Heliyon 5 (2019) e02808

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Heliyon

Review article

Mesenchymal stem cell-based bone tissue engineering for veterinary practice

Sirirat Nantavisai [a,b], Hiroshi Egusa [c], Thanaphum Osathanon [d], Chenphop Sawangmake [a,b,e,*]

[a] Veterinary Stem Cell and Bioengineering Innovation Center (VSCBIC), Veterinary Pharmacology and Stem Cell Research Laboratory, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand
[b] Veterinary Clinical Stem Cell and Bioengineering Research Unit, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand
[c] Division of Molecular and Regenerative Prosthodontics, Tohoku University Graduate School of Dentistry, Sendai, 980-8575, Japan
[d] Department of Anatomy and Center of Excellence for Regenerative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, 10330, Thailand
[e] Department of Pharmacology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

ARTICLE INFO

Keywords:
Bone tissue engineering
Mesenchymal stem cells
Canine
Biomedical engineering
Veterinary medicine
Stem cell research

ABSTRACT

Bone tissue engineering has been widely studied and proposed as a promising platform for correcting the bone defects. The applications of mesenchymal stem cell (MSC)-based bone tissue engineering have been investigated in various *in vitro* and *in vivo* models. In this regard, the promising animal bone defect models have been employed for illustrating the bone regenerative capacity of MSC-based bone tissue engineering. However, most studies aimed for clinical applications in human. These evidences suggest a knowledge gap to fulfill the accomplishment for veterinary implementation. In this review, the fundamental concept, knowledge, and technology of MSC-based bone tissue engineering focusing on veterinary applications are summarized. In addition, the potential canine MSCs resources for veterinary bone tissue engineering are reviewed, including canine bone marrow-derived MSCs, canine adipose-derived MSCs, and canine dental tissue-derived MSCs. This review will provide a basic and current information for studies aiming for the utilization of MSC-based bone tissue engineering in veterinary practice.

1. Introduction

Reconstruction of critical-sized bone defects is a challenging procedure for orthopedic surgeon. Various procedures have been introduced for bone defect treatment in clinic for example, autologous bone grafts, allogeneous bone grafts, and xenogeneous bone grafts, but the clinical outcomes are varied and lead to donor site morbidities (Shahgoli and Levine, 2011; Silber et al., 2003). Osteoconductive biomaterials are also available in clinical treatment. However, a therapeutic outcome of bone repair and regeneration in large defects or in compromised host is still unsatisfied. Hence, bone tissue engineering has been proposed as a promising tool for correcting simple and complicated bone defects (Black et al., 2015). Studies supporting the potential application of mesenchymal stem cell (MSC)-based bone tissue engineering employing *in vitro* and laboratory animal models have been reported (Perez et al., 2018). However, most of the evidences are focused on human application. In this review, fundamental principles and potential applications of MSC-based bone tissue engineering for veterinary practice are summarized. In addition, the canine models exhibited many features which are valuable as model manners for further human application (Pascual-Garrido et al., 2018). Animal models for bone tissue engineering have involved in various bone defect and bone disease models which are able to accelerate the translation of knowledge to clinical practice in both human and veterinary applications (McGovern et al., 2018).

2. Bone tissue engineering

Tissue engineering is a multidisciplinary study founded on the basis of cell biology, developmental biology, bioengineering, and biomaterial science. Tissue engineering approach aims to develop the biological substitutes that restore, maintain, or improve function of target tissues or organs (Bartold et al., 2006; Caddeo et al., 2017; Langer and Vacanti, 1993). Conventionally, three main components are comprised of matrix (scaffolds), applied cues (biochemical or biophysical cues), and cell resources which are required for an establishment of successful tissue engineering (Bartold et al., 2006; Caddeo et al., 2017).

* Corresponding author.
E-mail addresses: chenphop.s@chula.ac.th, chenphop@gmail.com (C. Sawangmake).

https://doi.org/10.1016/j.heliyon.2019.e02808
Received 21 May 2019; Received in revised form 29 October 2019; Accepted 7 November 2019

3. Scaffolds in bone tissue engineering

Scaffolds are functioned as the supportive extracellular matrix (ECM). Scaffolds should support cell adherence, spreading, proliferation, differentiation, maturation, communication, and ECM production (Caddeo et al., 2017). Scaffolds in bone tissue engineering are usually osteoconductive materials which provide an environment for bone formation *in vivo*. However, osteoinductive materials are also utilized as bone tissue engineering scaffolds since they could promote osteogenic differentiation leading to bone regeneration (Barradas et al., 2011; Blokhuis and Arts, 2011; Habibovic and de Groot, 2007).

3.1. Biomaterials for bone scaffolds

Different materials exhibit specific advantages and disadvantages for bone scaffold utilization. In this regard, natural polymers demonstrate high biocompatibility and contain biological activity. However, low mechanical strength and fabrication limitation are main disadvantages. Synthetic materials like ceramics with calcium phosphate-based materials exhibit osteoinductive abilities (Barradas et al., 2011). Calcium and phosphate ions have been shown to induce osteogenic differentiation in various cell types (Ali Akbari Ghavimi et al., 2018; An et al., 2012). Though, some bioceramic materials have a slow degradation rate, resulting in the remaining of materials in newly regenerated bone tissues (Sheikh et al., 2015). Due to flexible mechanical properties, ease for fabrication, and relatively low cost, biodegradable synthetic polymers have been used as a material of choices for bone tissue engineering scaffolds. However, these materials are lack of bioactivities and some materials are toxic upon degradation (Pastino et al., 2018).

To overcome some limitations of specific materials, advanced hybrid biomaterials have been developed. For example, poly(lactic-co-glycolic acid) (PLGA) and PLGA-polycaprolactone (PCL) co-polymer are developed to achieve a designed degradation time (Ulery et al., 2011). PLGA-polyphosphazenes polymer blends are generated to prevent biological incompatibility of the degradation products (Deng et al., 2008; Krogman et al., 2009). Further, polymer-bioceramic composite materials are initiated to improve osteoinductive properties of polymer materials and to control degradation ability of bioceramics. The exemplification of these polymer-bioceramic composite materials is HA-poly (lactic acid) (PLA), HA-PLGA, HA-gelatin, HA-Chitosan, HA-collagen (Chesnutt et al., 2009; Kim et al., 2005; Kim et al., 2006; Rodrigues et al., 2003; Wei and Ma, 2004; Zhang et al., 2003).

3.2. Scaffold characters for bone regeneration

Physical scaffolds' characters have been shown to participate in the osteoinductive and osteoconductive properties. The examples of those physical parameters are porosity, pore size, surface topography, surface roughness, and surface chemical composition. It has been proposed that these physical parameters could directly interact with cells and also indirectly control cells by the absorbed protein on the surface (Polo-Corrales et al., 2014). Scaffolds with suitable microporosity (i.e. pore size, volume, and interconnectivity) support cell proliferation and migration, nutrient and oxygen flow, vascularization, and ECM production (Logeart et al., 2005; Zeltinger et al., 2001). It has been shown that scaffolds with mean pore size >300 μm could efficiently enhance osteoblast proliferation and differentiation throughout the scaffold due to the thorough delivery of oxygen and nutrient within the scaffold (Holtorf et al., 2005; Kühne et al., 1994; Tsuruga et al., 1997; Volkmer et al., 2008). However, scaffolds with small pore size (<200 μm) could only illustrate peripheral osteoblast survival and bone formation (Karageorgiou and Kaplan, 2005). Nano-scale topography of scaffold plays a crucial role in osteoinduction, osteoconduction, and osseointegration (Porter et al., 2009; Ward and Webster, 2006). In this regard, it has been shown that rough surface promotes human osteoblast-like cell spreading and proliferation (Osathanon et al., 2011). Surface chemistry has been shown to participate to cell response. Phosphoric acid treated titanium surface demonstrates similar surface roughness parameters, but a high phosphate and oxygen content is observed on the surface (Osathanon et al., 2016). Results demonstrate that phosphoric acid treated surface promotes cell attachment and cytoskeletal orientation in murine pre-osteoblast cells (Osathanon et al., 2016). Taken all evidences together, physical characteristics of scaffolds exhibit a crucial role in biological responses.

