REVIEW

Fracture Healing and Current Adjuvant Approaches to Fracture Healing

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Abstract: Despite many scientific studies on the subject of fracture healing, the effective treatment of lesions in this tissue is still a major problem. Although there have been recent remarkable developments in the treatment methods applied, they remain insufficient for the complete treatment of lesions occurring in bones. Fracture healing is a regenerative process that includes the proliferation of mesenchymal cells, chondrogenesis, angiogenesis and bone formation. Different systemic and local variables which affect the regaining of the physical and mechanical properties of the bone tissue where the lesion has occurred play a role in this process. In the light of previous scientific research, the aim of this paper was to provide information about the options for different applications to be able to obtain rapid treatment through an understanding of the many complex processes in fracture healing.

INTRODUCTION

Although osteosynthesis methods used in orthopaedic surgery have undergone remarkable developments in recent years, they are not sufficient for the complete treatment of lesions in bone tissue[1]. Therefore, subjects such as the regeneration of lost or damaged bone tissue, fragmented diaphyseal fractures of long bones, bone defects occuring for various reasons[2], osteomyelitis, non-union, arthrodesis[3], and regaining bone function continue to be significant medical problems. Bone is a metabolically dynamic biological tissue formed from active cells. Fracture healing is a regenerative process including the proliferation of mesenchymal cells, chondrogenesis, angiogenesis and bone formation. Different systemic and local variables which affect the restoration of the original physical and mechanical properties of the damaged bone tissue also play a role in fracture healing. Not only fracture healing, but also all the biomechanical, cellular, hormonal, and pathological, and especially biochemical factors can be examined in scientific studies which take fracture healing as their subject. As a result of scientific studies, it is possible to understand the many complex processes underlying fracture healing and to obtain rapid healing[4].

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Bone Development and Osteogenesis

Bone formation (ossification)

Bone develops in the place of the pre-existing connective tissue in the embryonic period. At this time, ossification occurs in two forms. The first of these is intramembranous ossification and the other is endochondral ossification. The mechanism of the bone matrix formation is the same in both of these processes. First, a primary trabecular network or primary spongious tissue occurs. These tissues then transform to mature bone tissue. However, the difference between the two processes is that in endochondral ossification, the bone matrix replaces cartilage tissue[5].

Intramembranous ossification: In this type of ossification, mesenchymal cells come together directly. This process is controlled when signals are formed from hedgehog, fibroblast growth factor (FGF), and transforming growth factor- β (TGF- β) polypeptides. Mesenchymal cells transform to osteoblasts by differentiation. Osteocytes cleave to each other within the developing bone blastema and the osteoblasts are arranged to cover the surface of this bone blastema. The arranged osteoblasts then deposit osteoid, in other words, the bone matrix. Calcium coming to the region through blood vessels is used for mineralisation, and thus the primary bone tissue is formed. Osteocytes are retained within the calcified osteoid. On the osteoid surface, osteoblasts continue to deposit as an apositional matrix

containing primarily type I collagen and non-collagen proteins. The embryonic mesenchyma converts to vascular rich connective tissue. Mesenchymal cells similar to fibroblasts embedded in a gelatinous extracellular matrix containing collagen fibres collect together. Mesenchymal cells take on the typical prismatic shape of osteoblasts and start to express bone matrix. Several ossification centres develop, and by combining, these centres form a primary spongious trabecular network, resembling a sponge. The continuation of bone deposition on trabecular surfaces provides filling of the intertrabecular spaces, and thus compact bone is formed. The formation of spongious bone occurs with the continuation of primary spongiousness. The frontal and parietal bones, occipital and temporal bones, and the mandibular and maxillary bones develop with intramembranous ossification[5].

