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Biomimetic Tissue-Engineered Bone Substitutes for Maxillofacial and Craniofacial Repair: The Potential of Cell Sheet Technologies

*Fabien Kawecki, William P. Clafshenkel, Michel Fortin, François A. Auger, and Julie Fradette**

Maxillofacial defects are complex lesions stemming from various etiologies: accidental, congenital, pathological, or surgical. A bone graft may be required when the normal regenerative capacity of the bone is exceeded or insufficient. Surgeons have many options available for bone grafting including the "gold standard" autologous bone graft. However, this approach is not without drawbacks such as the morbidity associated with harvesting bone from a donor site, pain, infection, or a poor quantity and quality of bone in some patient populations. This review discusses the various bone graft substitutes used for maxillofacial and craniofacial repair: allografts, xenografts, synthetic biomaterials, and tissue-engineered substitutes. A brief overview of bone tissue engineering evolution including the use of mesenchymal stem cells is exposed, highlighting the first clinical applications of adipose-derived stem/stromal cells in craniofacial reconstruction. The importance of prevascularization strategies for bone tissue engineering is also discussed, with an emphasis on recent work describing substitutes produced using cell sheet-based technologies, including the use of thermo-responsive plates and the self-assembly approach of tissue engineering. Indeed, considering their entirely cell-based design, these natural bone-like substitutes have the potential to closely mimic the osteogenicity, osteoconductivity, osteoinduction, and osseointegration properties of autogenous bone for maxillofacial and craniofacial reconstruction.

1. Introduction

Bone is the most commonly transplanted solid tissue.[1,2] Indeed, more than 1.5 million autologous or allogeneic bone grafts are required annually for musculoskeletal surgical procedures in the USA.[3] Bone grafting is usually essential to reconstruct bone defects[4] or improve healing after maxillofacial trauma. Moreover, resection of maxillofacial tumors and correction of severe congenital malformations necessitate volume restoration that relies on bone substitution. In the field of maxillofacial reconstruction, the main goal is to restore functions, in addition to facial contours and aesthetics.[5] Selection of the graft option will be dependent on bone quality, the volume needed, associated morbidity, accessibility to the substitute, cost, and the patient's activity level.

This review provides a brief discussion of etiologies leading to maxillofacial bone defects and the description of several bone substitute options available to repair tissues in this specific anatomic region. Commercially available bone graft materials based on the ideal properties of the osteogenicity, osteoconductivity, osteoinduction, and osseointegration will be described. Standard approaches to bone repair are summarized along with recent advances in bone tissue engineering, namely, the use of human adipose-derived stem/stromal cells (ASCs). These cells are an interesting mesenchymal stem cell (MSC) source with osteoblast-lineage potential and a pro-angiogenic secretome promoting in vitro prevascularization and/or stimulating neovascularization for bone tissue engineering. Special attention is given to the suitability of MSCs for the development of bone-like tissue using cell sheet-based tissue engineering technologies. Ultimately, these methods aim to improve osteogenicity, osteoconductivity, osteoinduction, and osseointegration to stimulate efficient repair.

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adhm.201700919.

DOI: 10.1002/adhm.201700919

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2. The Etiologies of Maxillofacial Bone Defects

Diverse etiologies can affect normal maxillofacial bone structure and function. Nonurgent situations, such as birth defects, deformities induced by pathologies, as well as tumor development, allow the surgeons time to devise an effective surgical approach.

Some congenital malformations may develop during embryogenesis. For example, the cleft palate is the most common congenital deformity observed in newborns. It results from the lack of fusion between the maxilla bone structures. A cleft palate can have detrimental psychological impacts during childhood[6] and cause feeding problems.[7] Its reconstruction often requires multiple surgeries that consist of repairing the palate and closing the lip in newborns that are between three and six months old. Infants between 9 and 12 years of age will have additional surgery to create a functional palate.[8]

Acute trauma (e.g., gunshots, traffic, or industrial accidents) are the most common and severe events requiring maxillofacial surgical procedures for bone loss of substance.[9] The injury of multiple tissue types requires rapid repair of life-threatening damage. Thus, these complicated situations involve multi-disciplinary surgical teams.[10]

The jaw is also a common site for the development of tumors in diseases such as ameloblastoma. This benign lesion arises from the odontogenic epithelium and results in the development of a critical intraosseous mass that presents an aggressive biological behavior with risk of recurrence.[11,12] Resection of the tumor prevents metastasis and surgical reconstruction of the bone structure allows normal facial contour and function.[13]

Maxillofacial degenerative deformities can also develop at adult age from imbalances in the bone remodeling process. After teeth extractions, the alveolar ridge undergoes a remodeling process that promotes osteoclastogenesis and bone resorption.[14,15] Pathophysiological bone resorption, such as osteoporosis, can progress considerably without treatment, including inside the jaw. Oral health is a great concern in osteoporotic women undergoing dental procedures. For example, postmenopausal women are particularly susceptible to alveolar osteitis (e.g., dry socket) after teeth extractions.[16–18] Despite the high incidence of alveolar osteitis, an autologous bone graft is often inadequate or contraindicated by the poor quality of bone in this patient population.[19]

From these examples, it is obvious that the quantity/quality of bone donor sites necessary for autologous reconstruction can often cause severe morbidity. This clinical need leads to the development of various types of bone substitutes.