3.3. Fabrication and scaffolding techniques

As scaffold architecture is an important factor to regulate cell responses. Thus, various technologies are introduced in order to tightly control the fabrication of scaffolds' architecture favoring bone formation. Variety of fabrication techniques have been employed for the production of nano-featured scaffolds i.e. electrospinning (Lao et al., 2011; Tuzlakoglu et al., 2011), molecular self-assembly (Mata et al., 2010; Matson and Stupp, 2012), fiber bonding (Tuzlakoglu et al., 2011), and phase separation (Liu and Ma, 2009). With computer-based technology, computer assisted design (Caddeo et al., 2017) computer assisted technology (Black et al., 2015) systems have been utilized for the production of personalized and anatomical-shaped scaffolds to achieve the complex architecture bone tissues (Grayson et al., 2010).

The additional aspect for designing the scaffold is the orthopedic tissue interface (hard tissue-soft tissue interface) which is very important for graft stability and bone regeneration process (Lu et al., 2010). Bone-soft tissue (ligament, tendon, and cartilage) interface is such a complex and multifactorial organization of distinct tissue types required for musculoskeletal homeostasis and physiological functions (Lu et al., 2010; Qu et al., 2015). Designing of layered scaffolds mimicking natural structure and molecular components of bone-soft tissue interface is introduced. It has been reported that three-phase biomaterials composing of ligament-fibrocartilage-bone-like matrixes can support the maintenance of three cell types (fibroblasts, chondrocytes, and osteoblasts) *in vitro* (Spalazzi et al., 2006a, b). In term of bone-cartilage interface, osteochondral interface is a key component organizing load bearing and force distribution (Lu et al., 2010). Various material-forming techniques have been reported for the production of osteochondral interface-mimicking biomaterials including biphasic scaffolds fabricated with polyglycolic acid (PGA) mesh and PLGA/polyethylene glycol foam (Schaefer et al., 2000), hyaluronan sponge and porous calcium phosphate scaffold (Gao et al., 2001), and photo-polymerized polyethylene glycol-diacrylate hydrogel (Alhadlaq and Mao, 2005). One side of these layered materials is seeded with osteoblasts and another side is seeded with chondrocytes. This co-culture of two cell types on layered materials could simulate the bone-cartilage interface *in vitro*.

To further enhance the bone regenerative capacity of scaffolds, the ability to induce cell homing has been proposed as another key factor. In this regard, cell homing induction could be achieved by two approaches, scaffold-based and/or cell-based strategies (Shin et al., 2003). Scaffolds can be engineered to be more attractive for the cells, leading to the effective angiogenesis and subsequently bone regeneration. The integration of these strategies has been illustrated in the customized cytokine microdelivery system that was incorporated with PCL-based scaffold (Schantz et al., 2007). The system could sequentially deliver vascular endothelial growth factor (VEGF), stromal cell-derived factor (SDF)-1, and bone morphogenetic protein (BMP)-6 resulting in the enhanced angiogenesis, MSCs migration, and matrix mineralization, respectively (Schantz et al., 2007). Moreover, constructing a bio-adhesive surface on scaffold by immobilizing short protein motifs (e.g. arginine-glycine-aspartic acid (RGD motif) or glycine-phenylalanine-hydroxyproline-glycine-glutamate-arginine (GFOGER) shows the significant effects on cell adhesion, proliferation, and osteogenic differentiation (Garcia and Reyes, 2005).

4. MSC-based bone tissue engineering for veterinary practice

Applying of appropriate cell resources is necessary for enhancing bone regeneration and neovascularization (Amini et al., 2012). Many studies suggest the use of pluripotent and multipotent stem cells as cell sources to enhance bone regeneration. In this regard, pluripotent stem cells (such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) possess a high differentiation potency and are able to differentiate toward osteogenic lineage upon an appropriate stimulation (Ardeshirylajimi and Khojasteh, 2017; Buttery et al., 2001; Kawaguchi et al., 2005; C. Zhang et al., 2017). However, there are some concerns according to their tumorigenicity and immunological incompatibility after transplanted (Amini et al., 2012). Hence, the application of mesenchymal stem cells (MSCs) has been investigated for bone tissue engineering utilization (Amini et al., 2012). Isolations of MSCs from various tissue resources have been reported for example bone marrow (Bianco et al., 2001; Macrin et al., 2017), adipose tissues (Zuk et al., 2002), dental tissues (Miura et al., 2003; Shi and Gronthos, 2003), umbilical cord blood (Rosada et al., 2003), peripheral blood (Kuznetsov et al., 2001), amniotic fluid (In 't Anker et al., 2004), brain (Crisan et al., 2008; Lojewski et al., 2015), skin (Joannides et al., 2004), heart (de Paula et al., 2017), kidney (Leuning et al., 2017a, b), and liver (El-Kehdy et al., 2016). In this review, potential application of three main MSCs in veterinary bone tissue engineering namely a) bone marrow-derived MSCs (BM-MSCs), b) adipose-derived MSCs (AD-MSCs), and c) dental tissue-derived MSCs, is discussed.

Stemness and surface marker expression along with osteogenic gene marker expression upon an *in vitro* induction by these three canine mesenchymal stem cells were summarized in Table 1 and Table 2, respectively.