Endochondral ossification: This type of ossification develops in the form of bone replacing skeletal cartilage models. The primary ossification centre formed in endochondral ossification originates from chondrocytes which deposit an extracellular matrix containing type II collagen. Then, the chondrocytes in the central region of the cartilage start to mature and synthesize a matrix containing type X collagen. Vascular endothelial cell growth factor (VEGF) expressed by hypertrophic chondrocytes induces the formation of blood vessels from the perichondrium. Osteoprogenitor and hematopoietic cells reach the region through these vessels.

As a result of these events, a primary ossification centre is formed. Matrix calcification occurs in the midline of the cartilage model and apoptosis is seen in hypertrophic chondrocytes. Osteoprogenitor cells of the perichondrium form a periosteal collar, which contains reticulated bone. When areas are filled by hypertrophic chondrocytes and spaces are surrounded by blood vessels, these vessels extend as far as the two ends of the ossification centre. The periosteal collar and primary bone ossification centre first organise in the diaphysis. Secondary ossification centres then develop in the epiphyses. Other than on the joint surfaces, all the epiphyseal cartilage is replaced by bone tissue[5].

Bone Vascularisation

To be able to achieve problem-free treatment of lesions occurring in bone tissue, good knowledge of vascularisation and the stages of fracture healing is necessary. Vascularisation is of great importance in ensuring the physiological functions of bone tissue. The vascularisation of bone tissue is provided by the three systems of the afferent vascular system, the intermediate vascular system, and the efferent vascular system. The afferent system has the function of transporting arterial blood. Periosteal bone is fed with minor components of this system and these components reach the periosteum from the muscle connections. These vessels are responsible for the feeding of the outer layer of compact bone. Vascularisation of the diaphyseal region of the extremity long bones is provided by nutritional vessels entering the bone tisssue from the foramina nutricia. These vessels reach the medullar canal by crossing the Volkman canals to Haver's canal. The nutritional vessels separate into branches forming the vascular system of the distal and proximal medullar canals. These branches then establish the link between the periosteal and metaphyseal arteries[6,7].

Fracture Healing

The disruption of the integrity of bone tissue for whatever reasons is known as fracture. Fracture healing occurs directly (primary) or indirectly (secondary) depending on the gap between the fracture fragments and the stabilisation method applied. Fully anatomic and rigid fixation encourages direct fracture healing. In this type of healing, there is compression and contact between the fragments, as a result of which a rapid remodelling stage can be observed. Reduction of the fracture line is applied but if there is a gap between the fragments or micro movement, the healing is in the form of indirect fracture healing[8].

Direct (primary) healing: The possibility of direct fracture healing occurring in natural processes is very slight. Primary fracture healing occurs in two forms; when there is full contact, defined as a gap of <0.01mm between fracture fragments, or a minimal gap, defined as a gap of 800µm-1mm. The formation of lamellar bone is observed in both types of healing[9].

Indirect (secondary)healing: The healing of many fractures occurs in this way. Secondary healing is seen if there is movement between the fragments after the formation of the lesion. Following trauma, an inflammatory response starts with hematoma in and around the medullar canal. The hematoma forms a source of hematopoietic, stem cells and growth factors. An increase in polymorphonuclear leukocytes, macrophages, and lymphocytes, and vasodilatation mediated by TNF-α, TGF-β, IL1-β, IL6, IL17F, IL18, and IL23 inflammatory mediators is seen in the region. The acute inflammatory response reaches a maximum level within the first 24 hours and continues for 7 days. Interleukins have an effective function in the healing process. Increasing IL1 from macrophages and increased IL6 expression from osteoblasts, stimulate callus formation and vascularisation mediated by ILR1 and ILR2. Following the formation of hematoma after the occurrence of the lesion, fibrin-rich granulation tissue forms in the region. Endochondral ossification starts between fragments and outside the periosteum. In this process, hard callus tissue replaces the mineralised cartilage tissue. During the replacement of soft callus with hard callus, resorption of mineralised cartilage starts with the effect of macrophage colony-stimulating factor (M-CSF), nuclear kappa ligand receptor activator (RANKL), osteoprotegerin (OPG) and TNF- α. Apoptosis of mesenchymal stem cells and chondrocytes is stimulated by TNF- α. As the amount of hard callus formed in the region increases, mineralised cartilage tissue is replaced by woven bone tissue. Thus the healing tissue becomes more rigid, but however rigid the structure is, it does not have the physiological structure of bone. The remodelling stage which starts from the 3rd-4th week during lesion repair continues even after clinical healing is obtained and this process can take several years. While osteoclasts provide hard callus resorption in the remodelling stage, the formation of lamellar bone occurs with osteoblasts. It has been reported that this activity continues for many years[9].