3. General Anatomy and Embryogenesis of the Craniomaxillofacial Skeleton

The main function of the bones composing the craniomaxillofacial skeleton is to protect the brain and the sense organs. The human skull is composed of 22 bones divided into two regions that include the neurocranium (eight bones) and the facial skeleton (14 bones).[20] The neurocranium is composed of the frontal bone, the ethmoid, the sphenoid, two parietal bones, the occipital bone, and the temporal bones. The anatomy of the facial skeleton is defined by two palatine bones, two lacrimal bones, the maxilla bones, the mandible, the vomer, the zygomas, two inferior nasal turbinates, as well as two nasal bones. All these bones have specific shapes, different volumes, and they provide a frame on which the soft tissues of the face can act to facilitate facial expression, eating, breathing, and speech.[21] All these key parameters will have to be considered when selecting the ideal graft for craniomaxillofacial reconstruction.

During the early stage of embryogenesis (week 12), craniomaxillofacial bones arise from different processes of bone development.[22] For example, flat bones composing the skull (except the mandible) are derived from an intramembranous ossification process that consists of the replacement of a fibrous membrane, called center of ossification, by a spongy bone. This is followed by the formation of red marrow within the new bone tissue and the surrounding cortical bone. By comparison, the mandible also arises from an intramembranous ossification at the primary growth center of the two pieces of Meckel's cartilage, but an endochondral ossification process takes place at the inferior portions of the mandible to create the future condyles. Indeed, the hyaline cartilage is invaded by vascularization, innervation, and bone cells (osteoblasts and osteoclasts),

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and replaced by a spongy bone within red marrow, enclosed by compact bone and cartilage tissue.

After their maturation, all bones are comprised of spongy structures filled by bone marrow and surrounded by a hard tissue, termed compact or cortical bone. This bone constitutes the outside surfaces of the bones and gives the mechanical properties to the tissue. It is formed of cylindrical units called osteons containing concentric layers of lamellae that are formed by hard, calcified matrix with lacunae containing bone cells called osteocytes. Smaller canals, named canaliculi, radiate outward from a central channel, which contains microvasculature and nerve fibers. Osteocytes develop extensions to connect with each other within an osteon and the central canal. Nutrients, oxygen, and waste are exchanged between the osteocytes and the microvasculature through these cellular extensions. Perforating canals provide microchannels that allow microvasculature migration from the periosteum.

Spongy bone is composed of irregularly shaped thin plates called trabeculae, arranged in a porous structure. The micro and macroarchitecture of this porous material will strongly influence the Young's modulus of the compact bone.[23] As in osteons, canaliculi located in trabeculae allow the connections between each osteocyte. However, since each trabecula is only a few cell layers thick, each osteocyte can exchange nutrients and oxygen with the nearby microvasculature, eliminating the need of a central canal.

4. Characteristics of the Ideal Bone Graft

Several specific parameters characterize different bone graft subtypes and their substitutes. Indeed, the ideal bone substitute should replicate the essential traits of native bone:

Biocompatibility: According to the definition recorded by the European Society for Biomaterials at Chester (UK) in 1986, bone graft biocompatibility is defined as "the ability of the material to provoke an appropriate response of the host organism in a specific application".[24] The material has to present excellent chemical properties (e.g., resistance to corrosion and complete inertness to the body environment) to allow adequate reactions (e.g., tissue integration) from the tissue after grafting.

Osteogenicity: This property is defined as the capacity of a reconstructed tissue to form a new bone tissue.[25] It is the key requirement for potential clinical use of a tissue-engineered material in bone regeneration.

Osteoconductivity: This quality is defined by Moore et al. as "the ability to provide a 3D configuration for in-growth of host capillaries, perivascular tissue, and osteoprogenitor cells into the graft".[26]

Osteoinduction: This property is defined as the ability of the material to stimulate the specialization of undifferentiated cells toward an osteoblastic lineage.[27] For example, calcium ions or differentiation factors can be released progressively from the material to promote osteogenesis.[28]

Osseointegration: It is the ability of the material to integrate and fuse with the native bone tissue after grafting and bone remodeling.[29,30] This very important property will depend on the adequate combination of the osteoconductivity and osteoinduction properties of the graft.

