Comparing the effectiveness of different scaffolds (ceramic scaffolds, polymers scaffolds, composite scaffolds, native scaffolds, and metal scaffolds) in regenerating critical sized mandibular bone defect.

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August 2017

A thesis submitted to McGill University in partial fulfilment of the requirements of the degree of

Master of Dental Sciences

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DEDICATION

This project is dedicated to my small family (husband - kids) for their endless love and support throughout my graduate studies.

ACKNOWLEDGMENTS

Firstly, the most special thanks and gratitude must be extended to my supervisor **Dr. Simon Tran**. Thank you so much for accepting me as one of your students, and for taking me under your wing. It was an immense pleasure to work with you, and thank you for helping me to finish my Master's program.

Also, a special mention to my colleague Dr. **Murali Ramamoorthi** who Co-supervised this thesis. Thank you so much for your unfailing support and assistance during my studies.

I would also like to extend my gratitude to **Mrs. Maria Palumbo** for her assistance during my studies.

Also, many thanks to **Kevin Wu** for helping me in my project.

A very special thank you goes out to the **Libyan Ministry of Higher Education** for their financial support to help me obtain my Master's.

To my life-coach, my **husband,** and my **father**: I owe this all to you. Many thanks for your support.

To my forever curious, encouraging, and enthusiastic **mother**: she was always keen to know what I was doing and how I was proceeding, although it is likely that she never fully understood this project! I will miss your cries of joy whenever a significant milestone was reached.

I am grateful to my **siblings**, who have given me moral and emotional support in my life. I am also grateful to other family members and friends who have supported me along the way, especially my best **Hanan Moussa**.

Also, many thanks to my colleagues who worked with me as a reviewer and helped me to finish my project, specifically **Dr. Faez Alhamed, and Dr. Balqees Almufleh.**

Last but not least, I extend my appreciation to my **colleagues** in Dr. Tran's laboratory thank you so much for helping me during last two years.

Thanks for all your encouragement!

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ACS	- Absorbable Collagen Sponge.
AdEGFP	- Adenovirus vector encoding Enhanced Green Fluorescent Protein
ASCs	- Adipose Derived Stem Cells.
β-TCP	- β -Tricalcium Phosphate
BMP	- Bone Morphogenetic Protein.
BMSCs	- Bone Mesenchymal Stem Cells
CBCT	- Cone Beam Computerized Tomography.
CBOs	- Cryopreserved Bone-derived Osteoblasts
CHA	- Coralline Hydroxyapatite
CRM	- Compression-Resistant Matrix.
CT	- Computerized Tomographic
FBOs	- Fresh Bone-derived Osteoblast
HA	- Hydroxyapatite
IGF-I	- insulin-like growth factor-I
nHA	- nanohydroxyapatite.
NO-sig	- Not Significant.
NR	- Not Reported
PCL	- Polycaprolactone.
PDLLA	- Polylactide carrier.
PGE	- Prostaglandin E1
PLA	- Polylactic Acid

PLAGA	- Poly Lactic-co Glycolic Acid
PRP	- Platelet Rich Plasma.
TGF-β1	- transforming growth factor-beta one
Ti	- Titanium
VEGF	- Vascular Endothelial Growth Factor.

Preface and Contributions of the Authors

This project is about a systematic review completed by five authors, namely:

Mabrouka Almatlub, Murali Ramamoorthi, Balqees Almufleh, Faez Alhamed, and Hanan Moussa.

The main author is Mabrouka Almatlub who completed all the steps in the systematic review including the review questions review protocols with (Murali Ramamoorthi), and search strategy. She completed the first step in the systematic review which is to screen the titles and abstracts. Mabrouka Almatlub and Balqees Almufleh, later conducted the full text evaluation. All the authors conducted to the data extraction and quality assessment.

Abstract

Introduction:

Regeneration of large, 'critical-size' bone defects remains a clinical challenge. Bone tissue engineering (BTE) is emerging as a promising alternative to autogenous, allogeneic, xenogeneic, and biomaterial-based bone grafting. A critical size mandibular defect is characterized by a loss of continuity in the mandible and is the result of a segmental resection performed by maxillofacial surgeries. Also, critical sized mandibular defect can result in devastating functional disability, cosmetic deformity, and psychological impairment. The ideal scaffold for tissue engineering is a scaffold made from both organic and synthetic materials.

Objective:

The present study systematically reviewed the existing literature in order to answer the following "PICO" (population, intervention, comparison, outcome) question: In critical sized mandibular defect of animals and humans, are ceramic scaffolds more effective when compared with natural polymers/ synthetic polymers/ native scaffolds/composite scaffolds in enhancing histomorphometric bone regeneration and formation? The present study thus aimed (1) to systematically review preclinical *in vivo* and clinical literature regarding bone tissue engineering for critical size mandibular defects. (2) to determine if ceramic scaffolds are clinically superior to other types of scaffolds; and (3) to compare the effectiveness of different scaffolds.

Results:

Ceramic scaffolds were found to significantly improve bone regeneration when compared to polymer scaffolds. Meanwhile, adding the growth factors to tricalcium phosphate scaffolds and polymer scaffolds improved bone regeneration, but adding the growth factors to hydroxyapatite

scaffolds did not have the same effect. However, a clinical human study of 34 patients showed that growth factor alone is not enough to improve bone healing. Additionally, coating metal scaffold with hydroxyapatite or bioglass resulted in significantly better bone regeneration than uncoated metal.

Conclusion:

The reconstruction of a critical size defect is very challenging. This systematic review evaluated the effectiveness of different tissue engineered scaffolds and autologous bone in reconstructing of critical size mandibular defects. Most of the studies on this topic were animal studies with only one study performed using human participants. More studies on humans are therefore needed to evaluate the effectiveness of these scaffolds in the clinical setting.

Résumé

Introduction:

La régénération des défauts osseux de « taille critique » reste un défi clinique. La régénération tissulaire osseuse (BTE) apparaît comme une alternative prometteuse au greffe d'os autogène, allogénique, xénogénique ou synthétique. Un défaut de taille critique mandibulaire est caractérisé par une perte de continuité de la mandibule, et est le résultat d'une résection segmentaire. De plus, le défaut mandibulaire de taille critique peut entraîner un handicap fonctionnel dévastateur, une déformation cosmétique et des effets psychologiques. L'échafaudage idéal pour la régénération tissulaire est un échafaudage qui possède les propriétés biologiques et matérielles.

Objectif:

La présente étude consiste à réviser systématiquement la littérature existante afin de répondre à la question suivante: "PICO" (population, intervention, comparaison, résultat): dans les défauts mandibulaires de taille critique chez les animaux et les humains, les échafaudages en céramique sont plus efficaces comparativement aux polymères naturels / polymères synthétiques / échafaudages natifs / échafaudages mixtes pour améliorer la régénération et la formation des os? Le but de la présente étude était (1) d'examiner systématiquement la littérature préclinique in vivo et clinique concernant l'ingénierie des tissus osseux pour les défauts mandibulaires de la taille critique, (2) de déterminer si les échafaudages en céramique sont cliniquement supérieurs aux autres types d'échafaudages, et (3) de comparer leur efficacité.

Résultats:

Échafaudages en céramique ont été trouvés pour améliorer de manière significative la régénération osseuse par rapport aux échafauds pol-ymer. Pendant ce temps, l'ajout des facteurs de croissance

aux échafaudages de phosphate tricalcique et aux échafaudages de polymère a amélioré la régénération osseuse, mais l'ajout des facteurs de croissance aux échafaudages d'hydroxyapatite n'a pas eu le même effet. Cependant, une étude clinique chez l'homme de 34 patients a montré que le facteur de croissance seul n'est pas suffisant pour améliorer la cicatrisation osseuse. De plus, l'enrobage des scaffes métalliques avec de l'hydroxyapatite ou du bioverre a entraîné une régénération osseuse significativement meilleure que le métal non revêtu.

Conclusion:

La reconstruction d'un défaut de taille critique est très difficile. Cet examen systématique a évalué l'efficacité de différents échafaudages et de l'os autologue dans la régénération osseuse d'un défaut mandibulaire de taille critique. La plupart des études sur ce sujet sont encore en phase pré-clinique avec une seule étude réalisée chez l'humain. Nous recommandons fortement d'autres études cliniques pour évaluer l'applicabilité des résultats des études animales chez les êtres humains.

1. Introduction

The reconstruction of critical-sized segmental bone defects in the mandibular region remains a clinical challenge for Maxillofacial and Craniofacial Surgeons alike. These bone defects are mostly secondary effects of the resection of benign or malignant tumors, maxillofacial trauma, congenital conditions, or osteonecrosis (Goessler et al., 2007). The mandible is crucial not only for chewing, speaking and swallowing movements, but it is also an essential component of facial aesthetics. Critical sized mandibular defect can result in devastating functional disability, cosmetic deformity, and psychological impairment (Chiapasco, Colletti, Romeo, Zaniboni, & Brusati, 2008). The current gold standard for reconstruction of critical sized mandibular defect is autologous bone transplantation (i.e. free vascularized tissue transfer) (Corbella, Taschieri, Weinstein, & Del Fabbro, 2016). This approach stimulates three-stage healing process similar to fracture healing, which results in a reliable union (Pilia, Guda, & Appleford, 2013). Autogenous free vascularized flaps are commonly harvested from the fibula, iliac crest, scapula, serratus anterior rib, and radius. Autogenous bones (a.k.a native scaffolds) have several advantages for reconstructing bone defects. They are osteogenic as the graft contains osteoprogenitor cells from the host. They are also osteoinductive because they contain bone morphogenetic proteins (BMPs) which induce stem cell differentiation and proliferation. They are osteoconductive as they support vascular ingrowth and infiltration of osteogenic precursors. Moreover, they are histocompatibility and non-immunogenic (Vaccaro, 2002). Nevertheless, there are several drawbacks for autologous bone transplantation. First, free vascularized tissue transfer can lead to significant donor site morbidity (Nkenke & Neukam, 2014). Moreover, autogenous bone flaps to address large defects do not replace anatomically similar structures given the limitations of donor site morphology and anatomy. Certain critical sized defects require a larger amount of tissue that cannot be drawn entirely from the donor site (Fretwurst,

Gad, Nelson, & Schmelzeisen, 2015). With this approach, the restoration of continuity, dentition, soft tissue, sensation, function, and aesthetics cannot be completely achieved (Wong, Tideman, Kin, & Merkx, 2010). Alternatives to autologous bone have included allogeneic, xenogeneic, and alloplastic bone substitutes (Bilal Al-Nawas, 2014), however, none of these have similar effectiveness in comparison with autogenous bone (Milinkovic & Cordaro, 2014).

With the limitations of autologous bone transplantation, bone tissue engineering presents a promising alternative to the current reconstruction techniques. Tissue engineering was defined as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function (Langer & Vacanti, 1993). This novel approach can produce a precise, three-dimensional hard and soft tissue scaffolds that fits the complex mandibular anatomy without invasive harvesting procedures. Moreover, it is not restricted by the amount of available tissue at the donor site. This translates clinically into improved aesthetic outcomes and oral rehabilitation. The components (triad) of tissue engineering approach include osteogenic cells, osteoinductive signals, and osteoconductive scaffolds (Oppenheimer, Mesa, & Buchman, 2012). The scaffolds act as templates for tissue formation by allowing cells to migrate, adhere, and produce tissue. For these reasons, the scaffolds play a critical role in the tissue engineering process. The scaffolds can be manufactured from three groups of biomaterials: ceramics, synthetic polymers, and natural polymers. Composite scaffolds comprised of different materials have also been used by previous clinical studies. Several in-vitro and in-vivo studies have been performed to investigate the characteristics and suitability of specific biomaterials in tissue engineering. However, no consensus has been reached in terms of what the optimal scaffold material is for reconstructing of mandibular critical sized defects.

