INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact Factor: 6.078
(Volume 7, Issue 3 - V7I3-1456)
Available online at: https://www.ijariit.com

# Recent advancement in adipose stem cell-based bone tissue engineering

*Raksha M.*
*raksha51mohan@gmail.com*
*RV College of Engineering, Bengaluru, Karnataka*

*Shravani K. S.*
*shravaniks4u@gmail.com*
*RV College of Engineering, Bengaluru, Karnataka*

## ABSTRACT

***Adipose stem cells (ASCs), a variety of mesenchymal stem cells (MSCs) are an essential element within bone tissue engineering (BTE). ASCs offer several advantages in isolation, culturing, maintenance, differentiation, repair etc. Because of all these advantages of ASCs along with high paracrine activity, lacking major histocompatibility complex class II expression autologous ASCs are attracted towards treatment for organs damaged due to disease or injury and also in regenerative medicine. Bone is very compound tissue and it takes part in various processes which includes endocrine functions, haematopoiesis, mineral homeostasis and storage, body movement. Bone tissue engineering approaches involves combining cells and engineering materials with signalling molecules which is crucial for effective bone repair. ASCs are vital element in BTE. BTE requires 3 important elements: (1) osteoprogenitor cells, (2) bioactive factors, (3) scaffold. In this review, we will be focusing on the recent discoveries which have employed the use of scaffolds, ASCs, bioactive factors in BTE. This review first outlined the advantages of ADCs in terms of isolation, culture, differentiation etc. The different Growth factors involved in osteogenesis, which are the key segments in BTE. The available and recent innovations in the biological, mechanical and structural necessities in the design of a profitable scaffold and their effects, which is succeeded by summary of regular materials intended for scaffold fabrication were then briefed.***

***Keywords***— *Adipose Stem Cells, Cell Therapy, Bone Tissue Engineering, Growth Factors, Scaffolds*

## 1. INTRODUCTION

Stem cells are ones which have the capacity to differentiate into specific cell types. In order for a cell to be called as "stem cell," they must exhibit two important features, which includes continuous self-renewal to yield a progeny which is accurately same as the mother cell and their capacity to differentiate into a specified adult cell type [1]. Stem cells are divided into "embryonic," "adult", induced pluripotent stem cells depending on the developmental stage of an organism/animal from which they have come.

Adult stem cells one of the categories of stem cells are undifferentiated cells which are found in specific differentiated tissues inside our bodies which can produce or refurbish themselves to replenish damaged (due to injury, infection) or dead tissue. Adult stem cells are found all over an individual's lifetime in various tissues which includes skin, fat tissue, umbilical cord, gut, muscle, placenta, bone marrow, brain, etc. ASCs were initially extracted and used for production of blood in 1948. Later this procedure was extended in 1968 when adult bone marrow cells were first used in clinical therapies for blood related diseases. (https://www.unmc.edu/stemcells/educational-resources/types.html)

Categories of adult stem cells include Mesenchymal Stem cells (MSCs), Epithelial, Blood, Skin and Neural stem cells. Adipose - derived stem cells which is subcategory of MSCs is easily obtained from adipose tissues and they hold several regenerative properties when compared to the remaining MSCs. Some of the major advantages of ASCs include they are easily available in enormous quantities by means of a minimally invasive procedure, they can stick to plastic culture flask easily and multiply in vitro, differentiate into multiple cell lineages and hence they can be used to maintain, repair or enhance various tissues and immune-privileged as they lack major histocompatibility complex class II expression (hence they have the capacity to suppress proliferation of allogenic activated lymphocytes). Because of all these advantages along with their paracrine activity (secretion of various bioactive molecules), autologous ASCs are attracted towards treatment for organs damaged due to disease or injury and also in regenerative medicine. Some of the applications of ASCs include they are effective against pathological wound healing, immunological disorder, treating severe refractory acute graft-versus host disease, haematological disorder [2]. ASCs can

 

differentiate into numerous cell type and lineage-specific differentiation is directly associated to the expression of mature tissue genes and specific phenotypic markers.

