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Review Article

Bone Regeneration: Emerging Paradigms and Existing Snags

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- Gene therapy

Abstract

Bone loss owing to trauma, congenital defects and sports-related injuries has become a major health quandary all over the world. Existing therapies, although somewhat successful, do not provide the optimum remedy to orthopedic disorders. Conventional treatments typically rely on donor tissues obtained either from the patient or from another source, which raises the issue of donor-cell-supply, immune rejection and disease transfer. This has incited orthopaedic surgeons to look for viable alternatives. A smart option to overcome these problems is served by a tissue-engineered bone. Bone regeneration can be attained by an appropriate combination of cells, scaffold and growth factors. To regenerate full functional bone, researchers worked for decades to find suitable combination of cells, biomaterials for scaffold fabrication, scaffold structure and growth factor: the work is still in progress. Various biomaterials including bioceramics have emerged as an effective module for fabricating scaffold for bone tissue engineering. Stem cells have gained importance as a potent cell source for bone regeneration. Stem cells along with multiple growth factor approach are applied nowadays to regenerate bone. The delivery of these growth factors in conjunction with gene therapy has come forward as an ideal approach for augmenting bone tissue. This review highlights the advances in bone tissue engineering by focusing on three key components cell sources, scaffold biomaterials as well as growth factors used in bone tissue engineering. It also reflects an array of problems and future perspectives to overcome the existing stumbling blocks in bone regeneration.

INTRODUCTION

Bones are organs of the skeletal system that execute both biochemical and metabolic functions. Biomechanically, bone serves to maintain the shape of the skeleton, protect soft tissues in the cranial, thoracic and pelvic cavities, transmit the forces of muscular contraction during movement, and supply a framework for bone marrow. Metabolically, bone provides reservoir for ions, especially calcium ions, and contributes to the regulation of the extracellular matrix (ECM) composition. We realize the importance of bone in the case of diseases such as osteogenesis imperfecta, osteoarthritis, osteomyelitis and osteoporosis in which bone does not function properly [1]. Bone tissue loss due to disease such as osteoporosis or trauma, has been managed with bone grafts. To address the need for bone substitutes, current clinical therapies include autograft, allograft and inert implant. Autografting involves harvesting of 'donor' bone from a non-load-bearing site in the patient's body but this can cause morbidity at harvested site with problems such as pain, infection, blood loss. Allograft implants are attributed to immunogenic reactions in the patient's body. Inert implants, including metals, plastics, and ceramics, represent approximately 8% of bone substitutes. However, these materials are often subjected to fatigue, fracture, toxicity, and do not remodel with time, e.g., a metal bone implant cannot grow with the patient and thus do not serve as a lifelong therapy. For all these reasons, there is an intense need to find alternative bone substitutes. This has incited the scientists to opt for tissue engineering approach to address the need.

Tissue engineering is an "interdisciplinary field that involves the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain or improve tissue functions" [2]. Tissue engineering of bone and other tissues/organs involves three building blocks called tissue-engineering-triad (Figure 1): cells, biomaterial-scaffold (a 3D polymeric network) and growth factors (signaling molecules). There have been a number of approaches to engineer bone, by using composite polymeric scaffold, osteoprogenitor cells and multiple osteoinductive growth factors. This review provides an update on various osteogenic cell sources, scaffold biomaterials including bioceramics and growth factors, and their delivery through gene therapy approach. It also reviews the current challenges and recent progress in the field of bone tissue engineering.

CELLS FOR BONE TISSUE ENGINEERING

Osteoblast cells are highly responsible for bone regeneration.

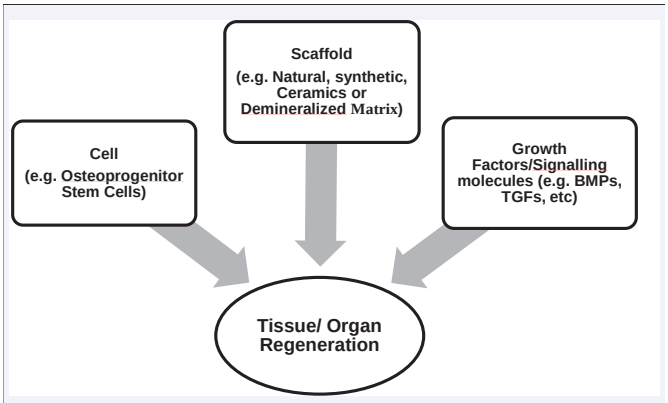

Figure 1 Tissue engineering triad - appropriate combination of cell, scaffold and growth factor generates a bio-functional tissue/organ.

Stem Cells, e.g., embryonic stem cells (ESCs), fetal stem cells, adult (including umbilical cord blood)-derived stem or progenitor cells, and adult-derived inducible pluripotent stem cells (iPSCs), are very important for bone tissue engineering as osteoblasts can be differentiated by inducing them with a suitable environment [3]. A list of the cells used for bone tissue engineering is summarized in (Figure 2).

SCAFFOLD CHARACTERISTICS FOR BONE TISSUE ENGINEERING

Scaffold provides shelter to the cells and behaves like ECM similar to that present in our body. The scaffold must possess some important characteristics for the regeneration of bone as summarized below:

a) Osteoinductive- Capable of promoting the differentiation of progenitor cells down to an osteoblastic lineage.

b) Osteoconductive-To support bone growth and encourage the in growth of surrounding bone.

c) Highly porous- To maximize the space for cellular adhesion, growth, ECM secretion, revascularization, adequate nutrition and oxygen supply.

d) Biocompatible- Biologically compatible to host tissue i.e. should not provoke any rejection, inflammation, and immune responses.

e) Biodegradable- The rate of biodegradation has to be adjusted to match the rate of bone tissue formation.

f) Three-dimensional structure- For reconstructing 3D tissues.

g) Adequate mechanical strength- To withstand in-vivo stimuli during bone formation.

h) Sterilizable - To avoid toxic contamination.

i) Cost-effective - Affordable to all

BIOMATERIALS FOR BONE TISSUE ENGINEERING

Various types of biomaterials used for bone tissue engineering can be divided into 4 main classes: synthetic polymers, natural polymers, decellularized tissue matrices and ceramics: these have been summarized in Figure 3 [4-9].

