International Journal of Orthopaedics Research
www.orthopaedicsjournal.in
E-ISSN: 2663-0923, P-ISSN: 2663-0915
Received Date: 08-05-2019 Accepted Date: 11-06-2019; Published: 18-06-2019
Volume 1; Issue 3; 2019; Page No. 32-37

Bone tissue engineering in fractures and bone defects: Potential applications

Dr. Kavyansh Bhan
Senior Clinical Fellow (Trauma & Orthopaedics) Medway Maritime Hospital Gillingham England

Abstract
Exploring new Bone Tissue Engineering approaches to generate new bone for repair or Replacement of bone defects in the clinical setting relies on the combination of scaffolds, cells and growth factors. Understanding whether such approaches are suitable and optimised for the translation from bench to bedside requires preclinical testing in animal models. Through this review article, efforts have been made to enumerate different bone tissue engineering approaches, draw comparisons where relevant, and discuss advantages and disadvantages of each.

Keywords: bone, bone tissue engineering, scaffold, PRP

Introduction

Bone fractures and segmental bone defects are a significant source of patient morbidity and place a staggering economic burden on the healthcare system. The annual cost of treating bone defects in the US has been estimated to be $5 billion, while enormous costs are spent on bone grafts for bone injuries, tumours, and other pathologies associated with defective fracture healing. Autologous bone grafts represent the gold standard for the treatment of bone defects. Bone grafts are utilized in a wide array of clinical settings to augment bone repair and regeneration. Bone grafts are utilized in a wide array of clinical settings to augment bone repair and regeneration. Bone defect repair using the tissue engineering approach is perceived as a better approach because the repair process may proceed with the patient's own tissue by the time the regeneration is complete. (Ami R. Amini *et al*, 2013) [2] However, they are associated with variable clinical outcomes, postsurgical morbidity, especially at the donor site, and increased surgical costs. In an effort to circumvent these limitations, tissue engineering and cell-based therapies have been proposed as alternatives to induce and promote bone repair. (Jose R. Perez *et al*, 2018) [14] Bone tissue engineering is an exciting approach to directly repair bone defect or engineer bone tissue for transplantation. Biomaterials play a pivotal role in providing a template and extracellular environment to support regenerative cells and promote tissue regeneration. A variety of signalling cues have been identified to regulate cellular activity, tissue development, and the healing process. Numerous studies and trials have shown the promise of tissue engineering, but successful translations of bone tissue engineering research into clinical applications have been limited, due in part to a lack of optimal delivery systems for these signals. Biomedical engineers are therefore highly motivated to develop biomimetic drug delivery systems, which benefit from mimicking signalling molecule release or presentation by the native extracellular matrix during development or the natural healing process. Engineered biomimetic drug delivery systems aim to provide control over the location, timing, and release kinetics of the signal molecules according to the drug's physiochemical properties and specific biological mechanisms. (Ming Dang *et al*, 2018) [12].This study focuses on the recent advances in Bone Tissue Engineering (BTE), specifically looking at its role in treating Acute Fractures and Bone Defects.

Epidemiology

Bone loss or damage can result from various causes, including degenerative diseases, surgery, and trauma, significantly compromising patient quality of life. Bone possesses an intrinsic ability to repair itself, but there are many situations where complete bone regeneration cannot occur and needs to be stimulated. The worldwide incidence of bone disorders and conditions has trended steeply upward and is expected to double by 2020, especially in populations where aging is coupled with increased obesity and poor physical activity. Engineered bone tissue has been viewed as a potential alternative to the conventional use of bone grafts, due to their limitless supply and no disease transmission. (Ami R. Amini *et al*, 2013) [2]. Millions of patients suffering from bone defects require bone grafts or substitutes. The market of bone grafts and substitutes was valued at over 2.3 billion US dollars in 2015 and is expected to reach over 3.6 billion US dollars between 2016 and 2024. (Ming Dang *et al*, 2018) [12].

