Future of Bone Repair

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ABSTRACT: Bone can suffer from various conditions such as fractures and diseases such as osteoporosis, osteogenesis imperfecta and tumors. Osteoporosis globally causes >8.9 million fractures each year. Current epidemiological data relevant to this and other diseases urge us to focus critically on promising and efficacious treatments for bone injury. Because of the limitations of conventional treatments for bone fracture, such as limited quantity for autograft, there is a demand to investigate better alternatives for bone healing. The main aim of this review is to highlight repair of bone injury, particularly focusing on several new research methods studied in preclinical trials and in vitro. New research methods such as low-level laser therapy, mesenchymal stem cell-based therapy, nanomaterials, biodegradable hydrogels, extracellular matrix-mimetic materials, and controlled delivery of growth factors from polymer scaffolds look promising for bone healing, and further clinical studies are suggested that use them in routine bone repair treatment in the near future.

KEYWORDS: fracture, bone healing, osteoblast, nanomaterials, growth factors, scaffolds

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Introduction

Biologically, bone is a living tissue made mostly of collagen and performs various functions within the body.[1] The collagen in the bone is a protein, which provides a soft framework, with minerals like calcium phosphate strengthening and hardening this framework.[2] Because of the combination of calcium phosphate and collagen, bone is strong and flexible enough to resist stress.[3] Bone is also involved in the homeostatic regulation of ions in the circulating fluids of the body.[4] It provides structural support and helps in maintaining the acid–base balance by absorbing or releasing alkaline salts that buffer the blood against excessive pH changes.[5] Being a living tissue, bone needs a constant supply of nutrients and oxygen, and, therefore, there is a limit to the size of a defect or fracture it is able to restore to healthy working tissue.[6] Consequently, bone can suffer from a number of pathological conditions like cancer, and is likely to degenerate as a result of aging and disease, for example, osteoporosis.[7]

Several bone diseases can occur, such as osteogenesis imperfecta, osteochondroma, osteoporosis, etc., and the most common occurrence is bone fracture. Bone fracture is a condition in which there is a disruption in the continuity of the bone. It can be a result of a large applied force or impact, or a marginal trauma injury as a result of some conditions that weaken the bones, such as bone cancer, osteogenesis imperfecta, or osteoporosis.

Classification of Bone Injury

Bone can suffer from various pathological conditions such as the following:

- Bone can develop infections and cancer;
- Osteoporosis and low bone density can make bones weak and liable to breaking;
- In osteogenesis imperfecta, bones become brittle;
- In Paget's disease, bone become weak;
- Other diseases of bone are caused by genetic factors, poor nutrition, or problems associated with bone growth rate or rebuilding, making the bone more easy to break.

Epidemiology

The prevalence of osteoporosis-related fractures includes aspects linked to the underlying osteoporosis and also those linked to injury, such as falling and age. The incidence of vertebral fracture increases sharply from 50 years of age and thereafter, whereas hip fracture increases from 70 years above.[8] Most of the fractures are osteoporotic, where the risk of fracture increases with decrease in bone density, with the few exceptions being fractures of fingers, toes, and skull.[9] Globally, osteoporosis causes >8.9 million fractures each year, resulting in an osteoporotic fracture every 3 seconds.[10] Also, it is estimated that by the year of 2050 the global incidence of hip fracture will increase by 310% in men and 240% in women.[11] The progressive loss of bone and increased risk of falling are two of the factors responsible for the growing risk of fracture with advancing age. Though traumatic features, such as those related to motor vehicle accidents, could be more usual in people with lower bone mass, these are commonly not considered to be osteoporotic.[12] Also, it is observed that, in most parts of the world, women have a greater risk of fractures than men.[8] One of the extremely important predictors of the

risk of future fractures is a history of prior fractures.[13] A past record of vertebral fracture increases the risk of a following spine fracture by 5 times and occurrence of non-spine fracture by 2 times.[14,15]