## 2. BONE TISSUE ENGINEERING (BTE)

Tissue Engineering (TE) methods targets to combine cells and engineering materials with the signalling biomolecules which is crucial for an effective tissue repair. Bone tissue engineering approaches involves combining cells and engineering materials with signalling molecules which is crucial for effective bone repair. ASCs are vital element in BTE. Bone is a very compound tissue and it participates in various physiological processes which includes endocrine functions, haematopoiesis, mineral homeostasis and storage, body movement [3]. Bone fractures stay amongst the utmost common organ injuries and they can have deleterious effect on the wellbeing of patients. However, due to the presence of self- repair and renewal capacity of bone tissue using the surrounding osteoprogenitor cells results in scar less healing. But sometimes the damage can exceed the self-repair capacity and cause delayed healing, persistent bone defects, scar formation, and non-union. This condition may be due to reduced number of progenitor cells, patients' comorbidities, genetic factors or lifestyle. Hence understanding mechanism of bone repair is of utmost importance for patients' economy and health. One of the treatments highly used in this situation is autologous bone graft, but this treatment has many drawbacks and hence researchers are targeting on unconventional treatment options like BTE. TE–based bone grafts are evolving as a doable treatment where the composition and structure of scaffolds stimulate the conventional osteogenic pathways from an active procedure which takes place in MSCs. BTE involves reconstructing bone substitutes that are readily get-at-able, significant regenerative potential and highly biocompatible. BTE involved first fruitful usage of ASCs back to a decade ago. However, a superior understanding of ASC physiology, their subpopulations, paracrine actions and differentiative mechanisms has led to constant development of innovative applications for ASCs usage in designing tissue engineering products (TEPs). TEP combines 3 factors:

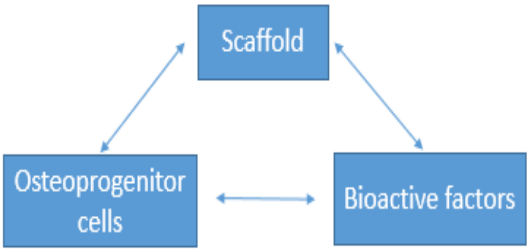


**Fig 1: Three Important factors necessary for BTE**

### 2.1 Osteoprogenitor cells

The above-mentioned advantages and the phenotypic study of ASCs have enhanced the vasculogenic and osteogenic capacity of ASCs. They have improved vascularization and also integration of the scaffolds. However, various studies are carried out to improve scaffolds' materials and design enhance the osteogenic attributes of ASCs and decreasing the requirement for any peripheral bioactive factors. ASCs secrete exosomes that are incredible source of pro-osteogenic, bioactive factors. The secreted exosomes show paracrine effects in the atmospheres, even when primary cells are absent and enhance regeneration.

### 2.2 Bioactive factors

Bioactive factors provide signals at the location of injury hence letting the inflammatory and progenitor cells to travel and commence the healing process. To upsurge the osteoconductive action, scaffolds will be bio activated by administering chemical condition like molecules [4]. Growth factors is one of the important bioactive factors that have chief regulatory tasks from stimulation of cell recruitment until differentiation of osteogenic precursor to a mature osteoblast. The cells will be seeded in scaffold enriched with growth factors (GFs) such as, bone morphogenetic proteins (BMPs), platelet-derived GFs, insulin-like GFs, fibroblast GFs, vascular endothelial growth factors, parathyroid hormones, Wnts/beta-catenins, Hedgehog, prostaglandins and transforming growth factor β (TGFβ) [5]. The most commonly used growth factor is BMP. They initiate the fracture repair cascade; they trigger the differentiation of osteoprogenitors (ASCs) and recruit them to the spot of injury [6]. Particularly BMP-2 plays a crucial part in the appearance of osteogenic markers. Controlled release technology has been advanced over last years and this technology permits the encapsulation of GFs in carrier structures so as to protect, prolong and localize their bioavailability. Combined delivery of several GFs are being studied lately in order to summarize the natural frequency of GF bioavailability [7].

**Table 1: Different growth factors, their delivery system and their effect for bone tissue engineering applications: [7]**

| Growth factor | Author (year) | Union approach | Carrier substance/ scaffold | Releasing profile | Result |
|---|---|---|---|---|---|
| VEGF and BMP-2 | Young S. Et al, (2009) | Microparticle encapsulation | Gelatin microparticles in Poly propylene fumarate scaffold | first 24h of burst releasing and 27 days of sustained release. | There was no increase in bone formation due to concurrent release of VEGF and BMP-2 over BMP-2 alone at 12 weeks in cranial critical size rat defect model.[8] |
| BMP-2 and GDF-5 | Bessa PC. Et al, (2010) | Nanoparticle encapsulation. | Thermoresponsive elastin nanoparticles | initial burst release for 24h and then 14 days of gradual release. | Increase in mineralization and ALP activity in C2C12 cells. [9] |

 

| | | | | | |
|---|---|---|---|---|---|
| IGF-1 and BMP-2 | Kim S. Et at, (2012) | microparticle /Adsorption encapsulation | chitosan gel scaffold with gelatin microspheres | Initial release of BMP-2 later slow and sustained release of IGF-1. | Superior alkaline phosphatase (ALP) action was observed in W-20-17 cells which were treated with successive delivery system as contrasted to other treatment after a week of culture.[10] |
| GDF-5 | Seiichi Yamano. et al, (2014) | Physical entrapment | Collagen scaffold/ membrane | Sustainable release for about 21 days | GDF-5 encouraged fresh bone formation in a dose-dependent fashion. Hence it suggested that this approach improved the existing clinical treatment of various bone defects. [11] |
| BMP-2 | Hankenson. Et al, (2015) | adsorption | Polyelectrolyte multilayer coating upon PLGA tube | Quick burst | formation of mineralized and vas-cularized bone in a rat critical-size flaw model.[12] |
| TGF-β3 and BMP-2 | Di Luca A. et al, (2017) | Covalent. | PCL-POEGMA scaffolds | No discharge of BMP-2 and TGF-β3 from scaffold. | increased osteochondral differentiation of hMSCs. [13] |