Methodology

Five Key Papers on Bone Tissue Engineering Caplan [4], 1991 -- This author postulated that isolation, mitotic expansion, and site-directed delivery of autologous stem cells can govern the rapid and specific repair of skeletal tissues. Friedenstein [9] *et al*., 1987 - The authors showed that a specific set of cells (colony forming unit fibroblasts—CFU-F or MSC) existing in bone marrow can differentiate to different cell types, including osteoblasts. Quarto *et al*., 2001 -- The first clinical paper to report repair of large bone defects with the use of autologous bone marrow stromal cells. Schimming *et al*., 2004 --The first study in humans showing that

periosteum- derived osteoblasts can form lamellar bone within three months after transplantation. Urist, 1965 -- The author showed that bone tissue contains specific growth factors that can induce bone formation in ectopic sites. Although, the abovesaid papers are widely regarded as Milestones in Bone Tissue Engineering, a more standardised approach of comprehensive review of the literature was required. Hence, Rodger's Evolutionary method (Coughlan, Cronin and Ryan, 2013) of concept analysis was utilized for this purpose. The National Library of Medicine was searched with key words "bone tissue engineering", "tissue engineering", "scaffolds", "stem cells", "regenerative medicine" and "3D Printing". A total of 39 results matched this search. For the purpose of this essay, only articles with available full texts and published in the last 6 years were considered, leading to a total number of 16 articles. Further references were obtained by hand search of Campbells Textbook of Operative Orthopaedics (13th edition) and Principles of Tissue Engineering (4th edition).

Discussion

Understanding Bone Tissue Engineering

Bone tissue engineering is concerned with creating implantable bone substitutes for critical skeletal defects that cannot heal on their own. These defects are common clinical scenarios in orthopaedics and craniofacial surgery, for the treatment of bone loss due to trauma, infection, and tumour resection. In the conventional tissue-engineering paradigm, combinations of cells and bioactive molecules are seeded onto three-dimensional biomaterial scaffolds to create an implantable 'osteogenic' implant. To date and despite numerous exciting advances in preclinical models, regulatory approval barriers, business challenges, and related intellectual property lifecycle issues have impeded clinical translation from the bench to the bedside. (Hani A Awad *et al*, 2014) The concept of BTE involves the integration of various concerting components: stem cells held together by a tri-dimensional biomaterial framework which provides the shape and initial mechanical strength, and molecular signals that induce differentiation of progenitor cells into the osteoblastic phenotype. The resulting construct can then be mechanically pre-conditioned *in vitro* to acclimate the growing structure to *in vivo* conditions, thus improving the functional coupling to the host bone (Jose R. Perez *et al*, 2018) [14]. This review will mainly focus on four fundamental components that take part in BTE in different settings, specifically:

- Stem cells
- Biomaterials
- Growth factors/Morphogens
- Mechanical stimulation

Fractures and Bone Defects

Fracture healing typically occurs uninterrupted during the first 6–8 weeks following an injury, although this process can be delayed by structural parameters such as the presence of thick cortices, which require more time to heal, as well as unfavourable mechanical and biological environments generated from excessive fracture site movement and/or gaps to general factors including aging, alcohol, tobacco, and steroid abuse and medical conditions such as infection, type 1 diabetes, anaemia, and deficient nutrition. (Kostenuik *et al*, 2017) In an effort to treat these defects**,** different types of bone grafts have been used including autografts, allografts, and synthetic grafts. Allografts have several drawbacks including graft rejection and disease transmission, while some synthetic grafts show an increased susceptibility to wear and tear. Autologous bone grafts, on the other hand, are considered the gold Standard to treat bone defects due to their established osteoinductive and osteoconductive properties, obviating the histocompatibility issue. When compared to allografts, auto grafts result in shorter time to union. However, after 10 years of incorporation, as high as 60% of grafts may fail to integrate leading to non-union. In an effort to find alternative therapies to treat bony defects and the complications associated with them, bone tissue engineering (BTE) has grown in popularity.

Stem Cells -- Tissue-specific cells (e.g., osteoblasts) can be used as the cellular component of engineered bone implants. However, technical difficulties associated with their harvesting, expansion into meaningful numbers and phenotypic maintenance undermine the benefits of using primary cells. Consequently, various types of stem cells have been largely proposed as a viable and easy source of osteoblast progenitors during the creation of engineered bone implants.