4.1. Bone marrow-derived mesenchymal stem cells (BM-MSCs)

Human and mouse BM-MSCs have been isolated and well characterized. Human BM-MSCs have been identified as CD10+, CD13+, CD29+, CD44+, CD59+, CD71+, CD73+, CD90 (Thy1)+, CD105+, CD106 (VCAM)+, CD146+, CD166 (ALCAM)+, STRO-1+, CD11a-, CD14-, CD19-, CD31 (PECAM)-, CD34 (C-18)-, CD45-, CD48-, CD135-, and HLA-DR- (Arvidson et al., 2011; Macrin et al., 2017; Peister et al., 2004). Canine BM-MSCs (cBM-MSCs) have been recently isolated and described as the clonogenic cells containing self-renewing and multilineage differentiation capacities. cBM-MSCs are able to differentiate toward osteogenic, chondrogenic, and adipogenic lineages (Chung et al., 2012; Sawangmake et al., 2016; Screven et al., 2014; Spencer et al., 2012). These cells express pluripotent stem cell markers, *zinc finger protein 42 (ZFN42 or Rex1)* and *POU class 5 homeobox 1 (Pou5f1)/octamer-binding transcription factor 4 (Oct4)* (Sawangmake et al., 2016). Immunophenotyping characterization by flow cytometry demonstrates that cBM-MSCs express CD44+ and CD90 + but lack of CD14-, CD29-, CD34-, and MHC II- (Screven et al., 2014). Additional analysis of cell surface markers by real-time reverse transcription-polymerase chain reaction (qRT-PCR) revealed that cBM-MSCs were negative for *CD45* and positive for *CD14, CD29, CD44, CD73, CD90, CD105,* and *MHC II* (Screven et al., 2014). PCR array has been used to analyze the pattern of gene expression in cBM-MSCs comparing with peripheral blood mononuclear cells (PBMCs), and the results suggested the set of upregulated genes *(matrix metallopeptidase 2; Mmp2,* and *platelet-derived growth factor receptor β; Pdgfrβ*) and down regulated genes (*protein tyrosine phosphatase receptor type C; Ptprc or Cd45, tumor necrosis factor; Tnf, integrin subunit alpha X; Itgax, beta-2-microglobulin; β2m, interleukin 10; Il10, peroxisome proliferator activated receptor gamma; Ppary, transforming growth factor beta 1; Tgfβ1)*, indicating the mesenchymal specific lineage characterization of cBM-MSCs compared with PBMCs (Screven et al., 2014).

In vitro and *in vivo* osteogenic differentiation potential of cBM-MSCs has been illustrated (Arinzeh et al., 2003; Chung et al., 2012; Sawangmake et al., 2016; Screven et al., 2014). An *in vitro* osteogenic induction is performed by employing the culture medium containing 40 μg/mL to 50 mg/mL ascorbic acid, 20 nM–100 nM dexamethasone, and 10 μM to 10 mM β-glycerophosphate. The induction period is also varied from 7 to 21 days (Bearden et al., 2017; Chung et al., 2012; Sawangmake et al., 2016; Screven et al., 2014; Spencer et al., 2012). At the end of the induction

Table 1
Stemness and surface marker expression of canine mesenchymal stem cells.

	cBM-MSCs	cDPSCs	cAD-SCs
STRO-1	− (Bearden et al., 2017)	+ (Dissanayaka et al., 2011)	− (Bearden et al., 2017)
Nanog	+ (Bearden et al., 2017)	+ (Dissanayaka et al., 2011)	+ (Bearden et al., 2017)
OCT4	+ (Bearden et al., 2017)	N/D	+ (Bearden et al., 2017)
Sox2	+ (Bearden et al., 2017)	N/D	+ (Bearden et al., 2017)
CD8	+ (Russell et al., 2016)	N/D	+ (Russell et al., 2016)
CD4	+ (Russell et al., 2016)	N/D	+ (Russell et al., 2016)
CD9	+ (Bearden et al., 2017)	N/D	+ (Bearden et al., 2017)
CD11b	N/D	N/D	− (Bahamondes et al., 2017)
CD14	+ (Russell et al., 2016)	N/D	+ (Russell et al., 2016)
CD29	+ (Russell et al., 2016)	N/D	+ (Russell et al., 2016)
CD34	− (Bearden et al., 2017)	N/D	− (Bearden et al., 2017)
CD44	+ (Bearden et al., 2017)	+ (Khorsand et al., 2013)	+ (Bahamondes et al., 2017)
CD45	− (Russell et al., 2016)	− (Dissanayaka et al., 2011)	− (Bahamondes et al., 2017)
CD73	+ (Russell et al., 2016)	+ (Dissanayaka et al., 2011)	+ (Russell et al., 2016)
CD90	+ (Bearden et al., 2017)	+ (Dissanayaka et al., 2011)	+ (Bahamondes et al., 2017)
CD105	+ (Bearden et al., 2017)	− (Dissanayaka et al., 2011)	+ (Bearden et al., 2017)
CD146	N/D	+ (Dissanayaka et al., 2011)	N/D

cBM-MSCs, canine bone marrow-derived mesenchymal stem cells; cDPSCs, canine dental pulp stem cells; cAD-SCs, canine adipocyte stem cells; N/D, no data.

Table 2
Osteogenic gene marker expression by canine mesenchymal stem cells upon an *in vitro* induction.

	cBM-MSCs	cDPSCs	cAD-SCs
Runx2	Upregulation (Russell et al., 2016)	N/D	Upregulation (Russell et al., 2016)
Osteocalcin	Upregulation (Chung et al., 2012)	N/D	Upregulation (Requicha et al., 2012)
Collagen 1A1	Upregulation (Chung et al., 2012)	Upregulation (Khorsand et al., 2013)	Upregulation (Chung et al., 2012)
Osterix	Upregulation (Kisiel et al., 2012)	N/D	Upregulation (Kisiel et al., 2012)
Osteopontin	Upregulation (Russell et al., 2016)	Upregulation (Khorsand et al., 2013)	Upregulation (Russell et al., 2016)
ALP	Upregulation (Kisiel et al., 2012)	N/D	Upregulation (Russell et al., 2016)

cBM-MSCs, canine bone marrow-derived mesenchymal stem cells; cDPSCs, canine dental pulp stem cells; cAD-SCs, canine adipocyte stem cells; N/D, no data.

period, cells were positively stained with Alizarin Red S or *Von Kossa* silver staining. The upregulation of osteogenic gene markers (*core-binding factor alpha 1 (Cbfa1)/Runt-related transcription factor 2 (Runx2), bone gamma-carboxyglutamic acid-containing protein (Bglap)/osteocalcin (Ocn), collagen type 1 alpha 1 (Col1A1), osterix (Osx),* and *bone sialoprotein I (Bsp-1)/osteopontin (Opn)*) along with the increase of alkaline phosphatase activity are illustrated (Bearden et al., 2017; Sawangmake et al., 2016; Screven et al., 2014; Spencer et al., 2012). These evidences imply the osteogenic differentiation ability of cBM-MSCs.

Various factors are shown to influence the osteogenic differentiation ability of cBM-MSCs *in vitro*. In this respect, β-glycerophosphate is a source of inorganic phosphate for mineralization *in vitro*. It is cleaved by alkaline phosphatase enzyme resulting in the release of phosphate ions. Increasing of β-glycerophosphate concentration to 20 and 40 mM enhances an upregulation of *Osx* and *Col1A1* mRNA expression without significant effects on alkaline phosphatase activity and mineral deposition ability compared with 10 mM β-glycerophosphate supplementation (Sawangmake et al., 2016). Supplementation of recombinant human bone morphogenic protein-2 (rhBMP-2) in osteogenic inductive medium increases an *in vitro* alkaline phosphatase activity and mineralization in dose-dependent manner (Bearden et al., 2017).

Potential application of cBM-MSCs in bone tissue engineering has been studied in various canine bone defect models. Segmental long bone defects treated with cBM-MSCs loaded HA-tricalcium phosphate has been reported (Arinzeh et al., 2003). Results show that callus formation is observed throughout the length of the defect at eight weeks after transplantation (Arinzeh et al., 2003). New bone formation entire the implant was found at sixteen weeks post-transplant (Arinzeh et al., 2003). Further, transplantation of cBM-MSCs in canine mandibular segmental bone defect has been investigated (Yuan et al., 2007). In this regard, osteogenically induced autologous cBM-MSCs seeded on biodegradable β-tricalcium phosphate (β-TCP) scaffold could regenerate the mandibular defects compared with the control (Yuan et al., 2007). At 32 weeks post-operation, the completed bone union of cBM-MSCs seeded β-TCP scaffold transplant is achieved as confirmed by morphological, radiographical, and biomechanical analyses (Yuan et al., 2007). Another success application has been shown in canine orbital wall defect model. In this study, a combination of autologous cBM-MSCs and canine bone marrow (cBM) aspirate is seeded onto β-TCP scaffolds. After 24 weeks, a successful bone repair of orbital wall bone defects is observed compared with β-TCP scaffold and blank control (Wang et al., 2014).