GROWTH FACTORS FOR BONE TISSUE ENGINEERING

Growth factors enhance the repairing/regenerating of damaged bone tissue. The important growth factors used for bone regeneration have been depicted in Table 1.

GENE THERAPY FOR ENHANCING BONE REGENERATION

The conjunction of tissue engineering with gene therapy is a hybrid approach (Figure 4) that provides means of delivering genes for growth factors, transcription factors, and extracellular matrix molecules to the targeted tissue which results in a good therapeutic effect. Table 2 describes various therapeutic genes used for bone tissue engineering.

RECENT ADVANCES IN BONE TISSUE ENGINEERING

There is a marvelous advancement in bone tissue engineering in combination to gene therapy and cell mediated bone regeneration, which encouraged the researchers to explore the potential for regeneration of bone. The recent advances in bone tissue engineering, from year 2006 to date, are discussed here.

Jiang and coworkers had successfully explored the capacity of one of the human bone morphogenetic proteins (BMPs): they combined BMP-4 gene therapy with tissue engineering technique to improve the mandibular osseous defects in rabbits [30].This was the first report to describe the transfection of bone marrow stromal cells with BMP-4 using plasmid-based technology to repair mandibular osseous defects [30]**.** Kiebl and coworkers investigated bone healing by administering growth factor BMP-2 embedded with autologous adipose derived stem cells (ADSCs) in a locally applied fibrin matrix [31]. In this study, a 2 mm transcortical drill hole in the femur of male rats served as a small non-critical size defect model for fracture simulation. The most significant result found in this model is that the combination of ADSCs and BMP-2 in a fibrin matrix significantly reduces callus formation after 2 weeks compared to BMP-2 alone. BMP-2 alone significantly increased callus formation, while ADSCs, when embedded alone in the fibrin matrix, did not lead to increased bone regeneration. This study reflects the importance of gene therapy and growth factors in bone regeneration (Figure 5) [31]. Martínez-Sanz and associates tested injectable hyaluronic acid (HA) hydrogel as a BMP-2 carrier by injecting the BMP-2 loaded HA gel in the rat calvarium [32]. This showed bone formation in 8 weeks in correlation with the amount of BMP-2 loaded (0,1 and 30μg) within the gel. This study suggests that novel HA hydrogels could be used as a BMP-2 carrier and can promote bone augmentation for potential orthopedic applications [32]. Zhang and coworkers developed a mesoporous bioglass/silk fibrin scaffolds containing platelets derived growth factor (PDGF-b) and BMP-7 growth factors and evaluated this composite for the treatment of osteoporotic critical-sized femur defects in ovariectomised rats [33]. Growth factors loaded scaffold degraded over time and enhanced MSCs proliferation and initiated bone turnover/remodeling by releasing PDGF-b and BMP-7 in a controlled manner [33]. Dadsetan and coworkers fabricated scaffolds with three different calcium phosphate

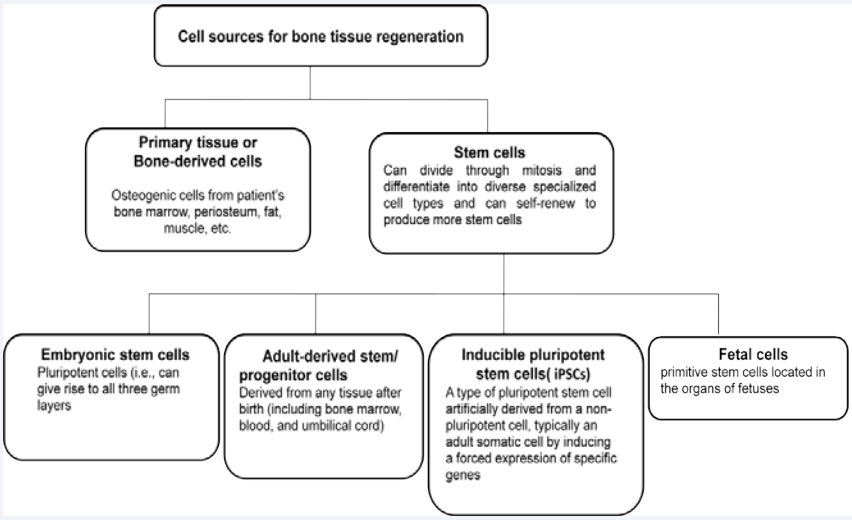

Figure 2 Cell sources for bone regeneration.