Factors affecting fracture healing

Growth factors: Growth factors are naturally formed agents which have a series of molecular variants. In vivo studies that have investigated the fracture healing process have shown that bone morphogenetic proteins (BMP), transforming growth factor (TGF)-beta, insulin-like growth factor (IGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) play a role in healing[10].

BMPs are members of the TGF-β super family. Although more than 20 different BMPs have been isolated to date, only a few (BMP-2, -4, -7) have the potential to trigger new bone formation. In adults, BMPs are synthesised by osteoblasts and osteocytes, and are primarily found in bone and dentin[10,11].

In fractures created experimentally, it has been proven that BMP-2 and BMP-7 have the capability to accelerate lesion healing[10,12]. In a study of tibia fractures, Govender et al (2002) investigated the reliability and efficacy of the use of recombinant human BMP-2 (rhBMP-2) applied with an absorbable collagen sponge carrier. As a result of the study, the use of rhBMP-2 carrier was reported to be reliable, reducing postoperative complication rates and the invasiveness of the procedure, and accelerating the fracture and wound healing[13].

TGF-βs are TGF-β1 and TGF-β2 proteins which function as growth-differentiation factors in connective tissue healing and bone regeneration. They are synthesised and transported in macrophages other than thrombocytes, osteoblasts, and some other cell types. TGF-βs not only stimulate bone healing, but also show growth factor activity in the remodelling stage of healing, providing long-term continuation of this process. The most important function of TGF-β1 and TGF-β2 is the stimulation of the formation of the collagen matrix with the aim of chemotaxis of osteoblast precursors, mitogenesis and bone formation. In addition, by inhibiting osteoclast formation and bone resorption, TGF-β provides greater bone formation than resorption[14].

The IGF family is formed of the two polypeptides, IGF-I and IGF-II. It is generally thought that IGF-I and IGF-II are expressed by osteoblasts during bone formation to increase the number of osteoblasts in the region and thereby accelerate bone deposition. Although IGF-II is found more abundantly in bone tissue, IGF-I has higher osteogenic potential. It has been proven in in-vitro studies that IGF-I stimulates chemotaxis and osteoblastic activity and in-vivo studies have shown that it increases bone formation[10,14]. It was reported in a previous study that the use of IGF and TGF-β together provided the maximum benefit in fracture healing[15].

FGF is formed from a family containing more than 20 growth factors such as acidic FGF (aFGF or FGF-1) and basic FGF (bFGF or FGF-2). It plays a role in mesenchymal stem cell proliferation, cartilage formation, vascularisation and osteoblastic activity. FGF-2 accumulates most in the bone matrix and is expressed from the early stages of bone formation. It has been determined that FGF-2 is significantly effective in fracture healing[16].

VEGF is expressed and deposited by osteoblasts and the endothelium. It has an important role in angiogenesis during fracture repair. Positive results have been reported to have been obtained in studies conducted on the combination of BMP and VEGF[17].

PDGFs provide migration of neutrophils, macrophages and other precursor cells to the fracture region. When PDGF is used with tricalcium phosphate, it has been reported to have a positive effect on fracture healing[9].