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that exhibit great differentiation potential into many different types of tissue lineages, including bone (osteoblasts), cartilage (chondrocytes), muscle (myocytes), and fat (adipocytes). Adult MSCs act as an inducible reserve force for tissue regeneration after injury, and therefore have been studied extensively for their therapeutic potential in fracture healing and bone regeneration. MSCs can be isolated from many different tissues including bone marrow, skeletal muscle, synovial membrane, and adipose tissue. (Jose R. Perez *et al*, 2018) [14].Biomaterials -- It is now nearly 50 years since Professor Hench in 1969 introduced the term "bioactivity" in biomaterials field, which is the characteristic chemical bondingbetween biomaterials and cells Hench, 2006). Specifically, the function of the biomaterial in BTE is to serve as a tri- dimensional framework for the stem cells to attach, grow and differentiate. There are several components of the biomaterial required for successful incorporation and functionality, including: (1) biocompatibility: incorporation into host tissues without eliciting an immune response; (2) biodegradability: as bone replaces the biomaterial, it provides supportive mechanical properties to withstand loading forces and uniformly distribute stresses; (3) proper surface properties and porosity: to influence cellular proliferation and differentiation; and (4) osteoinductive and osteoconductive properties: to recruit osteoprogenitors to the defect region and provide a controlled release of differentiation cues (Liu *et al*, 2013). The applicability of various biomaterials combined with MSCs for bone segmental defects treatment in preclinical settings is presented in Table. Ceramics -- Known for their effective biocompatibility, ceramic biomaterials are used more commonly in compressive loading conditions as they have very low wear rates due to their high hardness values. However, ceramics are also highly brittle. Because of their properties, ceramic-based biomaterials are commonly used on articulating surfaces, with calcium phosphate (CAP) and tricalcium phosphate being the most common Composites -- Composite biomaterials consist of polymers combined with ceramics, merging the benefits of both

classes while limiting their short-comings. They possess suitable properties for BTE such as mechanical toughness, improved biocompatibility, decreased creep-induced failure, load-bearing capabilities, host-implant interactions, and bioactivity (Niemeyer *et al*., 2004). By adding metals to these composites, additional benefits can be seen in bone interactions, strength, and osteogenesis. However, resorbable polymers degrade when expose to body fluid and therefore show poor mechanical properties for load-bearing orthopaedic applications

Table 1: Preclinical studies using Mscs and biomaterials for the treatment of bone segmental defects.

References	Cells	Biomaterials	Animal Model	Outcome
Bruder *et al*. (1998)	Canine BMSCs (7.5 × 10^6/ml)	Three groups used:	Segmental femoral bone defect (2.1 cm)	- At 16 weeks, radiographic union was established rapidly at the
		1. HA-TCP BMSCs,		interface between the host bone and the ha- tcp-bmscs implants
		2. HA-TCP, 3. Untreated	in canine model	only- Both woven and lamellar bone had filled the pores of the HA-TCP-BMSCs implants
Kon *et al*. (2000)	Ovine BMSCS (2.5 × 10^5/ml)	Two groups used: 1. HA-BMSCs 2. HA	Segmental tibial bone defect (3.5 cm) in ovine model	-At 2 months, extensive bone formation in HA-BMSCsmplants within the macropore space and around the implant- Stiffness higher in HA-BMSCs implant/bone complex compared to HA

Table 2

References	Cells	Biomaterials	Animal Model	Outcome
				control group
Arinzeh *et al*. (2003)	Canine BMSCs (7.5 × 10^6/ml)	Three groups used: 1) HA-TCP- allogeneic BMSCs 2) HA-TCP 3) Untreated	Segmental femoral bone defect (2.1 cm) in canine model	- No lymphocytic infiltration occurred and no antibodies against allogeneic cells were detected - At 16 weeks, new bone had formed throughout the HA-TCP-allogeneic BMSCs implant
Berner *et al*. (2015)	Ovine BMSCs (100 × 10^6)	Three groups used: 1) PCL-HA-allogeneic BMSCs 2) PCL-HA 3) Autologous bone graft	Segmental tibial bone defect (3 cm) in ovine model	- Minimally invasive percutaneous injection of allogeneic BMSCs into biodegradable composite biomaterials 4 weeks after the defect surgery led to significantly improved bone regeneration compared with preseeded biomaterial/cell and biomaterial-only groups
Masaoka et al. (2016)	Non-human primate BMSCs (1.3-4.1 × 10^6/ml)	Two groups used: 1) β-TCP-BMSCs 2) β-TCP	Segmental femoral bone defect (5 cm) in non-human primate model	-At 8–15 months, five of the seven animals treated with β-TCP-BMSCs implant showed successful bone regeneration