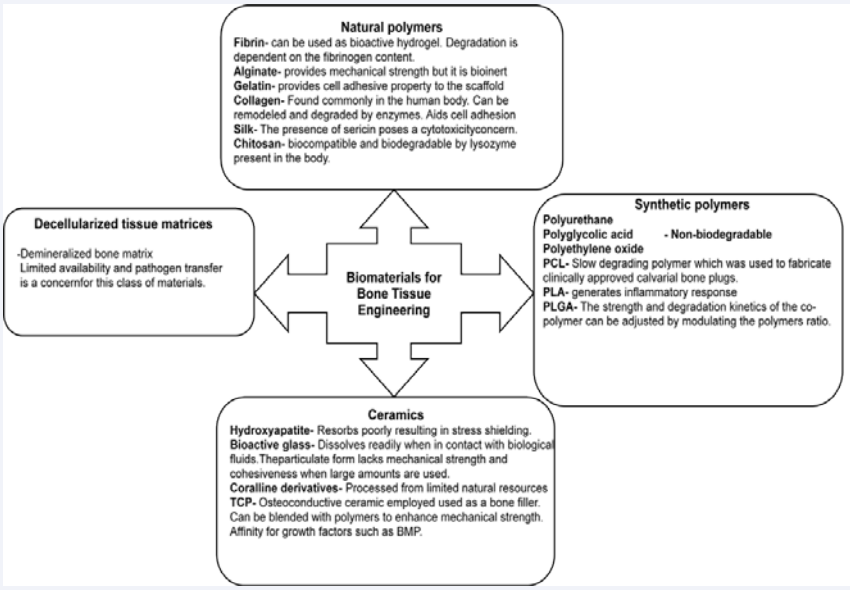

Figure 3 Biomaterials used in bone tissue engineering..

formulations: magnesium-substituted β-tricalcium phosphate (β-TCMP), carbonated hydroxyapatite (synthetic bone mineral, SBM) and biphasic calcium phosphate (BCP) coated porous polypropylene fumarate (PPF) scaffold loaded with recombinant human bone morphogenetic protein-2 (rhBMP-2) [34]. *In vivo* critical-sized rabbit calvarial defect treated with calcium phosphate coated PPF scaffolds showed that the β-TCMP and SBM in combination with rhBMP-2 improved osteoconductivity and osteointegration at the site of defect [34].

Insufficient vascularization often restrains new bone formation and delays bone healing, possibly because of deviations from the principles of vasculature functioning in osteogenesis. Endothelial cell seeded polycaprolactone-hydroxyapatite scaffold was found to assemble into microvascular networks which results formation of bony matrix and achieve a high level of vascularization. Zhou and associates fabricated a vascularized tissue engineered bone with mesenchymal stem cells (MSCs) and MSC-derived endothelial cells (ECs) co-cultured in porous β-tricalcium phosphate ceramic (β-TCP) to repair 1.5-cm ulnar defects in the rabbit was another achievement in the field of bone tissue engineering [35]. Mishra et al., in 2016, developed a novel poly (propylene fumarate)/fibrin composite scaffold for the development of vascularized neo-bone tissue was fabricated with human mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs) [36]. In vitro vascularized composite scaffold ensured early and uniform vascularization by following *in vivo* implantation [36].

Besides insufficient vascularization, another key obstacle on the way to successful bone implants is how to grow new bones

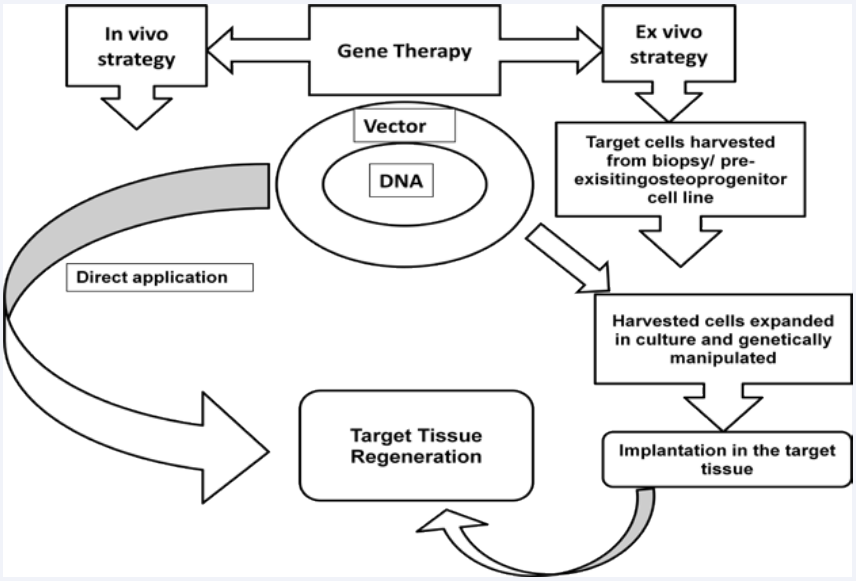

Figure 4 Gene therapy approach to deliver therapeutic gene to target tissue.

Table 1: Summary of several growth factors, their sources and role in bone tissue repair.

Growth Factors	Source	Receptor type	Function	Animal	Dose	Model	References
BMP-2	Osteoprogenitor, osteoblasts, ECM bone	Serine Thereonine Sulphate	Mesenchymal/ progenitor cells differentiation factor	Dog, rabbit, monkey	20µg/ day	Radius, Ulna, Spine, Tibia	[10]
BMP-7	Osteoprogenitor, osteoblasts, ECM bone	Serine Thereonine Sulphate	Mesenchymal/ progenitor cells differentiation factor	Monkey Human	2.5mg/day	Ulna, Tibia, Fibula	[11]
TGF-β1	Platelets, ECM bone, cartilage matrix	Serine Thereonine Sulphate	Mesenchymal cells proliferative factor	Rabbit	1-10 µg / day	Tibia	[12]
TGF-β2	Platelets, ECM bone, cartilage matrix	Serine Thereonine Sulphate	Mesenchymal cells proliferative factor	Rabbit	60/600 ng single shot	Tibia	[13]
FGF	Macrophages, mesenchymal cells,osteoblasts	Tyrosine Kinase	Mitogenic stimulus	Rat, dog, Monkey	0.4/1/3mg single dose	Tibia, Fibula	[14,15]
IGF-1	Bone matrix, Osteoblast, Chondrocytes	Tyrosine Kinase	Proliferation/ Differentiation osteoprogenitor cells	Rat	2mg	Skull	**[16]**
Growth Hormone	Pituitary gland cells	Pituitary gland	Regulation of skeletal growth	Rat, Rabbit	150µg/Kg	Tibia	[17]

Table 2: Summary of some gene therapy approaches for bone regeneration.

