REVIEW ARTICLE

INVESTIGACIÓN en DISCAPACIDAD

Vol. 8, No. 2
May-August 2022
pp 67-74
doi: 10.35366/105480

Tissue engineering in bone regeneration

La ingeniería de tejidos en la regeneración ósea

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Keywords:
Bone regeneration, bone tissue engineering, bone.

Palabras clave:
Regeneración ósea, ingeniería de tejidos ósea, hueso.

Abstract

The objective of regenerative medicine is to repair and replace damaged tissues or lost, initiating the process of natural regeneration, and using technologies such as tissue engineering. Bone tissue engineering requires a scaffold, a source of cells and growth factors, alone or in combination, to initiate the process of tissue regeneration. Several studies have developed safe and effective scaffolds for clinical use; some biomaterials used for bone reconstruction include ceramics, demineralized bone matrix, metals, and natural or synthetic biopolymers. The cells are an integral part of the strategy of Tissue Engineering, isolation, expansion efficiency, stability of the osteoblast phenotype, the ability of bone formation in vivo, as well as long-term security are essential requirements that must be met by any osteogenic cell type for successful clinical application in tissue engineering concepts. Growth factors are essential in tissue engineering because they function as signaling molecules that promote or prevent cell adhesion, proliferation, migration, and differentiation. This draft will mention each compound using tissue engineering strategy to repair and regenerate bone lesions and their clinical applications.

Resumen

El principal objetivo de la medicina regenerativa es reparar y reemplazar los tejidos dañados o perdidos, iniciando el proceso de regeneración natural y usando tecnologías como la ingeniería de tejidos, la cual requiere de un andamio, una fuente de células y factores de crecimiento, solos o combinados, para iniciar el proceso de regeneración de tejidos. Se han realizado diversas investigaciones para desarrollar andamios seguros y eficaces para el uso clínico, algunos de los biomateriales utilizados para la reconstrucción ósea son cerámicas, matriz ósea desmineralizada, metales y biopolímeros naturales o sintéticos. Las células son una parte integral en la estrategia de la ingeniería de tejidos, el aislamiento, la eficiencia de expansión, la estabilidad del fenotipo osteoblástico, la capacidad de formación ósea in vivo, así como la seguridad a largo plazo, son requisitos esenciales que deben ser cumplidos por cualquier tipo celular osteogénico, para el éxito de la aplicación clínica en los conceptos de ingeniería de tejidos. Los factores de crecimiento juegan un papel importante en la ingeniería de tejidos debido a que funcionan como moléculas de señalización. Ellos promueven o previenen la adhesión celular, proliferación, migración y diferenciación. En este artículo se mencionarán los elementos que utiliza la estrategia de ingeniería de tejidos para reparar y regenerar las lesiones óseas, así como sus aplicaciones en la clínica.

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Received: February 15, 2022
Accepted: March 28, 2022

How to cite: Ruvalcaba-Paredes EK, Brena-Molina A, Tamay de Dios L, González-Torres M. Tissue engineering in bone regeneration. Invest Discapacidad. 2022; 8 (2): 67-74. https://dx.doi.org/10.35366/105480

INTRODUCTION

Bone is a dynamic, vascularized, hard tissue with many vital functions in vertebrates. As the main component of the skeletal system, bone supports the body's weight, enables locomotion, and protects vital organs. Bone can heal naturally in response to injury; however, there are occasions when healing must be augmented, such as spinal fusion, to treat fractures or skeletal deformities caused by infection, trauma, or tumor resection. Especially if the bone lesions are of a critical size because they prevent the bone from repairing itself.[1,2]

Various methods can restore bone deficiency, including allografts, xenografts, and autografts. Although autologous bone is considered the gold standard for reconstruction of bone defects, its application is limited, as the process of obtaining autologous bone is associated with insufficient supplies, surgical morbidity, donor site pain, the possibility of infection, and the inability to react to physiologic conditions. In addition, other bone substitutes cannot promote bone formation satisfactorily because of the lack of osteoinductivity. It persists even though these biomaterials' biocompatibility and osteoconductive effect are proven. Therefore, we need bone regeneration methods to overcome these limitations and search for alternative solutions. One of these alternatives is regenerative medicine, which aims to repair and replace damaged or lost tissues, initiating the process of natural regeneration, combining several technologies, including molecular and cell biology, gene therapy, materials science, stem cell transplantation, tissue engineering, among others.[2-8]

Tissue engineering has been defined as the application of scientific principles to the design, construction, modification, and growth of living tissues using cells, inductive factors, and the synthesis of new biomaterials adapting structural, physical, and chemical properties to simulate natural physiological aspects, these compounds can be used in combination or alone *(Figure 1)*. While bone tissue engineering requires a scaffold conducive to cell adhesion and maintenance of cellular functions, together with osteoprogenitor cells in combination with osteoinductive growth factors, thus forming osteogenic constructs, it also requires a rich source of osteoprogenitor cells in combination with osteoinductive growth factors.[3,9,10] In this draft, mention will be made of each compound used by tissue engineering for bone regeneration and repair strategies.