Despite the evidences supporting bone regenerative potential of cBM-MSCs, some reports have suggested the burdens in cBM-MSCs application especially the invasive harvesting protocol and the difficulty of cell expansion *in vitro* (Bearden et al., 2017; Chung et al., 2012; Oryan et al., 2017). cBM-MSCs showed lesser *in vitro* capabilities comparing with cAD-MSCs according to initial cell isolation yield, colony forming unit (CFU) potency, and proliferation ability (Bearden et al., 2017). It has been reported that hypoxic conditions (5% and 10% oxygen tension) hampered an *in vitro* proliferation capacity of cBM-MSCs comparing with 21% oxygen tension (Chung et al., 2012). In this regard, strategy to enhance cell isolation yield and proliferation potential of cBM-MSCs is indeed necessitated for improving the potential application of the cells for bone tissue engineering. Our report has recently suggested the beneficial effects of simvastatin in low-dose range, 0.1 and 1 nM, on cBM-MSCs proliferation and pluripotent marker expression *in vitro* (Nantavisai et al., 2019).

4.2. Adipose-derived mesenchymal stem cells (AD-MSCs)

Canine AD-MSCs (cAD-MSCs) have been isolated and characterized (Screven et al., 2014; Spencer et al., 2012). According to the previous publications, morphology of the early passage cAD-MSCs has been described as a homogeneous spindle-shaped phenotype. The late passage morphology (after 5 passages) has changed to a wide and flat appearance (Spencer et al., 2012). The tri-lineage differentiation capacity of cAD-MSCs (adipogenicity, chondrogenicity, and osteogenicity) has been illustrated *in vitro* (Bearden et al., 2017; Screven et al., 2014; Spencer et al., 2012). Surface marker characteristics of cAD-MSCs analyzed by flow cytometry have been defined as $CD9^+$, $CD44^+$,$CD90^+$, $CD105^+$, MHC I^+, $CD14^-$, $CD29^-$, $CD34^-$, $CD45^-$, STRO-1^-, and MHC II^- (Bearden et al., 2017; Screven et al., 2014). The expressions of pluripotent markers (*Nanog homeobox; Nanog, Pou5f1/Oct4,* and *SRY-box 2; Sox2*) by cAD-MSCs have been illustrated using conventional PCR (Bearden et al., 2017). Canine-specific PCR array is used to identify the unique gene expression profiles of cAD-MSCs compared with canine peripheral blood mononuclear cells (cPBMCs). The results suggested that cAD-MSCs showed the similar gene expression pattern as found in cBM-MSCs. Set of upregulated genes comprised *Mmp2* and *Pdgfrβ* while set of down-regulated genes included *Ptprc or Cd45, Tnf, Itgax, β2m, Il10, Pparɣ, Tgfβ1* (Screven et al., 2014). Additionally, adipose tissues yielded lesser number of isolated cells per gram of tissue when compared with bone marrow source. However, cAD-MSCs showed greater performances comparing with cBM-MSCs in terms of CFU percentage, short- and long-term proliferation, number of population doublings, as well as cell number per culture plate (Bearden et al., 2017).

An *in vitro* osteogenic differentiation potential of cAD-MSCs has been investigated. Upon the maintenance with osteogenic induction medium containing the supplement combination as described previously in cBM-MSCs section, an *in vitro* osteogenic differentiation of cAD-MSCs is illustrated by an upregulation of osteogenic mRNA markers (*Cbfa1/Runx2, Bglap/Ocn, Col1A1, Osx,* and *BSP-1/OPN*) and the increase of alkaline phosphatase activity. The positive Alizarin Red S or *Von Kossa* staining was found in osteogenic induced cells compared to undifferentiated control suggesting the mineralization of extracellular matrix (ECM). The osteogenic differentiation potency between cAD-MSCs and cBM-MSCs is comparable especially the low responses of alkaline phosphatase activity of the both cell types upon an *in vitro* induction (Bearden et al., 2017; Chung et al., 2012; Screven et al., 2014; Spencer et al., 2012). rhBMP-2 supplement during osteogenic induction could enhance an *in vitro* alkaline phosphatase activity and mineralization in cAD-MSCs similar to those of cBM-MSCs (Bearden et al., 2017). Hypoxic conditions (5% and 10% oxygen tension) decreased an *in vitro* proliferation capacity and osteogenic differentiation potential of cAD-MSCs comparing with 21% oxygen tension (Chung et al., 2012).

Potential application of cAD-MSCs in veterinary bone tissue engineering has been investigated in canine cranial bone defect model (Cui et al., 2007). In this regard, cAD-MSCs isolated from inguinal subcutaneous fat pad are osteogenically induced and seeded on cuboid coral scaffolds (width 20 mm x height 20 mm x thickness 3 mm). After maintained for 7 days, the cells-seeded scaffolds and control scaffolds are transplanted into the bilateral critical-size parietal bone defects (20 mm $\times$ 20 mm) in each animal. Significant bone formation was observed at week 12 post-transplantation in the defects treated with cells-seeded scaffold. At 24 weeks post-transplant, the average bone formation area is 84.19 $\pm$ 6.45% and 25.04 $\pm$ 18.82% in the defects treated with cells-seeded scaffold and control scaffold, respectively. Histological analyses illustrate that the defects in cells-seeded scaffold transplant are repaired by a typical bone tissue while fibrous tissues are found in control group (Cui et al., 2007). Recent study demonstrates the advantages of technology that could preserve the cell-to-cell interactions and the endogenous ECM connections (Kim et al., 2016). These processes are necessary for osteogenicity and bone regenerative capability of cAD-MSCs (Kim et al., 2016). In this study, PCL/β-TCP composite scaffolds are wrapped with osteogenically induced cAD-MSCs sheets and transplanted into the 15 mm segmental defects of canine radial diaphysis. At 12 weeks post-transplant, the new cone-shaped bone formation is observed, and the newly formed bone mass in the defects treated with cAD-MSCs sheet-wrapped scaffolds is significantly greater than that in cAD-MSCs-seeded and control scaffolds. Histological examinations

revealed the newly formed bone had a woven, trabecular appearance surrounding by fibrous connective tissue with no obvious inflammation (Kim et al., 2016). However, it should be noted that the new bone formation in this study model at 12 weeks post-transplant did not completely cover the defect.

Although cAD-MSCs possesses osteogenic differentiation and bone regeneration potential, the study regarding clinical application of cAD-MSCs for bone tissue regeneration is still limited especially in terms of bone defect model variety and effectiveness comparison between autologous and allogenic transplants.