Growth factors -- Physiologically growth factors are usually stored in bone ECM, actively released after injury and play crucial role in bone repair with bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), and insulin-like growth factor 1 (IGF-1) being the major regulators of bone remodelling cascade. The therapeutic use of recombinant growth factors is based on the hypothesis that through appropriate signalling they induce and/or accelerate the bone healing process. (Jose R. Perez *et al*, 2018) [14]. Mechanical stimulation -- As clinical demand for bone grafts to treat congenital and trauma related skeletal defects continues to increase, the method of seeding hMSCs onto biological and synthetic biomaterials along with osteoinductive growth factors has been a significant advancement in the field of tissue engineering. However, the size of the tissue constructs that can be created under static conditions is greatly limited due to diffusional constraints of nutrients reaching bone cells which have very high metabolic requirements (Grayson *et al*., 2011). A solution to this problem is the utilization of perfusion bioreactors which can effectively disseminate nutrients and oxygen throughout graft constructs with a core larger than 200μm (generally thought to be the upper limit for oxygen diffusion and a bone graft in static culture). In addition to convective transport of nutrients and waste, the dynamic flow of perfusion bioreactors creates a mechanical stimulus that enhances osteogenesis and mineral deposition of cells in the graft (Gomes *et al*., 2003). It has been shown that use of a bioreactor allows for the cultivation of functional, clinically-sized bone grafts that can be used for transplantation (Grayson *et al*., 2011). Current Pre-clinical Scenario Bone defects are serious conditions in which a part of the bone is damaged or missing owing to trauma or surgery, and need to be repaired through interventional techniques such as bone grafting. There are many animal models being used to evaluate bone graft substitutes, but the main four types are the calvarial defect, long bone or segmental defect, partial cortical defect and cancellous bone defect models. Long Bone Segmental Defects -- Large animal models have been developed to assess the effectiveness of tissue engineering strategies in situations that more closely mimic the clinical scenario. In the majority of

reports, the CSD in long bones is created using an osteotomy approach whereby a drill or saw is used to remove the required segment from a predetermined site in the bone. Long-bone segmental defects have been modelled in several species, including dogs, sheep, goats and rabbits (Reichert *et al*., 2009), and a number of factors should be considered when selecting an animal species for long-bone defect modelling studies as detailed in Table 2. (Jacqui Anne McGovern *et al*, 2018) [10] Thus, the current preclinical scenario has mainly focused on Segmental bone Defects in Sheep and Rabbits.

Table 3: Depicting studies on Segmental Defects in Rabbit.

Method of Treatment	Site	Results	Study
Autogenous bone marrow with static magnet	Rabbit radial bone model	The bone that was induced by autogenous bone marrow concurrent with static magnetic field was shown to be superior than that was induced by only autogenous bone marrow.	Bigham A, Shadkhast M, Dehghani S. Autogenous bone marrow concurrent with static magnetic field effects on bone-defect healing: radiological and histological study. *Comp Clin Pathol* 2009; 18:163-8.
Bovine foetal growth plate	Rabbit radial bone model	Satisfactory healing occurred in rabbit radius defect filled with calf foetal growth plate. The use of calf foetal growth plate as a new xenograft is an acceptable alternative to cortical autogenous graft and could reduce the morbidity associated with harvesting autogenous graft during surgery.	Dehghani S, Bigham A, Nezhad ST, Shafiei Z. Effect of bovine foetal growth plate as a new xenograft in experimental bone defect healing: radiological, histopathological and biomechanical evaluation. *Cell Tissue Bank* 2008; 9:91-9.
Human Mineralized Bone Xenograft and Bone Marrow Mesenchymal Stem Cells	Rabbit Tibial Bone Model	new bone formation and step of maturity were significantly more, when the scaffold was used with MSCs.	Ai J, Ebrahimi S, Khoshzaban A, Jafarzadeh Kashi TS, Mehrabani D. Tissue engineering using human mineralized bone xenograft and bone marrow mesenchymal stem cells allograft in healing of tibial fracture of experimental rabbit model. *Iran Red Crescent Med J*. 2012; 14(2):96-103.

