Journal of Pharmaceutical Research International

32(34): 116-125, 2020; Article no.JPRI.62795
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Prospective Aspects of Regeneration in Orthopaedics: A Review

Amit Lakhani[1] and Ena Sharma[2*]

[1]Department of Orthopedics, Maharishi Markandeshwar Medical College and Hospital, Solan, India.
[2]Department of Periodontics, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana Ambala, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author AL wrote the protocol and managed the literature searches. Author ES wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3430971
Editor(s):
(1) Dr. Mohamed Fathy, Assiut University, Egypt.
Reviewers:
(1) Dennis Uba Donald, Adekunle Ajasin University, Nigeria.
(2) Bhanu K. Patibandla, Oregon Health & Science University, USA.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62795

Review Article

Received 22 September 2020
Accepted 27 November 2020
Published 12 December 2020

ABSTRACT

Musculoskeletal diseases affect millions of people worldwide and are one of the leading causes of long-term pain and physical disability. Traditional treatment methods for promoting healing and repair has always been consider gold standard, But the emergence of new therapeutic approaches aims to regenerate or repair musculoskeletal tissue. The recognition of a regenerative therapy in orthopaedics requires the demonstration of new Bone, Cartilage, ligament, tendons, healing of soft tissues injuries and Overuse conditions like plantar fasciitis or tennis elbow . Regenerative therapy boosts the body's ability to use its repair systems to heal diseased or damaged cells after a severe injury, or other degenerative condition. A diversity of regenerative strategies have been evaluated, including distraction osteogenesis, bone grafts and bone substitute materials, bone matrix proteins, growth/differentiation factors, combined therapies and, more recently, tissue-engineering approaches. This review aims to evaluate the current status of the therapies available and to discuss the challenges that must be faced in order to achieve predictable orthopaedic regeneration in clinical practice.

**Corresponding author: E-mail: dr.sharma_ena@yahoo.co.in;*

Keywords: Musculoskeletal diseases; regenerative therapy; cartilage; orthopedics.

1. INTRODUCTION

When injury occurs to muscular tissue and bone, the body tries to heal itself i.e. the injury through its own repair mechanisms. However, in some situations (especially in areas where there is lack of blood flow – such as inside and around the joints), the body cannot heal itself adequately. This can lead to continuing pain, disability and swelling permanent damage of the joint. With the concept of regenerative medicine, one can understand the natural healing process of the body or even "grow back" the damaged tissues.

Regeneration is defined as the reconstruction or reproduction of a lost or injured part in such a way that the architecture and function of the lost or injured tissues are completely restored.

Regenerative medicine is the emerging branch of medicine that tries to change and interfere with the course of chronic diseases. This branch has quickly become one of the promising treatment options for the patients with long-standing and non-healing tissues failures.

The field of orthopaedic surgery and traumatology has established significantly in the last century with the emergence of new surgical approaches, devices and products. Despite these advances there are still many pathological conditions of the musculoskeletal system as a result of congenital deformity, disease, injury or malignant process that are very difficult to treat by available standard therapeutic techniques and these often leads to unsatisfactory results.

To understand and manage such issues, regenerative medicine and tissue engineering has emerged as an important field of research and is paving the way for new developments in this zone.

In general, regenerative medicine provides promising approaches for successful repair or replacement of damaged tissues [1].

The objective of regenerative treatments in orthopaedics is to either encourage the regeneration of healthy tissue at the site of injury itself or grow new tissue outside the body (in a bioreactor, for instance) for implantation into the defect site at a later date.

Regenerative medicine has shown great potential for the effective treatment of various disabling orthopaedic disorders. Major research is going on bone healing, where various osteoconductive molecules, stem cells, gene therapies have shown possibly beneficial role. Genetic disorders like osteogenesis imperfecta are also being explored for an effective cure through regenerative medicine. Regenerative medicine has also shown a capable future treatment modality for spinal cord injury. Various inflammatory disorders like osteoarthritis and rheumatoid arthritis can also be treated through regenerative medicine. A significant opportunity exists to improve the cancer therapy beyond the capabilities of traditional cancer treatments such as chemotherapy and radiation.

Regenerative medicine is re-shaping these new therapies through the integration of its gene therapy, small molecule drug discovery and protein therapeutic capabilities.

2. HISTORY OF ORTHOPAEDIC REGENERATION

The development of metallic engineering in the last century produced various biocompatible alloys including stainless steel, cobalt chrome and titanium, which revolutionized fracture care. But due to marked difference in elasticity as compared to bone and ligaments, they can cause nonunion, pseudoartherosis and hypersensitivity.