The present study aimed to systematically review the existing literature in order to answer the following "PICO" (population, intervention, comparison, outcome) question: In critical sized mandibular defect of animals and humans, are ceramic scaffolds more effective when compared with natural polymers/ synthetic polymers/ native scaffolds/composite scaffolds in enhancing Histomorphometric bone regeneration and formation? The purpose of the present study was (1) to review systematically preclinical *in vivo* and clinical literature regarding bone tissue engineering for critical size mandibular defects (including segmental defect, continuity defect, and hemi-mandibulectomy), (2) to determine if the ceramic scaffolds are clinically superior to other types of scaffolds; and (3) to compare the effectiveness of different scaffolds.

2.Literature review

2.1 The mandible

The mandible is a U-shaped bone that supports the lower teeth and makes up the lower facial skeleton. The superior aspect of the mandible consists of the alveolar process that supports the lower teeth. The body of the mandible extends posteriorly to form the mandibular angle and the ascending ramus. The ascending ramus is formed by the vertical plate of bone which extends upward as two processes: coronoid process (anteriorly) and condyle (posteriorly) (Okeson, 2014). The condyle is the portion of the mandible that connects with temporal bone of the cranium. This area is called the temporomandibular joint (TMJ) and is the key structure around which movement occurs. From the anterior view, the condyle has medial and lateral projections, which are called poles (Okeson, 2014).

The mandible does not have any bony attachment to the skull. It is suspended below the maxilla by the muscles of mastication, namely: 1- the masseter, which functions to elevates and protrude the mandible; 2- the temporalis, which elevates and retrudes the mandible; 3- the medial pterygoid, which elevates and protrudes the mandible; 4- the lateral pterygoid, which stabilizes the condyle and the disc during mandibular loading, and protrudes the mandible; and 5- the digastric muscle, which depresses the mandible. The following ligaments also suspend the mandible: the collateral ligaments, the capsular ligaments, the temporomandibular ligaments, the sphenomandibular ligaments, and the stylomandibular ligaments. This unique characteristic of the mandible provides the mobility necessary for its function (e.g. mastication, swallowing and speech) (Okeson, 2014).

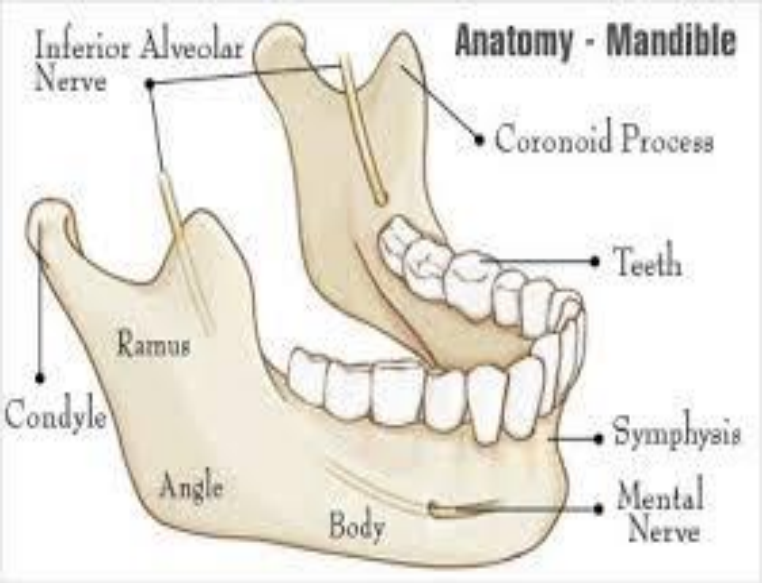

Figure 1: Anatomy of the mandible

Mastication represents the initial stage of digestion. It is made up of rhythmic and well-controlled separation and closure of the maxillary and mandibular teeth. Each opening and closing movement of the mandible represents a chewing stroke. The closing movement is further subdivided into the crushing phase and the grinding phase. The chewing strokes are repeated as food is broken down, and this activity is controlled by the Central pattern generators (CPG) of the brainstem (Okeson, 2014)The frontal view of the chewing stroke can be described as tear-shaped. The amount of force placed on the teeth during physiological mastication varies from individual to individual. The grinding phase of the closure stroke averages at 58,7lb on the posterior teeth, however, the maximum biting load for males varies from 118 to 142lb.

Swallowing (deglutition) is a series of coordinated muscular contractions that move a bolus of food from the oral cavity through the esophagus to the stomach. In a normal swallowing motion, the lips are closed to seal the oral cavity, and the mandibular teeth touch the maxillary teeth in the maximum intercuspal position to stabilize the mandible (Okeson, 2014). The average tooth contact

during deglutition is 0,683 second (Suit, Gibbs, & Benz, 1976). The average force applied to the teeth during deglutition is 66,5lb (Suit et al., 1976). The average frequency of swallowing in a normal individual is approximately 590 times per day.

Speech is another major function of the masticatory system. By moving the lips and tongue to the palate and teeth, a person can produce a wide range of sounds. Several sounds involve the masticatory system, such as the letters "M", "B" and "P" (produced when the lips to come together and touch), the letter "S" (produced when the incisal edges of the maxillary and mandibular incisors to closely approximate but not touch), and the letter "D" (produced when the tongue and the palate touch) (Okeson, 2014).

2.2 What is a critical size defect?

The size of the missing section which is large enough that bone will not completely heal over the natural lifetime of an individual, and this is classified as a "critical size defect" (Schmitz & Hollinger, 1986). A critical size bone defect is the smallest tissue defect in which tissue regeneration will not be completed during the animals life and additional intervention in thus required. Therefore, the surgical reconstructive procedures are used. Furthermore, a defect can be classified as "critical size" when its length deficiency is more than two to three times its diameter (Gugala, Lindsey, & Gogolewski, 2007).

2.3 Bone repair

Bone repair involves three distinct stages: the early inflammatory stage, the repair stage, and the remodeling stage. The early inflammatory stage lasts approximately three to five days. Initially, a hematoma develops around the injured site within hours of sustaining an injury. After, inflamma-

tory cells (including monocytes, macrophages, lymphocytes, and polymorphonuclear cells) infiltrate the wound through the blood. Fibroblasts also infiltrate the bone in a prostaglandin mediated fashion. This mixture results in the formation of granulation tissue, vascular ingrowth into the tissue, and migration of mesenchymal cells to the injury site. After the early inflammatory stage, the repair phase begins and will typically last four to six weeks. Fibroblasts in the granulation tissue produce fibrocartilage and stroma, which supports further vascular ingrowth. The vascular tissue continues to grow and distribute nutrients to all areas of the wound while supplying osteoclasts to remove necrotic tissue from the fracture site. Subsequently, osteoblasts infiltrate the bone and secrete osteoid (nonmineralized collagen matrix). Mineralization of the osteoid leads to the formation of a soft callus around the wound area. The soft callus is a weak structure that hardens as the callus ossifies and is subsequently replaced by woven bone, (i.e. a hard callus). Eventually, woven bone formation bridges the opposite bone extremities. The last stage of repair is the remodeling phase, which takes a minimum of three months and continues for the life of the bone. This process is facilitated by adequate mechanical loading on the bone, which direct bone resorption by osteoclasts and bone formation by osteoblast and in different areas. Over time, woven bone is replaced by lamellar bone, which has an excellent mechanical strength (Pilia et al., 2013).

2.4 Tissue engineering and biomaterials:

The components (triad) of the tissue engineering approach include osteogenic cells (i.e. mesenchymal stem cells harvested from an autologous source), osteoinductive signals (provided chemically by growth factors or physically by bioreactors), and osteoconductive scaffolds (Oppenheimer et al., 2012). The scaffolds act as the templates for tissue formation by allowing cells to migrate, adhere, and produce tissue.

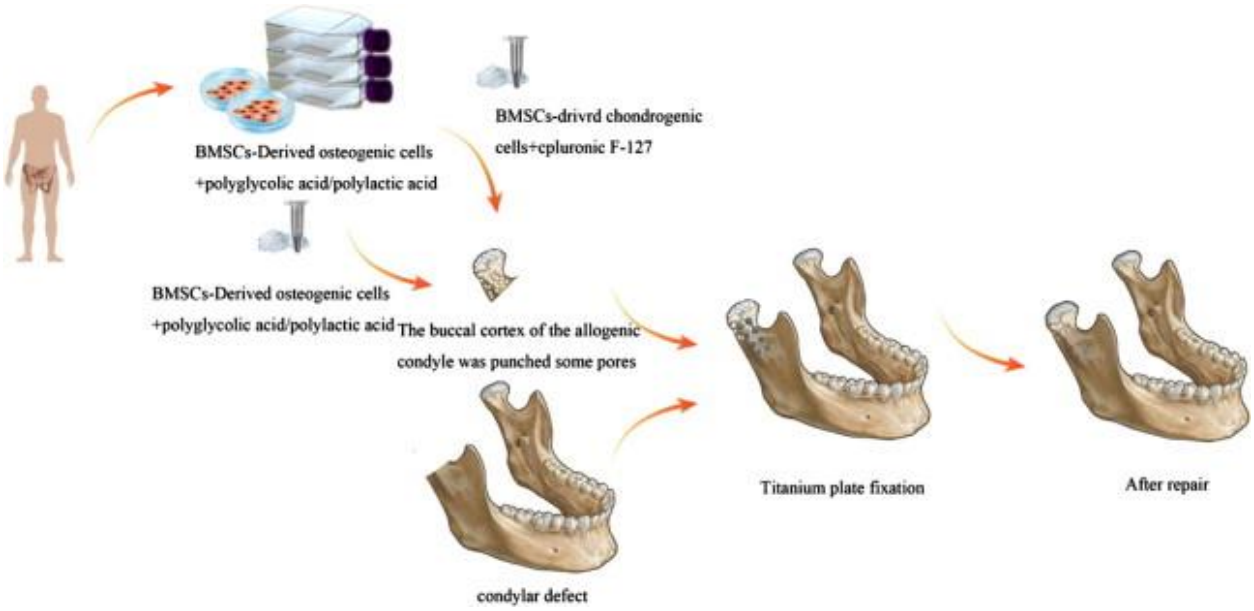

Figure 2: scaffolds act as templates for tissue formation

The ideal scaffold for tissue engineering is one that possesses both organic and synthetic materials (Pilia et al., 2013).

The biological properties described in the literature include biocompatibility (allowing cells to adhere and function normally without causing significant immune reaction), osteogenicity (ability to produce new bone in presence of osteoprogenitor cells), osteoinductivity (promoting stem cell differentiation through the release of local growth factors), osteoconductivity (promoting cellular attachment, migration, and proliferation), and the ability to promote vasculogenesis. The material properties of ideal regenerative bone scaffolds include biodegradability (which allows cells to produce their own extracellular matrix), mechanical integrity (must be strong enough to allow surgical handling during implantation, and mechanical properties consistent with the anatomical site into which it is to be implanted), appropriate scaffold architecture (must mimic the architecture of natural bone), and appropriate pore size and porosity (high porosity to ensure cellular penetration and

diffusion of nutrients to cells and waste products out of the scaffold). The ideal porosity ranges from 70% to 95%, while the ideal pore diameter ranges from 200-900 μm (Pilia et al., 2013) The scaffolds for tissue engineering can be manufactured from three groups of biomaterials: ceramics, synthetic polymers, and natural polymers.