Pathophysiology

The process of bone fracture healing has three phases that enable the protection and proliferation of the areas surrounding dislocations and fractures. These three phases are 1) the reactive phase, 2) the reparative phase and 3) the remodeling phase.[16] In the reactive phase, which happens after the fracture, there is presence of blood cells inside the tissues neighboring the injury site and this initial change is seen by electron and light microscope. After the fracture, bleeding stops as the blood vessels constrict. After a few hours, a blood clot, known as hematoma, is formed. All the cells within hematoma degenerate and die. Some of the cells that are adjacent to the site of injury but outside the blood clot also degenerate and die. The fibroblasts survive in this area and replicate. These fibroblasts build loose aggregates of cells scattered with small blood vessels, identified as the granulation tissue.[17] Also, there is migration of the mesenchymal cells and ingrowth of vascular tissue. Then the reparative phase starts, where there is formation of cartilage callus and lamellar bone deposition.[18] Many days after the fracture, periosteum cells start replicating and transforming. Some of these cells that are closest to the fracture gap start developing into chondroblasts that later on form the hyaline cartilage. The fibroblasts, which are present within granulation tissue, also develop into chondroblasts, further forming hyaline cartilage. These new tissues continue to grow in size till they start uniting with their counterparts from the other parts of fracture. Such processes culminate in the formation of a new mass of heterogeneous tissue, which is known as the fracture callus. Ultimately, the fracture gap is linked by woven bone and hyaline cartilage, and some of the original strength is restored. Subsequently, there is replacement of woven bone and hyaline cartilage with lamellar bone, and this process of replacement is called bony substitution with reference to woven bone and endochondral ossification with reference to hyaline cartilage. The formation of lamellar bone is seen after the collagen matrix of either tissue becomes mineralized. This mineralized matrix starts penetrating by channels, and each contains numerous osteoblasts and a microvessel. There is formation of new lamellar bone from the osteoblasts, which is in the form of the trabecular bone. Subsequently, the trabecular bone replaces the woven bone and cartilage of the original fracture callus. The compact bone substitutes the trabecular bone in the remodeling phase. This phase takes 3–5 years depending on factors such as the general condition and age.[19] There are three main complications for fracture healing. In delayed union, there is infection or poor blood supply; in non-union, wound contamination or bone loss is found; and in fibrous union, there is improper immobilization.[20] Therefore, before initiating any new research idea for the treatment of bone injury, it is important to keep these complications in mind.

Diagnosis

A fracture or other bone injuries can be diagnosed based on the physical examination and the history given. To view the fractured bone, imaging by X-ray is performed. Also, in some situations where X-ray alone is not sufficient, CT scan or MRI is performed.

Conventional Treatments

The treatment for bone fracture is classified as surgical or conservative, where conservative treatment includes pain management, nonsurgical stabilization, and immobilization. For pain management for arm fractures in children, ibuprofen is as effective as a combination of codeine and acetaminophen.[21] In immobilization, the fractured pieces of bones are aligned to their natural positions, called reduction, and X-ray is used to verify the position, but this method of treatment is very painful without anesthesia. Surgical methods are used only when conservative treatment fails, but they have their own risks and benefits. Due to the nature of recrudescence of bone infection, infection is particularly dangerous in bones. Bone tissue is extracellular matrix, and the few blood vessels required to maintain this low metabolism are able to bring only a limited number of immune cells to an injury site to fight the infection. Therefore, osteotomies and open fractures require prophylactic antibiotics and very careful antiseptic measures.[22] Occasionally, bone grafting is also used, which is a surgical method that replaces the missing bone so as to repair fractures that are very complex. This procedure enhances bone's ability to regenerate itself by using methods such as autologous, allograft, or synthetic variants. But this method also has limitations like related chronic donor site pain and the limited quantity available for autograft.[23] These limitations of the conventional treatments urge us to look for more promising, newer ways of healing that will have a shorter treatment course (eg, single-pulsed electromagnetic field) and will accelerate the bone fracture healing process (eg, low-level laser therapy).