Back in the 1950s, Professor Sir Charnley, a pioneer British orthopaedic surgeon, stated that 'practically all classical operations of surgery have now been explored, and unless some revolutionary discovery is made which will put the control of osteogenesis in the surgeon's power, no great advance is likely to come from modification of their detail' [2]. Since that time, the understanding of regeneration at cellular and molecular level has advanced immensely and still going on. The concept of orthopaedics regeneration came forward in the year 2000.

Regeneration of the skeletal system similar to many other organs requires a morphogenetic signals, mesenchymal cells and matrices or scaffolds. Besides, mechanical stimuli are of additional importance as they believe to induce morphogenic signals.

In the past, major efforts were expanded in developing traditional tissue engineering technologies for growing new tissue extra corporally before implantation. These usually

involved harvesting autologous cells from the patient, expanding them in culture, seeding them onto a scaffold, and incubating them in a bioreactor. Considerable success has been logged by this approach [3]. There is increasing interest in the use of technologies that do not require ex-vivo cultivation of autologous cells for each patient or more than one invasive procedure. This can be achieved with allograft cells, rapid isolation and manipulation techniques that can be used intraoperatively, or by provoking and facilitating endogenous repair processes [4].

3. BONE REGENERATION

Bone regeneration comprises of a well-orchestrated series of biological events of osseous induction and formation involving numerous cell types, intracellular and extracellular molecular signalling pathways with a definable temporal and spatial sequence, in an effort to optimise the skeletal repair and restore skeletal function [5].

Standard approaches widely used in clinical practice to augment bone regeneration includes distraction osteogenesis and the use of a number of different bone-grafting methods, such as autologous bone grafts, allografts, and bone-graft substitutes or growth factors [6].

During distraction osteogenesis, bone regeneration is induced between the gradually distracted osseous surfaces. A variety of methods are currently used to treat bone loss or limb-length discrepancies and deformities, including Rail fixators and the Ilizarov technique, combined undreamed intramedullary nails with external monorail distraction devices, or intramedullary lengthening devices. However, these methods are technically challenging and have several disadvantages.

Bone grafting is a commonly performed surgical procedure to augment bone regeneration in a variety of orthopaedic and maxillofacial procedures, with autologous bone being considered as the 'gold standard' bone-grafting material, as it combines all properties required in a bone graft material: osteoinduction, bone morphogenetic proteins (BMPs) and other growth factors, osteogenesis (osteoprogenitor cells) and osteoconduction (scaffold) [7]. It can also be harvested as a tricortical graft for structural support, or as a vascularised bone graft for restoration of large bone defects or avascular necrosis.

Bone-graft substitutes have also been developed as alternatives to autologous or allogeneic bone grafts. They consist of scaffolds made of synthetic or natural biomaterials that promote the migration, proliferation and differentiation of bone cells for bone regeneration. A wide range of biomaterials and synthetic bone substitutes are currently used as scaffolds, including collagen, hydroxyapatite(HA), b-tricalcium phosphate (b-TCP) and calcium-phosphate cements, and glass ceramics [8], and the research into this field is still on-going. Specifically for reconstruction of large bone defects, for which there is a need for a substantial structural scaffold, an alternative to massive cortical autograft or allografts is the use of cylindrical metallic or titanium mesh cages as a scaffold combined with cancellous bone allograft, DBM or autologous bone. Though these grafts are providing good results still they have many disadvantages.

4. BMPs AND OTHER GROWTH FACTORS

With improved understanding of fracture healing and bone regeneration at the molecular level, various key molecules regulating this complex physiological process have been identified, and are already in clinical use or under investigation to enhance bone repair. Of these molecules, BMPs so far have been the most extensively studied, as they are potent osteoinductive factors. They induce the mitogenesis of mesenchymal stem cells (MSCs) and other osteoprogenitors, and their differentiation towards osteoblasts. Since the discovery of BMPs, a number of experimental and clinical trials have supported the safety and efficacy of their use as osteoinductive bone-graft substitutes for bone regeneration. With the use of recombinant DNA technology, BMP-2 and BMP-7 have been licensed for the clinical use since 2002 and 2001 respectively [9].

These two molecules have been used in a variety of clinical conditions including non-union, open fractures, joint fusions, aseptic bone necrosis and critical bone defects.[9] Extensive research is still going on to develop injectable formulations for minimally invasive application, and novel carriers for the prolonged and targeted local delivery .