4.3. Dental tissue-derived mesenchymal stem cells (dental-MSCs)

Isolation of various human dental-MSCs types have been previously reported e.g. human dental pulp stem cells (hDPSCs), human periodontal ligament stem cells (hPDLSCs), and stem cells from human exfoliated deciduous teeth (SHED) (Egusa et al., 2012a). These dental-MSCs contained plasticity and differentiation potency toward various lineages including osteogenic, chondrogenic, adipogenic, pancreatic, and neurogenic lineages (Egusa et al., 2012a; Osathanon et al., 2014, Sawangmake et al., 2014a, b). In veterinary practice, canine dental pulp stem cells (cDPSCs) from canine premolars and canine periodontal ligament stem cells (cPDLSCs) have been isolated and reported (Dissanayaka et al., 2011; Tsumanuma et al., 2016). cDPSCs illustrate clonogenicity and contained higher proliferation rate compared with hBM-MSCs. Expression of *Nanog* mRNA, a pluripotent marker, is also observed. The expression of cell surface markers of cDPSCs were not quite correlated with those expressed in hDPSCs. Surface molecule immunophenotyping of cDPSCs using flow cytometry has been illustrated as $CD90^+$ (relative low), $STRO\text{-}1^+$ (relative low), $CD45^-$, $CD73^-$, and $CD105^-$. *CD146* mRNA expression was found as analyzed by RT-qPCR (Dissanayaka et al., 2011). In addition, previous research has shown that cPDLSCs expressed $CD29^+$, $CD90^+$, $CD34^-$ and $CD45^-$ (Tsumanuma et al., 2016). The inconsistent surface marker expression might due to the specificity of antibodies used in flow cytometry analysis.

Osteo/odontogenic differentiation potential of cDPSCs has been investigated *in vitro*. Upon the maintenance with osteo/odontogenic induction medium containing 10 nM 1,25 dihydroxyvitamin D_3, osteo/odontogenic differentiation of cDPSCs is illustrated by the increase of alkaline phosphatase enzymatic activity, mineral deposition, and dentin sialoprotein (DSP) expression. Neurogenic and adipogenic differentiation potential of cDPSCs is also illustrated *in vitro* (Dissanayaka et al., 2011). There is no further analysis regarding osteo/odontogenic mRNA marker expression of cDPSCs undergone an *in vitro* induction in this study.

This evidence has preliminarily suggested the osteogenic differentiation potential of cDPSCs *in vitro*. However, according to the regenerative craniofacial/bone therapies in human, two main issues (i.e. material/scaffold technology and dental stem cell technology) have to be addressed to accomplish the veterinary clinical application using dental-MSCs-based bone tissue engineering (Egusa et al., 2012b).

5. Conclusion

Advantages and disadvantages of three canine mesenchymal stem cells are summarized in Table 3. Most of the studies regarding bone tissue engineering in various animal bone defect models were human application-aimed studies. However, there were numerous of promising studies that supported the application of MSCs for veterinary bone tissue engineering, especially cBM-MSCs. For cAD-MSCs, despite the evidences supporting osteogenic differentiation potential *in vitro*, the studies regarding an *in vivo* bone regenerative capacity and material/scaffold technology are indeed necessitated. Additionally, the promising evidences supporting the *in vitro* and *in vivo* osteogenic differentiation and bone regenerative potential of dental-MSCs are still lacking. Studies regarding these issues will fulfill the development of technologies required for the cMSC-based bone tissue engineering for veterinary practice.

Table 3
Advantages and disadvantages of canine mesenchymal stem cells.

	Advantages	Disadvantages
cBM-MSCs	- Self-renewal - Multilineage differentiation potential - High yield of isolated cells - Many supporting evidences both *in vitro* and *in vivo*	- Invasive harvesting procedure - *In vitro* cell expansion difficulty
cAD-SCs	- Self-renewal - Multilineage differentiation potential - High proliferation rate - Many supporting evidences *in vitro* - Simple harvesting procedure - Multiple site collections	- Morphological change (after 5 passages) - Low number of isolated cells - Less supporting evidences *in vivo*
cDPSCs	- Self-renewal - Multilineage differentiation potential - High proliferation rate - High osteogenic differentiation performances	- Low number of isolated cells - Less evidences both *in vitro* and *in vivo* - Sample collection difficulty

cBM-MSCs, canine bone marrow-derived mesenchymal stem cells; cDPSCs, canine dental pulp stem cells; cAD-SCs, canine adipocyte stem cells.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Funding statement

CS was supported by the Veterinary Clinical Stem Cell and Bioengineering Research Unit, Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University, Thailand; the Chulalongkorn University Office of International Affairs Scholarship for Short-term Research, Thailand; the Government Research Fund, Thailand; and the Asahi Foundation research grant, Japan. TO was supported by the Chulalongkorn Academic Advancement into Its 2nd Century Project, Thailand.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Alhadlaq, A., Mao, J.J., 2005. Tissue-engineered osteochondral constructs in the shape of an articular condyle. J Bone Joint Surg Am 87 (5), 936–944.
Ali Akbari Ghavimi, S., Allen, B.N., Stromsdorfer, J.L., Kramer, J.S., Li, X., Ulery, B.D., 2018. Calcium and phosphate ions as simple signaling molecules with versatile osteoinductivity. Biomed. Mater. 13 (5), 055005.
Amini, A.R., Laurencin, C.T., Nukavarapu, S.P., 2012. Bone tissue engineering: recent advances and challenges. Crit. Rev. Biomed. Eng. 40 (5), 363–408.
An, S., Ling, J., Gao, Y., Xiao, Y., 2012. Effects of varied ionic calcium and phosphate on the proliferation, osteogenic differentiation and mineralization of human periodontal ligament cells in vitro. J. Periodontal. Res. 47 (3), 374–382.
Ardeshirylajimi, A., Khojasteh, A., 2017. Synergism of electrospun nanofibers and pulsed electromagnetic field on osteogenic differentiation of induced pluripotent stem cells. Asaio J. 64 (2), 253–260.
Arinzeh, T.L., Peter, S.J., Archambault, M.P., van den Bos, C., Gordon, S., Kraus, K., Kadiyala, S., 2003. Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. J Bone Joint Surg Am. 85-a (10), 1927–1935.

Arvidson, K., Abdallah, B.M., Applegate, L.A., Baldini, N., Cenni, E., Gomez-Barrena, E., Finne-Wistrand, A., 2011. Bone regeneration and stem cells. J. Cell Mol. Med. 15 (4), 718–746.

Bahamondes, F., Flores, E., Cattaneo, G., Bruna, F., Conget, P., 2017. Omental adipose tissue is a more suitable source of canine Mesenchymal stem cells. BMC Vet. Res. 13 (1), 166.

Barradas, A.M., Yuan, H., van Blitterswijk, C.A., Habibovic, P., 2011. Osteoinductive biomaterials: current knowledge of properties, experimental models and biological mechanisms. Eur Cell Mater 21, 407–429 discussion 429.

Bartold, P.M., Xiao, Y., Lyngstaadas, S.P., Paine, M.L., Snead, M.L., 2006. Principles and applications of cell delivery systems for periodontal regeneration. Periodontology 2000 41 (1), 123–135.

Bearden, R.N., Huggins, S.S., Cummings, K.J., Smith, R., Gregory, C.A., Saunders, W.B., 2017. In-vitro characterization of canine multipotent stromal cells isolated from synovium, bone marrow, and adipose tissue: a donor-matched comparative study. Stem Cell Res. Ther. 8 (1), 218.