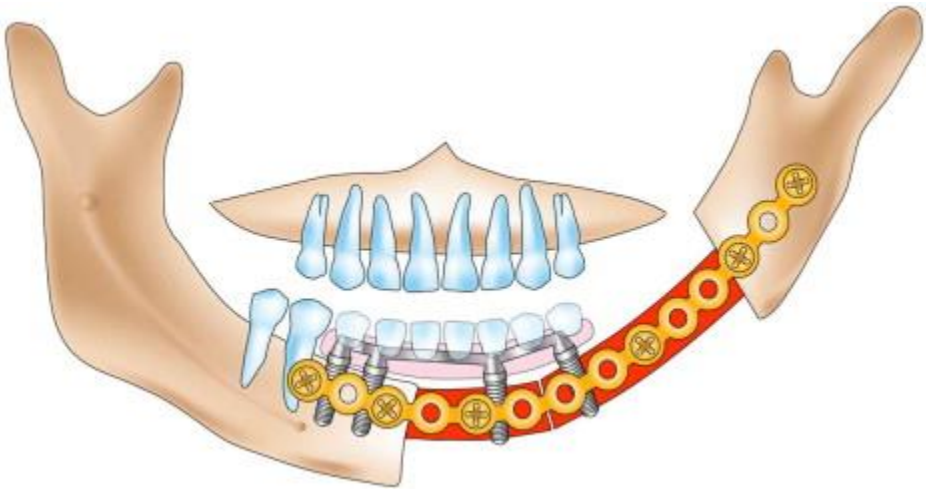

Figure 3: scaffold shape

Scaffold design and fabrication are major areas of biomaterial research and is also important for tissue engineering and regenerative medicine research. Furthermore, it defined as three- dimension porous solid biomaterials.

The materials selected for scaffolds should be evaluated in terms of material chemistry, shape/structure, high porosity, biodegradation, and mechanical property.

2.4.1 Scaffold requirements:

The scaffold used for tissue engineering must be: -

1-Biocompatible

Any scaffold for tissue engineering must be biocompatible; As such the material needs to coexist in contact with the tissues without causing deleterious effects that could compromise the health and function of the tissues.

2-Biodegradable

The scaffold must be biodegradable to allow cells to produce their own extracellular matrix. It should also be non-toxic and be able to exit the body without interfering with other organs. The surrounding tissue should also be able to absorb the material, so that surgical removal is not required (O'Brien, 2011).

3-Mechanical properties

Scaffolds should have good mechanical properties. They must be strong enough to allow surgical handling during implantation. The implanted scaffold must have sufficient mechanical integrity to function from the time of implantation to the completion of the remodeling process.

4-It should have the ability to form complex shapes with (**high porosity).**

2.4.2 Types of polymers for tissue engineering

Depending on the intended use, scaffold materials can be synthetic or biologic. As well as degradable or nondegradable.

2.4.2.1 Natural polymers:

It can be considered as the first biodegradable biomaterials used in clinics.

Natural Polymers Classification:

- Protein (i.e. silk, collagen, gelatin, fibrinogen, elastin, keratin, actin, and myosin) (O'Brien, 2011).

- Polysaccharides (i.e. cellulose, amylose, dextran, chitin, and glycosaminoglycan)

- Polynucleotides (i.e. DNA, RNA)

Numerous natural polymers have been used to produce scaffolds including collagen, proteoglycans, alginate-based substrates, and chitosan. These materials were successful in promoting excellent cell adhesion and growth due to their excellent bioactivity, and their degradation characteristics allowed host cells to produce their own extracellular matrix to replace the degraded scaffold. However, this material has its limitations (e.g. poor mechanical properties). In addition, the fabrication of homogeneous and reproducible structures from natural polymers presents a challenge (O'Brien, 2011).

2.4.2.2 Synthetic polymers:

They are highly useful in biomedical because of their properties, namely: -

- High porosity

- Shorter degradation time

- Stronger mechanical characteristics

- Cheaper than natural polymers

- Produced in large uniform quantities

- Longer shelf life

The most common synthetic polymers are

- PLA (polylactide)

- PGA (polyglycolide)

- PLGA (poly l-lactide- co- glycolide)

Synthetic polymers that have been used to produce scaffolds include polystyrene, poly-l-lactic acid (PLLA), polyglycolic acid (PGA) and poly-dl-lactic-co-glycolic acid (PLGA). These biomaterials possess the properties (i.e. architecture, porosity, degradation time, and mechanical characteristics) that can be tailored for specific applications. In addition, they tend to be less expensive than other types of scaffolds. Synthetic polymers can be produced in large quantities with predictable and reproducible mechanical and physical properties, and they have a long shelf life. However, they have a reduced bioactivity, which increases the risk of rejection. Moreover, their degradation process (hydrolysis) involves the production of carbon dioxide and the reduction of local pH, which can result in cell and tissue necrosis (O'Brien, 2011).

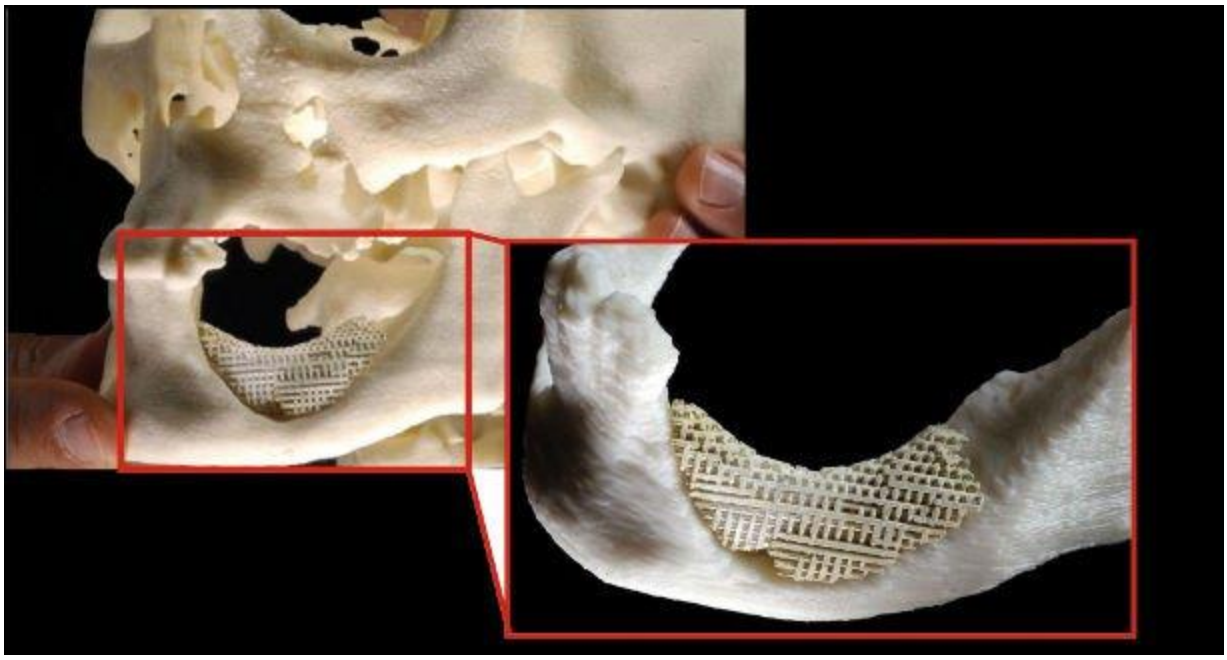

Figure 4: synthetic polymer

2.4.2.3 Ceramics:

Ceramics have been used in dental and orthopedic surgery to fill bone defects and to coat metallic implant surface to improve implant integration with the host bone.

Ceramic scaffolds are typically characterized by:

- High mechanical stiffness

- Very low elasticity

- Hard brittle surface

- Excellent biocompatibility due to their chemical and structural similarity to the native bone

The most common ceramic scaffolds are:

1- HA (hydroxyapatite)

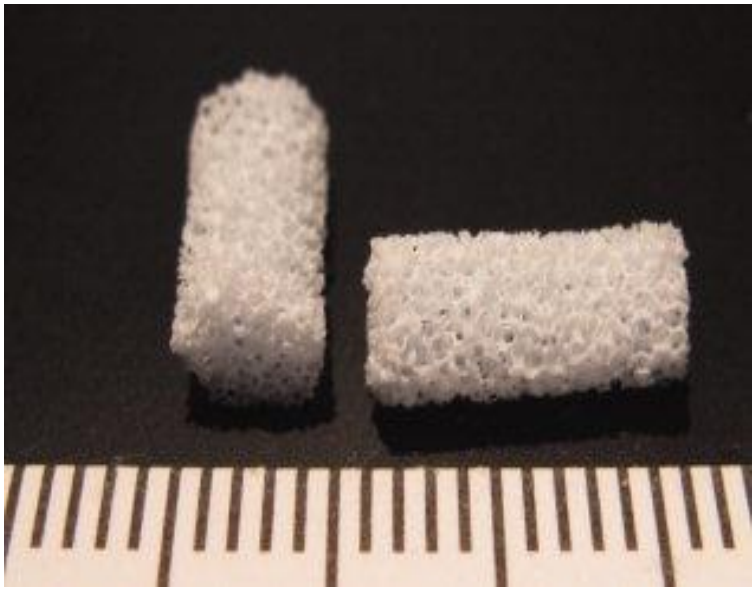

Figure 5: Cylindrical, interconnected 5 × 10 mm2 hydroxyapatite (HAp) scaffold for distal femoral condyle plug defect in New Zealand white rabbits

2- TCP (tri-calcium phosphate)

3- Certain composition of silicate

4- Phosphate glasses (bioactive glasses)

5- Glass ceramic

The available ceramic scaffolds include calcium phosphate (CaP), calcium sulfate (CS) and bio-active glass (BG). Ceramic scaffolds are generally used for hard tissue regeneration since they possess high mechanical stiffness, low elasticity, hard surfaces, and biocompatibility due to how its composition is similar to bone. Specifically, calcium phosphate biomaterials have the ability to promote cellular function and expression leading to the formation of a strong bone-biomaterial

interface (Li Shue, Zhang Yufeng, & Ullas Mony, 2012). In addition, the osteoconductive properties of calcium phosphate support osteoprogenitor cell growth, tissue ingrowth, and bone formation by promoting the attachment, proliferation, differentiation, and migration of bone cells. After in-vivo implantation, its surfaces can form a strong and direct bond with the native bone which mediates the exchange of calcium and phosphate ions between the cell matrix and the substrate (Pilia et al., 2013). Moreover, calcium phosphate biomaterials have been shown to be osteoinductive (enhancing osteoblast differentiation and proliferation), since they can bind to and draw out endogenous bone morphogenetic proteins (BMP) in circulation (L. Shue, Z. Yufeng, & U. Mony, 2012). The osteoinductive and osteoconductive properties of calcium phosphate biomaterials significantly enhance bone regeneration potential. The disadvantages of ceramic-based materials are their brittleness, difficulty in shaping for implantation and less controllable its degradation rate. Therefore, their clinical applications for tissue engineering is limited (O'Brien, 2011).