Hormonal factors: Parathyroid hormone (PTH) has an important role in bone mineral density by affecting calcium, phosphate, and Vitamin D metabolism. It is known that continuous release stimulates osteoclastic activity, whereas intermittent and balanced release affects osteoprogenitor cells and accelerates fracture healing by increasing osteoblastic activity. In studies of patients where PTH has been used, there has been reported to be an increase in bone mass, accelerated fracture healing, and a lower risk of fracture[18].

Calcitonin is a PTH antagonist, which increases the formation of both compact and trabecular bone. By inhibiting osteoclasts, it reduces bone resorption. The formation of new bone is directly proportional to the calcitonin dose, but the mechanism of the positive effect on healing has not yet been clarified[18].

Growth hormone (GH) deficiency has been reported in some experimental studies to have a negative effect on fracture healing, and replacement to have a positive effect on healing. In some studies, a greater amount of GH has been shown to have little or no effect on fracture healing[19].

Oestrogen has been reported to have both positive and negative effects on bone resorption[18].

Corticosteroids prevent bone formation by inhibiting collagen synthesis and increasing resorption. They delay fracture healing by slowing down the synthesis of the building blocks required for the development of osteoblasts from mesenchymal cells and matrix formation. Cortisone also decreases callus formation and has negative effects on fracture healing by creating an atagonist effect on FGF, EGF, and PDGF[18].

The thyroid functions in the bone remodelling stage by triggering more bone resorption than bone formation[18].

Ghrelin is a hormone of lipopeptide structure expressed from the stomach fundus. Ghrelin has been reported to shorten the fracture healing period and has been determined to biomechanically provide the formation of better bone tissue[20].

Vitamins: Vitamin A has positive effects on fracture healing by stimulating mesenchymal cell differentiation. However, when there is a deficiency of Vitamin A, endochrondral growth stops through the slowing down of bone formation and less development of epiphyseal cartilage. An excess slows down cell differentiation and erosion develops in the cartilage. A deficiency of Vitamin D lowers the Ca^{+2} level and weakens calcification. The expression of calcium from the bone to the blood increases. At normal doses fracture healing is stimulated, but at high doses, there are negative effects on fracture healing. Vitamin C is effective in fracture healing and in cartilage modelling. By stimulating collagen synthesis, it has a positive effect on fracture healing, and a deficiency has negative effects. Vitamin E has been shown to have an antioxidant effect on free oxygen radicals formed in fracture hematoma, and it has been reported that there could be positive effects in the early stage of fracture healing. When there is a deficiency of Vitamin K and Vitamin B6, the fracture healing process is affected negatively[20].

Elements: Deficiencies of zinc and selenium cause a slowing down of fracture healing. A deficiency of calcium, which is of critical importance in bone metabolism, similarly reduces fracture healing[21].

Infection: Infection is one of the factors negatively affecting fracture union. In this case, the inflammatory phase is prolonged, and cellular activity is directed to fighting the infection instead of fracture union. The inflammatory state which emerges in the fracture line against the micro-organism leads to necrosis in the tissue around the bone, insufficient callus, and an increase in the distance between the fracture ends. Thus, there is increased movement of the fracture ends and fracture healing is delayed[21].

Smoking and alcohol consumption: Nicotine, which is a component of cigarettes, reduces blood flow in the fracture region with a vasoconstriction effect. Smoking increases thrombocyte aggregation and the blood viscosity, while reducing collagen synthesis and levels of prostacyclin in the blood. All these effects have a negative impact on the fracture healing process. Chronic alcohol use reduces osteoblastic activity. Bone mineral density is also reduced with the effect of alcohol. Fracture healing has been reported to be reduced after ethanol use[21].