Bianco, P., Riminucci, M., Gronthos, S., Robey, P.G., 2001. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 19 (3), 180–192.

Black, C.R., Goriainov, V., Gibbs, D., Kanczler, J., Tare, R.S., Oreffo, R.O., 2015. Bone tissue engineering. Curr. Mol. Biol. Rep. 1 (3), 132–140.

Blokhuis, T.J., Arts, J.J., 2011. Bioactive and osteoinductive bone graft substitutes: definitions, facts and myths. Injury 42 (Suppl 2), S26–29.

Buttery, L.D., Bourne, S., Xynos, J.D., Wood, H., Hughes, F.J., Hughes, S.P., Polak, J.M., 2001. Differentiation of osteoblasts and in vitro bone formation from murine embryonic stem cells. Tissue Eng. 7 (1), 89–99.

Caddeo, S., Boffito, M., Sartori, S., 2017. Tissue engineering approaches in the design of Healthy and Pathological in vitro tissue models. Front. bioeng. biotechnol 5, 40.

Chesnutt, B.M., Yuan, Y., Buddington, K., Haggard, W.O., Bumgardner, J.D., 2009. Composite chitosan/nano-hydroxyapatite scaffolds induce osteocalcin production by osteoblasts in vitro and support bone formation in vivo. Tissue Eng Part A 15 (9), 2571–2579.

Chung, D.J., Hayashi, K., Toupadakis, C.A., Wong, A., Yellowley, C.E., 2012. Osteogenic proliferation and differentiation of canine bone marrow and adipose tissue derived mesenchymal stromal cells and the influence of hypoxia. Res. Vet. Sci. 92 (1), 66–75.

Crisan, M., Yap, S., Casteilla, L., Chen, C.W., Corselli, M., Park, T.S., Peault, B., 2008. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3 (3), 301–313.

Cui, L., Liu, B., Liu, G., Zhang, W., Cen, L., Sun, J., Cao, Y., 2007. Repair of cranial bone defects with adipose derived stem cells and coral scaffold in a canine model. Biomaterials 28 (36), 5477–5486.

de Paula, D.R.M., Capuano, V., Filho, D.M., Carneiro, A., de Oliveira Crema, V., de Oliveira, L.F., da Silva, V.J.D., 2017. Biological properties of cardiac mesenchymal stem cells in rats with diabetic cardiomyopathy. Life Sci. 188, 45–52.

Deng, M., Nair, L.S., Nukavarapu, S.P., Kumbar, S.G., Jiang, T., Krogman, N.R., Laurencin, C.T., 2008. Miscibility and in vitro osteocompatibility of biodegradable blends of poly[(ethyl alanato) (p-phenyl phenoxy) phosphazene] and poly(lactic acid-glycolic acid). Biomaterials 29 (3), 337–349.

Dissanayaka, W.L., Zhu, X., Zhang, C., Jin, L., 2011. Characterization of dental pulp stem cells isolated from canine premolars. J. Endod. 37 (8), 1074–1080.

Egusa, H., Sonoyama, W., Nishimura, M., Atsuta, I., Akiyama, K., 2012a. Stem cells in dentistry–part I: stem cell sources. J Prosthodont Res 56 (3), 151–165.

Egusa, H., Sonoyama, W., Nishimura, M., Atsuta, I., Akiyama, K., 2012b. Stem cells in dentistry–Part II: clinical applications. J Prosthodont Res. 56 (4), 229–248.

El-Kehdy, H., Pourcher, G., Zhang, W., Hamidouche, Z., Goulinet-Mainot, S., Sokal, E., Dubart-Kupperschmitt, A., 2016. Hepatocytic differentiation potential of human fetal liver mesenchymal stem cells: in vitro and in vivo evaluation. Stem Cell. Int. 2016.

Gao, J., Dennis, J.E., Solchaga, L.A., Awadallah, A.S., Goldberg, V.M., Caplan, A.I., 2001. Tissue-engineered fabrication of an osteochondral composite graft using rat bone marrow-derived mesenchymal stem cells. Tissue Eng. 7 (4), 363–371.

Garcia, A.J., Reyes, C.D., 2005. Bio-adhesive surfaces to promote osteoblast differentiation and bone formation. J. Dent. Res. 84 (5), 407–413.

Grayson, W.L., Frohlich, M., Yeager, K., Bhumiratana, S., Chan, M.E., Cannizzaro, C., Vunjak-Novakovic, G., 2010. Engineering anatomically shaped human bone grafts. Proc. Natl. Acad. Sci. U. S. A. 107 (8), 3299–3304.

Habibovic, P., de Groot, K., 2007. Osteoinductive biomaterials–properties and relevance in bone repair. J Tissue Eng Regen Med. 1 (1), 25–32.

Holtorf, H.L., Datta, N., Jansen, J.A., Mikos, A.G., 2005. Scaffold mesh size affects the osteoblastic differentiation of seeded marrow stromal cells cultured in a flow perfusion bioreactor. J. Biomed. Mater. Res. Part A 74 (2), 171–180.

In't Anker, P.S., Scherjon, S.A., Kleijburg-van der Keur, C., de Groot-Swings, G.M., Claas, F.H., Fibbe, W.E., Kanhai, H.H., 2004. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells 22 (7), 1338–1345.

Joannides, A., Gaughwin, P., Schwiening, C., Majed, H., Sterling, J., Compston, A., Chandran, S., 2004. Efficient generation of neural precursors from adult human skin: astrocytes promote neurogenesis from skin-derived stem cells. The Lancet 364 (9429), 172–178.

Karageorgiou, V., Kaplan, D., 2005. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 26 (27), 5474–5491.

Kawaguchi, J., Mee, P.J., Smith, A.G., 2005. Osteogenic and chondrogenic differentiation of embryonic stem cells in response to specific growth factors. Bone 36 (5), 758–769.

Khorsand, A., Eslaminejad, M.B., Arabsolghar, M., Paknejad, M., Ghaedi, B., Rokn, A.R., Jahangir, S., 2013. Autologous dental pulp stem cells in regeneration of defect created in canine periodontal tissue. J. Oral Implantol. 39 (4), 433–443.

Kim, H.W., Kim, H.E., Salih, V., 2005. Stimulation of osteoblast responses to biomimetic nanocomposites of gelatin-hydroxyapatite for tissue engineering scaffolds. Biomaterials 26 (25), 5221–5230.

Kim, S.S., Park, M.S., Gwak, S.J., Choi, C.Y., Kim, B.S., 2006. Accelerated bonelike apatite growth on porous polymer/ceramic composite scaffolds in vitro. Tissue Eng. 12 (10), 2997–3006.

Kim, Y., Lee, S.H., Kang, B.-j., Kim, W.H., Yun, H.-s., Kweon, O.-k., 2016. Comparison of osteogenesis between adipose-derived mesenchymal stem cells and their sheets on poly–caprolactone/-tricalcium phosphate composite scaffolds in canine bone defects. Stem Cell. Int. 2016.

Kisiel, A.H., McDuffee, L.A., Masaoud, E., Bailey, T.R., Esparza Gonzalez, B.P., Nino-Fong, R., 2012. Isolation, characterization, and in vitro proliferation of canine mesenchymal stem cells derived from bone marrow, adipose tissue, muscle, and periosteum. Am. J. Vet. Res. 73 (8), 1305–1317.