Therapeutic gene	Delivery approach	Experimental Model	Experimental Outcomes	References
BMP-2	Recombinant protein (RP)	Rat/Mouse; Rabbit; Non-Human primates, Human	Healed critical defects; Hydrogel carrier as scaffold; Laparoscopic anterior lumbar interbodies arthrodesis; Better fusion rate than autograft; No complications	[18]
	In vivo	Rat/ Mouse; Rabbit	Mixed results in immunocompetent animals, Healed femoral critical defects	[19]
	Ex vivo	Rat/Mouse	Better fusion rate than autograft; No complications	[19,20]
	Non-Viral	Rat/Mouse	Bones healed faster from adenovirus then liposomes	[19]
BMP-4	RP	Rat/Mouse	Pharmacokinetics study	[21]
	In Vivo	Rat/Mouse	Repaired segmental defects	[21,22]
	Ex vivo	Rat/Mouse	Healed Critical defects	[23]
	Non- Viral	Rat/Mouse	Electroporation	[24]
BMP-6	RP	Rat/Mouse	Pharmacokinetics study	[25]
	In Vivo	Rat/Mouse	Possibly more potent then BMP-2	[21]
	Ex Vivo	Rat/Mouse	Ectopic bone formation	[25]

BMP-7	RP	Rat, Canine, Rabbit, Sheep, Human	Different effects based stages of osteoblast differentiation	[26]
	In Vivo	Rat/Mouse	Repaired segmental defects	[27]
BMP-9	In Vivo	Rat/Mouse	Spinal fusion and etopic bone formed	[28]
	Ex Vivo	Rat/Mouse	Fusion attained/No toxicities	[29]

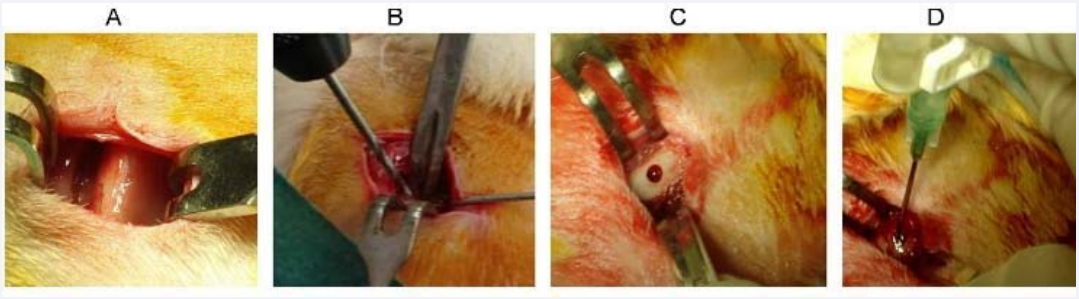

Figure 5 Drill hole model: (A) Surgical approach; (B) drilling; (C) 2mm drill hole; (D) filing (Reproduced with Permission from Reference [31]).

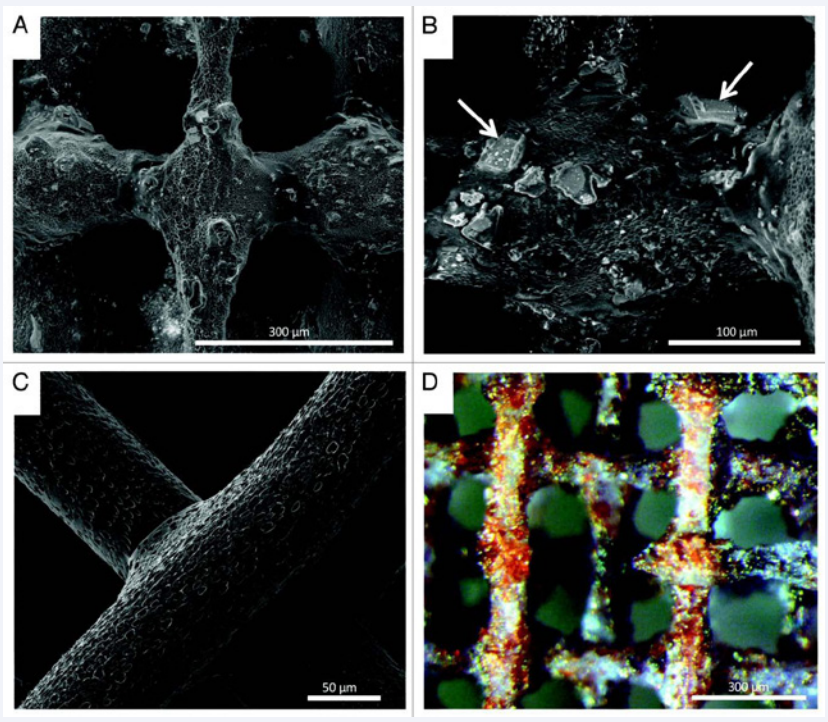

Figure 6 SEM images of PLA/CaP glass composite scaffolds (A and B) showing glass distribution and glass/polymer interface, white arrows indicate glass particles; (C) Struts of a PLA scaffold showing the micro and nanoporosity left after solvent evaporation; (D) PLA/CaP glass scaffold after Alizarin red staining. Red colored areas denote the CaP inorganic phase indicating the glass particles exposed on the scaffold surface (Adapted with permission from reference [39]).