Hydroxyapatite (HA) is one of the most widely used calcium phosphate biomaterials. Due to its similar composition and structure to native bone mineral, it can chemically bond to the bone without fibrous tissue interposition after implantation. Microscopically, de novo bone formation was observed on the biomaterial surface. Tricalcium phosphate (TCP) biomaterial is the second most widely used ceramic-based biomaterial. Tricalcium phosphate has two phases: a and β phases. Due to its alkaline nature, it is often used for hybrid scaffolds to counteract the acidity resulting from polymer breakdown. Highly purified β-tricalcium phosphate (β-TCP) degrades faster than hydroxyapatite (HA) and is shown to exhibit excellent biocompatibility and osteoconductivity that promotes the proliferation and differentiation of cells (Pilia et al., 2013). In order to improve the biodegradability of calcium phosphate biomaterials, a composite of hydroxyapatite and β-tricalcium phosphate was developed, namely: biphasic calcium phosphate (BCP) (L. Shue et al., 2012). This

combination is known for its biocompatibility, osteoconductivity, bioactivity, and degradability. It also has a controllable degradation rate (Ramay & Zhang, 2004).

Other ceramic-base materials include calcium sulfate (CS), bioactive glass (BG), and calcium phosphate cement (CPC). Calcium sulfate has a good compressive strength, it is commonly used as barrier membrane for periodontal regeneration. Bioactive glass is manufactured from heat treated MgO-CaO-SiO2-P2O5 glass, which results in a glass ceramic that contains crystalline apatite [Ca10(PO4)6O, F2)] and β-wollastonite (CaO SiO2) in an MgO-CaO-SiO2 glassy matrix. Bioactive glass possesses high mechanical strength and great bioactivity. In the periodontal literature, bioactive glass has been shown to promote cementogenesis by inducing cementoblasts proliferation (L. Shue et al., 2012) Calcium phosphate cements (CPC) are obtained from the mixture of soft-form dicalcium phosphate (DCP) and tetracalcium phosphate (TTCP), which hardens when the two are combined. The advantage of calcium phosphate cements (CPC) is that it allows surgeons to fill in the gap between two bone endings and conform to the shape of the defects. However, this biomaterial does not provide a porous structure and does not support bone growth well (Pilia et al., 2013).

2.4.2.4 Composite scaffold for tissue engineering

Compared to the strengths of metals and ceramics for medical applications, the strengths of biodegradable polymers are very low. With the introduction of pores in the polymers to form tissue engineering scaffolds, the strengths of porous structures are further decreased, as materials strengths decrease drastically with an increase in porosity.

The composite strategy provides a means for achieving stronger bioactive scaffolds as compared to conventional polymer scaffolds.

Traditional scaffolds based on biodegradable polymers (e.g. poly lactic acid and collagen) are weak and non-osteoconductive. For bone tissue engineering, polymer-based composite scaffolds containing bioceramics, such as hydroxyapatite can be produced and used. The bioceramics can be either incorporated in the scaffolds as a dispersed secondary phase or as a thin coating on the pore surface of polymer scaffolds. This bioceramics phase strengthens and renders the scaffolds bioactive. There are several methods that can be used to produce bioceramics-polymer composite scaffolds

1- Particulate Bioceramics for Composite Scaffolds
2- Incorporating Bioceramics Particles in the Scaffolds
3- Coating the Polymer Scaffold with an Apatite Layer

Each biomaterial group has its own advantages and drawbacks; as such composite scaffolds comprised of different materials are commonly used. The term "composite material" refers to the combination of two or more materials in order to obtain the desired chemical, physical and mechanical properties. The resulting composite material should possess a combination of the best properties of their constituents (Gloria, De Santis, & Ambrosio, 2011). One of the most investigated composite scaffolds is a hybrid scaffold composed of CaP and other polymeric materials. As discussed earlier, the drawbacks of CaP include mechanical instability (brittleness) and difficulty in shaping for implantation. These drawbacks can be counteracted by the excellent mechanical properties of synthetic polymers. By combining the osteoconductivity of CaP with the workability and elasticity of polymers, this hybrid scaffold possesses excellent properties for bone tissue engineering (Kang et al., 2011). In fact, this type of scaffold best simulates the original structure of natural bone, which is composed of the organic (collagen type-1) and the inorganic (hydroxyapatite) compounds. Similarly, hybrid scaffolds composed of polymeric matrices are paired with ceramics. The

polymers act as organic collagens to counteract the brittleness of hydroxyapatite in the scaffold, while the hydroxyapatite ensures osteoconductivity and biocompatibility of the construct. Examples of composite scaffolds include HA with PDLLA/PLLA/PLGA, β-TCP with PLLA-co-PEH or PPF, and bioglass with PLLA, /PLGA/PDLLA (Rezwan, Chen, Blaker, & Boccaccini, 2006).

Currently, the greatest challenge with HAs/polymers composite scaffolds are the creation of a porous architecture while maintaining mechanical integrity (Pilia et al., 2013).

2.5 Bone morphogenetic proteins (BMPs) as osteoinductive signals

As mentioned previously, the osteoinductive signal is one of the three critical components in tissue engineering (Oppenheimer et al., 2012). Osteoinductive signals can be provided chemically by several growth factors, including bone morphogenetic proteins (BMPs), transforming growth factor beta, fibroblast growth factor and insulin-like growth factor (Issa et al., 2008) The bone morphogenetic proteins (BMP) are the most extensively studied growth factors and most promising osteoinductive substances for bone formation. BMP are cytokines of the transforming growth factor-β (TGF-β) superfamily. Its efficacy for promoting the healing of critical size bone defects in multiple animal models has been clearly established (Das et al., 2016; Fan et al., 2014; Issa et al., 2008). (Lee et al., 2015). (Seto, Asahina, Oda, & Enomoto, 2001). Most clinical trials have used BMP-2 to stimulate bone regeneration because they can induce mesenchymal stem cells (MSCs) differentiation into bone-forming cells (osteoblasts) and cartilage-forming cells (Wozney, 2002), while stimulating bone resorption by promoting the differentiation of osteoclasts and activating mature osteoclasts (Poynton & Lane, 2002). Other BMP family growth factors such as BMP-2, BMP-4, BMP-6, and BMP-7 also have been shown to induce bone formation (Seto et al., 2001).

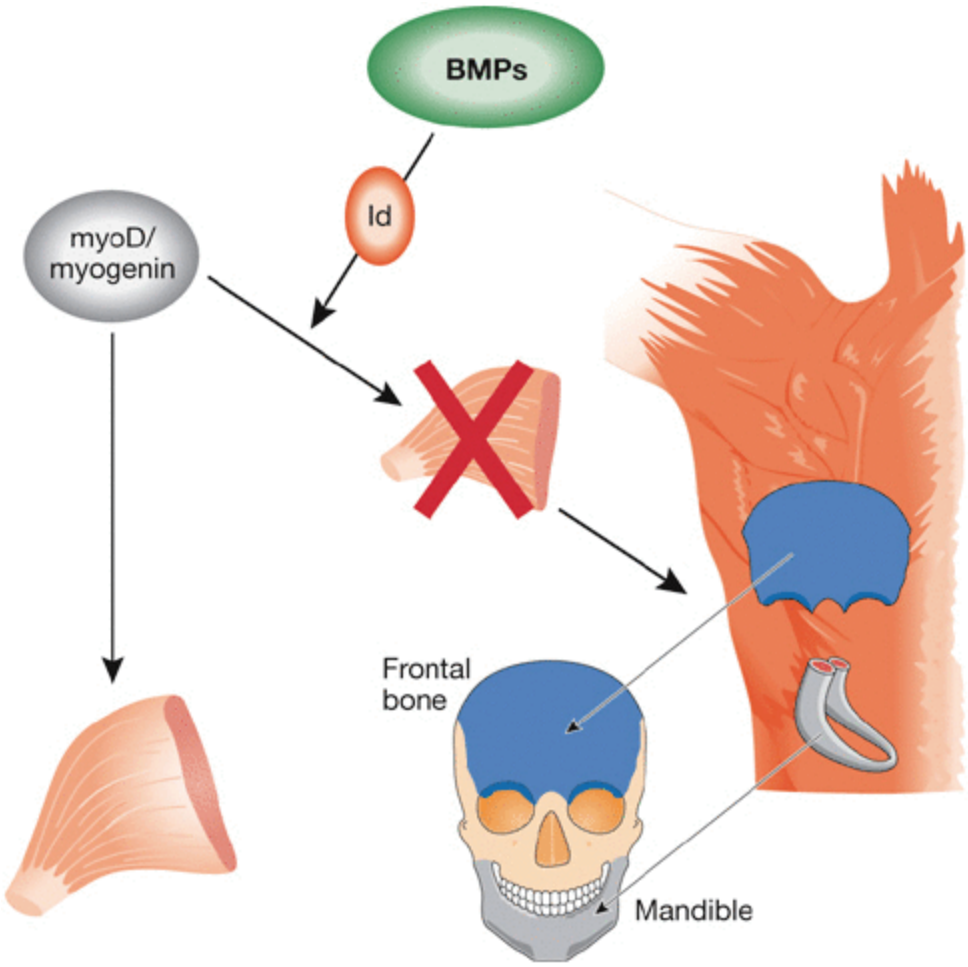

Figure 6: bone morphogenetic proteins in tissue engineering

3. Methodology

3.1 Review protocol

We focused our review to address the following question: What is the effectiveness of different scaffolds (i.e. ceramic scaffolds, natural polymers, synthetic polymers, native scaffolds, and composite scaffolds) in bone regeneration of the critical sized mandibular bone defect?

3.2 Outcomes measure

- The percentage of new bone formation (%NBF) in the defect area.

- Histological, Histomorphometric or Microscopical data were the outcome variables for human and animal reports on the bone defect amount.

- Radiographic evidence for human reports.

3.3 Search strategy

The article databases that were used include:

- Ovid Medline, Embase, and PubMed.

- Cochrane registry, Scopus.

- Journal of tissue engineering(A/B/C)

- Clinical trials, Google scholar, journal databases [Wiley, Elsevier, Quintessence, Sage pub, and nature].

Also, the search strategy involved using a combination of medical subject headings (MeSH) terms and keywords for Medline, PubMed, and EMBASE. The keywords and MeSH terms used for the

search were Ceramic scaffold, hydroxyapatite, bone regeneration, tissue engineering, bone reconstruction, bone defect, tissue scaffolds, synthetic polymer, critical size mandibular defect, defect closure, and hemi mandibulectomy.

Specific search strategies include the following: -

- PubMed Search: -

((((((((((((((((mandibular neoplasm) OR mandibular neoplasms) OR mandibular disease) OR mandibular diseases) OR mandibular injuries) OR oromandibular defect) OR oro mandibular defects) OR oromandibular defects) OR oro-mandibular defects)) AND ((((((loss of continuity) OR continuity defects) OR critical size defect) OR critical size defects) OR critical sized defect) OR critical sized defects))) OR ((((((mandibular continuity defect) OR mandibular continuity defects) OR hemimandibulectomy) OR partial mandibulectomy) OR segmental mandibular defect) OR segmental mandibular defects))) AND (((((((porous ceramics) OR bioactive ceramics) OR ceramic scaffold) OR bio inspired ceramics)) OR ((((((((((((((((((((((((((((((bone substitutes) OR calcium phosphate) OR durapatite) OR bioactive glass) OR polylactate) OR polyglycolic) OR titanium) OR peek) OR tricalcium phosphate) OR silicon carbide) OR methacrylate) OR polycaprolactone) OR chitosan) OR hyaluronic acid) OR natural scaffold) OR synthetic scaffold) OR composite scaffold) OR polymer scaffold) OR metal scaffold) OR composite) OR polymer) OR surgical mesh) OR autograft) OR allograft) OR xenograft) OR carbon scaffold) OR plla) OR PGA) OR plga) OR collagen scaffold) OR collagen)) OR silk fibroin))) OR ((((((tissue engineering) OR bone tissue engineering) OR scaffold based engineering)) OR bone regeneration) OR mandibular regeneration)))) AND (((((treatment outcome) OR complications) OR bone formation) OR failure) OR bone repair)).