Other growth factors besides BMPs that have been implicated during the bone regeneration are platelet-derived growth factor (PDGF), transforming growth factor- b (TGF-b), insulin-like

growth factor-1 (IGF -1), vascular endothelial growth factor and fibroblast growth factor [10]. These have been used either alone or in combinations in a number of in-vitro and in-vivo studies with controversial results.

One current approach to enhance bone regeneration and soft-tissue healing by local application of growth factors is the use of platelet-rich plasma, a volume of the plasma fraction of autologous blood with platelet concentrations above baseline, which is rich in many of the above-mentioned molecules. Platelets contain hundreds of proteins called growth factors,(TGF, PDGF, IGFetc.) which are very important in healing injuries.

PRP procedures are conducted in the clinics unless it is being used as an addition to a surgical procedure. It begins with a standard blood withdraw from a patient, where we withdraw 15 millilitres of blood. PRP is then prepared by separating the platelets from other blood cells and increasing their concentration in a process called centrifugation. The use of ultrasound is important as it allows in placement of PRP [11].

'Orthobiologics' and the overall concept to stimulate the local 'biology' by applying growth factors (especially BMPs, because these are the most potent osteoinductive molecules) which could be advantageous for bone regeneration or even for acceleration of normal bone healing to reduce the length of fracture treatment. Their clinical use, either alone or combined with bone grafts, is constantly increasing. However, there are several issues about their use, including safety (because of the supra-physiological concentrations of growth factors needed to obtain the desired osteoinductive effects An adequate supply of cells (MSCs and osteoprogenitors) is important for efficient bone regeneration. The current approach of delivering osteogenic cells directly to the regeneration site includes use of bone-marrow aspirate from the iliac crest, which also contains growth factors. It is a minimally invasive procedure to enhance bone repair, and produces satisfactory results.

Cell-based approaches for regenerating bone also have a substantial history. The clinical use of unfractionated autologous bone marrow as a source of osteoprogenitors goes back more than 20 years. Hernigou et al. improved the efficiency of the procedure by enriching for MSCs with a cell sorter. Their data suggests that the injection of a minimum average of approximately 55,000 osteoprogenitors is required to achieve union. Harvesting of these cells may be aided by the development of improved recovery devices, such as the "reamer-irrigator-aspirator" [12]. The cells recovered by this device have proved to be successful clinically as adjuncts to the healing of difficult nonunions and segmental defects. Osteonecrosis has also been treated clinically with marrow-derived MSCs, or the stromal vascular fraction of fat. The MSC content of the marrow aspirates were measured, and better outcomes were associated with the administration of larger numbers of progenitor cells. Considerable emphasis is being placed on the use of MSCs to repair large segmental defects. Studies shows promising results in animal models.

In a related craniofacial application, autologous MSCs derived from adipose tissue were used successfully to repair calvarial defects in a 7-year-old child. Perhaps the most striking example of successful bone regeneration concerns regeneration of the entire distal phalanx of the human thumb [13]. This was achieved by implanting autologous, expanded osteoprogenitors from the patient's periosteum loaded onto a coral (porous hydroxyapatite) scaffold.

An additional type of biological osteogenic membrane was discovered by accident. After surgeons remove large segments of bone, they will sometimes implant an inert spacer until the time of subsequent reconstructive surgery. Masquelet and Begue [14] noted that the spacers became surrounded by highly osteogenic membranes, which are now used to aid the regeneration of difficult, large osseous defects. Their high osteoinductivity may reflect a unique combination of osteogenic cells and potent osteogenic factors.

Another innovative approach under development is the use of matrix laid down by MSCs as they undergo osteogenesis in-vitro. After devitalizing, the matrix is combined with MSCs that have been treated with an inducer of osteogenesis. Implantation of such a construct showed great efficacy in healing a murine calvarial defect.

Gene transfer offers an elegant way to combine cell therapy with the delivery of growth factors. As pioneered by Lieberman's group, marrow MSCs can be genetically modified with recombinant adenovirus vectors to express large amounts of BMP-2, thereby becoming potent

osteogenic cells when implanted into a defect. To expedite this strategy, this team now uses lentivirus vectors combined with buffy coat cells that can be isolated and transduced intraoperatively. Other gene therapy approaches includes the direct injection of vectors carrying osteogenic genes and the combination of vectors [15,16]. Gene transfer technologies can also deliver inhibitory RNA molecules; knockdown of chordin and noggin, two inhibitors of BMPs, enhances the osteogenic differentiation of MSCs.