Potential Future Treatments

Several risks and disadvantages of conventional treatments have led to the development of new potential research ideas for bone injury repair and have been carried out in preclinical studies or in vitro. These new methods look promising and should be further studied to use them as a regular way of treatment for bone repair.

Extracellular Matrix-mimetic Biomaterials[24]

Failure of implants such as bone screws, bone grafts, and arthroplasties is caused by the limited osseointegration of currently

Table 1. Summary of different new research methods of treatment for bone injury.

SR. NO.	MATERIALS	PROPERTIES AND USES	STUDIED IN	ADVANTAGES	REFERENCES
1.	Extracellular matrix-mimetic biomaterials	Adhesive, short peptide sequences, modulates responses of host cell-implant material to augment implant osseointegration and formation of bone	*In vitro*	No low solubility/large cost to extract and purify as by use of full-length natural extracellular matrix polymers	24
2.	Controlled delivery of growth factors (BMP-2) from polymer scaffolds	Strong osteo-inductive activity, long half-life	Mouse calvarial defect model	Overcomes problems like high cost and protein stability, which are associated with growth factor-loaded scaffolds. Longer half-life	51
3.	Cell therapy (Cultured MSCs)	Can differentiate into osteogenic cells	*In vitro* MSC culturing	Easily administered alone via percutaneous injection/ also can be implanted during an open surgery with biomaterials. Large quantity can be obtained unlike the natural one that is available from iliac-crest	62
4.	Low-level Laser Therapy	Stimulation of cells and molecules of body, improves the expression of osteogenic factors	Rat calvarial cells, *in vitro*, rat tibia	Accelerates the bone fracture healing process, causes callus increase in bone mineral density and volume	66–73
5.	Incorporation of Platelet-rich plasma into PLGA/CPC composite scaffold	CPC is extremely osteoconductive, PLGA on the scaffold enhances the mechanical properties of bioceramic scaffolds	Radial and femoral defects in rabbit model, *in vitro*	Enhances *in vitro* cell response like cell attachment, cell proliferation, and cell differentiation, improves bone formation and angiogenesis	74–80
5.	Biodegradable hydrogels (glycol-chitosan)	Enzymatic degradability, aqueous solubility, antibacterial activity biocompatibility	Mouse calvarial defect model	Locally releases BMP-2 in a bioactive form, by incorporating statin in the injectable gel, the bioactivity of hydrogel increases	81
6.	Administration of Sclerostin Antibody	Sclerostin neutralizing antibody exerts anabolic effect during bone fracture healing	Critical-sized femoral defect rat model, hindlimb-immobilization rat model, rat model of postmenopausal osteoporosis	Increases bone formation	82–98
7.	Nanomaterials	Nanostructured scaffolds regulates cell proliferation, cell differentiation, and migration, Nanomaterials increases surface area and wettability	Female adult sheep, noncritical-size calvarial defects in rabbits, rabbit subcutaneous and bilateral femoral defect model, calvarial bone defect rat model	Increased protein adsorption in comparison to conventional biomaterials	99–104
8.	Single-pulsed electromagnetic field	Osteogenic effects, improves bone repair and cell growth	*In vitro*, necrotic bone mice model	Shorter treatment course, short daily application, quickens osteogenic differentiation of hBMSCs	105–109

available orthopedic biomaterials, which present a great socioeconomic cost. The use of full-length natural extracellular matrix (ECM) polymers has several disadvantages, such as low solubility, high cost to extract and purify it in large quantities, suffering from batch-to-batch variation, and potentially suffering from immunogenicity. Additionally, it is a great challenge to alter, characterize, and maintain the presentation of natural ECM polymers. This emphasizes the need for adhesive biomaterials that mimic the extracellular matrix and which will modulate responses of host cell–implant material to augment implant osseointegration and formation of bone. Osteoprogenitors and osteoblasts play an important role in complex processes like host reactions to implants and bone remodeling. The protein signals identified by the osteoprogenitor and osteoblast receptors found on the implant surface significantly influence the host responses to implants. Only a few short peptide sequences out of the thousands of amino acids found in natural ECM polymers help in integrin recognition and binding sequences that trigger other responses like adhesion, signaling, and distribution. Because of this, short peptide sequences such as GFOGER,[25,26] RGD,[27]

