogenicity and ethical issues. At first the embryonic stem cells were separated and grown in culture more than 10 years ago [72]. Later it was recognized that when the embryonic stem cells are transferred to mouse embryos they can generate all cell types of the embryo [73].

The embryonic stem cells are normally isolated from rodents, primates, and humans. These cells have two main characteristics including the capacity to differentiate via precursor cells and the unlimited self-renewal capability [74]. Other properties of these cells are the expression of embryonic antigens, high alkaline phosphatase activity, high telomerase activity, and the expression of germ-line transcription factor Oct-4. It has well been documented that the haematopoietic cells, cardiomyocytes, hepatocytes, neurons, endothelial cells, adipocytes, chondrocytes, and pancreatic islets have been differentiated from embryonic stem cells [75]. Differentiation of osteoblasts from the embryonic stem cells in presence of dexamethasone was an interesting finding in bone tissue engineering [76]. Two issues still need to be solved before safely application of the embryonic stem cells: (i) to promise that the donated embryonic stem cells are not tumorigenic and (ii) the immunological incompatibility in the embryonic stem cells generated donor cells. This last issue can be solved by using the somatic nuclear cloning transfer procedure [77].

Adult stem cells

There are great assure to use the adult stem cells in the oral region. Adult stem cells are defined as undifferentiated cells found among the specialized cells in the post-natal state. They can differentiate to many types of cells [78]. The adipose derived stem cells, bone marrow mesenchymal stem cells, dental pulp stem cells, periodontal ligament stem cells, and stem cells from human exfoliated deciduous teeth are stem cells that can generate bone. Bone marrow contains a subgroup of non-hematopoietic cells. The bone marrow stem cells are well-characterized adult stem cell populations which can be differentiated to various types of cells, such as osteoblasts [79]. The adult stem cells are restricted to differentiate only into cell lineages from the original tissue. However, the recent experiments have reported that their degree of differentiation plasticity can be higher than what we expected [80]. There is a specific interest in the bone marrow derived mesenchymal stem cells in bone tissue engineering science. At first Petrakova., *et al.* (1963) reported that bone marrow contains osteogenic precursor cells; and it was showed that implanting pieces of autograft bone marrow in the renal subscapular portion resulted in a bony tissue structure. A procedure has been developed to isolate the fibroblast-like cells from the bone marrow, based on adhesion to tissue culture plastic dish [81]. About 30 years later, Caplan named these stem cells "mesenchymal stem cells". When the mesenchymal stem cells, are placed in adequate culture conditions, they could be differentiated into cartilage, bone, muscle, tendon, ligament, skin, fat, and other tissues of mesenchymal origin [82].

Differentiation of stem cells to osteogenic cells

Application of the stem cells in bone tissue engineering needs effective protocols to direct differentiation of the stem cells to the osteogenic cells. A proper protocol decreases the chance of spontaneous differentiation of the transplanted stem cells to divergent lineages and also decreases the risk of teratoma formation after application of the embryonic stem cells. In addition, such protocols can supply useful *in vitro* models for the study of osteogenesis and bone formation, and expedite the genetic stem cells manipulation for therapeutic implementation. Osteoinductive elements, growth factors, cytokines, and biomaterials should be used to direct differentiation of the osteogenic stem cells [83].

Cells from periosteum

Periosteum could be used as another source of primary osteogenic cells [84]. The main techniques in isolating the mesenchymal stem cells from this source are enzymatic release of progenitor cells from the periosteal layers or preparation of the explant cultures from the dissected tissues. The previous studies have shown differences in proliferation rates of the periosteal cells isolated by different procedure and originating from different donors and age-related declines in cell proliferation [85]. It has also been shown which periosteal populations have chondrogenic and adipogenic differentiation potential, as the primary periosteal cells of human that are cultured on porous scaffolds, produce bone-like tissue [86]. It has been reported that the primary osteogenic cells can be isolated from the tissues which are discarded during surgical procedures and used in bone tissue engineering. A small volume of tissue from the accessible sites such as jaw bones during placement of dental implants could be used for cell isolation and preparation of autografts up to several millimeters in diameter and length [87].

Growth factors for tissue engineering

Growth factors are cytokines which are produced by various cell types and act as signaling molecules. They enhance or prevent cell proliferation, adhesion, differentiation and migration by regulating the synthesis of proteins, receptors and growth factors. Growth factors are essential in bone regeneration and tissue engineering. In unisonous with osteoblasts and osteoprogenitor populations, a pile of growth factors has been involved in osteogenesis [88]. The osteoinductive growth factors have an eminent role in differentiation and proliferation of osteogenic cells. In addition, the growth factors can attract progenitor cells of the host bone to invade scaffold and also induce osteoblastic differentiation. There are so many of such proteins which stimulate differentiation and proliferation of osteogenic cells [89].