Presurgical factors	Surgical factors	Postoperative factors
Similarity in bone structure to the human bone	Anatomic site	Animal availability
Similarity in bone physiology to the human bone	Ease of access to the defect site due to the soft tissue	Animal cost
Similarity in bone mechanics to the human bone	Availability of suitable external and internal fixators	Animal housing
Social concerns		Established analysis tools
Lifespan of the animal		
Nutritional requirements		Tolerance to external fixation devices

Fig 2

Reference	Defect size	Biomaterial	Endpoint	Experimental outcome
Reichert et al., 2012b	3 cm (mid-diaphyseal tibia) stabilised with a modified 10-hole dynamic compression plate	mPCL-TCP scaffold±autologous MSC; mPCL-TCP scaffold±rhBMP-7; ABG (gold standard); empty defect	3 months	Empty defect had a 0% union rate, ABG and rhBMP-7 resulted in 100% bone bridging of the defect, mPCL-TCP±MSC: 37.5% with bridging in defect. CT measurement of TBV (mm^3): mPCL-TCP +rhBMP-7 resulted in largest TBV, followed by ABG, mPCL-TCP+MSC, mPCL-TCP alone then the empty defect.
			12 months	CT measurement of TBV (mm^3): mPCL-TCP +rhBMP7 resulted in largest TBV, followed by ABG, mPCL-TCP alone, then the empty defect.
Kirby et al., 2016	3 cm (mid-diaphyseal tibia) stabilised with a modified 10-hole dynamic compression plate	mPCL scaffold loaded with PLGA microparticles containing either: BMP-2 alone, BMP-2 in combination with VEGF and PDGF or empty microparticles	6 months	Segmental defects loaded with microparticles alone failed to bridge. Bridging was observed in BMP-2 microparticle-loaded and PDGF+VEGF +BMP-2 combined microparticle-loaded groups as measured by X-ray and CT. No observable differences in mechanical properties in BMP-2 alone or PDGF+VEGF +BMP-2 combined groups. Enhanced detection of blood vessels in PDGF +VEGF+BMP-2 combined group compared with BMP-2 and empty microparticle group, as determined by IHC.
Berner et al., 2013	3 cm (mid-diaphyseal tibia) stabilised with a modified 10-hole dynamic compression plate	mPCL-TCP scaffold generated by FDM, alone or seeded with autologous MSCs, allogenic MSCs or ABG	3 months	No difference in TBV between mPCL-TCP scaffold alone, or in combination with allogenic or autologous MSCs. All were lower than ABG as determined by CT. No signs of immunological reaction with allogenic MSCs as determined by histology.
Reichert et al., 2012b	3 cm (mid-diaphyseal tibia) stabilised with a modified 10-hole dynamic compression plate	Defects were left empty or reconstructed with mPCL-TCP or silk-HA scaffolds, or ABG	3 months	Empty defect did not heal, defects bridged with bone in ABG group, partial bridging in mPCL-TCP group and smaller amount of bone formation in silk-HA group, as determined by CT.
Reichert et al., 2011	2 cm tibial defect model stabilised with a limited contact locking compression plate	mPCL-TCP and (PLDLLA)-TCP-PCL scaffolds, empty defect or ABG	3 months	Defect was determined to be sub-critical (bridging was observed in empty defect group). Full bridging was observed in ABG group and partial bridging in mPCL-TCP and PLDLLA-PCL-TCP groups.
Maissen et al., 2006	1.8 cm segmental tibia defect in the mid-diaphysis stabilised by an external fixator	PLDLLA scaffold±rhTGFβ-3, compared with empty defect and ABG	3 months	Transient local inflammation in PLDLLA+rhTGFβ-3 group, but not in other groups (up to 3 weeks postoperation). CT analysis determined that the highest extent of bridging was observed in the ABG, followed by PLDLLA+TGFβ3, PLDLLA alone and then empty scaffold groups.
Berner et al., 2017	3 cm (mid-diaphyseal tibia) stabilised with a modified 10-hole dynamic compression plate	mPCL-TCP alone, or with allogenic mesenchymal-origin tOB, neural crest-origin mOB, or MPCs	6 months	Trend for slightly higher bone formation in mPCL-TCP+MPC, but overall no differences in TBV between all groups as determined by CT. In all groups, new bone detected external and external to scaffold and in the endosteal scaffold area. No differences between groups with biomechanical testing.
Cipitria et al., 2013	3 cm (mid-diaphyseal tibia) stabilised with a modified 10-hole dynamic compression plate	mPCL-TCP alone or combined with 1.75 mg or 3.5 mg rhBMP-7, compared with empty defect or ABG	3 months	CT analysis demonstrated defect bridging in ABG and both rhBMP-7 groups. Significantly higher TBV in both mPCL-TCP-rhBMP-7 groups as compared with mPCL-TCP scaffold alone. No differences in TBV between ABG and both mPCL-TCP+rhBMP-7 groups. Significantly higher torsional movement and torsional stiffness in both mPCL-TCP-rhBMP-7 groups compared with mPCL-TCP scaffold alone. No differences in mechanical properties between ABG and both mPCL-TCP+rhBMP-7 groups.
Cipitria et al., 2015	3 cm (mid-diaphyseal tibia) stabilised with a modified 10-hole dynamic compression plate	mPCL-TCP alone or with 3.5 mg rhBMP-7	3 months	Significantly higher BV in mPCL-TCP+rhBMP-7 group compared with mPCL-TCP alone group. Full defect bridging in mPCL-TCP+rhBMP-7 group.
			12 months	Significantly higher BV in mPCL-TCP+rhBMP-7 group compared with mPCL-TCP alone group. Full defect bridging in mPCL-TCP+rhBMP-7 group. Still only partial bridging in mPCL-TCP only group.