in the anatomical shape of the original one: this problem has been solved by Grayson et al., in 2010 [37]. A Jaw bone known as the temporomandibular joint (TMJ) was created from (patient's own) stem cells. Grayson and coworkers turned the bare scaffold into living tissue by putting it into a chamber molded to its exact shape, and adding human cells, typically isolated from bone marrow. The outmost advantage of this research is that, it can allow a patient's own cells to produce the final tissue for implantation, thereby eliminating any fear of rejection by the immune system [37]. Shim and coworkers fabricated a dual cell-laden 3D polycaprolactone-alginate composite scaffold by 3D printing technology as a model for bone tissue engineering [38]. Cell laden construct consists of both osteoblasts and chondrocytes that had successfully undergone proliferation within the composite scaffold up to 7 days [38]. This research work is a fruitful example that proves that fabricating scaffold in an appropriate way could potentially allow growth and proliferation of more than one type of cells simultaneously. Serra

and co-workers synthesized a new biodegradable 3D printed composite scaffold from polylactic acid (PLA), polyethylene glycol (PEG) and bioactive calcium phosphate (CaP) glass with well-defined architecture, which showed good mechanical stability and biocompatibility for growth and proliferation of MSCs—stimulating bone tissue regeneration (Figure 6) [39]. In another study conducted by Bendtsen and associates fabricated 3D printed composite scaffold from hydroxyapatite, polyvinyl alcohol and alginate, which showed well-defined shape fidelity and excellent osteoconductivity [40]. Inzana and co-worker synthesized collagen-calcium phosphate composite scaffold by 3D printing method [41]. *In vivo* bone healing performance of the composite scaffold in curing a critically sized murine femoral defect in 9 weeks showed that the implants were osteoconductive with new bone formation [41].

A major problem in the reconstruction of large oral and craniofacial defects includes the neogenesis of osseous and ligamentous interfacial structures. Currently, oral regenerative medicine strategies are fickle for repair of tooth-supporting tissues destroyed as a consequence of trauma, chronic infection or surgical resection. Park et al. established multi-scale computational design and fabrication of composite hybrid polymeric scaffolds for targeted cell transplantation of genetically modified human cells for the formation of human tooth dentin-ligament-bone complexes *In vivo* [42]. They used polycaprolactone (PCL)-poly (glycolic acid) (PGA) designed constructs in which the newly-formed tissues grow and traverse within the scaffold, forming tooth cementum- like tissue, ligament, and bone structures. This approach offers prospective for the clinical performance of tailored periodontal scaffolds that may enable regeneration of multi-tissue interfaces required for oral, dental and craniofacial engineering applications.

For bone tissue regeneration, Shaui and coworkers fabricated composite scaffold of polylactic-co-glycolic acid (PLGA)/n-HAP (nano-hydroxyapatite) by selective laser sintering technology, and obtained well-controlled pore architectures with high exposure of the bioactive ceramics to the scaffold surface [43]. Soumya et al., reported that the biocomposites of alginate with nanobioactive glass ceramics enhanced mineralization and protein adsorption for bone tissue engineering [44]. Vo and coworkers fabricated composite hydrogels by combining copolymer macromers of N-isopropylacrylamide and gelatin microparticles and applied for the delivery of encapsulated MSCs at the damaged bone tissue site [45]. Encapsulated MSCs maintained viability up to 28 days under in vitro condition. *In vivo* critical size cranial defect of 8 mm in rat also showed that the gelling cell-laden composite hydrogels can facilitate bone ingrowth and integration, mineralization of regenerated bone tissue [45]. Tang and associates developed trimodal macro/micro/nano-porous scaffold (TMS) with mesoporous bioactive glass (MBG) as matrix loaded with recombinant human bone morphogenetic protein-2 (rhBMP-2) by casting method [46]. TMS induced excellent cell attachment, ingrowth and osteogenesis under *in vitro* condition. *In vivo* ectopic bone formation and orthotopic rabbit radius critical size defect treated with TMS showed rhBMP-2 delivery and biodegradability, and finally the regenerated tissue with good osteoconductivity and osteoinductivity [46]. Recently, in 2017, Lee and co-workers reported that the natural origin β-tricalcium phosphate scaffold reinforced with silk fibroin have remarkable mechanical strength, stability and porosity [47]. *In vitro* study showed higher proliferation and osteogenic differentiation of BMSCs over the scaffold. *In vivo* calvarial defects in model also showed bone mineralization, tissue formation and collagen production at the scaffold implanted wound site [47]. Xu and coworkers developed a polylactic acid (PLA) and chitosan (CS) composite scaffold via electrospinning accompanied by an automatic phase separation and crystallization [48]. This scaffold provided a good platform for cell adhesion and proliferation of pre-osteoblast (MC3T3-E1) cells [48]. This study provides a new approach to design PLA scaffolds with combined topographic and bioactive modification effects at the interface between cells and materials for biomedicine.

Fu and co-workers synthesized a novel three-component biomimetic hydrogel composite scaffold consisting of tri-block PEG-PCL-PEG copolymer (PECE), collagen and nano-hydroxyapatite (n-Hap) for bone tissue regeneration [49]. *In vivo* implantation of the composite construct in cranial defects in rabbit model showed better bone tissue healing with biocompatibility and better performance as compared to the self-healing process [49]. Pieri et al., showed that the transplantation of ADSCs (adipose derived stem cells) with an anorganic bovine