BIOMATERIALS USED IN BONE TISSUE ENGINEERING

Ideally, the designed scaffold is directed to reproduce all macro, micro, and nanoscale signals corresponding to the tissue, cell, and molecular scales in a specific tissue to promote cell adhesion, proliferation, and desired differentiation towards specific cell phenotypes. It must also be easy to handle, biocompatible, osteoinductive (material capable of inducing the transformation of undifferentiated cells into osteoblasts or chondroblasts), and osteoconductive (bone growth from and on the existing bone). In this context, several factors must be considered, such as the chemical nature of the scaffold material, the physical structures at various size scales, and the fabrication method.[7,11,12]

Several kinds of research have been carried out to develop safe and effective scaffolds for clinical use. Some biomaterials used for bone reconstruction are ceramics, demineralized bone matrix, metals, and natural or synthetic biopolymers.[13] Some of them will be briefly described below.

Calcium phosphate ceramics. The most used calcium phosphate (CaP) ceramics are hydroxyapatite (HA), tricalcium phosphate (TCP), and biphasic calcium phosphate (BCP), often combined with biodegradable polymers to produce better structures, that exhibits chemical and mineralogical similarity to the inorganic component of bones. It is recognized as the better implantable materials in bone surgery due to its biocompatible, osteoinductive, non-inflammatory and bioresorbable nature. Calcium phosphates have excellent tissue compatibility and are osteoactive, radiolucent, and readily available.[14] These biomaterials

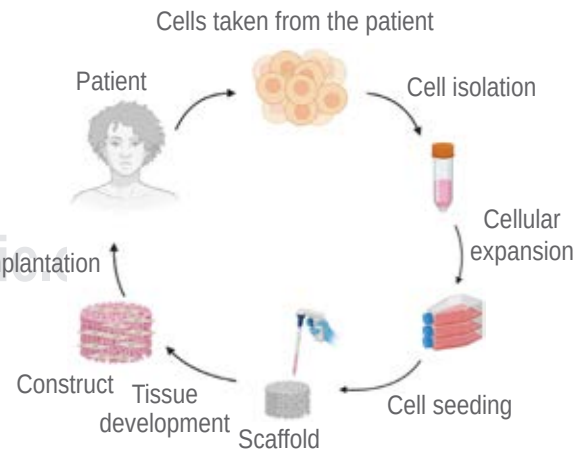

Figure 1: Fundamental principle of tissue engineering.

have excellent properties, as they support adhesion osteoblast proliferation and bind strongly to tissue, thus improving the fixation of implants.[15]

As a significant component of bone, HA [$Ca_{10}(PO_4)_6(OH)_2$] is one of the most common forms of calcium phosphate in clinical use.[1,14] It is widely used for bone repair applications because of its similarity to the mineral phase of the original tissue. We can find it in powder form, which has been used as a filling material to complement the injured part of the bones.[16]

HA derivatives have been used in the ceramic form to repair craniofacial bone defects since the 1970s. Friedman *et al*. have also reviewed several clinical applications in which they report excellent results using HA cement paste for craniofacial reconstruction. In 1994 HA was approved by the US FDA (Food and Drug Administration) for clinical use. In addition, it has been successfully used to repair cerebral spinal fluid leaks and has recently been used in otologic surgery in the form of a hybrid implant (HA-titanium).[14,17,18]

Although various calcium phosphate ceramic materials have been used clinically for more than two decades, these biomaterials are limited due to their insufficient mechanical properties. Therefore, calcium phosphate ceramics are mainly used in non-load bearing applications (e.g., middle ear implants) and bone filler material.[1]

Demineralized bone matrix. Demineralized bone matrix (DBM) is the approved medical device used in bone defects. Commercially available as injectable gels, putty, paste, sheets, and flexible strips, all of which lack structural strength but possess osteoconductive and osteoconductive agents.[1,7] DBM is an acid-extracted organic matrix, allowing the organic and protein components originating from bone to be retained, with small amounts of calcium-based solids, inorganic phosphates, and some cellular detritus. Many of the protein components of DBM are known to be potent osteogenic agents, such as bone morphogenetic proteins (BMP-2, BMP-4, BMP-7), the residual type I collagen in DBM contributes to the essential physical and biological properties of the matrix.[19,20]