- Scopus:

(TITLE-ABS- KEY (mandibular AND critical AND size AND defect) OR TITLE-ABS-KEY (mandible AND critical AND size AND defect) OR TITLE-ABS-KEY (mandibular AND segmental AND defect) OR TITLE-ABS-KEY (mandibular AND segmental AND defects)

- Journal of tissue engineering(A/B/C))

[All mandibular tissue engineering] AND [All mandible regeneration] AND [All mandible critical size defect] AND [All mandibular critical sized defects] AND [All scaffolds for mandible regeneration] – JTE, A, B, C In addition, a hand search strategy was performed by the authors from the citation/reference list of the primary studies and reviews.

3.4 Inclusion and Exclusion

The inclusion criteria were:

- All animal experimental studies (in vivo)

- Human studies.

- All papers were published in English language.

- Papers that included the following as keywords:

Critical Size Mandibular Defect, Segmental Defect, Continuity Defect, and Hemimandibuloctomy.

- Randomized Clinical Trials, Controlled clinical trials, as well as Retrospective, and Prospective Studies.

The exclusion criteria were:

- Case series, case studies, Systematic reviews, and Meta-analyses.

- Studies that included less than 10 patients and 3 animals.

- Observation period of less than 1 year for human studies

- Studies that did not report on at least one of the outcomes defined earlier.

- In vitro studies.

- Periodontal defects.

- Expert opinion, letter to editor, narrative reviews, overview.

- Retrospective studies without clinical follow up.

- descriptive studies (No quantitative outcomes data).

- No clinical outcome reviewed at the follow up visit.

3.5 Study selection process

Titles and abstracts from the search-identified studies were screened by two authors (Mabrouka Almatlub and Balqees Almufleh). Both reviewers had to agree before a study was included for the present review. Also, the same reviewers completed the full text evaluation.

3.6 Data extraction process

Data was extracted from the full texts of the selected articles, four independent reviewers did this [M.A, B.A, FS. A, and H.M] and information on the following aspects were retrieved Reference number, Study type, Study design, Study quality, animal species sample Age/gender, Sample size, Defect type, dimension, Follow-up time, Source induction, Cells count, and Outcome measured.

3.7 Quality assessment (critical appraisal)

The quality of the articles was assessed by the researchers using the Risk of bias (RoB) assessment, which performed using a modification of the systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) RoB tool for animal studies. Subsequently, the selected articles were judged as ‘high’, ‘low’ or ‘unclear’ in terms of bias level (Shanbhag, Pandis, Mustafa, Nyengaard, & Stavropoulos, 2016).

4. Results:

4.1 Study Selection

A total of 1466 relevant articles were identified from the literature search. As 293 of these papers were later excluded because of duplication, only 1173 articles were eligible for title and abstract screening process. During the vetting process, a further 1103 articles were excluded from this study as they did not meet the inclusion criteria. At this point 70 references were qualified for a full-text evaluation. However, 37 of these articles were excluded after the evaluation as they provided: no quantitative data, were non- English, were descriptive, had less than 10 patients; (or 3 animals), or were in vitro studies. The remaining 33 articles were ultimately included in this systematic quantitative review, and data extraction was conducted by the four researchers (M-A, B-A, FS-A, H-M). The articles selection process is summarized in the following flow chart Figure 7.

Flow chart Diagram

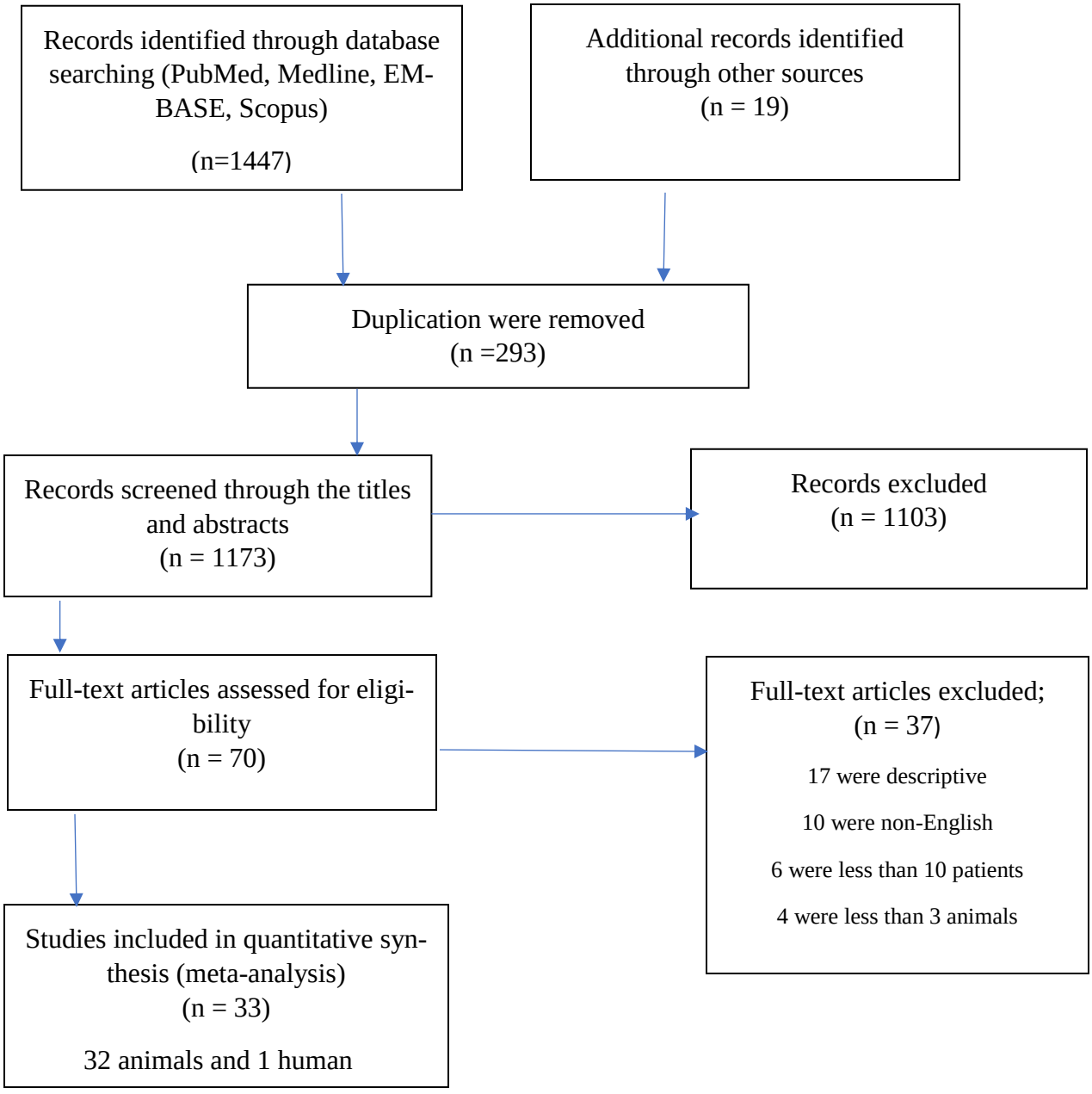

Figure 7: selection process.

4.2 Animal studies

In total 32 out of 33 studies reported on animal experimental quantitative outcomes from 7 different species and 557 animals were included in the systematic review. Large-animal models included goats (one study, n = 24), dogs (eight studies, n = 98), monkeys (two studies, n =37), and minipigs (two studies, n = 37), and sheep (four studies, n= 39). Small-animal models included rats (nine studies, n =250), and rabbits (six studies, n = 72). Sample sizes ranged from 8-56 and 4-24 respectively for the small and large animal models. The follow-up times varied between species, namely: 2-12 weeks for rats, 2-24 weeks for rabbits, 12 weeks for sheep, 5–12 weeks for minipigs, 3-6 weeks for goats, 3weeks-18 months for monkeys, and 3-9 weeks for dogs. Most of the study designs were either prospective animal studies or randomized/ pilot studies. Animal of both genders were included in the studies.

4.3 Human study

In our systematic review, we only included one human study (Marx & Harrell, 2014) which contained 40 patients and investigated how the bone marrow–derived CD34+ cell recombinant human bone morphogenetic protein (rh-BMP), and crushed cancellous allogeneic bones can be used to treat the mandibular continuity defect clinically.

4.4 The effectiveness of different scaffolds

Of the selected articles, only one study compared the effectiveness of different scaffolds in the bone regeneration. This study (Arosarena & Collins, 2003) compared ceramic (hydroxyapatite) with polymer scaffold (polylactic acid) with or without BMP-5 or PGE. The HA scaffold with BMP-5 was found to significantly improve bone regeneration when compared to polymer scaffolds. In general, most ceramic scaffolds significantly improved bone regeneration when compared to polymer scaffolds. Meanwhile, adding the growth factors to tricalcium phosphate scaffolds and

polymer scaffolds improved bone regeneration, but adding the growth factors to hydroxyapatite scaffolds did not have the same effect. However, a clinical human study of 34 patients showed that growth factor alone is not enough to improve bone healing. Additionally, coating metal scaffold with Hydroxyapatite or bioglass resulted in significantly better bone regeneration than un-coated metal.

4.5 Types of scaffold

The 32 animal experiment studies utilized different kinds of scaffolds. 11 papers used ceramics scaffolds(Al-Fotawei et al., 2014; Alfotawei et al., 2014; Appleford, Oh, Oh, & Ong, 2009; Busuttil Naudi et al., 2012; Du et al., 2015; Henkel, Gerber, Dorfling, Gundlach, & Bienengraber, 2005; Lemperle, Calhoun, Curran, & Holmes, 1996; Saad, Abu-Shahba, El-Drieny, & Khedr, 2015; Schliephake et al., 2009; S. Wang et al., 2015; Zhao et al., 2010), 11 papers used (polymers-copolymers) scaffolds (Abu-Serriah, Ayoub, Boyd, Paterson, & Wray, 2003; Abu-Serriah et al., 2006; Das, Segar, Hughley, Bowers, & Botchwey, 2013; Fennis, Stoelinga, & Jansen, 2005; Hussein et al., 2013; Issa et al., 2008; Lee et al., 2015; Marx & Harrell, 2014; H. Wang et al., 2004; Yuan et al., 2010; Zellin, Gritli-Linde, & Linde, 1995), 8 papers used composite (Arosarena & Collins, 2003; Chanchareonsook, Tideman, Lee, et al., 2014; Deppe & Stemberger, 2003; Deppe, Stemberger, & Hillemanns, 2003; Herford et al., 2012; Hirota et al., 2016; Rai et al., 2007; Zhang et al., 2010). three papers used native scaffolds (Dorafshar et al., 2014; Gallego et al., 2015; Huh et al., 2006), and one paper used metal scaffold (Schouman, Schmitt, Adam, Dubois, & Rouch, 2016).

4.5.1 Ceramic scaffolds:

There were 11 articles that contained ceramic scaffolds (Al-Fotawei et al., 2014; Alfotawei et al., 2014; Appleford et al., 2009; Busuttil Naudi et al., 2012; Du et al., 2015; Henkel et al., 2005;

Lemperle et al., 1996; Saad et al., 2015; Schliephake et al., 2009; S. Wang et al., 2015; Zhao et al., 2010).