Chemical stimuli upsurge the therapy effectiveness but there are some restrictions which includes tumours formation. [4]

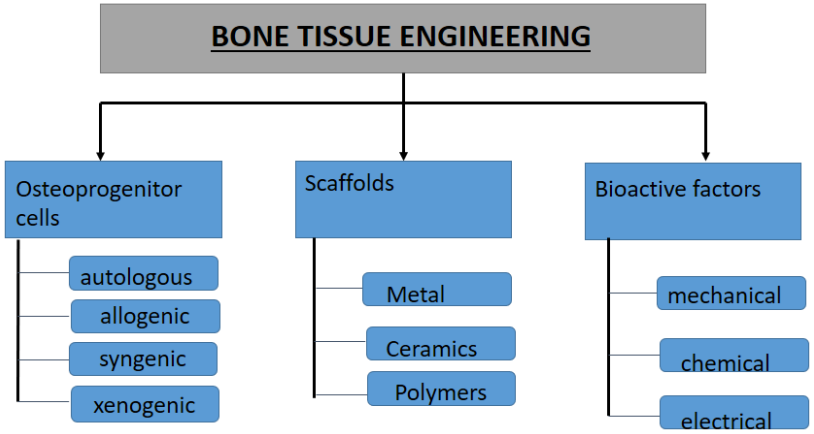


**Fig 2: Overview of BTE**

### 2.3. Scaffolds

The main aim of BTE is to cultivate 3D scaffolds that are able to imitate extracellular matrix (ECM) physical properties and offer mechanical support in order to assist cells employment, adhesion, proliferation and differentiation [15]. These Scaffolds provide a template for cell attachment and stimulate functional osteocyte differentiation in-vivo. Some of the important characteristics of scaffold include porous, biodegradable and chemically biocompatible so that they encourage vascularization, provide biochemical, physical and mechanical stimuli. An optimum scaffold should promise bioactivity, dimensional stability, easily manufactured, biodegradability. The advantages of using acellular scaffold in BTE include longer shelf-lives, ease of sterilization, immunogenicity or low potential for infection. A great range of scaffolds have been established according to the main necessities of osteo-inductivity, cytocompatibility and osteo-conductivity.

**Table 2: Some of the important requirements of scaffolds for effectiveness in BTE: [14]**

| Requirement | Description |
|---|---|
| Bioactivity | They should communicate with the tissue according to osteoconductive and osteo-inductive principles. |
| Cytocompatibility | products released have to be non-inflammatory and non-toxic. |
| Biodegradability | It should degrade by external-enzymatic/biological process in a controlled way. |
| Tunable properties | Should have customizable properties. |
| Mechanical features | they have to reproduce fatigue and elastic strength of the bones tissue spot. Young's modulus of approximately 0.1 -5GPa. Compressive strength of approximately 2-12 MPa. |
| Easy processing, manufacturing and handling | Easy fabrication, sterilized and clinical handling is essential. |
| Porosity | It should have micro porosity so that it guarantees adequate surface area for communication with tissues. micro porosity is essential for cell growth and also cell migration. While, porosity should not disturb the mechanical stability. Pore sizes normally ranges from approximately 100 µ m-300 µ m. [16] |

 

**2.3.1 scaffold materials**

In order to meet the structural, mechanical, biological requirements for fruitful BTE, different categories such as metals, polymers and ceramics have been calculated for their capacity to support the development of fresh, functional bone and also their osteogenic properties. Natural polymeric scaffolds are made of extracellular biological materials of 3 groups: 1) proteins (elastin , silk, collagen, keratin, fibrinogen, gelatin); 2) polysaccharides (glycosaminoglycans, dextran, amylose, chitin, cellulose); and 3) polynucleotides (RNA and DNA) [17],[18].