Urist demonstrated cartilage and heterotopic bone formation after allogeneic DBM implantation in intramuscular sites in rodents.[21] While, Borrelli *et al*. evaluated the efficacy of DBM in the reconstruction of cranial defects in the patients, they found it to be a biocompatible biomaterial with significant osteoinductive potential. They also observed multiple areas of bone neoformation and complete filling of the cranial defect two years after DBM implantation in the lesion.[4]

DBM provides a non-immunogenic matrix and is gradually degradable, facilitating the endogenous release of these compounds to the wound sites of the bone in which it is surgically placed to fill bone defects, inducing the formation of new bone and accelerating healing.[19,20]

The various commercially available formulations of DBM exhibit a wide range of biological functionality due to variations in the BMP content associated with each preparation.[21,22]

Materials metal-based. Porous metal scaffolds have been investigated for bone-related applications due to their excellent physical properties and their ability to promote tissue ingrowth. The most used materials in this category are titanium (Ti) and tantalum (Ta). The Ti exhibits biocompatibility along with mechanical and corrosion resistance. Xue *et al.* generated Ti scaffolds ranging from 17 a 58% de porosity, with a pore size of 800 um. These structures exhibit mechanical properties more similar in consistency to bone and enhanced osteoblast adhesion, proliferation, and differentiation. In addition, Ti has frequently been incorporated into alloys. Structural modification of Ti surfaces has been shown to increase osteoconductivity, as observed in Das *et al*., also, Li *et al*. evaluated various porous Ti6Ta4Sn, surface treatments, noting that a sol-gel-coating of HA produced optimal adhesion of osteoblast-like cells.[15]

Despite higher cost and difficulty in fabrication, Ta has recently gained attention because it possesses more favorable qualities than Ti. Zhang *et al*. fabricated a Ta scaffold ranging from 27 to 55% porosity that demonstrated superior adhesion, proliferation, and differentiation capabilities compared to its porous Ti counterparts.[15]

Unlike Ti and Ta, magnesium (Mg) has the additional characteristic of biodegradation. Witte *et al.* investigated the porous effects of Mg alloys *in vivo*, observing that bone formation and resorption are accompanied by scaffold degradation. However, possible problems with corrosion and ionic removal may restrict the use of these metal-based constructs, and some biomedical reports indicate that Mg alloys may contain aluminum (Al) and impurities, which could cause some damage. For example, Al is harmful to neurons and osteoblasts, it has also been associated with dementia and diseases such as Alzheimer's. It can also cause hepatotoxic damage, as well as damage

to gene expression. For these reasons, other suitable elements have been sought for alloys with Mg.[15,23]

Natural or synthetic biopolymers. Polymers have been employed for bone tissue engineering applications, because they possess physical properties like fibrous proteins found in soft and hard connective tissues. Natural and synthetic polymeric materials, can be fabricated with defined geometries and formats (e.g., porous foams) and chemically modified to modulate cell adhesion and degradation characteristics.[1]

Collagen, which is the most abundant protein in the extracellular matrix of vertebrates, is a logical choice as a biomaterial for tissue regeneration. Collagen is the most widely used natural polymer for regenerative therapies because its biological properties favor cell adhesion and differentiation. Collagen is rarely used alone, but is commonly combined with other biopolymers in bone repair. Synthetic polymers for bone regeneration include polyfumarates and polyesters, poly (lactic acid), poly (glycolic acid), and polycaprolactone. These resorbable materials offer versatile alternatives to natural polymers (e.g., collagen), and can be processed into three-dimensional biopolymers. However, synthetic biopolymers often provoke inflammatory responses as a result of acidic degradation products.[1] Natural polysaccharides, such as chitosan, agarose and alginate, are other types of natural polymeric scaffolds. These materials possess positively charged amino groups on their surface that allow for interactions with anions. The cationic nature of chitosan promotes interactions with glycosaminoglycans and proteoglycans which are known to stimulate cytokines and growth factors for tissue regeneration. Silk fibroin, another natural polymer, has also demonstrated ability to support cell proliferation, induce osteogenesis *in vitro* and bone formation *in vivo* calvarial defect models.[12]

CELLS USED IN BONE REGENERATION

Cells are an integral part of the tissue engineering strategy; different osteogenic cells are used, although it is unknown which cell type will be the most suitable of bone tissue engineering. Isolation, expansion efficiency, stability of the osteoblastic phenotype, *in vivo* bone formation capacity, as well as long-term safety, are essential requirements that must be fulfilled by any osteogenic cell type for the future success of the clinical application in Tissue Engineering concepts. For immunological and safety reasons, autologous cells are currently considered the first choice for osteogenic constructs,[24] which can be obtained from different human body sites; some cells used in bone regeneration are mentioned below.