ABG, autologous bone graft; BMP-2, bone morphogenetic protein-2; FDM, fused deposition modelling; HA, hydroxyapatite; IGF-I, insulin-like growth factor-I; IHC, immunohistochemistry; mOB, orofacial skeleton-derived osteoblasts; mPCL, medical grade polycaprolactone; MPCs, bone marrow-derived mesenchymal progenitor cells; MSCs, mesenchymal stem cells; PDGF, platelet-derived growth factor; PLDLLA, poly(L-lactide-co-D,L-lactide); PLGA, poly(lactic-co-glycolic acid); TBV, total bone volume; TGFβ-3, transforming growth factor beta 3; tOB, axial skeleton-derived osteoblasts; VEGF, vascular endothelial growth factor.

Fig 4: depicting Bone Tissue Engineering concepts in Ovine Segmental Defects (Jacqui Anne McGovern *et al*, 2018)

Potential Applications

Exploring new Bone Tissue Engineering approaches to generate new bone for repair or replacement of bone defects in the clinical setting relies on the combination of scaffolds, cells and growth factors. Understanding whether such approaches are suitable and optimised for the translation from bench to bedside requires preclinical testing in animal models. Over recent years, an emphasis has been placed on the optimisation of small and large animal preclinical models of bone loss and regeneration due to the rapidly expanding field of Tissue Engineering. Large animal models offer a suitable system for the testing of TE products used to restore bone defects, whereas small animal models are being explored to model primary and secondary bone-related malignancies.The motivation for the future of preclinical *in vivo*testing must now be to standardise these procedures at every level, from animal species choice to surgical practice. Such standardisation will shrink the gap between the creation of bone TEC to their regulatory approval and clinical testing. This will allow for greater translation of novel experimental TE scaffolds into the clinical practice of restoring traumatic bone loss.

Conclusion

The evidence presented strongly suggests that Regenerative Medicine for Fractures and Bone Defects is still in its nascent stages and further research is required to achieve desirable outcomes which will meet patient expectations in humans. Its promising and the potential to translate the current research to humans exists.