PHSRN,[28] REDV,[29] and LDV[30] with ECM-derived protein fragment such as FNIII7-10 are utilized to biofunctionalize bone tissue engineering scaffolds and titanium surfaces. Also, the structure and conformation of the ligand is a vital factor in their capacity to bind to the integrin receptors as well as to trigger the signaling pathways. Covalent immobilization and simple adsorption onto titanium surfaces are two of the common methods of protein fragment functionalization for titanium implants. RGD is an adhesive protein sequence that is found on many ECM molecules and can bind to several integrins, though for some integrins binding to RGD is greatly controlled by additional sequences like the PHSRN synergy site for $\alpha 5\beta 1$.[31,32] Many biomaterial approaches have used RGD as an adhesive ligand because it serves as a potent binding site. But according to different studies performed, RGD does not stimulate bone formation and bone repair in vivo.[33] Also, fibronectin-mimetic peptide fragments such as FNIII7-10 improve both osteoblast differentiation and adhesion strength.[34] Other ECM-derived proteins that have been found to improve osteoblast differentiation and adhesion strength in vitro include KRSR, which is a heparin binding site (HBP) located on several ECM proteins;[35–40] FHRRIKA, which results from the HBP of bone sialoprotein (BSP);[35,41–44] an osteopontin-derived peptide;[45] HBP12;[46] and the human vitronectin peptide.[47–50] Though these ECM-derived protein fragments have shown potential as bone materials in vitro, many more studies need to be carried out to validate their osteogenic capacity in vivo also.

Controlled Delivery of Growth Factors from Polymer Scaffolds[51]

Natural bone repair process can be stimulated by using growth factors in bone tissue engineering.[52] The use of a localized and sustained delivery approach can overcome problems like high cost and protein stability, which are associated with growth factor-loaded scaffolds.[53] Because of the controlled delivery profile of growth factors in scaffolds, cells can migrate to the area of the defect, proliferate, and differentiate, boosting tissue repair.[54] Bone morphogenetic proteins (BMPs), which are involved in maintaining differentiation processes of a variety of cells during fracture repair and skeletal development, are protein members of transforming growth factor-β superfamily.[55,56] BMP-2 proteins have strong osteo-inductive activity, but they exhibit a short half-life in vivo of 7 minutes.[57–61] Therefore, sustained and controlled delivery of BMP-2 to use as scaffold has been suggested. It was observed that, on average, 70% of BMP-2 into the scaffold was released in a mouse calvarial defect model by the end of 3 weeks. BMP-2 was shown to be active, and there was substantial increase (55%) in the new bone volume. Conversely, only 31% increase in new bone volume was found in scaffolds without BMP-2 in comparison to empty defect controls, suggesting the potential of novel scaffolds for sustained and controlled BMP-2 delivery for bone-regeneration purposes.

Cell Therapy[62]

Autologous bone grafting is preferred when the natural bone repair mechanism fails to work. Bone matrix and osteogenic cells in the graft provide the osteo-conductive and osteo-inductive activity required for proper bone repair. Bone marrow mesenchymal stem cells (MSCs) are able to differentiate into osteogenic cells. Treatment by MSC-based cell therapy has shown potential to enhance bone repair. The quantity of MSCs that is available from iliac-crest aspirates is very small to be useful clinically. Therefore, either culture or concentration must be used to increase the MSC population.[63] These MSCs can be easily administered alone via percutaneous injection, or can be implanted during an open surgery with biomaterials.[64] Patients with avascular necrosis of femoral head or delayed repair of long bone fractures have shown encouraging preliminary results.[65] In vitro MSC culturing on specific biomaterials such as β-calcium triphosphate granules or biphasic hydroxyapatite is used to obtain colonization of the biomaterials and cell differentiation. After that, the biomaterial–cell construct is implanted into the zone that is to be treated. As there are challenges to promoting implant vascularization and increasing cell survival, much work still remains to be done before knowing that this method is appropriate for the regular filling of bone tissue defects.