These properties can vary between bone graft and substitute options as discussed in the next sections.

5. Bone Graft Options: Autografts, Allografts, Xenografts, and Synthetic Substitutes

In the clinical context of maxillofacial reconstructive surgery, autologous bone grafting remains the "gold standard".[31,32] Vascularized bone flaps and nonvascularized bone grafts are both widely used for maxillofacial reconstruction. However, an important distinction must be provided between the two, considering the reliability of each type of surgery. Regarding the associated surgical procedures, nonvascularized bone grafts are more easily performed, with better donor sites availability (e.g., iliac crest, parietal bone, or mandibular symphysis). Nevertheless, the procedure of collection is not without some drawbacks and can sometimes be associated with a high level of morbidity after surgery.[33] Conversely, vascularized bone flaps cannot be easily harvested from fibula with minimal morbidity.[34] The significant difference in terms of osseointegration rates between vascularized flaps and bone grafts is related to the structure of the bone graft and the level of osteoblastic activity throughout the graft.[35] Sequelae associated with the harvest site include bone deformity, pain, and at times a continuous progressive resorption phenomenon.[36] As expected for such surgeries, the risks of infection, hematomas, prolonged pain, sensory loss, and scarring are prevalent, and sometimes result in multiple surgeries.[33,37]

The use of engineered bone substitutes may be more suitable for some patient populations, such as patients affected by osteoporosis or in children, based on the limitations and morbidities associated with the use of autografts. Surgeons do have a few options among alternative bone grafting materials,[38] including the use of allogeneic (e.g., human decellularized cadaver tissue), xenogenic (e.g., bovine or equine decellularized matrix), or synthetic biomaterials (e.g., hydroxyapatite, bioglass, or ß-tricalcium phosphate (ß-TCP) formulations), as shown in **Table 1**.

Various types of bioactive membranes are also available for guided bone regeneration.[48] This technique uses resorbable membranes and bone grafts to increase alveolar bone defects in patients.[49] The role of the membranes is to guide bone regeneration by releasing growth factors (e.g., BMP-2) and prevent the invasion of the surrounding soft tissue into the graft bed. Originally, nonresorbable expanded polytetrafluoroethylene (e-PTFE) membranes were used as barrier function materials for vertical ridge augmentation in human.[50] Nevertheless, e-PTFE membranes are not without some drawbacks, such as the need of re-operation to remove the membrane and a high rate of bacterial infection.[51,52] Recently, new types of resorbable membranes with a low rate of infection have been developed. They are either made of animal-derived collagen[53,54] or bioresorbable polymers, such as the poly(desamino tyrosyl-tyrosine ethyl ester-carbonate)[55] or the polylactic acid-polyglycolic acid co-polymer.[56] All of these membranes being acellular, they are not considered to be derived from the cell sheet technology.

Surgeons should consider the advantages and limitations of each type of substitutes when selecting a commercially available

Table 1. Examples of bone substitutes used for maxillofacial reconstruction.

Bone substitutes		Company (Country)	Advantages	Limitations	References
Allogeneic substitutes	Cadaveric human demineralized bone matrix	Various providers	No donor site from the patient, and excellent osteoconductive properties	Immunogenicity, and inflammatory reactions due to residual toxicity of the detergent	[39]
	Grafton®: Freeze-dried allograft from demineralized human bone tissue	Osteotech Inc. (USA)	No donor site from the patient, storage stability, and excellent osteoconductive properties	Suboptimal mechanical properties	[40]
Xenogeneic substitutes	Colloss®: Lyophilized equine demineralized bone matrix supplemented with BMPs and vascular growth factors	Ossacur AG (Germany)	No donor site from the patient, long-term conservation, osteoinductive, and enhanced neovascularization	High immunogenicity	[41]
	Bio-Oss®: Bovine derived xenograft	Giestlich Pharma AG (Switzerland)	No donor site from the patient, osteointegrative, and commonly used in the clinic	Immunogenic, and only sold in small volumes	[42]
Synthetic substitutes	M-TAM®: Micro titanium augmentation mesh	Stryker (USA)	No donor site from the patient, excellent mechanical properties, and tunable properties	Nonresorbable, nonosseointegrative, and nonosteoinductive	[43]
	Bioactive bioceramics (ionic exchanges)	Various providers	No donor site from the patient, osteoinductive, and excellent mechanical properties	Poor osseointegration	[44]
	Medpor™: A porous polyethylene implant	Stryker (USA)	No donor site from the patient, excellent mechanical properties, resistant to infections, and tunable properties	Nonresorbable, nonosseointegrative, and nonosteoinductive	[45]
	Norian®: A calcium phosphate bone cement mixed with reinforcing fibers and a sodium hyaluronate based solution	DePuy Synthes Companies (Switzerland)	No donor site from the patient, osteoinductive, excellent mechanical properties, and tunable properties	High risk of infection, and poor osseointegration	[46]
	Ceratite®: A hydroxyapatite-tricalcium phosphate (HA-TCP) composite	NGK Spark Plag Co. Ltd. (Japan)	No donor site from the patient, osteoinductive, and excellent mechanical properties	Poor osseointegration	[47]