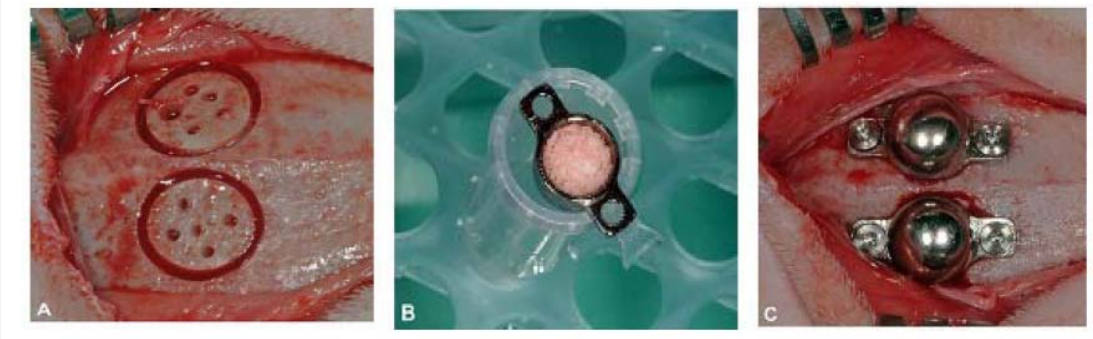

Figure 7 (A) Two circular 0.5-mm-deep slits were prepared in the calvarial bone, one on each side of the midline. Five cortical bone perforations were carried out inside the boundary of both slits. (B) Photograph showing the titanium dome grafted with ADSCs in combination with ABB immediately before implantation. (C) The titanium domes, one grafted with ASCs-ABB and one with ABB alone, were positioned on the slits and tightly fixed on the calvarial bone by means of two miniscrews (Reproduced with Permission from Reference [50]).

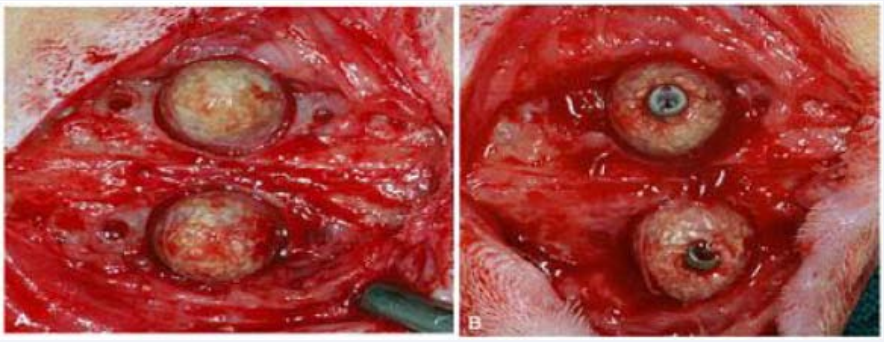

Figure 8 (A) The titanium domes were removed after 4 weeks of healing. Photograph of the vertically regenerated sites after dome removal. (B) Two 2x5-mm implants were placed into center of the regenerated sites (Reproduced with Permission from Reference [50]).

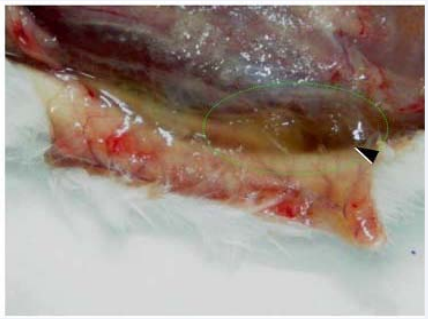

Figure 9 In vivo gel stability study: Representative macroscopic image of the post-mortal mouse showing the location and texture of iGel 24 h post-implantation (Reproduced with Permission from Reference [51]).

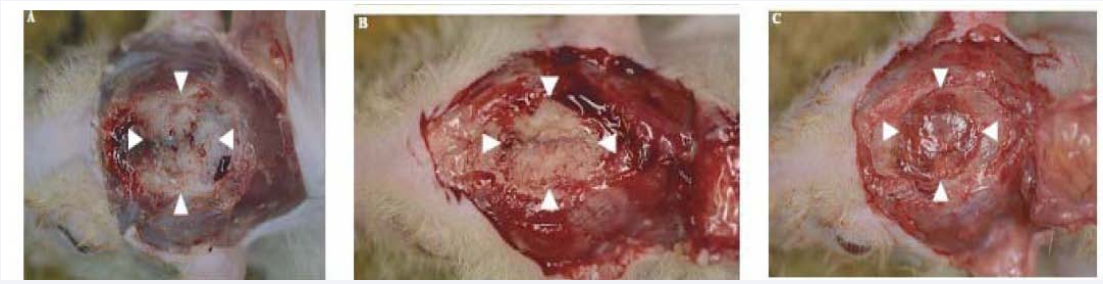

Figure 10 (A-C) Photographs showing representative calvarial bone defect of athymic rats 3 months after surgery. Animals with grafted fresh CP showed complete closure of calvarial defect (A). Animals, grafted with cryopreserved CP also showed complete closure of the defect, and there was no apparent difference between fresh and cryopreserved CP macroscopically (B). However, the bone defect of the control group without CP remained almost the same size as before grafting (C). Arrows indicate margin of the original bone defect (Reproduced with Permission from Reference [52]).

bone (ABB) scaffold enhances new bone formation and implant osseointegration following vertical bone augmentation on the calvarial bone of rabbits (Figures 7,8) [50]. Mishra and associates developed an enzymatically crosslinked carboxymethyl-chitosan/gelatin/nano-hydroxyapatite injectable gels (iGEL) for in situ bone tissue engineering application. The injectible gel may be used in treating irregular small bone defects with minimal clinical invasion as well as for bone cell delivery (Figure 9) [51]. Mase and associates studied the potential of periosteal cells to regenerate bone. It has been demonstrated that cultured periosteum (CP) in membrane form is an effective device to regenerate alveolar bone. The transplantation experiment showed that the calvarial bone defect of athymic rats were completely closed by grafting cryopreserved CP, which demonstrates that the osteogenic property of CP. This advanced research will provide a convenient and effective treatment option for bone regeneration in clinics (Figure 10) [52]. In 2007, an issue of obtaining highly porous structure with desired mechanical strength drives the scientists to focus on fabricating bionanocomposite scaffolds [53]. Wang et al., fabricated n-HA/PA (nanohydroxyapatite/polyamide) scaffold by using thermally induced phase inversion processing: this was employed for repairing critical defect on rabbit mandible, which showed very good biocompatibility and enhanced osteogenesis (Figure 11) [53]. Xu and co-workers

fabricated a porous β-calcium silicate (β-CS), which upon implantation in a rabbit calvarial defect, was found to stimulate bone regeneration (Figure 12) [54]. Salgado and team developed starch-based scaffolds which when implanted in rats, exhibited rapidly forming initial "connective tissue" around the scaffold—indicating early form of bone formation [55]. Steigman et al. tested the electrospun polylactic acid based scaffold to heal sternal repair by seeding amniotic mesenchymal stem cells in rabbit model and found to be successful in repairing the sternal repair (Figure 13) [56].