**2.3.1.1 Metals:** These might be considered as the oldest materials used and it was first recorded to be used in Egyptian times [15]. Till date there are various metallic materials used for ortopedic surgery so as to offer support for healing bones or substitute the damaged bone. Some of the standard metallic materials include Cobalt based alloys (ASTM F75, ASTM F799), stainless steel 316 L, titanium alloys. [20]. Most frequently used metallic biomaterials include titanium and its alloys because of their corrosion resistance, non-toxicity, biocompatibility. Some of the advantages of using metallic biomaterials as implants include corrosion resistance, good biocompatibility, and strength. However, there are certain disadvantages of metallic scaffolds which includes absence of biological identification on the material surface, not degradable etc. [22]-[23]

**2.3.1.2 Bio ceramics:** melting of inorganic materials to create crystalline or amorphous body, is known as bio ceramics [25].They can be classified into-

- Non resorbable (relatively inert)- alumina, silicon nitride, zirconia.
- Surface active or bioactive (semi inert) –ceramics, glass.
- Resorbable (non-inert)- aluminium calcium phosphates, calcium phosphates, tricalcium phosphate, coralline, zinc sulphate calcium phosphates, zinc calcium phosphorous oxides, calcium aluminates.

**2.3.1.3 Natural scaffolds:** It could be of 2 types i.e. cell-derived (cells are seeded onto a supporting matrix or used to generate new bone tissue) and tissue-derived (directly bone tissue is used). Natural polymers such as gelatin, alginate, chitosan, collagen, fibrins, levan, starch, emulsan, fibroin, dextran, cellulose, albumin, gellan, heparin, scleroglucan, silk, hyarulonic acid, curdlan and so on. Benefits of using natural polymers includes their easy processing, biocompatibility, they can more closely mimic the natural ECM of the tissues. However, there are limitations which include expensive, short supply, susceptible to cross-contamination. Some of the examples, advantages and disadvantages are mentioned in table 3.

**2.3.1.4 synthetic polymers:** The physiochemical properties can be easily controlled using these scaffolds. Some of the advantages of using synthetic scaffolds include no immunogenicity, easily available, easily formed with good mechanical strength. However, there are certain limitations which includes toxicity, uncontrollable shrinkage, lack cell recognition signals etc. [24]. They can be classified into and 2 types.

- Biodegradable-polyglycoside, polyphosphazene, lactide –co-glycolide, polycyanoacrylate, polydioxanone etc.
- Non-biodegradable- polyhydroxyethymethacrylate, polyvinyl alcohol, N-isopropylacrylamide etc.

**Table 3: Summary of different materials, key advantages and restrictions**

| Scaffold substance | Example | Advantages | Disadvantages |
|---|---|---|---|
| Metal scaffold | Titanium, magnesium alloy, NiTi, porous tantalum | High compressive strength<br>High young's modulus | Ion release,<br>Not degradable<br>[26]-[29] |
| Ceramics | TiO2, Bioglass, Hap, b-TCP, | Biodegradable,<br>Chemically biocompatible | Susceptible to to fracture and fatigue<br>Brittle. [30]-[33] |
| Natural polymer scaffolds | Collagen | Similar to ECM, Possible injectability, Cytocompatibility, FDA approved, Enzymatic biodegradability, Flexibility in processing to diverse physical forms, Cytocompatibility and cell-binding properties. | Difficult disinfection and handling, Low mechanical strength.<br>[34]-[35] |
| | Gelatin | Biodegradability, Cytocompatibility, Osteoconductivity and Porosity tunability | Poor mechanical properties.[36]-[37] |
| | Chitosan | Mucoadhesivity, Cytocompatibility, Antibacterial properties, Biodegradability, Easy tenability of properties, Cell-binding, differentiation and migration properties. | Rapid in vivo degradation rate, Poor stability and mechanical strength. [39] |

 

| | | | |
|---|---|---|---|
| | Silk fibroin | Immunogenicity, Cytocompatibility, Restricted biological adhesion, Flexible processability, Thermal stability, Easy chemical modification, excellent mechanical strength. | Spider silk production is very less. [38] |
| | Cellulose | Bioactivity, Hydrophilicity, Optical transparency, Cytocompatibility, Tuneable properties. | [39]-[43] |
| | Alginate | Cytocompatibility, Easy gelling, Tuneable properties. | Non-degradable in mammals, Difficult to sterilize as it causes degradation, Little cell adhesion. [44]-[45] |
| | Hyaluronic acid | Viscoelasticity, Cytocompatibility, Easy chemical functionalization, Biodegradability, Easy manipulation, Enzymatic biodegradability | Rapid degradation, reduced mechanical strength and water solubility.[46]-[49] |
| Synthetic polymers | PCL | Biodegradability, Cytocompatibility, Slow degradation rate | Low bioactivity, Hydrophobicity, Acidic degradation by-products.[50] |
| | PLGA | Tunability, Varied range of degradation rate, | Suboptimal mechanical belongings, poor cell adhesion, possibility of inflammatory response, Poor osteo-conductivity. [51]-[52] |
| | PLA | Cytocompatibility, excellent biodegradation rate, Thermal stability, good biosorable, Tuneable properties. | Poor cell adhesion, possibility of inflammatory response, poor compression strength. [53] |
| | Polyurethanes | good biocompatibility, Excellent mechanical properties. | degradation products can be toxic in nature.[46] |