Bone is formed physiologically by 2 different routes- direct or intramembranous osteogenesis occurs when osteoprogenitors differentiate directly into osteoblasts. Endochondral ossification occurs via a cartilaginous intermediate that is replaced by bone. Strategies for the regeneration of bone have tended to focus on the former, which has to confront the early need for a blood supply; thus explaining why vascular endothelial growth factor is a favoured growth factor in many studies.

However, it has proved difficult to engineer an effective vascular supply for regenerating bone. Under these conditions, the endochondral route is attractive because it relieves the physician of the need to provide a blood supply. Chondrogenesis does not require angiogenesis, and the biology of endochondral ossification supplies angiogenic signals spontaneously as the process evolves. Much recent attention is thus focused on the endochondral route to bone regeneration, especially as chondrogenesis is favoured by the hypoxic, acidotic conditions of major lesions. It may be particularly useful in regenerating bone at sites such as the diaphysis of long bones, where the endochondral process is the normal route of healing.

As noted, mechanical signals are important for osteogenesis, and clinical research has confirmed that a certain level of micromotion promotes fracture healing. The concept of dynamization has also proved attractive, whereby bones are fixed rigidly to initiate healing and then are allowed axial motion to promote maturation and remodelling. Bone regeneration has been reviewed in several studies but still research and optimal solution is in progress [17].

5. CARTILAGE REGENERATION

Cartilage is frequently damaged as a result of sporting injuries or other trauma and is eroded in joints with arthritis. Damaged cartilage often leads to joint pain and can predispose to osteoarthritis (OA). Cartilage repair is indicated for symptomatic, but otherwise healthy, joints. It is normally seen in sports persons. Repair is not normally attempted in arthritic joints because of the size and nature of the lesions and recognition that the concomitant disease process could impair restoration of cartilage. Strategies for articular cartilage regeneration may differ depending on whether the lesion is restricted to the cartilage itself or penetrates the underlying bone to form an osteochondral lesion. Unlike bone, articular cartilage has almost no intrinsic ability to regenerate. The lack of a repair process is usually ascribed to the low cellularity of cartilage and the absence of blood, lymph, or neural innervation.

One way to obviate this is to allow communication between the cartilaginous defect and the underlying marrow by piercing the subchondral bone. A clot forms where MSCs from the marrow differentiate and synthesize cartilaginous repair tissue. A simple arthroscopic procedure, such as microfracture, is often clinically effective, especially for lesions smaller than 2 cm, although the repair tissue is a fibrocartilaginous scar rather than true hyaline cartilage. The major reasons for failure are the inferior mechanical properties of the repair tissue and bone invasion. In an alternative clinical procedure, healthy cartilage is harvested from a non weight-bearing part of the joint as a source of autologous chondrocytes. These autologous chondrocytes are expanded in monolayer culture and, in the original ACI method, are reimplanted as a suspension under a flap of periosteum or fibrin glue.

Despite the phenotypic modulation of the chondrocytes during serial monolayer passage and the absence of a scaffold, this procedure is surprisingly effective and produces a clinical response arguably equivalent to microfracture and suitable for larger defects [18]. Improvements in the technology include using a collagen flap to replace the need for periosteum and seeding the chondrocytes onto a collagen scaffold before implantation in a procedure known as matrix associated chondrocyte implantation. Selection of the most chondrogenic cells before implantation may also improve outcomes. The major disadvantages of ACI are the need for two invasive procedures and the extensive expansion of cells for each patient.

The use of stem cells to generate a suitable matrix for repair has gained recent popularity

with the use of marrow stromal stem cells and perichondrial / periosteal progenitors most commonly employed in Cartilage repair.

Mesenchymal stem cells provide an attractive alternative to chondrocytes. Marrow-derived MSCs undergo chondrogenesis in vitro in response to TGF-b, but there is a concern that they will undergo further differentiation to osteoblasts, leading to the formation of bone where there should be cartilage.

Because cartilage is a highly hydrated tissue, many of the scaffolds being developed for cartilage repair are hydrogels. Materials include fibrin, hyaluronic acid, collagen, chitosan, silk, alginate, and synthetic polymers, such as polylactic acid (PLA) and polyglycolic acid. Newer hydrogels are based on polyethylene glycol, self-assembling peptides, and electrospun nanofibers.

Another scaffold of interest is devitalized cartilage, whose investigation is encouraged by the successful regeneration of a trachea, an organ that contains articular cartilage, from devitalized donor tissue. Another approach dispenses with matrices altogether and instead allows chondrocytes in culture to develop their own extracellular matrices, creating implantable grafts [19].