Osteoblasts. Osteoblasts can be found on the surface of the bone and are involved in bone repair and remodeling.[24] They possess a robust osteogenic potential and can be seeded for bone regeneration. As bone-forming cells, osteoblasts can synthesize and secrete bone matrix, promoting bone mineralization and bone formation.[25]

They can be isolated from fetal or adult bone samples using enzymatic digestion techniques; even without enzymatic digestion, osteoblasts can grow out of small pieces of trabecular bone under appropriate conditions. Under *in vitro* conditions, they show stable osteogenic differentiation, as evidenced by the expression of standard markers; *in vivo* osteoblasts reliably form bone in several matrices. Compared to stem cells, the main disadvantages of osteoblast application include less availability of donor tissue, less proliferative capacity *in vitro*, and longer incubation time.[24]

Mesenchymal stem cells. The term mesenchymal stem cells (MSC), was proposed by Caplan *et al.* Friedenstein *et al.* initially isolated bone marrow-derived MSCs, and demonstrated that MSCs grow as foci with fibroblast-like morphology and were termed as fibroblast colony-forming unit (CFU).[26,27] It was subsequently established that MSCs are undifferentiated cells with the ability to self-renew, produce more stem cells and differentiate into various cell lineages under appropriate conditions. Stem cells are classified into embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult MSCs according to their origin, also frequently correlated with their plasticity.[24,25]

For this review, we will refer to Adult MSCs, which are defined as undifferentiated cells that are among the specialized cells after birth, are capable of self-renewal, and are characterized by their ability to differentiate into mesenchymal tissues such as bone, cartilage, and fat.[25,28,29] The potential of adult MSCs for bone generation includes:

1. Bone Marrow Stem Cells (BMSCs),
2. Adipose-derived stem cells (ASCs),
3. Dental pulp stem cells (DPSCs),
4. Human pulp stem cells from exfoliated deciduous exfoliated teeth (SHED),
5. Periosteum-derived stem cells (PDCs), and
6. Periodontal ligament stem cells (PDLSCs).[25,30]

These cell populations possess a proliferative capacity and multipotent differentiation potential comparable to bone marrow-derived MSCs, suggesting that adult MSC populations share a similar ontogeny.[29,30]

They are also heterogeneous and may contain differentiated cells, and variations in morphology, growth rate, proliferation potential, and differentiation capacity have been reported to be specific to the tissue from which MSCs are derived.[26,30-32]

MSCs are already used clinically to repair bone defects. However, to generate sufficient MSCs for therapeutic applications, they must first be significantly expanded *in vitro*.[30]

In 2006 Sotiropoulou *et al.* conducted a study in which they attempted to identify the optimal culture conditions for effective clinical-scale production of large numbers of MSCs for transplantation in cell therapy, immunotherapy, and regenerative medicine. They found that serum quality, glucose concentration, plastic surface quality affect the outcome. They also observed that using the basic fibroblast growth factor (b-FGF) increases the proliferation rate and the differentiation capacity to different lineages, favoring the differentiation towards the osteogenic lineage. This study establishes the general parameters for the standardization of a protocol to produce numerous high-quality MSCs to be used in preclinical studies and clinical protocols.[33]

Periosteal cells. It has been identified as a niche for various cells involved in endochondral, and intramembranous ossification during prenatal development, postnatal development, and fracture healing. The mixed periosteal cell population contains fibroblasts, osteoblasts, MSCs, and pericytes.[27,29,34,35] Ball *et al.* in 2011 determined that the periosteum has many undifferentiated cells that can differentiate and be maintained in culture up to pass ten without losing the ability to form mineralized tissue.[36]

These cells are derived from the inner layer of the periosteum. There is no significant difference between these cells and osteoblasts concerning *in vitro* osteoblastic phenotype and *in vivo* osteogenic properties.[24] An advantage for future clinical applications, may be that periosteum could be obtained from the oral cavity with minimal donor site morbidity.