**Table 4: Recent literature about different scaffolds and their result.**

| Author, (year of publication) | Type of cells | Type of scaffold | Experimental model | Result |
|---|---|---|---|---|
| Sándor GK, (2012) | hASCs | resorbable scaffolds | 23 patients with large craniofacial osseous flaws. | 23 patients were ASC seeded with resorbable scaffolds along with rhBMP-2 and was positively implanted into humans so as to recreate their jaws and found 3 failures (1 case of infection and 2 cases with inadequate bone formation).[54] |
| Daei-farshbaf. et al, (2013) | hASCs | Bio-Oss and type 1 collagen gel | Nine male Wistar rats (6–7 weeks old ) | No sign of inflammation or mortality was seen after 8 weeks of implantation at the site of defect. Rats were randomly divided into 3 clusters 1) control cluster (with zero defect) 2) experimental cluster where defect is filled with only scaffold (Bio-Oss+typeI collagen gel) 3) experimental cluster where fault is filled with scaffold i.e. Bio-Oss+typeI collagen gel loaded with AT-MSCs. Results of imaging analysis and histological staining showed that scaffold i.e. Bio-Oss+type I collagen gel loaded with AT-MSCs showed highest level of bone regeneration, and impressive osseointegration.[55] |
| Carvalho. et al, (2014) | hASCs | Wet-spun starch + PCL i.e. SPCL | Invitro +murine calvarial CSD | Micro-CT imaging and Histological analysis showed enhanced fresh bone deposition, osseointegration with SPCL loaded with hASCs engrafted calvarial defects when equated with control.[56] |
| Vériter S. et al., (2015) | Human ASCs | Human demineralized bone scaffold | Clinical case study of 17 patients who had not experienced any positive result with | 16 patients had no oncological recurrences or no adverse effects (follow up of about 54 months). And the other patient had spontaneous bone healing and hence did not undergo grafting. [57] |

 

| | | | | |
|---|---|---|---|---|
| | | | ordinary/traditional therapy. | |
| Eunkyung Ko. Et al, (2016) | hASCs | *Nanostructured Tendon-Derived Scaffolds* | Mouse Model which has Calvarial Bone Defect. | *Nanostructured Tendon-Derived Scaffolds* made of decellularized tissue matrices enhanced osteogenesis of ASCs. [58] |
| Chi Zhang. et al, (2017) | hASCs | biofabricated small intestinal submucosa (SIS) scaffold | C57BL/6 mice | Biofabricated SIS scaffold coated with osteogenic ECM provided bony microenvironment for ADSCs and enhanced their differentiation, proliferation, bone repair and bone regeneration.[59] |
| Duan W. et al., (2018) | Equine ASCs | PEG/PLLA/ HA /TCP (36: 24 :16: 24 ) or PEG/PLLA (60: 40), TCP/HA (40 : 60). | murine ectopic ossification+ In vitro model | PEG/PLLA/HA/TCP and TCP/HA upheld osteogenic differentiation of ASCs even where there is lack of any differentiating factors. [60] |
| Lee. Et al, (2019) | immortalized murine adipocyte (iMAD) | PPCN-gelatin | Twelve mice (Eight-week-old male,nude, athymic, mice) | PPCN-gelatin scaffold combined with iMADs support in vivo osteogenesis and it was considerably great when correlated with the control. [61] |

## 3. CONCLUSIONS AND FUTURE CHALLENGES

ASCs established a chief role in BTE by furnishing high versatility of application, many novel solutions which is evident in both in vivo and in vitro. Usage of ASCs has revealed to be more advantageous over other MSCs with respect to isolation, differentiation, interactions with microenvironment etc. for regenerative purposes. BTE research has considerably progressed, in terms of encouraging strategies for GF transport and scaffold fabrication. This review article primarily focuses on key segments of BTE which includes defined the advantages of ADCs in terms of isolation, culture, differentiation etc. and the diverse GFs engaged in osteogenesis. The available and recent advancements in the biological, structural and mechanical requests in the design of a fruitful scaffold and their effects, and after that summary of regular materials used for scaffold fabrication were then briefed. In spite of these findings, BTE also face numerous challenges with respect to clinical implementation. Firstly, increased workload is necessary to progress strong and highly porous biomaterials with a good and controlled biodegradation and decreasing present disadvantages of the available scaffolds. The field must augment and accurately control scaffold physical, biological and mechanical properties. The selection of appropriate elements could help in the forthcoming in streamlining the design by decreasing necessity of non-physiological and strong differentiative stimuli.

**ACKNOWLEDGEMENT**

The authors would like to thank R.V. College of Engineering staff for their technical support towards this review paper.