Biological and mechanical integration of engineered cartilage grafts in articular cartilage is problematic, exacerbated by the fact that, unlike bone, articular cartilage does not remodel rapidly or extensively. One strategy for improving biological integration involves local digestion of the surrounding cartilage using enzymes or catabolic cytokines. Mechanical integration may be helped by recent research suggesting that exposure of neocartilage to a combination of fibroblast growth factor-2 and TGF-b promotes rapid maturation with enhanced mechanical properties [20]. Another approach subjects chondrocytes to hydrostatic pressure in a bioreactor to accelerate maturation before implantation [21].

Rather than implant rigid, preformed tissue grafts, there is interest in applying soluble materials that can take on the shape of the defect and then solidify in situ. This leads to greater filling of the defect and enhanced integration with surrounding cartilage. One approach uses a hyaluronan-based polymer that is liquid at room temperature and solidifies at body temperature [22].

Gene therapy could also be used in conjunction with MSCs from various sources.

As the field of cartilage regeneration develops, it confronts the need to restore very large lesions or even resurface entire joints. Repair of cartilage defects in OA is more challenging because there is a concomitant disease process creating an unfavourable repair environment that marrow-derived MSCs from individuals with OA are intrinsically less chondrogenic. One approach to regenerating cartilage in joints with OA, already in clinical trials (gov.in identifier NCT01671072), involves the intra-articular injection of genetically modified allogeneic chondrocytes expressing elevated amounts of TGF [23].

The influences of mechanical forces on chondrogenesis are widely appreciated but incompletely understood. Recent data suggest that shear forces are important in this regard, promoting the endogenous production of TGF-b. These are the sorts of forces that could be produced by a modified continual passive motion machine of the type already used in rehabilitation after knee surgery [24].

Strategies are being developed to deliver appropriate bioactive factors that may optimize this regenerative process. These involve either direct delivery of the factors or delivery of the transgene coding for the factors. Cartilage tissue engineering is another promising approach of regenerative medicine to tackle this problem

6. SPINE REGENERATION

Intervertebral Disc degeneration. The intervertebral disk (IVD) is a major load-bearing structure of the spine, and its degeneration is associated with back pain. It is currently treated surgically by removing the degenerate disk and fusing the adjacent vertebrae, often using BMP-2 to enhance the process. Prosthetic disks have been developed but are not a clinical success. Attempts to regenerate the disk have to accommodate the anatomy of the disk, where a highly hydrated, gelatinous nucleus pulposus exists in a fibrous, collagenous annulus fibrosus. These 2 structures differ considerably in biology and mechanical properties. Attempts to regenerate the nucleus pulposus by the intra-discal injection of growth factors has met with some success in animal models, and the FDA has given permission for clinical trials using BMP-7 and growth differentiation factor (GDF)-5 (also BMP-14) in this manner [25]. Recognizing

the probably transient effect of injected growth factors, there is interest in using gene transfer to provide sustained delivery. Because the disk is so physiologically isolated and cell turnover is low, it is possible to obtain remarkably long periods of transgene expression, even using highly antigenic vectors, such as adenovirus. Cell therapy is attractive because IVD degeneration is associated with cell death; disk cells and MSCs have been evaluated in this regard. Because the nucleus pulposus of the disc is highly acidotic and hypoxic, there are concerns about the survival of transplanted cells. Preconditioning has been suggested to prepare cells for this environment and to help engraftment. In a small clinical trial, suspensions of autologous, expanded, nucleus pulposus cells were injected into disks after surgery for disc prolapsed [26].

The discovery of progenitor cells in the disc adds new possibilities for this type of therapy.

One impediment to research into cellular therapies for IVD regeneration is the lack of good markers for the relevant disk cells. Survival and function of cells could be aided by a suitable scaffold, and a variety of hydrogels based on chitosan, hyaluronan, alginate, cellulose, and composites of collagen/ hyaluronan and chondroitin sulfate have been investigated. The annulus fibrosus has much greater tensile strength, and materials explored in annulus regeneration include PLA, poly(1,8- octanediol malate), gelatin, silk, and polycaprolactonetriol malate. Electrospun PLA has been examined as a way of forming the alternating lamellar structure of the annulus. IVD degeneration is associated with calcification of end plates, which limits diffusion. Another limitation to progress in this area is the lack of good models and clinical trials [27].

Spinal fusion- spinal fusion is a commonly performed yet often unsuccessful procedure. Strategies to enhance spinal fusion include use of extracted and partially purified proteins including BMPs, recombinant BMP-2 & 7 and gene therapy i.e. delivery of gene or osteoinductive factor itself.