bone graft such as those indicated in Table 1. Additional important characteristics to consider when selecting a bone substitute include their biochemical properties (e.g., osteogenic and pro-angiogenic factors), which are intimately related to their osseointegration capacity. Moreover, the success of bone grafting may be determined by the substitute's mechanical properties, which are influenced by their micro and macroarchitecture. For example, cortical bone from the skull exhibits an average porosity of 54% with a mean Young's modulus estimated to be at 19 GPa.[57] The implanted substitutes should ultimately closely mimic the structural properties of the surrounding native bone to preserve the normal mechanical behavior of the host tissue and their own integrity.[58,59]

Several studies have reviewed the development of various biomaterials subtypes engineered to display improved osteoconductive and osteoinductive properties.[28,60–62] Nonetheless, in general, the osseointegration of synthetic substitutes usually remains suboptimal compared to autografts or allografts.[63–65] To circumvent this, researchers focused on improving graft osseointegration, as well as osteoinduction, by modifying the composition and architecture of biomaterials, and also by adding growth factors (e.g., bone morphogenetic proteins (BMPs)) or/and stem cells to enhance bone formation.[66–69] Indeed, combining osteoprogenitor cells with an appropriate scaffold has demonstrated a significant improvement in the use of bone substitutes, and in some case, it might even be considered as a better substitution for autologous bone autografts.

6. Emergence and Evolution of Bone Tissue Engineering

6.1. Mesenchymal Stem Cells

Tissue engineering was initially defined as an approach that "applies the principles of biology and engineering to the development of functional substitutes for damaged tissue".[70] As it expanded, tissue engineering combined the use of biomaterials, stem cells, and growth factors in order to produce a reconstructed tissue that mimics the physical and functional characteristics of a native tissue.[71,72] By using tissue engineering techniques and appropriate cell types, researchers have the potential to produce human bone-like tissues that eliminate or reduce the requirement of harvesting a bone tissue graft from a secondary site in the patient.[73]

From 1970 to 1980, Friedenstein et al. validated the in vitro clonogenic potential of bone marrow cells. Bone marrow extracted from guinea pigs and rats was cultured on plastic culture dishes. Nonadherent cells were separated from spindle-like cells that demonstrated plastic adhesion.[74,75] These adherent cells were heterogeneous in appearance but capable of forming

fibroblastic colony-forming units. Since then, bone marrow mesenchymal stem cells (BM-MSCs) were identified as a population of progenitor cells distinct from hematopoietic stem cells. Indeed, they can be phenotypically distinguished by their specific expression of cell-surface molecules used as markers. Over ≥95% of the cell population that is adherent to plastic culture surfaces positively express CD73, CD90, and CD105 surface markers.[76] Moreover, this population usually lacks the expression of CD11b, CD14, CD19, CD34, CD45, CD79-α, and human leukocyte antigen (HLA) class II,[77] which are hallmarks of hematopoietic cells. However, endothelial cells (ECs) can also express CD90 and CD105.[78] Consequently, these markers should be associated with others, such as CD13 and CD73, when attempting to select human MSCs.

In the early 1990s, researchers explored the ability of BM-MSCs to differentiate into several cell types such as bone, cartilage, muscle, and adipose cells.[79] By using specific induction cocktails, BM-MSCs were successfully differentiated into cells exhibiting features of these specialized cell types, such as osteoblast-like cells.[80–82] In addition to their multipotency, MSCs have a paramount role in normal healing, angiogenesis, and tissue regeneration via their primary trophic property to secrete growth factors and chemokines, including hepatocyte growth factor (HGF), transforming growth factors (TGF-α or TGF-ß), insulin-like growth factor-1 (IGF-1), epithelial growth factor, basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF).[83–86] Murphy et al. also reviewed the anti-inflammatory, immunomodulatory, and anti-apoptotic properties of MSCs.[87] Indeed, many studies have shown that MSCs can secrete growth factors and anti-inflammatory proteins in response to inflammatory molecules such as interferon-gamma, interleukin (IL)-1, IL-2, IL-12, as well as tumor necrosis factor-α. Undifferentiated MSCs present a low or medium expression of HLA class I and a low expression of HLA class II on their cell surface, making them less detectable by the host immune system and suggesting their successful use for allogeneic therapies.[77,88] Finally, MSCs also have the ability to protect surrounding cells from apoptosis including damaged neurons, cardiomyoblasts, and lung fibroblasts.[89–91] Although not fully understood, such protective effects are thought to result from the actions of several proteins including IGF-1 and IL-6, which increase the expression of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and Akt (protein kinase B).[92] Moreover, it was demonstrated that elevated levels of Akt increase secreted frizzled-related protein 2 (Sfrp2) expression, which would contribute to prosurvival effects and protect cells from apoptosis.[93]