Low-level Laser Therapy

Low-level laser therapy (LLLT) is a technique that supplies biostimulative light energy to cells of the body. The light that is absorbed leads to the stimulation of cells and molecules of the body.[66] LLLT has shown potential for its positive effects on fracture repair and bone metabolism.[67–70] This therapy improves the expression of osteogenic factors in the bone repair process.[71] Moreover, it also accelerates the bone fracture healing process and causes callus increase in bone mineral density and volume.[72,73] Though LLLT seems to have great advantages, the biomechanical properties of bones do not show any improvement.[71] Therefore, further research on the LLLT is suggested to prove its efficiency.

Incorporation of Platelet-rich Plasma into PLGA/CPC Composite Scaffold[74]

Calcium phosphate cement (CPC) is extremely osteoconductive and biocompatible, as has been demonstrated by the rapid deposition of new bone on the CPC surface.[75,76] Coating a polymer such as poly(lactic-*co*-glycolic acid) (PLGA) on the scaffold surface has proven to be a successful approach to enhance the mechanical properties of bioceramic scaffolds.[77–80] The incorporation of platelet-rich plasma (PRP) into a PLGA/CPC scaffold with unidirectional pore structure has shown positive effects in improving bone repair of radial and femoral defects in a rabbit model. It was observed that the introduction of PRP into PLGA/CPC scaffold enhanced in vitro cell responses such as cell attachment, cell proliferation, and cell differentiation. It also boosted bone formation and angiogenesis.

Therefore, this scaffold with a unidirectional pore structure seems to be a potential candidate for bone repair.

Biodegradable Hydrogels[81]

Hydrogels, which are biodegradable and injectable, have proven to be effective candidates as cell delivery vehicles to sustain tissue regeneration. Glycol–chitosan has several intrinsic properties such as enzymatic degradability, aqueous solubility, antibacterial activity, and biocompatibility. Because of such properties, glycol–chitosan is one of the most preferred natural scaffolds for bone tissue engineering. This gel has been observed to have the ability to locally release BMP-2 in a bioactive form to stimulate bone formation at the implantation site. Moreover, it has also been demonstrated that by incorporating statin in the injectable gel, the bioactivity of hydrogel increases. Therefore, the incorporation of statin in the injectable glycol–chitosan seems to be a potential way in the process of bone repair.

Administration of Sclerostin Antibody[82]

The glycoprotein sclerostin is expressed by osteocytes and acts as a negative regulator of bone formation and osteoblast development.[83,84] Sclerosteosis and Van Buchem disease are caused by mutations in the gene coding for sclerostin and are described by bone thickening and high bone mass due to amplified bone formation.[85–88] Although the mechanism for inhibition of bone formation by sclerostin is still under investigation, it has been suggested that sclerostin inhibits the canonical Wnt signaling pathway and/or BMP pathway by modulating their receptors.[89–94] The preclinical studies performed in models of osteoporosis, as well as a clinical trial, have shown that systemic administration of the sclerostin neutralizing antibody increases bone formation and prevents bone loss.[95–98] It was also observed that systemic administration of the sclerostin neutralizing antibody leads to increased bone formation and enhances bone repair in a critical-sized femoral defect in a rat model.