Furthermore, five articles evaluated bone healing using histology slides to show the rate of bone formation (Appleford et al., 2009; Busuttil Naudi et al., 2012; Lemperle et al., 1996; S. Wang et al., 2015; Zhao et al., 2010) and the other six articles evaluated bone healing using different scales at different time intervals (Al-Fotawei et al., 2014; Alfotawei et al., 2014; Busuttil Naudi et al., 2012; Du et al., 2015; Henkel et al., 2005; Schliephake et al., 2009). Summaries of these studies are provided in Tables 1 and 2.

Additionally, six of these papers showed no significant difference between the various sizes of hydroxyapatite scaffolds and hydroxyapatite scaffolds with or without the VEGF growth factor (Al-Fotawei et al., 2014; Alfotawei et al., 2014; Appleford et al., 2009; Du et al., 2015; Henkel et al., 2005; Schliephake et al., 2009). However, the other studies showed there was a significant difference between TCP seeded with different growth factors (BMP, BMCs, FBO and CPO) versus TCP alone or an autologous graft with the seeded TCP (Busuttil Naudi et al., 2012; Lemperle et al., 1996; Saad et al., 2015; H. Wang et al., 2004; Zhao et al., 2010).

Table 1: Main characteristics of the included studies that used ceramic scaffolds.

Reference	Study Design	Animal species	Age / gender	Sample size	Treatment Groups	Dimensions size	Follow-up
Lemperle, et al. 1997	Randomized, parallel,	Dog, Mongrel	Adult, NR	18	1.Coralline HA blocks. 2.Iliac crest graft. 3.Titanium Mesh.	30mm	8-16 weeks
Appleford, et al. 2008	Randomized, split mouth	Dogs, Foxhound	2 y, male	10	1.Micro size HA. 2.Nano size HA. 3.Control (empty) for both groups.	5mm	3-12 weeks
Zhao, et al. 2009	Prospective	Rat, Fisher 344	12 weeks, Male	11	1.B-TCP alone. 2.B-TCP with untreated bMSCs. 3.B-TCP with bMSCs transduced with AdEGFP. 4.B-TCP with bMSCs transduced with AdBMP-2.	5mm	8 weeks
Wang, et al. 2013	Randomized, parallel	Dog, beagle	12-18 months, male	16	1.CBOs/B-TCP 2.FBOS/B-TCP 3.Resected autologous mandibular segment 4.B-TCP alone	30 mm	51 weeks
Khedr, et al. 2014	Controlled, parallel	Rabbit, New Zealand white	Adult, NR	16	1.B-TCP with autogenous bone marrow derived mesenchymal stem cells 2.B-TCP	10 x 15mm	2-24 weeks
Alfotawei, et al. 2014 (1)	Experimental animal study	rabbit, New Zealand White	Male	8	1.β -Tricalcium phosphate 2.β -Tricalcium phosphate loaded with Bone marrow stem cells	20 mm	12 weeks
Alfotawei, et al. 2014 (2)	Experimental animal study	New Zealand rabbits	NR	10	Calcium sulphate and hydroxyapatite cement in masseter muscle flap	20 x15 mm	4, 8 and 12 weeks
Du, et al. 2015	Experimental animal study	Beagle dogs	Male aged 12-15 months	4	1.Nano hydroxyapatite/ coralline alone.	9mm x 6 mm x 4 mm	3 and 8 weeks

	split mouth design				2.Nano hydroxyapatite/ coralline coated with recombinant vascular endothelial growth factor (VEGF)		
Naudi, et al. 2012	experimental Animal study	Rabbits, New Zealand white	Adult	9	1.β -Tricalcium phosphate 2.β -Tricalcium phosphate loaded with rhBMP-7 in a type-I bovine bone collagen carrier	30 mm	12 weeks
Schiephake , et al. 2009	Prospective	Rat, thymic nude	5-7 weeks, NR	30	1.Empty control scaffold kept under static condition for 24h. 2.Scaffolds seeded with human bone cells and cultivated under static condition for 24h. 3.Scaffolds seeded with human bone cells and cultivated for 14 days under static condition. 4.Scaffolds seeded with human bone cells and cultivated for 14 days in bioreactor.	5mm	6 weeks
Henkel, et al. 2005	Experimental Animal study	Mini-pigs	1-year old adults	16	1.Periosteum covering both defect sides 2.Periosteum covering and suspension of autologous osteoblasts from autogenous pieces of cancellous bone of the sternum 3.Periosteum covering and porous calcium phosphate biomatrix (60% hydroxyapatite and 40% β-tricalcium phosphate) 4.Periosteum covering, suspension of osteoblasts and biomatrix implanted into defect	5 cm^3	5 weeks

Table .2 Bone healing within the included studies that used ceramic scaffolds.

Reference	New Bone Formation	Measurement method	Follow up	Interven-tion	Control	Other groups	Main Findings	P-Value
Lemperle, et al. 1997	Bone volume (mm^3)	Histology	8 weeks	9.72(8.7)	31.5 (16.4)	30.6(5.9)	The empty defect showed the greatest amount of bone healing comparing with other materials	P = 0.03
			16 weeks	19.0(1.2)	47.3 (6.5)	34.8(6.4)		
Appleford, et al. 2009	New bone (%)	Histology	3 weeks	4.4(2.6)	7.2(6.6)		No significant difference between n-HA and M-Ha.	NR
			12 weeks	43.9(4.1)	50.4(8.8)			
Wang, et al. 2013	New bone (%)	Histology	12 months	G1 62.73 (12.28) G2 68.83 (14.52) G3 64.77 (17.75)	G4: Only small amount of bone for-mation was observed in B-TCP		No significant difference between G1, 2, or 3 Sig- between G1,2,3 and G4. Only small amount of bone formation was observed in B-TCP group.	P > 0.05 P < 0.05
Schiephae, et al. 2009	New bone (%)	Histomorphol-ogy	6 weeks	19.7% (for seeded scaf-folds) for 24h	15.1% for non-seeded scaffolds		No significant difference between the groups	P = 0.19
Khedr, et al. 2014	New bone (%)	Computed To-mography	2 weeks	0.65(0.37)			There is significant difference between study and control favoring the interven-tion group for the 2,4,12 weeks evalua-tion period	0.37
			4 weeks	0.88(0.28)				0.03
			12 weeks	0.80(0.28)				P < .001
			24 weeks	0.79(0.32)				P < .001

Alfotawei, et al. 2014 (1)	New bone (Clinical qualitative and quantitative score) Cook, et al.	Periapical radiograph	12 weeks	Qualitative score = 3 Quantitative score = 41.6	NR	No reference to statistical results. No- sig difference	NR
	Surface area (mm^2) Bone volume (mm^3)	CBCT		12.8 mm^2 221.4 mm^3			
Alfotawi, et al. 2014 (2)	New bone (%)	Radiographic assessment		46.6 (15)	36.2 (14)		NR
	Bone volume (mm^3)	Micro CT analysis		237.8 (50.9)	NR		
Naudi, et al. 2012	New bone (%)	Histology	12 weeks	37.4	9.2	BFR of HA-coated scaffolds was significantly higher than that of non-coated scaffolds	P < 0.05
			21week		.		P < 0.05
Du, et al. 2015	New bone (%)	Histomorphometric	3week	27.3 (8.1)	21.7 (3)	No- sig difference	P > 0.05
			8 weeks	39.3 (12.8)	32.6 (10.3)		
Henkel, et al. 2005	New bone (%)	2D radiographic evaluation	5 weeks	59	72	No- sign difference	

	Bone volume (cm^3)	3D macro-scopic evalua-tion		5.85	5.46		
Zhao, et al. 2009	New bone (%)	Histology	8 weeks	12.22 (3.63)	4.73 (1.74)	significant difference in the AdBMP-2 transuded bMSCs/B-TCP compared with B-TCP alone	$P < 0.01$

4.5.2 Polymer scaffold

Furthermore, 11 articles of the 33 selected studies used polymers scaffolds (Abu-Serriah et al., 2003; Abu-Serriah et al., 2006; Das et al., 2016; Fennis et al., 2005; Hussein et al., 2013; Issa et al., 2008; Lee et al., 2015; Marx & Harrell, 2014; H. Wang et al., 2004; Yuan et al., 2010; Zellin et al., 1995).

Three articles evaluated bone healing using the same radiographic methods (micro-CT) at different time intervals (Das et al., 2016; Lee et al., 2015; Yuan et al., 2010), while eight articles evaluated bone healing using different scales at different time intervals (Abu-Serriah et al., 2003; Abu-Serriah et al., 2006; Fennis et al., 2005; Hussein et al., 2013; Issa et al., 2008; Marx & Harrell, 2014; H. Wang et al., 2004; Zellin et al., 1995) furthermore, five of the 11 studies showed that the use of growth factors improved bone regeneration, especially when two types were combined (i.e.BMP and ASCs, or BMP and VEGF) (Das et al., 2016; Hussein et al., 2013; Lee et al., 2015; H. Wang et al., 2004; Yuan et al., 2010).

One study demonstrated 4 membrane types (e-PTFE, cellulose acetate, collagen, and poly glycolic and polylactic acid membrane) promoted significant bone formation when compared with the other 6 membrane types (Zellin, Gritli-Linde, and Linde 1995). regarding the best carrier for BMP in polymer scaffolds, using polymer gels and collagen sponges together was superior to using either materials alone (Issa et al., 2008). meanwhile, one article showed no significant difference between their control and experimental groups.

Tables 3 and 4 provides more details on the selected studies that used polymer scaffolds.

Table 3: Main characteristics of the included studies that used polymer scaffolds.

Reference,	Study Design	Animal species	Age / gender	Sample size	Treatment Groups	Dimensions size	Follow up
Das, et al. 2016	Prospective, Split mouth	Rat, Sprague Dawley	9 weeks, NR	32	1.poly lactic-co- glycolic acid (PLAGA) as control. 2.P^{VEGF} 3.p^{BMP-6} 4.$P^{VEGF+BMP-6}$ NB: The left side were left empty as a control for all groups.	4 mm	2-12 weeks
Issa, et al. 2008	Randomized, parallel	Rat, Wistar	NR, male	56	1.rhBMP-2. 2.rhBMP-2 + collagen sponge. 3.rhBMP-2 with poloxamer gel. 4.rhBMP-2 with poloxamer gel and collagen sponge.	4 x 4mm	2-4 weeks
Fennis, et al. 2005	experimental Animal study	Goats	Not mentioned	24	irradiated cortical scaffold, filled with a particulate cancellous bone graft, platelet rich plasma	4 cm	3 -6 weeks
Zellin, et al. 1995	Randomized, Split mouth	Rat, Albina Sragues-Dawley	Adult, Male	25	10 types of biodegradable and non-degradable membranes	5 mm	6 weeks
Yuan, et al. 2010	Non-randomized, split mouth	Canine, dog mongrel	Adult, 16months	24	1.BMSCs+ Coral (biodegradable). 2.Coral or normal.	Bilateral 30 mm	4-32 weeks
Wang, et al. 2003	Prospective, Split mouth	Minipigs, Gottingen	adult, Female	5	1.rhOp-1 (BMP) + Bovine collagen I 2.No intervention	50 mm	12 weeks

					3.Titanium plates		
Lee, et al. 2015	Prospective animal study	Rat, Lewis	Adult,	19	1.Blank scaffold (PLGA) 2.Scaffold conatain BMP-2 3.Scaffold containing adipose derived stem cells (ASCs) 4.Scaffold containing combination of ASCS and BMP-2	5 mm	12 weeks
Marx, et al. 2014	Human study		Over 18 years old	34	1.RhBMP-2 Absorbable collagen sponge Crushed cancellous allogeneic bone. 2.RhBMP-2 Absorbable collagen sponge Crushed cancellous allogeneic bone but Less CD34+ 3.RhBMP-2 Absorbable collagen sponge Crushed cancellous allogeneic bone with more CD34+	6 - 8 cm	12-24 weeks
Hussein, et al. 2012	Prospective animal study	Dog, foxhound	>2 years old	11	1.BMP-2 2.ACS.	35 mm	12 weeks
Abu-Serriah, et al. 2003 (1)	experimental Animal study	Scottish Grey Face sheep	Adult female	6	Type-I collagen carrier rhOP-1 ($1mg/cm^3$)	35 mm	0, 2, 4, 8, 12 weeks
Abu-Serriah, et al. 2004 (2)	experimental Animal study	Scottish Grey Face sheep	Adult female	6	Type-I collagen carrier rhOP-1 ($1mg/cm^3$)	35 mm	1 day, 2, 4, 8, 12 weeks

Table 4: Bone healing within the included studies that used polymer scaffolds.