Spinal cord injury-Ability of stem cells to incorporate into the spinal cord, differentiate and to improve locomotor recovery. Stem cells have the ability to remyelinate the demyelinated injured neurons. SCs have neurotrophic, ECM, and cell adhesion properties that are favourable to axonal regeneration in the peripheral nervous system [28].

7. MENISCUS REGENERATION

The menisci are responsible for load transmission through the knee and are frequently injured. At one time, it was common to remove the offending menisci; realization that this predisposed to OA spurred efforts to repair or regenerate menisci. The outer third of the meniscus (the red zone) is attached to the synovium and has a blood supply, providing it with some potential for repair. The inner two-thirds (the white zone) lacks a blood supply and cannot regenerate.

There are three main methods of modern surgical management of meniscus tears: arthroscopic partial meniscectomy; meniscal repair with or without augmentation techniques; and meniscal reconstruction. The enthusiastic evolution of meniscus surgery has seen a great change from interest in repair in the 1800s to total resection in the 1970s, and finally to protection, or reconstruction if resected, from the 1990s to the present day [29].

The rapid developments in cell biology and tissue engineering will advance new alternative biological methods in the treatment of meniscal tears in the future.

8. MENISCI SCAFFOLDS [30,31]

Highly porous, cell-free, and biodegradable meniscal scaffolds are used to fill the defect in the previously partially-resected meniscus and to develop the meniscal tissue by allowing migration and growth of vascular channels and precursor cells into the scaffold.

In clinical practice, there are two main types of meniscal scaffolds: the Collagen Meniscus Implant (CMI) and polyurethane-based scaffold. The indications and surgical techniques are similar for these two implants. These procedures can be performed arthroscopically, Currently, it is concluded that the mid-term survival rate of scaffolds is favourable compared with meniscal repair. In conclusion, the chondroprotective effects of these implants are still controversial, and long-term higher level of evidence comparative studies are required to clarify the clinical efficacy of these implants.

9. MAT

MAT (menisci allograft transplantation) procedure is not actually a new concept. The

interposition arthroplasty with autogenic fat pad was first reported at the beginning of the 1900s [26]. Since the more recent performance of MAT by Wirth in the 1980s, the MAT procedure has evolved to be what it is now; an increasingly performed, safe, reliable and highly specialized knee procedure, rather than an experimental or investigational surgery in patients whose meniscus is lost for any reason, and who have refractory persistent symptoms [32].

Currently, the MAT can be performed as an open procedure or arthroscopically in carefully selected patients by experienced surgeons. The malalignment, instability and limb length inequality of the patients must be corrected beforehand or concomitantly. Among four storage methods of meniscal allografts (fresh viable, fresh-frozen, cryopreserved and lyophilized), fresh viable and fresh-frozen allograft are recommended [33]. The optimal-sized allograft, according to the MRI or radiological knee films of the patient, can be fixed with or without bone plugs or block. In order to obtain conclusive results regarding the relative technical and clinical superiority, the relevant literature requires comparative studies, involving higher number of patients and longer follow-up periods.

10. LIGAMENT AND TENDON REGENERATION

Although ligaments and tendons are different tissues, they have a high incidence of injuries and usually lead to instability and loss of function with surgical intervention, the grafts (e.g. in ACL reconstruction) are gradually replaced by scar tissue which is of mechanical inferior quality. In case of tendon injuries, problem of healing as well as complication of adhesion development is also there. Regenerative medicine aims to potentiate the healing of natural ligaments and tendons with a more biologic plausible tissue and to prevent the above complications. Most preclinical research in this area uses injuries to the anterior cruciate ligament, Achilles tendon, or rotator cuff as experimental models. Two types of regenerative requirements exist, depending on whether the need is to regenerate tissue in the centre of the structure or in the insertion site to the bone or tendon muscle junction. The former is simplified by the relative homogeneity of the surrounding tissue, whereas the insertion and junction sites are complex, multi tissue graded entities [34].