6.2. Potential of Adipose-Derived Stem/Stromal Cells in Bone Tissue Engineering

Originally, tissue engineering endeavors that had focused on generating bone substitutes utilized BM-MSCs, because of their physical proximity to the native tissue and their multipotency. Thus, the discovery of different MSC sources from other body sites has greatly expanded the field of bone tissue engineering. Indeed, in 2001, Zuk et al. identified the benefits of using mesenchymal stem cells harvested from adipose tissues (ASCs) and their implication for cell-based therapies. Human ASCs are abundant in adipose tissue, readily harvested by a minimally invasive procedure such as lipoaspiration, and capable of multilineage differentiation.[94] ASCs and BM-MSCs share many similarities including the expression of cell surface markers and the potential to differentiate into osteoblastic-lineage. The accessibility and abundancy of adipose tissue compared to bone marrow, as well as the proportion of cells featuring stem cell-like features extracted from their respective tissue (**Table 2**), has generated a strong interest for ASC-based applications in recent years.[95,96] It is also recognized that the pro-angiogenic secretome of ASCs (e.g., angiopoietin-1 (Ang-1), VEGF, bFGF, and HGF)[97–99] has a great impact on EC proliferation and tubulogenesis, similar or even greater compared to that of BM-MSCs (Table 2),[100–102] which makes them excellent candidates to stimulate the vascularization of bone substitutes.

Table 2. Comparison of bone marrow and adipose-derived mesenchymal cells.

	BM-MSCs	ASCs	References
Harvest method	Needle biopsy	Liposuction or lipectomy	[103,104]
Average volume of tissue that can be harvested/procedure	40 mL	1000 mL	[95]
Donor-site morbidity	High	Low	[105]
% of stem cell-like cells reported to total cells	0.002%	2%	[95]
In vitro proliferation potential	Normal	Moderate–high	[101]
Angiogenic secretome	Pro	Pro	[100–102]

6.3. First Clinical Applications of Adipose-Derived Stem Cells for Maxillofacial Repair

The first reported use of human ASCs for craniofacial repair was published by Lendeckel et al. in 2004. A seven-year-old girl with poorly healed multifragment calvarial fractures was grafted with nondifferentiated autologous ASCs combined with autologous fibrin glue extracted from her plasma, and processed using the CryoSeal FS System.[106] A resorbable macroporous mesh (polylactic acid-based) was used to mechanically fix the bone graft mixture. After three months, calvarial healing was evaluated by micro-CT imaging and these successful results provided the first demonstration of autologous ASCs beneficial use for craniofacial repair (**Figure 1**).

In 2014, the BioMediTech Institute group (University of Tampere, Finland) further investigated this tissue engineering technique in a clinical study involving 13 patients presenting different types of craniomaxillofacial defects (frontal sinus (three cases), cranial (five cases), mandible (three cases), and nasal septum (two cases)).[107] Several biomaterials were included in the reconstruction strategies: bioactive glass (frontal sinus group), ß-TCP only (cranial group), ß-TCP with bone morphogenetic protein-2 (mandibular group), or ß-TCP incorporated into a polycaprolactone scaffold/matrix (nasal septum group). Autologous ASCs were harvested from each patient, osteogenically differentiated in vitro, then seeded into the biomaterials