Several works indicate that the periosteum can give rise to bone tissue under appropriate conditions, either by using culture media enriched with osteoinductive factors or by using biopolymers suitable for osteogenic differentiation. Zheng's group used a poly-l-lactic-co-glycolic acid (PLGA) biopolymer, finding that the mineralized matrix starts at early stages (7 days), but decreases when the culture is expanded (28 days), probably to insufficient nutrition of the *in vitro* culture with the PLGA polymer. While in the group of Honsawek *et al.*, they found that human periosteal cells treated with DBM express osteogenic genes.[10,37]

OSTEOINDUCTIVE FACTORS

Growth factors are cytokines secreted by various cells and function as signaling molecules.[9] Growth factors play an essential role in inducing bone formation and maintaining bone integrity.[24] They promote and prevent cell adhesion, proliferation, migration, and differentiation. These molecules play an important role in tissue engineering.[9]

Growth factors. Many growth factors stimulate osteogenic cell proliferation and differentiation *in vitro* and *in vivo*; many of these have been cloned and are available as recombinant proteins. The most popular osteoinductive factors in bone tissue engineering are the morphogenetic proteins (BMPs).[9,24]

BMPs are members of the TGF-β superfamily, and play an essential role in skeletal development, bone formation, and stem cell differentiation. These factors were discovered when it was found that DBM could induce de novo bone formation.[38]

Osteogenic BMPs include BMP-2, 4, 6, 7, and 9. BMPs aid in the healing process by recruiting bone-forming cells to the injury site. The use of BMPs is currently FDA approved to treat acute tibial fractures.[39] In addition, BMPs have been combined with biomaterials for clinical use, as is the case with Infuse™ Bone Graft (Medtronic IS; Wyeth, UK), containing rhBMP-2 (BMP-2recombinant) and Osigraft (Stryker Biotech), containing rhBMP-7, these two products are collagen-based and were approved by the FDA for clinical human consumption in the treatment of long bone fractures.[37,40] Nowadays, intensive studies are carried out in order to better understand how growth factors, can be efficiently trapped by materials or immobilized at the surface and how they intract.[9,40]

Chemical compounds. In addition to BMPs, *in vitro* MSC differentiation can be directed toward an osteoblastic lineage by adding soluble factors, including chemical compounds and hormones, in the cell culture medium.[32] Chemical compounds have been tested to promote osteogenic differentiation of MSC cells *in vitro*. These chemical compounds tend to be less labile and have a long active

half-life compared to cytosine-based proteins and growth factors, which can be beneficial in prolonging *in vitro* cell culture for several days or even weeks. In addition, these chemical compounds can be manufactured by chemical reactions in the laboratory. TAK-778 is a potent inducer of osteogenesis *in vitro* and *in vivo* studies as a new synthetic compound. While statins, a family of synthetic chemical compounds, play an integral role in hepatic cholesterol biosynthesis, apart from their widespread pharmacological application for blood cholesterol reduction, they also have a profound ameliorating effect on osteogenesis.[25]

Studies indicate that stem cells were induced to differentiate *in vitro* into mineralized osteoblasts under the influence of a combination of various compounds such as prostaglandin E2, 1,25-dihydroxyvitamin D3, L-ascorbic acid, dexamethasone, beta-glycerol phosphate, TAK-778, teriparatide, and statin family compounds. prostaglandin E2, a naturally occurring eicosanoid derived from arachidonic acid metabolism, has been reported to increase the proliferation and osteogenic differentiation of BMSCs.[25]

An active form of vitamin D, 1,25-dihydroxyvitamin D3 (also known as calcitriol), has been shown to inhibit adipogenic differentiation of BMSCs for the promotion of osteogenic differentiation. Metzger *et al*. indicate that vitamin D induces osteocalcin expression from osteoblasts. While Metzger *et al.* indicate that the presence of vitamin D in cell cultures induces osteogenic differentiation.[41,42]

Dexamethasone (DEX), is a synthetic steroid used in cell culture experiments to induce proliferation, maturation, and extracellular matrix (ECM) mineralization of adult stem cells and ESCs. DEX is an inducer of osteogenesis and is often used in combination with L-ascorbic acid (vitamin C), and beta-glycerol phosphate,[25] the latter two being required for extracellular matrix mineralization.[41]

CONCLUSION

Tissue engineering plays a vital role as an alternative in regenerating and repairing bone lesions. Regeneration of tissue defects in the bone requires an understanding of complex developmental processes, molecular pathways, physiology, and remodeling characteristics. Although it is difficult to mimic nature, recent scientific and technological findings show great potential to achieve bone scaffolds. Continued growth of this field hinges in part on the development of new materials and improved scaffold processing techniques. With exciting current advances in stem cell biology, genetics, gene therapy, matrix synthesis and nanotechnology offers the development of a flexible, biomimetic osteogenic cellular scaffold will also have application in the future in our Institute.

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