Reference	New bone formation	Measurement methods	Follow-up	Intervention	Control	Main Findings	P-Value
Fennis, et al. 2005	New bone (%)	Histology and Histomorpho-metric	6 weeks	29.06 (10.4)	21 (15)	-	NR
Abu-Ser-riah, et al. 2006 (1)	New bone (%)	CT scan	2 weeks	25.7 (3.5)	17.8 (1.7)	All parameters of the operated side were sig-nificantly larger than on the non-operated side	P < 0.05
			4 weeks	19.0(4.0)	11.2 (0.7)		
			8 weeks	326 (114.9)	130.8 (14.4)		
			12 weeks	10.5 (3.3)	5 (0.8)		
Abu-Ser-riah, et al. 2003 (2)	New bone (%)	Ultrasound im-aging	2 weeks	25	NR	-	NR
			4 weeks	71.8			
			8 weeks	87.5			
			12weeks	100			
Lee, et al. 2015	New bone (%)	Micro-CT Bone union	12 weeks	2.0	0.0 only scaf-fold	Scaffolds contain BMP-2 or ASCs or both are significantly better than scaffold PLGA alone in bone regeneration	0.01 0.34 0.01
Marx, et al. 2014	Bone volume (mm^3)	Radiographic CT scan	4 weeks	424 (115)	731 (98)	All patients proceeded through the postopera-tive course without sig and showed evidence of new bone regeneration by 6 months.	P = 0.01
				36 (10)	67 (13)		
Hussein, et al. 2012	New bone (%)	Histomorpho-metric analysis	12 weeks	56.3 (5.5)	38.5 (10.8)	The percent of regenerated bone in group 1 was significantly higher than that of group 2.	P < 0.05
Yuan, et al. 2010	Bone volume (mm^3)	Radiogram Micro. CT (DBV)	12 weeks	562.76 (85)	474.04 (86.85) normal	The BMSCs+ Coral group showed better healing than the coral alone.	P < 0.05 P < 0.02

					90.95 (20.5) Coral group		
			32 weeks	554.3 (59.43)	469.36 (67.74) nor-mal 47.51 (6.41) coral group		P < 0.05 P < 0.02
Wang, et al. 2003	New bone (%)	CT-scan	12 weeks	29.81	8.85	Collagen type-1 with rhOP-1 + CMC scaffold is significantly better than controls	NR
		Histomorpho-metric		84.81%	95.08%		
Das, et al. 2016	Bone volume (mm^3)	Micro-CT	2 weeks	11	4	The $P^{VEGF+BMP-6}$ has significantly enhance bone healing, while either P^{VEGF} or P^{BMP-6} had no significant effect	P < 0.02 For week 2, 8 P < 0.05 For week 12
			8 weeks	15	7		
			12 weeks	17	8		
Issa, et al. 2008	New bone (%)	Histology	2 weeks	Not reported	Not reported	There was sig. difference between all groups and the two periods of time. It is shown that the use of poloxamer and collagen as a carrier for rhBMP-2 enhance bone healing	P < 0.01
			4 weeks				
Zellin, et al. 1995	New bone (%)	Histology and SEM	6 weeks	Not reported	Not reported	4 membrane types (e-PTFE, Cellulose acetate, collagen, and poly glycolic and polylactic acid membrane) showed significant bone for-mation compares with other 6 types	P < 0.05

4.5.3 Composite scaffold

Eight articles utilized composite scaffolds (Arosarena & Collins, 2003; Chanchareonsook, Tideman, Lee, et al., 2014; Deppe & Stemberger, 2003; Deppe et al., 2003; Herford et al., 2012; Hirota et al., 2016; Rai et al., 2007; Zhang et al., 2010). Table 5 provides more details on these studies.

Four of these articles evaluated bone healing using micro- CT scans at different time intervals (Chanchareonsook, Tideman, Feinberg, et al., 2014; Herford et al., 2012; Hirota et al., 2016; Rai et al., 2007). Three of the studies reported a significant difference between the groups of each study (Herford et al., 2012; Hirota et al., 2016; Rai et al., 2007). Five article evaluated bone healing using histological methods with different scales at different time intervals (Arosarena & Collins, 2003; Deppe & Stemberger, 2003; Deppe et al., 2003; Schliephake et al., 2009; Zhang et al., 2010). Additionally, four of the 8 articles found no significant difference between the groups (Deppe and Stemberger 2003; Deppe, Stemberger, and Hillemanns 2003; Schliephake et al. 2009; Arosarena and Collins 2003). One article reported a significant difference between the intervention and the control group (Zhang et al. 2010). The details of these are shown in Table 6.

Composite scaffolds included those made from a combination of ceramic and polymers (Herford et al., 2012; Rai et al., 2007; Zhang et al., 2010), metal coated ceramics (Chanchareonsook, Tideman, Feinberg, et al., 2014; Hirota et al., 2016), and metal coated polymers (Deppe & Stemberger, 2003; Deppe et al., 2003).

Table 5: Main characteristics of the included studies that used composite scaffolds.

Reference	Study Design	Animal species	Age/ gender	Sample size	Treatment Groups	Dimensions size	Follow up
Rai, et al. 2007	Pilot study Split mouth	Dogs, Mongrel	1-2 y, Mixed	8	1. Polycaprolactone20%-tricalcium phosphate scaffold (PCL-TCP). 2. PCL-TCP + PRP 3. Control	18 x 10 x 7 mm	24-36 weeks
Hirota, et al. 2016	Experimental animal study	Rabbits, Japanese, White	19-21 weeks old, male	27	1.Titanium fiber mesh scaffold 2.Titanium fiber mesh scaffold coated with submicron thin hydroxyapatite	10 mm	9 -21 weeks
Arosarena, et al. 2003	Experimental animal study	Sprague Dawley rats	Retired male breeder	29	1.Collagen/Polylactic acid PLA 2.Collagen/Polylactic acid PLA with BMP-5 3.Collagen/Polylactic acid PLA with PGE 4.Collagen/hydroxyapatite cement 5.Collagen/hydroxyapatite cement with BMP-5 6.Collagen/hydroxyapatite cement with PGE 7.Unfilled defect (control)	Bilateral 5x5 mm	12 weeks
Herford, et al. 2012	Experimental Animal study split mouth design	Rhesus Macaque monkeys	Skeletally mature male	13	1.ACS stabilized by Ti crib 2.ACS combined with ceramic granules stabilized by Ti crib 3.CRM stabilized by reconstruction plate with 2 mg/ml rhBMP-2 4.CRM stabilized by reconstruction plate with 0.75 mg/ml rhBMP-2 5.CRM alone (control)	Bilateral 2.5 cm	24 weeks
Zhang, et al. 2010	Randomized, spilt mouth	Rabbits, New Zealand	Adult, NR	15	1.Porous nano-hydroxyapatite/polyamide (nHA/PA). 2.No intervention.	15 x 10mm	4 – 24 weeks

Deppe, et al. 2003 (1)	experi-mental Ani-mal study	Sprague-Dawley rats	female	24	1.titanium membranes coated with polylactide carrier (PDLLA) 2. titanium membranes coated with PDLLA and clindamycin 3. titanium membranes coated with PDLLA mixture of TGF-β1 and IGF-I 4. Six titanium membranes coated with PDLLA and clindamycin mixture of TGF-β1 and IGF-I	Bilateral 5 mm	28 days
Deppe, et al. 2004 (2)	pilot Animal study	Sprague-Dawley rats	female	24	1, 2, 3, and 4 are the same as Deppe 2003 control group uncoated titanium membranes.	Bilateral transosse-ous defects, 5 mm in diame-ter	28 days
Chanchareonsook, et al. 2014	Pilot Animal study	Macaca fas-cicu-laris mon-keys,	Adult male 4-6 years old	9	1. Ti6Al4V endoprosthesis with hydroxy-apatite and bioglass coating 2. Ti6Al4V endoprosthesis with hydroxy-apatite coatings	15 mm	6 months

Table 6: Bone healing within the included studies that used composite scaffolds.

Reference	New bone formation	Measurement methods	Follow up	Intervention	Control	Main Findings	P-Value
Herford, et al. 2012	Bone volume (mm^2)	Histology	24 weeks	1. 19 2. 15.5	3. 13 4. 16	group showed significantly higher amounts of new bone, bone density, and reduced voids when compared with group 1 and 2	P < 0.05
	New bone (%)	Micro-CT		1. 10 2. 14.5	3. 17 4. 14		
Arosarena, et al. 2003	Bone volume (mm^3)	Histology	12 weeks	0.445 (0.363)	0.646 (0.300)	None of the other experimental groups differed significantly from control group	P < 0.02
Hirota, et al. 2016	Bone volume (mm)	Computed tomography	9 weeks	9	3	The survival rate of HA-coated scaffolds are significantly higher than that of non-coated scaffolds. Mean volume outside the scaffold in HA-coated scaffolds was significantly higher than that in non-coated scaffolds.	P < 0.01
	Bone volume (cm^3)			0.2	0.075		
Rai, et al. 2007	Bone volume (mm3)	Micro-CT	6 months	10.1 (3.24)	5.07 (2.13)	PRP-treated defects combined with scaffold had more fraction of bone volume compared with the scaffold alone	P < 0.05
			9 months	15.5 (1.89)	9.78 (1.11)		
Deppe, et al. 2003 (1)	New bone (%)	Histomorphometric	28 days	1. 22 (11.7) 2. 38 (11.7) 3. 32 (16.4) 4. 33 (13.6)	5. 25 (10.6)		NR
Deppe, et al. 2004 (2)	New bone (%)	Histomorphometric	28 days	1.22 (11.7) 2.38 (11.7) 3.32 (16.4) 4.33 (13.6)	Control 25(17.2)	No- sig difference	NR

Chanchareonsook, et al. 2014	New bone (%)	Histology	6 months	For the whole groups: 45.56 (range: 21.1-66.5)	NR	For all the specimens (no results for each group) Buccal, lingual, and inferior region of anterior stem: 55.6(32.8), 56.2(27.6), 59.9(39.4). buccal lingual and inferior of posterior region: 77.18(30.7), 75.2(35), 63.3(21.7).	NR
Zhang, et al. 2010	New bone (%)	Micro-CT evaluation	4 weeks	95	62	(nHA/PA) is significantly enhance bone formation than controls	$P < 0.05$
			12 weeks	140	92		
			24 weeks	165	120		

4.5.4 Native scaffold:

Three articles from the 33 selected studies used native scaffolds (Dorafshar et al., 2014; Gallego et al., 2015; Huh et al., 2006) Main characteristics of the included studies shown in Table 7. Two of them reported a significant difference between the experimental group and the control group (Gallego et al., 2015; Huh et al., 2006). However; one study showed no statistical significance for both groups (Dorafshar et al., 2014). These articles evaluated bone healing using different scales at different time intervals. The details of these are shown in Table 8.