**REFERENCES**

[1] Biehl, J. K., & Russell, B. (2009). Introduction to stem cell therapy. The Journal of cardiovascular nursing, 24(2), 98–105.
[2] Zizhen Si, Xue Wang, Changhui Sun, Yuchun Kang, Jiakun Xu, Xidi Wang, Yang Hui (2019).
[3] Porter J. R., Henson A., Popat K. C. Biodegradable poly(ε-caprolactone) nanowires for bone tissue engineering applications. *Biomaterials.* 2009;30(5):780–788.
[4] Donnaloja, F., Jacchetti, E., Soncini, M., & Raimondi, M. T. (2020). *Natural and Synthetic Polymers for Bone Scaffolds Optimization. Polymers, 12(4), 905*.
[5] De Witte, T.; Fratila-apachitei, L.E.; Zadpoor, A.A.; Peppas, N.A. Bone tissue engineering via growth factor delivery: From scaffolds to complex matrices. *Regen. Biomater.* **2018**, *5*, 197–211.
[6] Bose S, Vahabzadeh S, Bandyopadhyay A. Bone tissue engineering using 3D printing. Mater Today 2013;16:496–504.
[7] De Witte, T.-M., Fratila-Apachitei, L. E., Zadpoor, A. A., & Peppas, N. A. (2018). *Bone tissue engineering via growth factor delivery: from scaffolds to complex matrices. Regenerative Biomaterials, 5(4), 197–211*.
[8] Young S, Patel ZS, Kretlow JD, Murphy MB, Mountziaris PM, Baggett LS, Ueda H, Tabata Y, Jansen JA, Wong M, Mikos AG. Dose effect of dual delivery of vascular endothelial growth factor and bone morphogenetic protein-2 on bone regeneration in a rat critical-size defect model. Tissue Eng Part A. 2009 Sep;15(9):2347-62.
[9] Bessa PC, Machado R, Nu¨rnberger S et al. Thermoresponsive selfassembled elastin-based nanoparticles for delivery of BMPs. J Controlled Release 2010;142:312–8.
[10] Kim S, Kang Y, Krueger CA, Sen M, Holcomb JB, Chen D, Wenke JC, Yang Y. Sequential delivery of BMP-2 and IGF-1 using a chitosan gel with gelatin microspheres enhances early osteoblastic differentiation. Acta Biomater. 2012 May;8(5):1768-77.
[11] Yamano S, Haku K, Yamanaka T, Dai J, Takayama T, Shohara R, Tachi K, Ishioka M, Hanatani S, Karunagaran S, Wada K, Moursi AM. The effect of a bioactive collagen membrane releasing PDGF or GDF-5 on bone regeneration. Biomaterials. 2014 Mar;35(8):2446-53.
[12] Hankenson KD, Gagne K, Shaughnessy M. Extracellular signaling molecules to promote fracture healing and bone regeneration. Adv Drug Deliv Rev 2015;94:3–12
[13] Di Luca A, Klein-Gunnewiek M, Vancso JG et al. Covalent binding of bone morphogenetic protein-2 and transforming growth factor-b3 to 3D plotted scaffolds for osteochondral tissue regeneration. Biotechnol J 2017; 12.

 