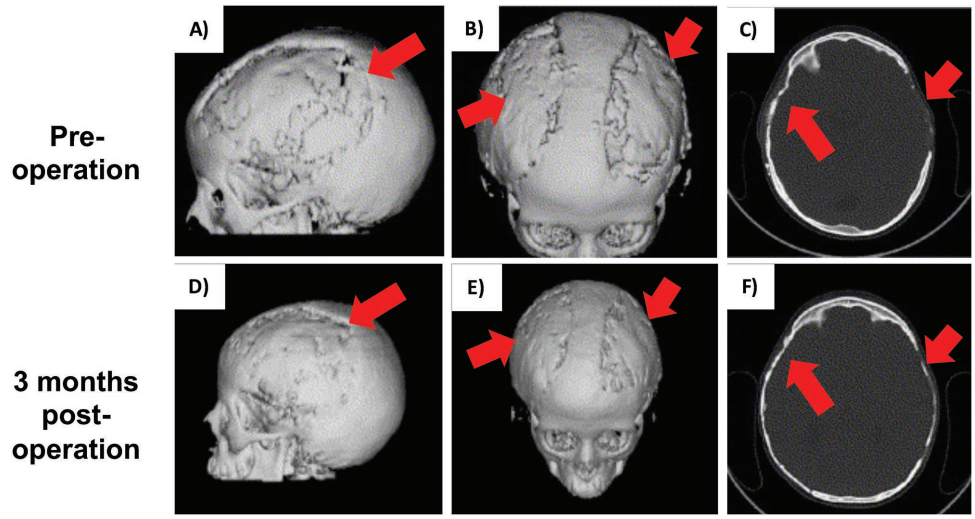

Figure 1. A seven-year-old girl suffering from closed multi-fragmental craniofacial fractures was treated with autologous ASCs combined with autologous fibrin glue extracted from plasma, and processed using the CryoSeal FS System. A) Axial view, left side; B) axial view, top; and C) axial cross-section CT scans of widespread calvarial bone defects preoperatively. D–F) CT scan of the same patient three months postoperatively showing improved healing in the treated regions. Red arrows indicate the main craniofacial defects. Reproduced with permission.[106] Copyright 2004, Elsevier.

before implantation. The early followup period ranged from 12 to 52 months depending on the case. The majority of frontal sinus patients had recurrent infections and two patients from the nasal septum group had perforations with chronic ulcerative wounds. Overall, a total of three patients in this group had their graft removed due to poor osseointegration.[107] Moreover, six-year clinical and radiological followups of the five patients who have received cranioplasty revealed unsatisfactory results.[108] Indeed, due to the radio-opacity of the biomaterial it is unclear that the procedure stimulated ossification or if the autologous ASCs generated new bone. Additionally, graft resorption was observed in three of five cases and another case was re-operated due to meningioma. Globally, these cases showed intriguing results suggesting that when associated with human ASCs, these biomaterials may not be appropriate for long-term maxillofacial bone healing.

7. Current Advances in Bone Tissue Engineering

7.1. Biomimetic Bone Substitutes: The Cell Sheet Technologies

Considering the results exposed in the previous sections regarding the limitations of certain types of biomaterials, successful graft osseointegration could likely be improved by the production of cellularized biomimetic bone substitutes devoid of exogenous or synthetic biomaterials. Cell-based tissue engineering techniques are established on the ability of the cells

Table 3. Overview of selected cell sheet models developed for bone tissue engineering.

	MSC origin	Use of thermo-responsive plate	Study type	Prevascularization	Length of culture	Ascorbic acid supplementation	Thickness
Kaibuchi et al. 2016[110]	Rat BM-MSCs	Yes	In vivo (empty socket associated with BRONJ in rats)	No	7 d	82 μg mL^{-1}	–
Xie et al. 2015[111]	Human ESMSCs	Yes	In vivo (rat critical-size calvarial defects)	No	14 d	100 000 μg mL^{-1} (for 7 d of cell expansion) 50 000 μg mL^{-1} (for 7 d of induction)	100–150 μm
Galbraith et al. 2017[112]	Human ASCs	No	In vitro	No	35 d	50 μg mL^{-1}	150 μm
Ueyama et al. 2016[113]	Rat BM-MSCs	No	In vivo (mandibular symphysis defect in rat)	No	14 d	82 μg mL^{-1}	30–50 μm
Pirraco et al. 2014[114]	Rat BM-MSCs	Yes	In vivo (ectopic graft in rat)	Yes (hUVECs)	21 d	50 μg mL^{-1}	<50 μm
Chen et al. 2016[115]	Human BM-MSCs	No	In vitro	Yes (hUVECs)	21 d	50 μg mL^{-1}	–