Chronic diseases: Associated with low oxygen carrying capacity in iron-deficiency anemia, the tensile strength of callus tissue has been

seen to be reduced causing delayed union or non-union. In diabetes, delayed union or non-union may develop with the negative effect of collagen production and osteoblast proliferation, associated with increased glycolisation end-products and low insulin level. In osteoporosis, bone healing takes longer because of the decreased contact surface in cortical and cancellous bone associated with reduced bone mass. The strength and stability between bone and screws is negatively affected by reduced bone mass, in which case insufficient union or non-union can be seen and the rate of failure of fixation increases. In type 1 osteoporosis, trabecular bone is affected causing vertebral fractures or distal radius fractures. In type 2 osteoporosis, trabecular and cortical bone are affected and hip fractures occur. In previous studies, there has been observed to be a decrease in mesenchymal cell count, vascularisation, growth factors and differentiation of osteoblasts and chondrocyte cells. Diseases such as tuberculosis and gastrointestinal diseases such as non-tropical sprue causing nutritional disorders, delay fracture healing[19].

Drugs: Chondroitin sulphate and hyaluronidase have a positive effect on fracture healing. In experimental studies, L-Dopa and clonidine have been shown to positively affect fracture healing by increasing growth hormone[18]. At high doses, indomethacin is known to halt fracture healing. Some studies have shown that non-steroid anti -inflammatory drugs (NSAID) slow down fracture healing. In conditions requiring long-term corticosteroid use, such as rheumatismal joint diseases and autoimmune diseases, there is a negative effect on fracture healing. There are publications showing that quinolone group antibiotics and aminoglycosides inhibit callus formation. Anti-epileptic drugs have also been reported to negatively affect fracture healing[19].

Some Supportive Treatment Methods for Fracture Healing

Ultrasound Therapy

Treatment methods for fracture healing defined in literature include systemic and local drug administration, and physical treatments such as low-intensity laser, electromagnetic field, extra-corporeal shock, mechanical stimulation and ultrasound therapy. Of these methods, ultrasound therapy has been used in fracture healing for more than half a century. The therapeutic use of ultrasound was first described by Pohlman in 1939[22]. Despite the complex characteristics of bone healing and the multiple effects of ultrasound on biological tissues, much still remains unknown about the mechanism of the effect of ultrasound treatment. Ultrasound waves penetrate tissues and create vibrations in all tissues and components, including intracellular and extracellular fluids and damaged cells. The mechanical stimulation caused by these vibrations creates a micro massage effect in the tissues. Acoustic vibration creates a thermal and non-thermal effect in tissues. High-intensity ultrasound signals create significant heat increases. Therefore, therapeutic ultrasound for bone healing is applied at an intensity of approximately 20-50 mW/cm^2 [22]. When ultrasound signals move within the bone, a biological response develops in the tissue. The application of low-intensity pulsed ultrasound (LIPUS) stimulates aggrecan messenger RNA expression and proteoglycan synthesis by chondrocytes, and increases PDGF synthesis and prostaglandin E_2 synthesis by osteoblasts. Ultrasound signals cause conformational changes in the cell membrane and alter ionic permeability and second messenger activity. Changes in second messenger activity accelerate the fracture repair process by regulating cartilage and bone-specific genes[22]. Previous studies have also shown that the application of ultrasound increased the synthesis of cytokines related to angiogenesis such as IL-8, FGF, and VEGF[23].

There are also studies related to successful LIPUS treatment in cases with delayed union or non-union, and it is thought that in the future, this patient group could be the main indication for the treatment of bone lesions with ultrasound[22].

Bone Grafts

Bone grafts or materials which can be substituted for these are useful in the treatment of bone lesions that have occurred for various reasons. If graft is used in treatment, generally autografts and allografts are used[24]. For the treatment to be successful, the graft material to be used must have one or more of the properties of osteointegration, osteogenesis, osteoconductivity or osteoinductivity[25].