[14] Donnaloja, F., Jacchetti, E., Soncini, M., & Raimondi, M. T. (2020). *Natural and Synthetic Polymers for Bone Scaffolds Optimization. Polymers, 12(4), 905.*
[15] Nava, M.M.; Fedele, R.; Raimondi, M.T. Computational prediction of strain-dependent diffusion of transcription factors through the cell nucleus. *Biomech. Model. Mechanobiol.* **2016**, *15*, 983–993.
[16] Karageorgiou, V.; Kaplan, D. Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials* **2005**, *26*, 5474–5491.
[17] Short AR, Koralla D, Deshmukh A, Wissel B, Stocker B, Calhoun M, et al. Hydrogels that allow and facilitate bone repair, remodeling, and regeneration. *J Mater Chem B.* 2015;3(40):7818–30.
[18] Lee SH, Shin H. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. *Adv Drug Deliv Rev.* 2007;59(4):339–59.
[19] Alvarez, K., & Nakajima, H. (2009). Metallic Scaffolds for Bone Regeneration. *Materials*, *2*(3), 790–832.
[20] Salehi-Nik, Nasim & Rezai Rad, Maryam & Nazeman, Pantea & Khojasteh, Arash. (2017). Polymers for oral and dental tissue engineering. 10.1016/B978-0-08-100961-1.00003-7.
[21] Babuska V., Moztarzadeh O., Kubikova T., Moztarzadeh A., Hrusak D., Tonar Z. Evaluating the osseointegration of nanostructured titanium implants in animal models: Current experimental methods and perspectives (Review) *Biointerphases.* 2016;11:030801.
[22] Turnbull G., Clarke J., Picard F., Riches P., Jia L., Han F., Li B., Shu W. 3D bioactive composite scaffolds for bone tissue engineering. *Bioact. Mater.* 2018;3:278–314.
[23] Liu J., Ruan J., Chang L., Yang H., Ruan W. Porous Nb-Ti-Ta alloy scaffolds for bone tissue engineering: Fabrication, mechanical properties and in vitro/vivo biocompatibility. *Mater. Sci. Eng. C.* 2017;78:503–512.
[24] Alaribe, F. N., Manoto, S. L., & Motaung, S. C. K. M. (2016). *Scaffolds from biomaterials: advantages and limitations in bone and tissue engineering. Biologia, 71(4).* doi:10.1515/biolog-2016-0056.
[25] 3D bioactive composite scaffolds for bone tissue engineering.*Turnbull G, Clarke J, Picard F, Riches P, Jia L, Han F, Li B, Shu WBioact Mater. 2018 Sep; 3(3):278-314.*
[26] Dabrowski B, Swieszkowski W, Godlinski D et al. Highly porous titanium scaffolds for orthopaedic applications. J Biomed Mater Res B Appl Biomater 2010;95:53–61.
[27] Witte F, Ulrich H, Palm C et al. Biodegradable magnesium scaffolds: part II: peri-implant bone remodeling. J Biomed Mater Res A 2007;81:757–65.
[28] Balla VK, Bodhak S, Bose S et al. Porous tantalum structures for bone implants: fabrication, mechanical and in vitro biological properties. Acta Biomater 2010;6:3349–59.
[29] Tiainen H, Lyngstadaas SP, Ellingsen JE et al. Ultra-porous titanium oxide scaffold with high compressive strength. J Mater Sci Mater Med 2010;21: 2783–92.
[30] Fu Q, Saiz E, Rahaman MN et al. Bioactive glass scaffolds for bone tissue engineering: state of the art and future perspectives. Mater Sci Eng C 2011;31:1245–56.
[31] Seitz H, Rieder W, Irsen S et al. Three-dimensional printing of porous ceramic scaffolds for bone tissue engineering. J Biomed Mater Res B Appl Biomater 2005;74:782–8.
[32] Bose S, Tarafder S. Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: a review. Acta Biomater 2012;8:1401–21.
[33] Panseri S, Cunha C, D'Alessandro T et al. Magnetic hydroxyapatite bone substitutes to enhance tissue regeneration: evaluation in vitro using osteoblast-like cells and in vivo in a bone defect. PLoS One 2012;7: e38710.
[34] Zhang, D.; Wu, X.; Chen, J.; Lin, K. The development of collagen based composite scaffolds for bone regeneration. *Bioact. Mater.* **2018**, *3*, 129–138.
[35] Ferreira, A.M.; Gentile, P.; Chiono, V.; Ciardelli, G. Collagen for bone tissue regeneration. *Acta Biomater.* **2012**, *8*, 3191–3200.
[36] Zarif, M.-E. A review of chitosan-, alginate-, and gelatin-based biocomposites for bone tissue engineering. *Biomater. Tissue Eng. Bull.* **2018**, *5*, 97–109.
[37] Echave, M.C.; Saenz del Burgo, L.; Pedraz, J.L.; Orive, G. Gelatin as Biomaterial for Tissue Engineering. *Curr. Pharm. Des.* **2017**, *23*, 3567–3584.
[38] Choi, J.H.; Kim, D.K.; Seong, J.E.; Oliveira, J.M.; Reis, R.L.; Khang, G. Silk Fibroin-Based Scaffold for Bone Tissue Engineering. In *Novel Biomaterials for Regenerative Medicine. Advances in Experimental Medicine and Biology*; Springer Nature Singapore Pte. Ltd.: Singapore, 2018.
[39] Saravanan, S.; Leena, R.S.; Selvamurugan, N. Chitosan based biocomposite scaffolds for bone tissue engineering. *Int. J. Biol. Macromol.* **2016**, *93*, 1354–1365.
[40] Garg, Tarun. (2011). Scaffold: Tissue engineering and regenerative medicine. International Research Journal of Pharmacy. 2.
[41] Luo, H.; Cha, R.; Li, J.; Hao, W.; Zhang, Y.; Zhou, F. Advances in tissue engineering of nanocellulose-based scaffolds: A review. *Carbohydr. Polym.* **2019**, *224*, 115144.
[42] Sharma, P.R.; Varma, A.J. Functional nanoparticles obtained from cellulose: Engineering the shape and size of 6-carboxycellulose. *Chem. Commun.* **2013**, *78*, 8818–8820.
[43] Klemm, D.; Heublein, B.; Fink, H.; Bohn, A. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. *Angew. Chem. Int. Ed.* **2005**, *44*, 3358–3393.
[44] Venkatesan, J.; Bhatnagar, I.; Manivasagan, P.; Kang, K.H.; Kim, S.K. Alginate composites for bone tissue engineering: A review. *Int. J. Biol. Macromol.* **2015**, *72*, 269–281.
[45] Kaczmarek-pawelska, A. Alginate-Based Hydrogels in Regenerative Medicine. In *Alginates—Recent Uses of This Natural Polymer*; Pereira, L., Cotas, J., Eds.; IntechOpen Limitid: London, UK, 2020.
[46] Chocholata, P., Kulda, V., & Babuska, V. (2019). Fabrication of Scaffolds for Bone-Tissue Regeneration. *Materials (Basel, Switzerland)*, *12*(4), 568.