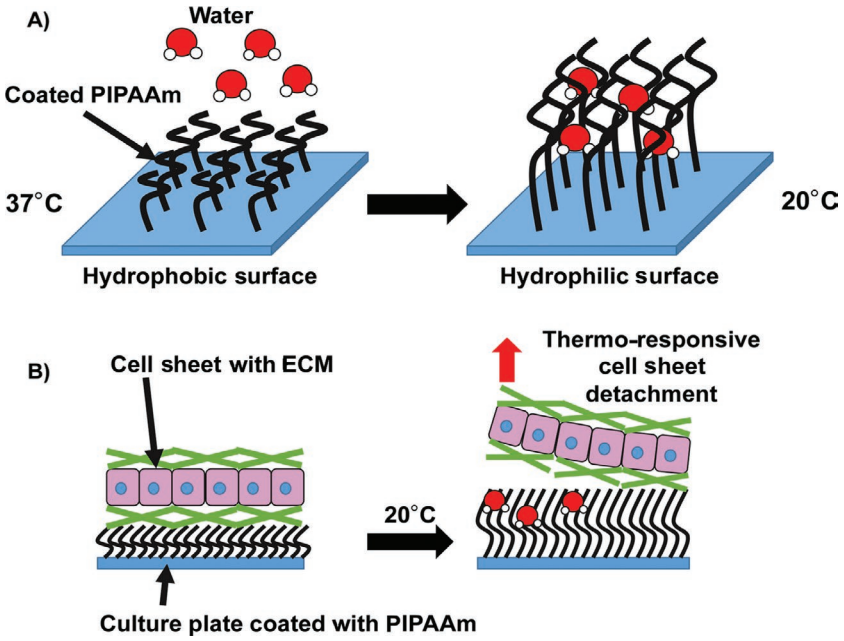

Figure 2. Physicochemical interactions on thermo-responsive culture surfaces. A) The structural conformation of the PIPAAm polymer layer is altered by lowering the environmental temperature to 20 °C in aqueous environments. At this temperature, the polymer layer becomes hydrophilic and captures water. B) The adsorbed water molecules make it more difficult for proper cell attachment, leading to the release of the cultured tissue. Nonetheless, without the use of an anchorage device, these tissues are susceptible to contraction once released. Adapted and modified with permission.[117]

to connect with each other via cell–cell junction proteins and assemble an autogenous extracellular matrix (ECM), when they are cultured to confluency.[109] The resulting constructs can be harvested with different methods, such as the use of a thermo-responsive surface or a supporting anchorage device. Selected studies are listed in **Table 3** and further discussed, in order to provide examples of the potential of using cell sheet technologies for in vitro and in vivo applications for maxillofacial reconstructive surgery.

In 1990, Yamada et al. developed thermo-responsive surfaces that are grafted with poly-*N*-isopropylacrylamide (PIPAAm), which has the capacity to become a hydrophilic surface at 20 °C (**Figure 2**A).[116] The alteration from a hydrophobic to hydrophilic surface makes it more difficult for cells to adhere, as they must displace the adsorbed water molecules, resulting in cell detachment. Subsequently, Dr. Teruo Okano's laboratory used this culture process in order to engineer cell sheets cultured at 37 °C that will then spontaneously detach from the culture surface after briefly reducing the temperature to 20 °C (Figure 2B). The use of thermo-responsive culture dishes has become common in cell sheet tissue engineering using different cell types and for various applications, such as skin, cartilage repair, as well as bone engineering.[117]

Cell sheets produced using thermo-responsive surfaces were evaluated for different types of indications, such as bisphosphonate-related osteonecrosis of the jaw (BRONJ). Indeed, Kaibuchi et al. produced tissue-engineered