Bone grafts can be grouped as autograft, isograft, allograft, and xenograft. Autografts may be osteogenic, osteoconductive, and osteoinductive at varying degrees in the cancellous or cortical structure. Autologous cancellous grafts are known to be the most effective graft material used in bone healing and in filling the defects in bone lesions with material loss. Together with the application of autologous cancellous graft, there is also the application to the region of osteogenic bone, bone marrow cells, osteoconductive collagen, and mineral matrix, matrix proteins, and osteoconductive matrix proteins[26]. However, the limited availability of this graft may cause problems in terms of applicability[27]. There is limited capacity on the subject of conformity of autologous cortical bone grafts to the area where they are applied. As there is little porous bone tissue in cortical grafts, there are limitations to the effect of the vascular structures within the tissue and a slowing down in the healing period. In addition, they include a lower rate of osteoblastic progenitor cells compared to cancellous bone grafts. Autologous cortical bone grafts may be preferred for lesions with >5cm material loss, as they are resistant to mechanical forces and can be easily applied in bone lesions with large defects[25,28].

Allografts or allogenic grafts, which are defined as transfer made between two living organisms of the same species but with different genetic structure, can be used in the forms of fresh prepared, frozen, or dried after freezing. The use of fresh prepared allografts is very rare as there is a greater risk of immune response and infection. Frozen and freeze-dried allografts are prepared through various processes to reduce the possibility of an immune response developing. Therefore, there are no living cells in these grafts. The most common indication for allografts is to replace a large amount of lost bone tissue. Allografts can be a good alternative in joint reconstruction after trauma and to fill the defects formed after resection of tumours involving the skeletal system. As xenografts are grafts taken from a different species, the antigenicity is extremely high. This type of graft has a limited role in producing effective callus formation. Deproteinised bovine bone and heat-treated bovine cortical bone are xenograft materials commonly used in orthopaedic applications[1].

Thrombocyte Concentrate Procedures (PRP and PRF)

Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are treatment methods which have been developed in recent years, and are used to accelerate the treatment process of lesions occurring in the bone. Platelets in concentrated form prepared from peripheral blood were first presented for use in the 1990s, and since then have been increasingly used in clinical applications. Previous studies have r eported positive results from PRP and PRF as platelets are cells that

secrete active growth factor, which initiates the wound healing process, especially connective tissue healing, fracture healing, fibroblast mitogenesis, angiogenesis, macrophage activation and cell proliferation. Platelet-rich fibrin (PRF) was developed by Choukroun et al in France in 2001 as a second-generation platelet-rich biomaterial, and it was introduced to the market by Dohan et al in 2006. PRF is rich in leukocytes, cytokines, fibrin, circulating stem cells, the proinflammatory cytokines of IL-1β, IL-6, and TNF-α, the anti-inflammatory cytokines of IL-4 and IL-10, VEGF, insulin-like growth factor-I and insulin-like growth factor-II, and PDGF[29]. The successful preparation of PRF depends on the blood withdrawn and the centrifugation rate. In a PRF procedure applied slowly, fibrin polymerisation occurs resulting in a fibrin clot smaller than required. Unlike PRP, PRF contains a fibrin clot matrix (PRF-M) and this can not be immediately broken down, but forms slowly similar to the blood clotting process. Various studies have reported that PRF-M has shown regeneration potential in bone and soft tissue without an inflammatory reaction forming[30,31].

Stem Cell Treatment

Stem cells are cell types with the properties of the ability for unlimited proliferation, regeneration, differentiation from other cells, and the repair of damaged tissue. They are preferred in regenerative type applications as they can be easily obtained, and can be safely and effectively transplanted to autologous and allogenic recipients. Stem cells are named totipotent, pluripotent, multipotent, oligopotent, or unipotent according to the differentiation capacity[32]. According to the place from where they are obtained, they can be examined in 3 groups as embryonal (fetal), adult (postnatal), and cells produced with genetic manipulation from somatic cells. Embryonic stem cells are totipotent and pluripotent cells with the capacity to create all tissues. Adult stem cells are cells found in undifferentiated form in a differentiated tissue, which can regenerate and transform to specific cells of the organ from which they originated. These cells are known as somatic stem cells and are multipotent cells which can convert to cell types of other tissues under special conditions[33]. The subjects of most studies of multipotent adult stem cells are hematopoietic and mesenchymal stem cells. In addition to the capacity to convert to different cell types such as bone, cartilage, and muscle, mesenchymal stem cells have the potential for immune suppression and a trophic effect as they produce several growth factors and cytokines[34,35].