 

[47] Chircov, C.; Grumezescu, A.M.; Bejenaru, L.E. Hyaluronic acid-based scaffolds for tissue engineering. *Rom. J. Morphol. Embryol.* **2018**, *59*, 1–6.
[48] Zhu, Z.; Wang, Y.M.; Yang, J.; Luo, X.S. Hyaluronic acid: A versatile biomaterial in tissue engineering. *Plast. Aesthetic Res.* **2017**, *4*, 219–227.
[49] Zhai, P.; Peng, X.; Li, B.; Liu, Y.; Sun, H.; Li, X. The application of hyaluronic acid in bone regeneration. *Int. J. Biol. Macromol.* **2019**, in press
[50] Dwivedi, R.; Kumar, S.; Pandey, R.; Mahajan, A.; Nandana, D.; Katti, D.S.; Mehrotra, D. Polycaprolactone as biomaterial for bone scaffolds: Review of literature. *J. Oral Biol. Craniofacial Res.* **2020**, *10*, 381–388.
[51] Gentile, P.; Chiono, V.; Hatton, P.V. An Overview of Poly ( lactic- co -glycolic ) Acid ( PLGA ) -Based Biomaterials for Bone Tissue Engineering. *Int. J. Mol. Sci.* **2014**, *15*, 3640–3659.
[52] Pan, Z.; Ding, J. Poly ( lactide- co -glycolide ) porous scaffolds for tissue engineering and regenerative medicine. *Interface Focus* **2012**, *2*, 366–377.
[53] Gregor, A.; Filová, E.; Novák, M.; Kronek, J.; Chlup, H.; Buzgo, M.; Blahnová, V.; Lukášová, V.; Bartoš, M.; Nečas, A.; et al. Designing of PLA scaffolds for bone tissue replacement fabricated by ordinary commercial 3D printer. *J. Biol. Eng.* **2017**, *11*, 31.
[54] Sándor GK. Tissue engineering of bone: Clinical observations with adipose-derived stem cells, resorbable scaffolds, and growth factors. Ann Maxillofac Surg. 2012 Jan;2(1):8-11.
[55] Daei-farshbaf, N., Ardeshirylajimi, A., Seyedjafari, E., Piryaei, A., Fadaei Fathabady, F., Hedayati, M., … Norouzian, M. (2013). *Bioceramic-collagen scaffolds loaded with human adipose-tissue derived stem cells for bone tissue engineering. Molecular Biology Reports, 41(2), 741–749.*
[56] Carvalho, P. P., Leonor, I. B., Smith, B. J., Dias, I. R., Reis, R. L., Gimble, J. M., & Gomes, M. E. (2014). *Undifferentiated human adipose-derived stromal/stem cells loaded onto wet-spun starch-polycaprolactone scaffolds enhance bone regeneration: Nude mice calvarial defectin vivostudy. Journal of Biomedical Materials Research Part A, 102(9), 3102–3111*.
[57] Vériter S., André W., Aouassar N., et al. Human adipose-derived mesenchymal stem cells in cell therapy: safety and feasibility in different “hospital exemption” clinical applications. *PLoS One*. 2015;10(10).
[58] Ko, E., Alberti, K., Lee, J. S., Yang, K., Jin, Y., Shin, J., … Cho, S.-W. (2016). *Nanostructured Tendon-Derived Scaffolds for Enhanced Bone Regeneration by Human Adipose-Derived Stem Cells. ACS Applied Materials & Interfaces, 8(35), 22819–22829.*
[59] Zhang, C., Li, M., Zhu, J., Luo, F., & Zhao, J. (2017). *Enhanced bone repair induced by human adipose-derived stem cells on osteogenic extracellular matrix ornamented small intestinal submucosa. Regenerative Medicine, 12(5), 541–552*
[60] Duan W., Chen C., Haque M., Hayes D., Lopez M. J. Polymer-mineral scaffold augments in vivo equine multipotent stromal cell osteogenesis. *Stem Cell Research & Therapy*. 2018;9(1):p..
[61] Lee, C. S., Bishop, E. S., Dumanian, Z., Zhao, C., Song, D., Zhang, F., … Reid, R. R. (2019). *Bone Morphogenetic Protein-9–Stimulated Adipocyte-Derived Mesenchymal Progenitors Entrapped in a Thermoresponsive Nanocomposite Scaffold Facilitate Cranial Defect Repair. Journal of Craniofacial Surgery, 30(6), 1915–1919.*

 