Because ligaments and tendons are collagenous, there is much interest in using growth factors that promote collagen synthesis, including TGF-b, PDGF, and insulin-like growth factors, as well as autologous conditioned serum and the ubiquitous PRP. Bone morphogenetic proteins-12, -13, and -14 (GDF-7, GDF-6, andGDF-5) [35] and the transcription factor scleraxis are of particular interest because they promote the differentiation of progenitor cells into tenocytes and ligament cells. Because scleraxis is an intracellular protein, gene transfer is a useful modality for its delivery that has been used successfully to promote tendogenesis *in vitro* [36]. Most cell-based approaches to regeneration have adopted ex vivo strategies using tenocytes, ligament cells, and skin fibroblasts. However, tendons and ligaments have been found to possess local populations of stem cells, raising the possibility of stimulating endogenous repair processes [37]. Collagen, synthetic polymers such as poly(lactic-co-glyocolic acid),and devitalized extracellular matrix preparations are frequently the scaffold of choice. Mechanical forces are important in the formation and maturation of ligament and tendon; tensile stress, for example, up-regulates the expression of scleraxis by MSCs. There is interest in harnessing mechanobiological processes to regenerate the region where tendon or ligament inserts into bone. This task is challenging because the area includes fibrocartilaginous intermediary zone. An alternative approach uses multiphasic scaffolds containing multiple cell types. In one example, scaffold was seeded with ligament cells at one end, osteoblasts at the other, and chondrocytes in between [38]. A more practical approach may be to use a single progenitor cell type and induce zone-specific differentiation by spatial differences in the matrix [39]. Ma et al1 recently engineered a scaffold free, bone-ligament-bone anterior cruciate ligament graft in vitro using marrow-derived.

11. CONCLUSION

Unlike the case with the other tissues, there have been no major clinical trials adopting the strategies of regenerative orthopaedics for healing ligaments and tendons. Hence it can be concluded that Biomaterials used in orthopedics regeneration include inorganic materials, polymeric materials and composites. While inorganic biomaterials are components used for bone regeneration due to their similar compositional and mechanical property, polymeric biomaterials are used for soft tissues

regeneration. The combination of inorganic and polymeric materials is used fabricate biomimetic scaffolds for tissues regeneration. To provide an ECM-mimicking microenvironment, biomimetic nanofibrous and multilayer scaffolds have been developed for orthopedics tissue regeneration in recent years. A few studies have been attempted to regenerate orthopedic tissues in Human trials, but achieved limited success. Despite all these challenges, orthopedic regeneration is an exciting and rapidly growing field. The advances of this field provide the promising potential to improve the health of patients in the near future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Berebichez-Fridman R, Gómez-García R, Granados-Montiel J, et al. The holy grail of orthopedic surgery: Mesenchymal stem cells - their current uses and potential applications. Stem Cells Int. 2017;1-14.
2. Urist MR, O'Connor BT, Burwell RG: Bone Graft Derivatives and Substitutes Oxford: Butterworth-Heinemann Ltd; 1994.
3. Grayson WL, Frohlich M, Yeager K, et al. Engineering anatomically shaped human bone grafts. Proc Natl Acad Sci U S A. 2010;107(8):3299-3304.
4. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med. 1994;331(14):889-895
5. Einhorn TA: The cell and molecular biology of fracture healing. Clin OrthopRelat Res. 1998;355(Suppl):S7-21.
6. Giannoudis PV, Dinopoulos H, Tsiridis E: Bone substitutes: an update. Injury. 2005;36(Suppl 3):S20-27.
7. Bauer TW, Muschler GF: Bone graft materials. An overview of the basic science. Clin OrthopRelat Res. 2000;371:10-27.
8. Finkemeier CG: Bone-grafting and bone-graft substitutes. J Bone Joint Surg Am. 2002;84(3):454-464.
9. Food and Drug Administration: Medical devices.
   Available:[[http://www.fda.govtMedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/default.htm]].
10. Dimitriou R, Tsiridis E, Giannoudis PV: Current concepts of molecularaspects of bone healing. Injury. 2005;36(12):1392-1404.
11. Alsousou J, Thompson M, Hulley P, Noble A, Willett K: The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. J Bone Joint Surg Br. 2009;91(8):987-996.
12. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions: influence of the number and concentration of progenitor cells. J Bone Joint Surg Am. 2005;87(7):1430-1437.
13. Vacanti CA, Bonassar LJ, Vacanti MP, Shufflebarger J. Replacement of an avulsed phalanx with tissue-engineered bone. N Engl J Med. 2001;344(20):1511-1514.
14. Masquelet AC, Begue T. The concept of induced membrane for reconstruction of long bone defects. Orthop Clin North Am. 2010;41(1):27-37:table of contents.
15. Virk MS, Sugiyama O, Park SH, et al. "Same day" ex-vivo regional gene therapy: a novel strategy to enhance bone repair. Mol Ther. 2011;19(5):960-968.
16. Betz OB, Betz VM, Nazarian A, et al. Direct percutaneous gene delivery to enhance healing of segmental bone defects. J Bone Joint Surg Am. 2006;88(2):355-365.
17. Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: current concepts and future directions. BMC Med. 2011;9:66.
18. Knutsen G, Engebretsen L, Ludvigsen TC, et al. Autologous chondrocyte implantation compared with microfracture in the knee: a randomized trial. J Bone Joint Surg Am. 2004;86(3):455-464.
19. Jubel A, Andermahr J, Schiffer G, et al. Transplantation of de novo scaffold-free cartilage implants into sheep knee chondral defects. Am J Sports Med. 2008;36(8):1555-1564.
20. Khan IM, Francis L, Theobald PS, et al. In vitro growth factorinduced bio engineering