Sayin et al., in 2017, demonstrated a guided human osetoblast and ADSCs proliferation on scaffold fabricated by microchannel patterned elastin-like recombinamer (ELR) with a nucleation sequence for hydroxyapatite incorporated into collagen-silk fibroin blend film, for bone tissue regeneration [57]. After 28 days in vitro incubation, the ADSCs showed higher attachment, proliferation, mineralization, osteogenic differentiation [57]. In order to enhance osteogenesis and angiogenesis of bone repair, co-cultures of human umbilical vein endothelial cells (HUVECs) and human bone marrow stromal cells (HBMSCs) was studied by Wu and associates, on a polymer-ceramic composite scaffold of polyhydroxybutyrate–polyhydroxyvalerate (PHBV) and bioglass (BG) [58]. In vitro study results showed that BG-PHBV enhanced the osteogenic differentiation and vascularization of co-cultured HBMSCs and HUVECs, by up regulating paracrine effects between the two types of cells compared to pure PHBV scaffolds. Similarly, *in vivo* implanted co-cultured PHBV-BG (10%) scaffold on the back of mice showed the strong stimulatory effects on osteogenesis and angiogenesis [58].

CHALLENGES AND FUTURE PERSPECTIVES IN BONE TISSUE ENGINEERING

- As we know that cells without blood supply will die, and in this reference, perhaps the biggest challenge in bone tissue engineering, is how to insure angiogenesis in a sensible mode within the scaffold construct. Several scientists all over the world have been trying to find a convenient way to engineer bone with a blood supply that can be easily connected to the blood supply of the host. Another major challenge is how to engineer a piece of bone with the right dimensions: this is critical for some of the bone defects.
- Cell based issue includes a readily available and safe supply of osteogenic cells, and to reduce necrosis/apoptosis of implanted cells.
- Fixation and preservation of the scaffold at the wound-site is another important point of consideration.
- Maintenance of sustained delivery of growth factors and nutrient transport throughout the scaffold is of outmost importance [59].
- A basic understanding of the spatial and temporal distributions of cells and growth factors required for osteogenesis subject to particular diseased conditions, is yet to be determined
- Choice of growth factor or combination of growth factors (multiple growth factor approach) is to be investigated and optimized for successful bone regeneration [60-61].
- Furthermore, it should be kept in mind that most of the studies in tissue engineering were performed using mostly young adult and even fetal animal cells and not with cells from elderly osteoarthritis patients. Therefore, extensive research on using the cells from elderly osteoarthritis

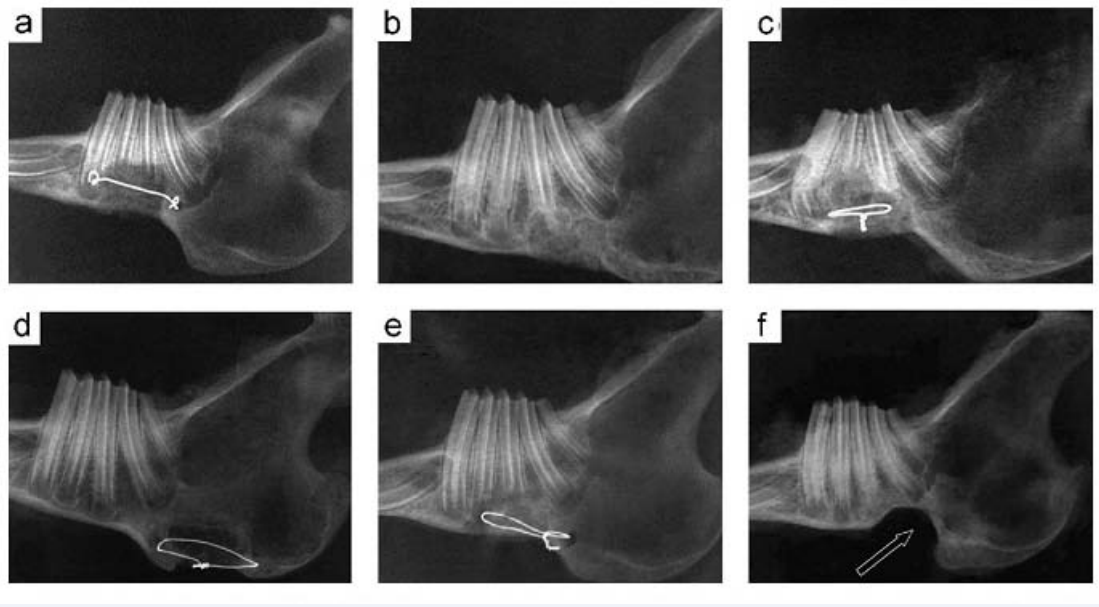

Figure 11 X-ray microradiographs of the defect area of the rabbit mandible after being implanted with MSCs hybrid n-HAp/PA scaffolds (a-c) and pure n-HAp/PA scaffolds (d,e), as well as the empty control group (f). Implantation time: (a) 4 weeks, (b,d) 8 weeks and (c,e,f) 12 weeks. Light area denotes the enhanced X-ray absorption that reveals more mineralization and higher bone density, whilst dark areas indicate less absorption of X-ray and lower bone density. The white cycles in some of the specimens are stainless-steel wires that are used to fix the scaffolds implanted in the mandible defects (Reproduced with Permission from Reference [53]).