References

1. Ai J, Ebrahimi S, Khoshzaban A, Jafarzadeh Kashi TS, Mehrabani D. 'Tissue engineering using human mineralized bone xenograft and bone marrow mesenchymal stem cells allograft in healing of tibial fracture of experimental rabbit model.', Iran Red Crescent Med J. 2012; 14(2):96-103.
2. Amini AR, Laurencin CT, Nukavarapu SP. 'Bone tissue engineering: recent advances and challenges.' Critical reviews in biomedical engineering. 2012; 40(5):363-408.
3. Aoyama T, Goto K, Kakinoki R, Ikeguchi R, Ueda M, Kasai Y. *et al*. An exploratory clinical trial for idiopathic osteonecrosis of femoral head by cultured autologous multipotent mesenchymal stromal cells augmented with vascularized bone grafts. Tissue engineering. Part B, Reviews. 2014; 20(4):233-242. doi:10.1089/ten.TEB.2014.0090
4. Arnold I Caplan, 'Mesenchymal stem cells'. Journal of Orthopaedic Research, 1991. https://doi.org/10.1002/jor.1100090504
5. Berner A, Henkel J, Woodruff MA, Steck R, Nerlich M, Schuetz MA. *et al*. Delayed minimally invasive injection of allogenic bone marrow stromal cell sheets regenerates large bone defects in an ovine preclinical animal model. Stem cells translational medicine. 2015; 4(5):503-512. doi:10.5966/sctm.2014-0244
6. Bigham A, Shadkhast M, Dehghani S. 'Autogenous bone marrow concurrent with static magnetic field effects on bone-defect healing: radiological and histological study.' Comp Clin Pathol. 2009; 18:163-8.

7. Cai J, Wu Z, Huang L, Chen J, Wu C, Wang S, 'Cotransplantation of bone marrow mononuclear cells and umbilical cord mesenchymal stem cells in avascular necrosis of the femoral head.', Transplantation Proceedings, 2014; 46:151-155. 10.1016/j.transproceed.2013.06.021
8. Dehghani S, Bigham A, Nezhad ST, Shafiei Z. 'Effect of bovine foetal growth plate as a new xenograft in experimental bone defect healing: radiological, histopathological and biomechanical evaluation, Cell Tissue Bank. 2008; 9:91-9.
9. Friedenstein AJ, Chailakhyan RK, Gerasimov UV. 'Bone marrow osteogenic stem cells: *in vitro* cultivation and transplantation in diffusion chambers, Cell and Tissue Kinetics. 1987; 20(3):263-72.
10. Jacqui Anne McGovern, Michelle Griffin, Dietmar Werner Hutmacher. 'Animal models for bone tissue engineering and modelling disease', Disease Models and Mechanisms, 2018. doi: 10.1242/dmm.033084
11. Masaoka T, Yoshii T, Yuasa M, Yamada T, Taniyama T, Torigoe I. *et al*. Bone Defect Regeneration by a Combination of a β-Tricalcium Phosphate Scaffold and Bone Marrow Stromal Cells in a Non-Human Primate Model. The open biomedical engineering journal. 2016; 10:2-11. doi:10.2174/1874120701610010002
12. Ming Dang, Laura Saunders, Xufeng Niu, Yubo Fan, Peter X Ma. 'Biomimetic delivery of signals for bone tissue engineering', Bone Research, 2018. https://doi.org/10.1038/s41413-018-0025-8
13. Mittwede PN, Gottardi R, Alexander PG, Ivan S, Turkin. Clinical Applications of Bone Tissue Engineering in Orthopedic Trauma', Current Pathobiology Reports. 2018; 6:99. https://doi.org/10.1007/s40139-018-0166-x
14. Perez JR, Kouroupis D, Li DJ, Best T M, Kaplan L, Correa D. *et al*. Tissue Engineering and Cell-Based Therapies for Fractures and Bone Defects. Frontiers in bioengineering and biotechnology. 2018; 6:105. doi:10.3389/fbioe.2018.00105
15. Roseti L, Parisi V, Petretta M, Cavallo C, Desando G, Bartolotti I. *et al*, 'Scaffolds for Bone Tissue Engineering: State of the art and new perspectives.', Materials Science and Engineering. 2017; 78:1246-1262. doi: 10.1016/j.msec.2017.05.017.