of mature articular cartilage. Biomaterials 2013;34(5):1478-1487.
21. Crawford DC, Heveran CM, Cannon WD Jr, Foo LF, Potter HG. An autologous cartilage tissue implant NeoCart for treatment of grade III chondral injury to the distal femur: prospective clinical safety trial at 2 years. Am J Sports Med. 2009;37(7):1334-1343.
22. Mortisen D, Peroglio M, Alini M, Eglin D. Tailoring thermoreversible hyaluronan hydrogels by "click" chemistry and RAFT polymerization for cell and drug therapy. Biomacromolecules. 2010;11(5):1261-1272.
23. Murphy JM, Dixon K, Beck S, Fabian D, Feldman A, Barry F.Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. Arthritis Rheum. 2002;46(3):704-713.
24. Salter RB. Continuous passive motion: from origination to research to clinical applications. J Rheumatol. 2004;31(11): 2104-2105.
25. An HS, Masuda K, Cs-Szabo G, et al. Biologic repair and regeneration of the intervertebral disk. J Am Acad Orthop Surg. 2011;19(7):450-452.
26. Hohaus C, Ganey TM, Minkus Y, Meisel HJ. Cell transplantation in lumbar spine disc degeneration disease. Eur Spine J. 2008;17(suppl 4):492-503.
27. Risbud MV, Guttapalli A, Tsai TT, et al. Evidence for skeletal progenitor cells in the degenerate human intervertebral disc. Spine (Phila Pa 1976). 2007;32(23):2537-2544.
28. Myckatyn TM, Mackinnon SE, McDonald JW. Stem cell transplantation and other novel techniques for promoting recovery from spinal cord injury. Transpl Immunol. 2004;12(3-4):343-58.
29. Verdonk R. The meniscus: past, present and future. Knee Surg Sports Traumatol Arthrosc. 2011;19:145-146
30. Verdonk R, Verdonk P, Huysse W, Forsyth R, Heinrichs EL. Tissue ingrowth after implantation of a novel, biodegradable polyurethane scaffold for treatment of partial meniscal lesions. Am J Sports Med. 2011;39:774-782.
31. Stone KR, Steadman JR, Rodkey WG, Li ST. Regeneration of meniscal cartilage with use of a collagen scaffold. Analysis of preliminary data. J Bone Joint Surg [Am]. 1997;79-A:1770-1777.
32. Grassi A, Zaffagnini S, MarcheggianiMuccioli GM, Benzi A, Marcacci M. Clinical outcomes and complications of a collagen meniscus implant: a systematic review. Int Orthop. 2014;38:1945-1953.
33. Verdonk PC, Demurie A, Almqvist KF, et al. Transplantation of viable meniscal allograft. Surgical technique. J Bone Joint Surg [Am] 2006;88-A(Suppl):109-118.
34. Smith L, Xia Y, Galatz LM, Genin GM, Thomopoulos S. Tissue- engineering strategies for the tendon/ligament-to-bone insertion. Connect Tissue Res. 2012;53(2):95-105.
35. Schweitzer R, Chyung JH, Murtaugh LC, et al. Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments. Development. 2001;128(19):3855-3866.
36. Longo UG, Lamberti A, Maffulli N, Denaro V. Tissue engineered biological augmentation for tendon healing: a systematic review. Br Med Bull. 2011;98:31-59.
37. Kuo CK, Tuan RS. Mechanoactive tenogenic differentiation of human mesenchymal stem cells. Tissue Eng Part A. 2008;14(10):1615-1627.
38. Spalazzi JP, Dagher E, Doty SB, Guo XE, Rodeo SA, Lu HH. In vivo evaluation of a multiphasedscaffolddesigned for orthopaedic interface tissue engineering andsoft tissue-to-bone integration. J Biomed Mater Res A.2008;86(1):1-12.
39. Phillips JE, Burns KL, Le Doux JM, Guldberg RE, Garcia AJ. Engineering graded tissue interfaces. Proc Natl Acad Sci U S A. 2008;105(34):12170-12175.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62795