Table 7: Main characteristics of the included studies that used native scaffolds.

Reference	Study Design	Animal species	Age/ gender	Sample size	Treatment Groups	Dimensions size	Follow-up
Gallego, et al. 2015	pilot animal study	Latxa Austrian Sheep	Adult (12-15 months) Female	15	Autologous Bone marrow-mesenchymal stem cells from iliac crest	30 mm	12 weeks 32 weeks
Dorafshar, et al. 2014	experimental animal study	Yorkshire pigs	3-month-old	16	Osseous Free Fibular Flaps from left leg	6-cm	12 weeks
Huh, et al. 2006	Prospective, Split mouth	Dog, Mongrel	Adult, Female	7	1.Platelet enriched fibrin glue with autologous G 2.Autologous G	15mm	6 weeks

Table 8: Bone healing within the included studies that used native scaffolds.

Reference,	New bone formation	Measurement method	Follow up	Intervention	control	Main Findings	P-Value
Gallego, et al. 2015	New bone (%)	microcomputed tomography	32 weeks	89.36(2.81)	11.35(1.85)	mean ±SD BV/TV, was significantly higher in the Experimental group than the control group	P=0.00
Doraf-shar, et al. 2014	New bone (%)	Computerized tomographic	12 weeks	72 (3.3)	71% (4.5%)	Both groups showed no statistical significance	P = .6
		CT volumetric analysis		30(4.5)	26.2(2.3)		p>.05
Huh, et al. 2006	New bone (%)	Radiographic	6 weeks	2.7	1.7	Platelet enriched fibrin glue with particulate bone significantly enhance bone healing than control group	0.023
		Histology		41.7	30.8		0.018

4.5.5 Metal scaffold:

Only one of the 33 selected studies used a metal scaffold (Schouman et al., 2016). This was a prospective parallel study, and bone healing was evaluated using micro- CT scans. A statistically significant result was obtained when comparing porous implants with control implants. More details on this study can be found in Tables 9 and 10 below.

Table 9: Main characteristics of the included study that used metal scaffold.

Reference	Study design	Animal species	Age/ gender	Sample size	Treatment Groups	Dimensions size	Follow-up
Schouman, et al. 2016	Prospective, Parallel	Sheep, NR	Adult, NR	12	1.Load bearing rigid porous implants 2.Flexible porous implants 3.Control for 1st group. 4.Control for 2nd group	18mm	12 weeks

Table 10: Bone healing within the included study that used metal scaffold.

Reference,	New bone for-mation	Measurement methods	Follow up	Intervention	control	Main Findings	P-Value
Schouman, et al.2016	New bone (%)	Micro CT (BV/TV)	12 weeks	2 1.6	2.9 4.5	BV/TV of porous implants was statistically significant when compared with control implants	0.014 0.004

4.6 Quality assessment:

As mentioned previously, the quality of the studies included in our systematic review was assessed by researchers using Risk of bias (RoB) assessment. This assessment was performed using a modification of the systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) RoB tool for animal studies, which labelled the studies as 'high', 'low', or 'unclear' in terms of bias (Shanbhag et al., 2016).

The SYRCLE RoB showed that all of the animal studies included in our review had unclear RoB in terms of sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregiver, random outcome assessment and blinding of outcome assessor. On the other hand, low RoB was obtained in other areas (i.e. incomplete outcome data, selective outcome reporting and Other sources of bias). See Table 11 for the details.

As (Marx & Harrell, 2014), was a randomized clinical trial, the RoB assessment was carried out using Cochrane's RoB assessment tool for randomized clinical trials.

From the assessment, this study was unclear in terms of random sequence, allocation concealment, and, blinding of participants; it was also deemed at high RoB in terms of personnel blinding of outcome, and in attrition bias selective reporting.

Study ID	Sequence generation	Baseline characteristic	Allocation concealment	Random housing	Blinding of caregiver	Random outcome assessment	Blinding of outcome assessor	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Zhang, et al. 2010	1	1	3	3	3	3	3	1	1	1
Zellin, et al. 1995	1	1	3	1	3	3	3	1	1	1
Yuan, et al 2010	2	1	2	3	3	3	3	1	1	1
Lemperle, et al. 1997	3	1	3	3	3	3	3	1	1	1
Huh, et al. 2006	3	1	3	3	3	3	1	1	1	1
Wang, et al. 2003	3	1	3	3	3	3	3	1	1	1
Schouman, et al. 2016	3	1	3	3	3	3	3	1	1	1
Rai, et al. 2007	3	1	3	3	3	3	3	2	1	1
Das, et al. 2016	3	1	3	3	3	3	3	1	1	1
Issa, et al. 2008	3	1	3	3	3	3	3	1	1	1
Appleford, et al. 2008	3	1	3	3	3	3	3	1	1	1
Zhao, et al. 2009	3	1	3	3	3	3	3	1	1	1
Schiephake, et al. 2009	3	1	3	3	3	3	3	1	1	1
Wang, et al. 2013	3	1	3	3	3	3	3	1	1	1

Khedr, et al. 2014	3	1	3	3	3	3	3	1	1	1
Lee, et al. 2015	3	1	3	3	3	1	3	1	1	1
Hussein, et al. 2012	3	1	3	3	3	3	3	1	1	1
Gallego, et al. 2015	3	3	3	3	3	3	3	3	1	1
Fennis, et al. 2005	3	3	3	3	3	3	1	3	1	1
Deppe, et al. 2003 (1)	3	3	3	3	1	3	3	3	1	1
Deppe, et al. 2004 (2)	3	3	3	3	1	3	3	3	1	1
Dorafshar, et al. 2014	3	3	3	3	3	3	3	3	1	1
Chancharo-ceseek, et al. 2014	3	3	3	3	3	3	2	3	1	1
Abu -Se-riah, et al. 2003 (1)	3	3	3	3	3	1	1	3	1	1
Abu-Ser-riah, et al. 2006 (2)	3	3	3	3	3	1	1	3	1	1
Alfotawei, et al. 2014 (1)	3	3	3	3	3	3	3	3	1	1
Alfotawei, et al. 2014 (2)	3	3	3	3	3	3	3	1	1	1
Naudi, et al. 2012	3	3	3	3	3	3	3	1	1	1
Hirota, et al. 2016	3	3	3	3	3	3	3	1	1	1

Arosarena, et al. 2003	3	3	3	3	3	3	3	1	1	1
Du, et al. 2015	3	3	3	1	3	3	1	1	1	1
Herford, et al. 2012	3	3	3	3	3	3	3	1	1	1
Henkel, et al. 2005	3	3	3	3	3	3	3	1	1	1

Table 11: SYRCLE tool for risk of bias

Yes = low risk of bias =1
No =high risk of bias =2
Unclear = unclear risk of bias =3

5. Discussion

Regenerating critical size mandibular defects is very challenging. This systematic review evaluated the effectiveness of different tissue engineered scaffolds and autogenous bone in regenerating of critical size defects.

The size of the critical size defect was different for each animal species due to their stature; additionally, there may be variation between animals of the same species. However, the general ranges were goats 4cm, dogs 5mm- 35mm, pigs 50mm-6cm, sheep 18mm-35mm, monkeys 15mm-2.5cm, rats 4mm-5mm, and rabbits 10mm-30mm). In the human study, the size of the defect was 6cm-8cm.

Of the selected articles, only one study compared the effectiveness of different scaffolds in the bone regeneration. This study (Arosarena & Collins, 2003) compared ceramic (HA) with polymer scaffold (polylactic acid) with or without BMP-5 or PGE. The HA scaffold with BMP-5 was found to significantly improve bone regeneration when compared to polymer scaffolds. However, more studies are needed to compare the efficacy of different scaffolds.

Most studies on this topic are still in the animal experiment stage; in fact only one study involved human participants i.e. (Marx & Harrell, 2014). This study with human participants evaluated the effect of the count of CD34+ cells count BMP-2in added to absorbable collagen and crushed autologous bone. The study later found that a minimum of 200/ml of CD34+ cells count is needed for clinically successful bone regeneration result (Marx & Harrell, 2014).

The result of the studies using ceramic scaffolds showed that adding the growth factors to TCP scaffolds improved bone regeneration, but adding the growth factors to hydroxyapatite scaffolds did not have the same effect (Al-Fotawei et al., 2014; Alfotawei et al., 2014; Appleford et al.,

2009; Busuttil Naudi et al., 2012; Du et al., 2015; Henkel et al., 2005; Lemperle et al., 1996; Saad et al., 2015; S. Wang et al., 2015; Zhao et al., 2010).

The studies that used polymer scaffolds showed that adding growth factors can improve bone regeneration, especially when two different growth factors were combined (Das et al., 2016; Hussein et al., 2013; Lee et al., 2015; H. Wang et al., 2004; Yuan et al., 2010).

However, a clinical human study of 34 patients showed that BMP alone is not enough to improve bone healing; CD34+ cell counts in a concentration of at least 200/ml in a composite graft was needed for successful bone regeneration (Marx & Harrell, 2014). More human studies are ultimately needed to evaluate the effects of growth factors with polymer scaffolds.

On the other hand, one study (Abu-Serriah et al., 2006) showed that polymer scaffolds with rhOP did not result in significantly better results than untreated critical size defects (i.e. the control group).

The results of the studies used composite scaffolds showed that coating titanium scaffolds with polymers provided no significantly different results than simply using uncoated metal with or without growth factors or clindamycin.

Additionally, coating metal scaffold with Hydroxyapatite resulted in significantly better bone regeneration than uncoated metal (Hirota et al., 2016), but the results were not significant different than metal coated with HA and bioglass (Chanchareonsook, Tideman, Feinberg, et al., 2014).

Compared to the control (i.e. no treatment), no significant bone regeneration was observed when ceramic-polymer composite scaffolds were used (Zhang et al., 2010). On the other hand, adding platelet rich plasma to ceramic-polymer scaffolds resulted in a significant improvement in bone healing when compared to the untreated defect as well as when the scaffold was used alone (Rai et al., 2007). However, only a few studies evaluated metal and native scaffolds which indicated

the need for further studies in these areas (Dorafshar et al., 2014; Gallego et al., 2015; Huh et al., 2006; Schouman et al., 2016).

Furthermore, the inadequate reporting by these studies resulted in an unclear RoB evaluation. Inadequate reporting mainly occurred in the following areas: - sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregiver, random outcome assessment, and blinding of outcome assessors. The use of ARRIVE reporting tools for animal studies may result in better reporting.

6. Conclusion

The following conclusions can be drawn from this systematic review:

1. Most of the reviewed studies showed that in general, seeding scaffolds (i.e. ceramics, polymers, or composites) with bone growth factors (including BMP, VEGF, PGE, and rh-OP) can significantly improve the scaffolds bone regeneration potential. However, the level of evidence in these studies is questionable as they had uncleared RoB results.

2. This review demonstrated the lack of comparative studies in which different scaffolds' bone regeneration abilities are compared and evaluated. In fact, only one study compared polymer scaffold with ceramic ones, and it indicated the superiority of ceramic hydroxyapatite scaffolds seeded with BMP.

3. Human studies involving these scaffolds were lacking.

Recommendations:

Based on the results of this review, the following recommendations were made:

1. More emphasis should be placed on the appropriate reporting of animal studies; this should be made easier with the availability of the ARRIVE tool.

2. Further studies that compare the effectiveness of different scaffolds for regenerating critical size mandibular defects should be carried out to guide clinical decision making

3. More human studies should be conducted to evaluate the applicability of animal studies results to human beings.

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