Krogman, N.R., Weikel, A.L., Kristhart, K.A., Nukavarapu, S.P., Deng, M., Nair, L.S., Allcock, H.R., 2009. The influence of side group modification in polyphosphazenes on hydrolysis and cell adhesion of blends with PLGA. Biomaterials 30 (17), 3035–3041.

Kühne, J.-H., Bartl, R., Frisch, B., Hammer, C., Jansson, V., Zimmer, M., 1994. Bone formation in coralline hydroxyapatite: effects of pore size studied in rabbits. Acta Orthop. Scand. 65 (3), 246–252.

Kuznetsov, S.A., Mankani, M.H., Gronthos, S., Satomura, K., Bianco, P., Robey, P.G., 2001. Circulating skeletal stem cells. J. Cell Biol. 153 (5), 1133–1140.

Langer, R., Vacanti, J.P., 1993. Tissue engineering. Science 260 (5110), 920–926.

Lao, L., Wang, Y., Zhu, Y., Zhang, Y., Gao, C., 2011. Poly(lactide-co-glycolide)/hydroxyapatite nanofibrous scaffolds fabricated by electrospinning for bone tissue engineering. J. Mater. Sci. Mater. Med. 22 (8), 1873–1884.

Leuning, D.G., Lievers, E., Reinders, M.E., van Kooten, C., Engelse, M.A., Rabelink, T.J., 2017a. A novel clinical grade isolation method for human kidney perivascular stromal cells. J. Vis. Exp.: J. Vis. Exp. 126.

Leuning, D.G., Reinders, M.E., Li, J., Peired, A.J., Lievers, E., de Boer, H.C., Rabelink, T.J., 2017b. Clinical-grade isolated human kidney perivascular stromal cells as an organotypic cell source for kidney regenerative medicine. Stem Cells Transl Med. 6 (2), 405–418.

Liu, X., Ma, P.X., 2009. Phase separation, pore structure, and properties of nanofibrous gelatin scaffolds. Biomaterials 30 (25), 4094–4103.

Logeart-Avramoglou, D., Anagnostou, F., Bizios, R., Petite, H., 2005. Engineering bone: challenges and obstacles. J. Cell Mol. Med. 9 (1), 72–84.

Lojewski, X., Srimasorn, S., Rauh, J., Francke, S., Wobus, M., Taylor, V., Schwarz, S., 2015. Perivascular mesenchymal stem cells from the adult human brain harbor no instrinsic neuroectodermal but high mesodermal differentiation potential. Stem cells transl med. 4 (10), 1223–1233.

Lu, H.H., Subramony, S.D., Boushell, M.K., Zhang, X., 2010. Tissue engineering strategies for the regeneration of orthopedic interfaces. Ann. Biomed. Eng. 38 (6), 2142–2154.

Macrin, D., Joseph, J.P., Pillai, A.A., Devi, A., 2017. Eminent sources of adult mesenchymal stem cells and their therapeutic Imminence. Stem Cell Rev. 13 (6), 741–756.

Mata, A., Geng, Y., Henrikson, K.J., Aparicio, C., Stock, S.R., Satcher, R.L., Stupp, S.I., 2010. Bone regeneration mediated by biomimetic mineralization of a nanofiber matrix. Biomaterials 31 (23), 6004–6012.

Matson, J.B., Stupp, S.I., 2012. Self-assembling peptide scaffolds for regenerative medicine. Chem Commun (Camb) 48 (1), 26–33.

McGovern, J.A., Griffin, M., Hutmacher, D.W., 2018. Animal models for bone tissue engineering and modelling disease. Dis. Model. Mech. 11 (4), dmm033084.

Miura, M., Gronthos, S., Zhao, M., Lu, B., Fisher, L.W., Robey, P.G., Shi, S., 2003. SHED: stem cells from human exfoliated deciduous teeth. Proc. Natl. Acad. Sci. U. S. A. 100 (10), 5807–5812.

Nantavisai, S., Rodprasert, W., Pathanachai, K., Wikran, P., Kitcharoenthaworn, P., Smithiwong, S., Sawangmak, C., 2019. Simvastatin enhances proliferation and pluripotent gene expression by canine bone marrow-derived mesenchymal stem cells (cBM-MSCs) in vitro. Heliyon 5 (10), e02663.

Oryan, A., Kamali, A., Moshiri, A., Baghaban Eslaminejad, M., 2017. Role of mesenchymal stem cells in bone regenerative medicine: what is the evidence? Cells Tissues Organs 204 (2), 59–83.

Osathanon, T., Bespinyowong, K., Arksornnukit, M., Takahashi, H., Pavasant, P., 2011. Human osteoblast-like cell spreading and proliferation on Ti-6Al-7Nb surfaces of varying roughness. J. Oral Sci. 53 (1), 23–30.

Osathanon, T., Sawangmake, C., Nowwarote, N., Pavasant, P., 2014. Neurogenic differentiation of human dental pulp stem cells using different induction protocols. Oral Dis. 20 (4), 352–358.

Osathanon, T., Sawangmake, C., Ruangchainicom, N., Wutikornwipak, P., Kantukiti, P., Nowwarote, N., Pavasant, P., 2016. Surface properties and early murine pre-osteoblastic cell responses of phosphoric acid modified titanium surface. J Oral Biol Craniofac Res 6 (1), 3–10.

Pascual-Garrido, C., Guilak, F., Rai, M.F., Harris, M.D., Lopez, M.J., Todhunter, R.J., Clohisy, J.C., 2018. Canine hip dysplasia: a natural animal model for human developmental dysplasia of the hip. J. Orthop. Res. 36 (7), 1807–1817.

Pastino, A., Steele, J., Kohn, J., 2018. Advancing biomaterials towards biological complexity. Biochem (Lond) 40 (1), 16–19.

Peister, A., Mellad, J.A., Larson, B.L., Hall, B.M., Gibson, L.F., Prockop, D.J., 2004. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood 103 (5), 1662–1668.

Perez, J.R., Kouroupis, D., Li, D.J., Best, T.M., Kaplan, L., Correa, D., 2018. Tissue engineering and cell-based therapies for fractures and bone defects. Front Bioeng Biotechnol 6, 105.

Polo-Corrales, L., Latorre-Esteves, M., Ramirez-Vick, J.E., 2014. Scaffold design for bone regeneration. J. Nanosci. Nanotechnol. 14 (1), 15–56.