The principal osteoinductive growth factor members in bone tissue engineering belong to the TGFβ superfamily particularly the bone morphogenetic proteins (BMPs); others included insulin growth factor I and II (IGF I/II), platelet derived growth factor (PDGF), and fibroblast growth factors (FGFs). Almost 30 of the BMPs have been recognized and they have much clinical efficacy as therapeutic elements through recruitment, bone formation, commitment and differentiation of the bone progenitor cells. A suitable carrier has not yet been identified for BMPs and this resulted in unsuccessful selection of dosage, delivery and maintenance of biological activity of growth factors in target tissues; therefore, super physiological dosing has been prescribed to achieve efficacy. Although several BMPs have been reported in the literature, BMPs 2, 4, 6 and 7 are considered to be the most osteoinductive bone morphogenetic proteins [76]. It has properly been known which of these BMPs can interfere in the expression of some growth factors, such as TGFβ. Their most important role is to recruit mesenchymal stem cells to the fractured site, and then differentiate them to the osteoblasts or osteogenic lineage. Their mechanisms of action on the mesenchymal stem cells are not understood yet, but it has been known that, BMP 2 plays an important role in the expression of osteogenic markers such as alkaline phosphatase and osteocalcin [90].

TGFβ stimulates cellular proliferation and differentiation and promotes cellular hypertrophy. TGFβ blocks or initiates cellular differentiation or migration and also stimulates osteoblast-like cells to promote, proliferate and initiate collagen production. Both IGF I and II have similar effects on the metabolism of bone, but IGF I is more potent than IGF II. They probably stimulate collagen synthesis [91]. FGF has important role in the bone remodeling. It is involved in regulation of the balance between bone resorbing cells and bone forming cells. It also enhances the development of new blood vessels.

PDGF is produced by platelets, monocytes/macrophages and osteoblasts and have a role in bone regeneration and in mesenchymal stem cell migration to the healing site [92]. Stem cells were induced in the previous studies to differentiate into osteoblasts under the influence of calcitriol, prostaglandin E2, dexamethasone, L-ascorbic acid, β-glycerol phosphate, teriparatide, and TAK-778, etc [77]. Calcitriol has been reported to inhibit differentiation of the adipogenic bone marrow derived mesenchymal stem cells to promote osteogenic differentiation. Prostaglandin E2 is an eicosanoid that is derived from the arachidonic acid metabolism. It has been demonstrated to enhance proliferation and osteogenic differentiation of mesenchymal stem cells of the bone marrow. Dexamethasone is a steroid drug which is used in cell culture to induce proliferation, maturation, and ECM mineralization in the adult stem cells [93]. TAK-778 has been proved to be an inducer of osteogenesis. The inorganic ions that are essential in bone formation process, should be added to biomaterial to assist osteogenic differentiation of stem cells. The minerals Ca, and Mg have been proved to assist the osteogenic differentiation of progenitor cells [94].

Conclusions

Several procedures have been applied to promote bone healing. Study of bone biology has been expanded with the improved knowledge at molecular science and this resulted in various treatment strategies [12]. Production of scaffolds to prepare sufficient mechanical support for replacing bone de¬fects in critical sized defects is the most important challenge in bone repair and tissue engineering. In spite of the fact that numerous scaffolds and ma¬terials have been tested, there are still some insoluble problems such as stimulation of vascularization, mechanical property, and good degradability that need to be solved to be applied in clinical settings. The degradable scaffolds have generated so many in¬terests, in bone regeneration, because their degradability allows them to avoid more surgery to remove the scaffold and this decreases pain and cost for patients [43]. Treatment of bone fracture is hindered by our understanding of bone stem cell fate, immuno-phenotype and choice criteria. There is an urgent need for safe, efficacious and facile method of stem cell isolation and expansion together [66]. Despite of great progress in this field, there is still surprisingly little knowledge regarding the cellular basis of mesenchymal stem cells mediated fracture healing and bone regeneration *in vivo* in humans [89]. Studies are ongoing in many relevant fields, and it is hoped that bone disorders due to trauma, bone resections in surgery, ageing, and genetic or metabolic bone disorders to be successfully treated with novel bone regeneration methods in near future.

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