Nanomaterials[99]

Nanostructured scaffolds regulate cell proliferation, cell differentiation, and migration, resulting in the formation of functional tissues. They also provide cells with structural support. Nanomaterials possess unique properties such as increased surface area and wettability, which result in increased protein adsorption in comparison to conventional biomaterials. Nanocomposites such as collagen/hydroxyapatite (HA) has three-gradient multilayer scaffolds, which are made of assembled collagen fibers with/without HA. This nanocomposite finds application in the repair of osteochondrial defects, as has been demonstrated in female adult sheep.[100] Also, the poly(lactic-*co*-glycolic acid)/tricalcium phosphate (PLGA/TCP) composite possesses unique properties like flexibility and mouldability, and has been shown to heal circular noncritical-size calvarial defects in rabbits.[101] The poly(propylene fumarate)/propylene fumarate diacrylate/carbon nanotube (PPF/PF-DA/CNT) composite has been demonstrated to heal rabbit subcutaneous and bilateral femoral defects.[102] Nanomaterials such as poly-L-lactic acid (PLLA) as a nanofibrous scaffold have found application in healing a critical-sized calvarial bone defect in rats.[103] Nanofibrous PLLA membrane with a collagenous-guided bone renewal membrane, ie, a bilayer membrane, was able to heal a defect in the anteromedial cortex of the proximal tibia in rabbits.[104] Ultimately, novel strategies that combine nanoscale properties and various compositions could be established in the near future.

Single-pulsed Electromagnetic Field

Pulsed electromagnetic field (PEMF) has been confirmed to have osteogenic effects for treatment of bone fractures.[105–108] But the main disadvantage of PEMF treatment is time utilization, which is a minimum of 10 h/day for the treatment duration, as suggested by the U.S. FDA (Federal Drug Administration). So there was a search for an efficient model for PEMF treatment. In a recent study, there was modification as a single-pulsed electromagnetic field (SPEMF), which required only a 3-minute daily treatment.[109] In an in vitro study, osteogenic differentiation and cell proliferation were observed in human bone marrow mesenchymal stem cells (hBMSCs), whereas in vitro revascularization and new bone formation were evaluated. There was no significant cytotoxic effect of SPEMF on hBMSCs in the in vitro study. Also, there was increase in osteogenic differentiation of hBMSCs after 3–7 days of treatment. Mineralization also increased after 10, 15, 20, and 25 days of SPEMF treatment. The study demonstrated that a 7-day short course has similar results on osteogenesis and proliferation as the 25-day SPEMF treatment.[109] This suggested that this novel SPEMF treatment quickens osteogenic differentiation of hBMSCs and improves bone repair and cell growth in the necrotic bone in mice. Therefore, there is a potential advantage of SPEMF due to shorter treatment course and short daily application. Also, it is a superior treatment to inductive coupling, where the disadvantage is the need for cooperation between the patient and the treating physician, as patient noncompliance may occur because of the heavy weight of the different units used in it. Additionally, SPEMF is also a better treatment than capacitive coupling, where the disadvantage that the units, although lightweight and small, can cause irritation to the skin from the electrodes.

Conclusion

Bone repair is a complex process, and the current conventional methods have their own disadvantages. Due to the increasing prevalence of bone injuries, there is a need to find a better alternative for bone repair. The current new approaches are studied in animal models or in vitro studies, and have shown to be efficient by demonstrating several advantages over the conventional treatments. The advantages of potential future treatments, such as shorter treatment course, low cost, and

increase in the rate of osteogenesis, will accelerate the bone-healing process and eventually decrease the prevalence of bone injuries. Therefore, future work needs to be done in direction of bone repair. And these new methods are to be studied in clinical trials as well to make them routine in the treatment of bone injuries.

Author Contributions

Conceived and designed the experiments: RS and GK. Analyzed the data: RS and GK. Wrote the first draft of the manuscript: RS and GK. Contributed to the writing of the manuscript: RS and GK. Agree with manuscript results and conclusions: RS and GK. Jointly developed the structure and arguments for the paper: RS and GK. Made critical revisions and approved final version: RS and GK. Both authors reviewed and approved of the final manuscript.

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