Porter, J.R., Ruckh, T.T., Popat, K.C., 2009. Bone tissue engineering: a review in bone biomimetics and drug delivery strategies. Biotechnol. Prog. 25 (6), 1539–1560.
Qu, D., Mosher, C.Z., Boushell, M.K., Lu, H.H., 2015. Engineering complex orthopaedic tissues via strategic biomimicry. Ann. Biomed. Eng. 43 (3), 697–717.
Requicha, J.F., Viegas, C.A., Albuquerque, C.M., Azevedo, J.M., Reis, R.L., Gomes, M.E., 2012. Effect of anatomical origin and cell passage number on the stemness and osteogenic differentiation potential of canine adipose-derived stem cells. Stem Cell Rev. 8 (4), 1211–1222.
Rodrigues, C.V., Serricella, P., Linhares, A.B., Guerdes, R.M., Borojevic, R., Rossi, M.A., . . . Farina, M., 2003. Characterization of a bovine collagen-hydroxyapatite composite scaffold for bone tissue engineering. Biomaterials 24 (27), 4987–4997.
Rosada, C., Justesen, J., Melsvik, D., Ebbesen, P., Kassem, M., 2003. The human umbilical cord blood: a potential source for osteoblast progenitor cells. Calcif. Tissue Int. 72 (2), 135–142.
Russell, K.A., Chow, N.H., Dukoff, D., Gibson, T.W., LaMarre, J., Betts, D.H., Koch, T.G., 2016. Characterization and Immunomodulatory effects of canine adipose tissue- and bone marrow-derived mesenchymal stromal cells. PLoS One 11 (12), e0167442.
Sawangmake, C., Nantavisai, S., Osathanon, T., Pavasant, P., 2016. Osteogenic differentiation potential of canine bone marrow-derived mesenchymal stem cells under different β-glycerophosphate concentrations in vitro. Thai J. Vet. Med. 46 (4), 617–625.
Sawangmake, C., Nowwarote, N., Pavasant, P., Chansiripornchai, P., Osathanon, T., 2014a. A feasibility study of an in vitro differentiation potential toward insulin-producing cells by dental tissue-derived mesenchymal stem cells. Biochem. Biophys. Res. Commun. 452 (3), 581–587.
Sawangmake, C., Pavasant, P., Chansiripornchai, P., Osathanon, T., 2014b. High glucose condition suppresses neurosphere formation by human periodontal ligament-derived mesenchymal stem cells. J. Cell. Biochem. 115 (5), 928–939.
Schaefer, D., Martin, I., Shastri, P., Padera, R.F., Langer, R., Freed, L.E., Vunjak-Novakovic, G., 2000. In vitro generation of osteochondral composites. Biomaterials 21 (24), 2599–2606.
Schantz, J.T., Chim, H., Whiteman, M., 2007. Cell guidance in tissue engineering: SDF-1 mediates site-directed homing of mesenchymal stem cells within three-dimensional polycaprolactone scaffolds. Tissue Eng. 13 (11), 2615–2624.
Screven, R., Kenyon, E., Myers, M.J., Yancy, H.F., Skasko, M., Boxer, L., Zhu, M., 2014. Immunophenotype and gene expression profile of mesenchymal stem cells derived from canine adipose tissue and bone marrow. Vet. Immunol. Immunopathol. 161 (1), 21–31.
Shahgoli, S., Levine, M.H., 2011. Introduction and overview of bone grafting. N. Y. State Dent. J. 77 (2), 30–32.
Sheikh, Z., Najeeb, S., Khurshid, Z., Verma, V., Rashid, H., Glogauer, M., 2015. Biodegradable materials for bone repair and tissue engineering applications. Materials 8 (9), 5744–5794.
Shi, S., Gronthos, S., 2003. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J. Bone Miner. Res. 18 (4), 696–704.
Shin, H., Jo, S., Mikos, A.G., 2003. Biomimetic materials for tissue engineering. Biomaterials 24 (24), 4353–4364.
Silber, J.S., Anderson, D.G., Daffner, S.D., Brislin, B.T., Leland, J.M., Hilibrand, A.S., Albert, T.J., 2003. Donor site morbidity after anterior iliac crest bone harvest for single-level anterior cervical discectomy and fusion. Spine 28 (2), 134–139.
Spalazzi, J.P., Dagher, E., Doty, S.B., Guo, X.E., Rodeo, S.A., Lu, H.H., 2006a. *In* Vivo Evaluation of a Tri-phasic Composite Scaffold for Anterior Cruciate Ligament-To-Bone Integration. In: Paper presented at the 2006 International Conference of the IEEE Engineering in Medicine and Biology Society.
Spalazzi, J.P., Doty, S.B., Moffat, K.L., Levine, W.N., Lu, H.H., 2006b. Development of controlled matrix heterogeneity on a triphasic scaffold for orthopedic interface tissue engineering. Tissue Eng. 12 (12), 3497–3508.
Spencer, N.D., Chun, R., Vidal, M.A., Gimble, J.M., Lopez, M.J., 2012. In vitro expansion and differentiation of fresh and revitalized adult canine bone marrow-derived and adipose tissue-derived stromal cells. Vet. J. 191 (2), 231–239.
Tsumanuma, Y., Iwata, T., Kinoshita, A., Washio, K., Yoshida, T., Yamada, A., Izumi, Y., 2016. Allogeneic transplantation of periodontal ligament-derived multipotent mesenchymal stromal cell sheets in canine critical-size Supra-alveolar periodontal defect model. Biores Open Access 5 (1), 22–36.
Tsuruga, E., Takita, H., Itoh, H., Wakisaka, Y., Kuboki, Y., 1997. Pore size of porous hydroxyapatite as the cell-substratum controls BMP-induced osteogenesis. J. Biochem 121 (2), 317–324.
Tuzlakoglu, K., Santos, M.I., Neves, N., Reis, R.L., 2011. Design of nano- and microfiber combined scaffolds by electrospinning of collagen onto starch-based fiber meshes: a man-made equivalent of natural extracellular matrix. Tissue Eng Part A 17 (3-4), 463–473.
Ulery, B.D., Nair, L.S., Laurencin, C.T., 2011. Biomedical applications of biodegradable polymers. J. Polym. Sci. B Polym. Phys. 49 (12), 832–864.
Volkmer, E., Drosse, I., Otto, S., Stangelmayer, A., Stengele, M., Kallukalam, B.C., Schieker, M., 2008. Hypoxia in static and dynamic 3D culture systems for tissue engineering of bone. Tissue Eng. A 14 (8), 1331–1340.
Wang, Y., Bi, X., Zhou, H., Deng, Y., Sun, J., Xiao, C., Fan, X., 2014. Repair of orbital bone defects in canines using grafts of enriched autologous bone marrow stromal cells. J. Transl. Med. 12 (1), 123.
Ward, B.C., Webster, T.J., 2006. The effect of nanotopography on calcium and phosphorus deposition on metallic materials in vitro. Biomaterials 27 (16), 3064–3074.
Wei, G., Ma, P.X., 2004. Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. Biomaterials 25 (19), 4749–4757.
Yuan, J., Cui, L., Zhang, W.J., Liu, W., Cao, Y., 2007. Repair of canine mandibular bone defects with bone marrow stromal cells and porous beta-tricalcium phosphate. Biomaterials 28 (6), 1005–1013.
Zeltinger, J., Sherwood, J.K., Graham, D.A., Müeller, R., Griffith, L.G., 2001. Effect of pore size and void fraction on cellular adhesion, proliferation, and matrix deposition. Tissue Eng. 7 (5), 557–572.
Zhang, C., Hu, K., Liu, X., Reynolds, M.A., Bao, C., Wang, P., Xu, H.H.K., 2017. Novel hiPSC-based tri-culture for pre-vascularization of calcium phosphate scaffold to enhance bone and vessel formation. Mater Sci Eng C Mater Biol Appl 79, 296–304.
Zhang, Y., Ni, M., Zhang, M., Ratner, B., 2003. Calcium phosphate-chitosan composite scaffolds for bone tissue engineering. Tissue Eng. 9 (2), 337–345.
Zuk, P.A., Zhu, M., Ashjian, P., De Ugarte, D.A., Huang, J.I., Mizuno, H., Hedrick, M.H., 2002. Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell 13 (12), 4279–4295.