In vitro studies of mesenchymal stem cells have shown the osteogenic potential, and in vivo studies have shown contributions to osteoblastic activities and bone formation. In a study by Taguchi et al . (2005), following fractures created in the femur of mice, a significant increase was seen in callus tissue formed associated with the administration of bone marrow cells to the tail vein and this was reported to be definitely related to mesenchymal stem cells[36]. In another study of tibia fractures created in rats, mesenchymal stem cells were found to contribute to callus formation through the expression of BMP-2[37]. In a femur fracture model in rats, Yörükoğlu et al applied stem cell membrane around the fracture line. As a result of macroscopic and radiological examinations, this was shown to have effects increasing fracture healing. Thus it was demonstrated that by creating a membrane, mesenchymal stem cells can be applied to regions of tissue damage without the need for scaffolds[38].

Gene Therapy

Recombinant human bone morphogenetic proteins (rhBMP) have recently been shown to be a bone graft substitute. There is evidence that BMPs are more effective than autograft in supporting fracture healing and spinal fusion. A high dose of growth factor is needed for the formation of sufficient callus. Currently, rhBMP is applied at doses greater than one million times the normal concentration in the bone. Concerns about both the cost and safety of these types of supraphysiological doses have directed the search for an alternative solution in application[39].

Gene therapy makes possible the ex vivo or in vivo transfer of nucleic acid materials (DNA or RNA) encoding growth factors into the cells in the fracture region[40]. This provides localised, continuous, and physiological production of osteogenic proteins such as TGF-β1, LMP-1, and BMP-2, which are effective in bone healing. The duration of the production of osteogenic proteins is largely dependent on the gene transfer technique used[39,41,42]. Therefore, both short-term and long-term protein production is possible. Short-term protein production is sufficient in most applications supporting bone fracture healing, but long-term protein production may be necessary for the treatment of larger bone lesions[39]. It is thought that gene therapy can increase fracture healing and spinal fusion rates by increasing the systemic or local bone production[41,42]. In the future, regional gene therapy may be a part of a comprehensive tissue engineering strategy which will include treatment options such as autologous bone grafting, the use of recombinant growth factors, autologous bone marrow injection, and stem cell treatments[39].

Plant Extracts

In recent years, the use of plants and natural products with antioxidant properties has become more popular and is accepted throughout the world as an alternative treatment method. In addition to the fixation of fragments, the systemic or local use of medicinal plants with antiseptic, antioxidant, anti-inflammatory, antimicrobial, and biostimulating properties has come to the fore for the stimulation of callus formation. These plants accelerate tissue healing by providing basic substances which are necessary at different regeneration and proliferation stages[43]. In a study of propolis by Karaman (2009), propolis was reported to have positive effects on fracture healing both radiologically and in terms of callus quality, associated with duration of use[44]. Ankaferd, which has anti-oxidant, hemostatic, anti-atherosclerotic, anti-tumoral, anti-inflammatory, and vasodilator effects, is obtained as a mixture of natural plants and has been reported to have the effect of increasing healing in the early stages of fracture healing[45]. In a 2018 study by Görmez et al, the effects on the process of fracture healing were evaluated of cotton seed oil, which is a natural plant molecule. The evaluation showed that cotton seed had a significant antioxidant effect in the early stage of fracture healing, radiological improvement was determined, and histologically, it was reported to have contributed to the whole healing process[21].