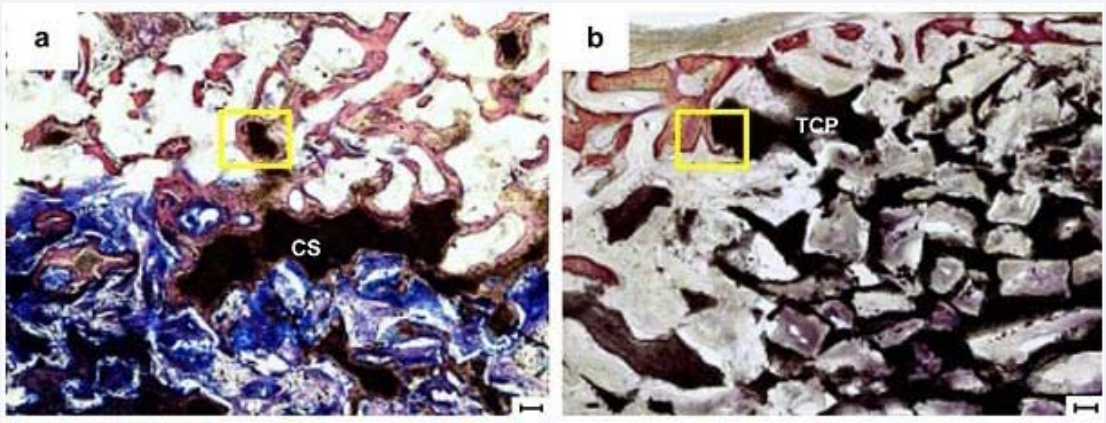

Figure 12 Histological morphologies of the interface between bone tissue and β-CS (a ,c) after implantation for 4 weeks. NB- newly formed bone, HB-host bone. Bars = 100μm (Reproduced with Permission from Reference [54]).

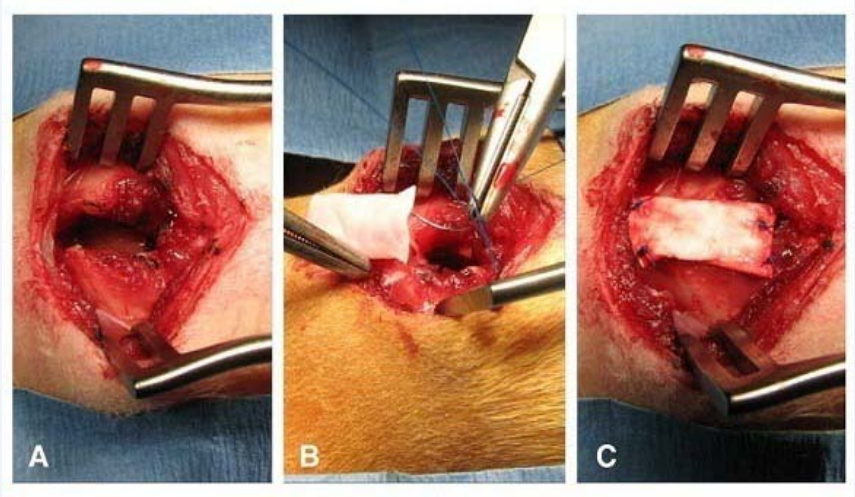

Figure 13 Sequence of repair of a surgically created full-thickness sternal defect with an engineered osseous graft, in a rabbit kit. Representative intraoperative photographs of (A) the sternal defect, (B) the graft being sewn into place, and (C) the repair of the defect nearing completion (Reproduced with Permission from Reference [56]).

patients will be needed to extend the results for treating human bone defects [62,63].

CONCLUSIONS

The field of tissue engineering is at its thrilling point with colossal research focused on producing new and improved biomimetic materials. Tissue-engineered bone constructs have the potential to reduce the demand arising from the shortage of suitable autograft and allograft materials for augmenting bone healing. They can also serve as controllable *In vitro* models of high biological fidelity for studies of bone development, disease or regeneration. However, the level of biological complexity that needs to be recapitulated within a synthetic 3-D environment is still uncertain. To this end, advanced scaffolds with molecular, structural and mechanical properties designed to mimic bone, are being developed to engineer bone grafts and to test the osteogenic capacity of stem cells. Specific bio functionality can be incorporated into the scaffold through polymer functionalization by inclusion of sequences recognized by cell membrane receptors. Each of the sources of osteogenic human cells (primary cells, MSCs, ESCs and induced pluripotent stem cells) has distinct advantages when used for bone tissue engineering, thus the pursuit for an 'ideal' cell source is still in progress. Moreover, as multiple growth factors are functional in a synchronized bone development, there is a need to focus on the releasing strategies of multiple growth factors at a time, to favour the production of more natural bone tissue. In this regard, gene therapy approach has the potential to provide control over the level of multiple regenerative factors that can be either simultaneously or sequentially expressed in a tissue-specific manner. Thus, the crucial combination of scaffold, cells and growth factors that can give rise to fully functional bone, still needs more focused research. It remains to be seen how much can be done in near future to obtain an ideal sized and anatomically shaped tissue engineered bone graft for implantation. As the scientists are still in the pursuit of finding new composites and best cell source to overcome pitfalls in bone repair, it is correct to point out that although, tissue engineering holds the promise of factual tissue

replacement but still this technology is years away from the true clinical use with huge success.

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