Biomaterials

The application of autogenous cancellous bone graft is the accepted gold standard treatment method in bone lesions with substance loss because of the osteogenic, osteoconductive, and osteoinductive properties. However the use of autologous bone has significant disadvantages such as difficulties of supply and variations in quality, hematoma, infection, prolonged operating time and bleeding, chronic pain in the donor region, and additonal costs. Therefore, some researchers have developed synthetic scaffolds to support osteoconduction for bone regeneration and to mimic the

physical and mechanical structure of the natural tissue. These scaffolds, which aim to protect living cell populations, are produced from various materials, primarily natural and synthetic polymers, ceramics, metals, and composites[46].

Polymers: Polymers can be natural or synthetic. Natural polymers, which can be broken down biologically, such as type-I collagen, fibrin, hyaluronic acid, and chitosan, have high biocompatibility and osteoconductive properties. Synthetic polymers, which can be broken down biologically, such as polyanhydrite, polypropylene, fumarate, polycaprolactone, polyphosphazene, polylactite, polyglycolide and related co-polymers such as polylactite-co-glycolide, are commonly used scaffolding materials in tissue engineering studies[46].

Ceramics: Ceramics are generally used as bone matrix in bone healing. Studies in the field of ceramic biomaterials have intensified on materials such as tricalcium phosphate from calcium-based ceramics, hydroxyapatite, calcium sulphate, and bioactive glass. As the inorganic component of the bone is formed of ceramic calcium hydroxyapatite, calcium phosphate in particular is an ideal candidate for use in bone healing. The absorption rates of calcium phosphate ceramics vary in inverse proportion to the calcium/phosphate ratio and also depend on the density, particle size, and porosity. Calcium phosphate and bioactive glass are accepted as biomimetic as they stimulate the formation, precipitation and deposition of calcium phosphate from solution. In addition, materials such as calcium sulphate have the potential to be used as transport materials in drug applications because of the high binding affinities between ceramics and proteins[46,47].

Metals: Scaffolds produced from metallic materials with a porous structure such as titanium are a relatively new biomaterial class with an area of use in clinical applications. Metallic scaffolds with a porous structure mimic trabecular bone which has an interdependent porous structure. Currently, titanium has a wide area of use as a metallic biomaterial, as it has high biocompatibility and is extremely resistant to corrosion. This material which is produced in a structure very similar to that of trabecular bone, cannot be broken down biologically, and can be prepared in different forms and tissues without affecting the biocompatibility. However, the bioinert character of the naturally occurring protective surface oxide does not allow the material to form an interface easily and strongly with the surrounding tissue. Moreover, as the structure of titanium is harder than that of the bone surrounding the material, this may lead to implant loosening. These types of metallic biomaterials are used to cover prosthetic implant surfaces to support implant stability and bone growth[48].

Composites: Composites are formed from the combination of materials with different properties. Therefore, benefit can be gained from the advantages of other materials to optimise the class of the material. For example, combinations of bioactive ceramics such as calcium phosphate with polymers improve the mechanical properties of the scaffolds formed. Or the addition of polymers to ceramics reduces the fragility of ceramics, whereas the addition of ceramics to polymers both increases the bioactivity of the material and increases the capacity of the effect on factor or therapeutic agents. The combination of natural polymers with calcium sulphate ceramics can increase the mechanical stability of these. In some recent studies, titanium surfaces have been combined with hydroxyapatite to decrease the bioinert character of the metal and increase bone osteointegration[46].

Conclusion

There has been a great increase in studies of bone healing in recent years and as a result of these studies, several new methods have been developed. Nevertheless, however many treatment options are defined, there are still problems related to the rapid and effective treatment of lesions occurring in bone tissue, which is a hard, living tissue formed from active cells. For the rapid and effective treatment of lesions in this tissue, it is vitally important to understand all the stages of the regeneration process and apply the correct treatment technique.

Conflict of interest

The authors declare that they have no conflict of interest.

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