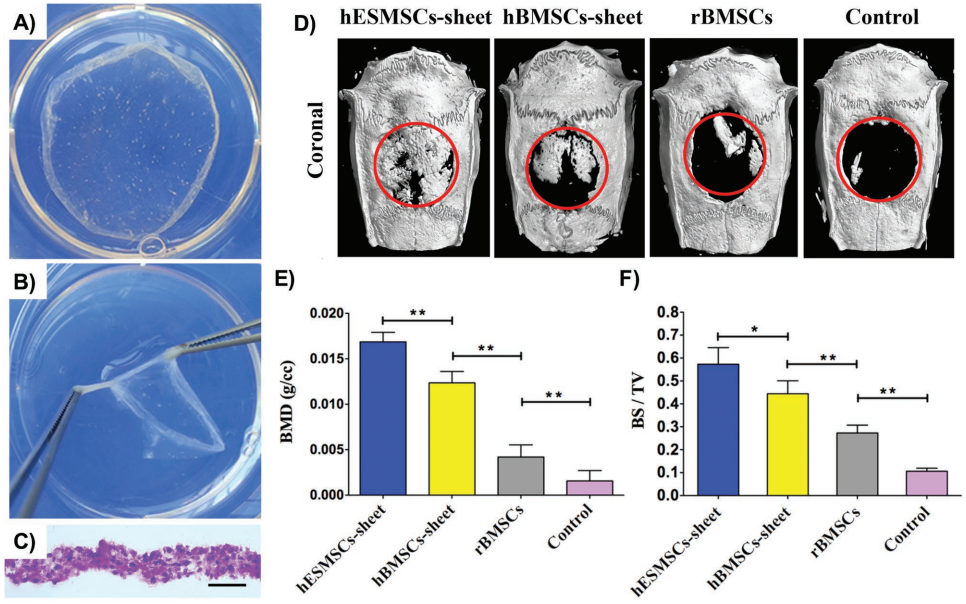

Figure 3. Ethmoid sinus progenitor cells were used as a new source of stem cells for the production of osseous cell sheets to stimulate bone regeneration. A,B) Macroscopic images of the generated osteogenic cell sheets detached from the temperature-responsive culture plates. C) Histological cross-section of the cell sheet reveals a tissue composed of five cell layers. Scale bar, 200 µm. D) Micro-CT images of calvarial bone defects eight weeks postimplantation demonstrated the greatest improvement in bone regrowth in animals treated with hESMSC sheets (red circle = original defect). E) Bone mineral density (BMD) and F) bone substance/total volume were quantified and were greatest when animals were treated with the hESMSCs-sheet/rBMSCs+PSeD constructs compared to other groups (*$P < 0.05$ and **$P < 0.001$). Adapted with permission.[111] Copyright 2015, Elsevier.

bone-like sheets using rBM-MSCs extracted from healthy rats.[110] The authors generated a pathological rodent model of BRONJ by injecting the animals with zoledronate (a bisphosphonate used for the treatment of osteoporosis and bone metastasis with a side effect of inducing osteonecrosis of the jaw) and dexamethasone three times per week for one month. Afterward, the first right molars of the maxilla were extracted for each animal. The empty sockets were either left untreated (sham animals), treated with rBM-MSC-derived bone-like sheets, or the rats were injected intravenously (IV) with rBM-MSCs. The authors observed, by immunohistochemical and histological analyses, better neovascularization and mucosal healing in the rBM-MSC sheet group compared to both untreated and IV-injected rBM-MSCs groups, two weeks after implantation. The impact on bone healing was observed by micro-CT and histological analysis in rat molar extraction sites treated with osteogenically induced cell sheets. The sites treated with rBM-MSC sheets had a lower occurrence of bone exposure compared to the IV-injected BM-MSCs and control groups, suggesting a better wound healing. These data suggest a promising role for rBM-MSC-derived bone-like cell sheet therapy for BRONJ and other bone pathologies.

Interestingly, Xie et al. were able to harvest, characterize, and differentiate human ethmoid sinus mucosa-derived mesenchymal stem cells (hESMSCs), and use them into the context of cranial bone regeneration.[111] In their study, they investigated the osteogenic potential of hESMSCs cultured on thermo-responsive dishes with or without the co-culture of rat BM-MSCs for 14 d under osteogenic conditions, in comparison to the co-culture of rat and human BM-MSC. Bone-like sheets composed of hESMSCs with a thickness of ≈100–150 µm were generated using a high concentration of ascorbic acid (100 000 µg mL^{-1}) for 7 d followed by an osteogenic treatment including ascorbic acid (50 000 µg mL^{-1}) for additional 7 d (**Figure 3**A–C and Table 3). Their results confirmed an enhancement of osteogenesis when rat BM-MSCs were co-cultured in vitro with hESMSC compared to their co-culture with human BM-MSCs or alone. Subsequently, they compared the bone healing potential of hESMSC sheets and rBM-MSC sheets, when they are combined with a porous poly-sebacoyl diglyceride (PSeD) scaffold seeded with rat BM-MSCs, in 8 mm critical-sized rat calvarial defects. New bone formation was assessed following histological, immunohistochemical, and micro-CT analyses and was greatest for the group of animals treated with hESMSC sheets (Figure 3D–F). The authors attribute this to the increased expression of cytokines secreted by hESMSCs including BMPs and bFGF, as well as the enhancement of BMP-regulated genes. Overall, this study showed the development of a bone-like sheet model derived from a unique stem cell source that could be combined with biomaterials and serve as a future alternative for bone regeneration.

Cell-based tissue engineering can also be performed in vitro without the use of thermo-responsive plates. Indeed, the core technology of the LOEX (Laboratoire d'organogénèse expérimentale de l'Université Laval/LOEX, Quebec City, Canada) is based on the self-assembly approach of tissue engineering. This method of tissue production relies on the capacity of mesenchymal cells to secrete and assemble their own ECM when they are stimulated with ascorbic acid and serum, resulting in a sheet-like tissue over long-term culture.[118–120] The generated tissues are easily raised with forceps from the culture surface, especially when using an anchoring device that protects from cell and tissue damage (**Figure 4**). The resulting cell sheets can further be combined to form a variety of 3D structure types (e.g., stacked sheets or tubes) that are resistant enough to be manipulated and sutured. Since the secreted ECM is abundant and serves as a natural support system for the developing tissue, this method leads to the production of thicker cell sheets and constructs, prevents excessive contraction when anchored, and allows easier handling compared to thermo-responsive based-cell sheets. However, the production of such tissues requires a longer culture period. Depending on the desired type of application, the balance between the rapid availability of the substitute and its increased mechanical properties should be considered.

Recently, our team demonstrated that osteogenic induction of human ASCs concomitantly to matrix production leads to the

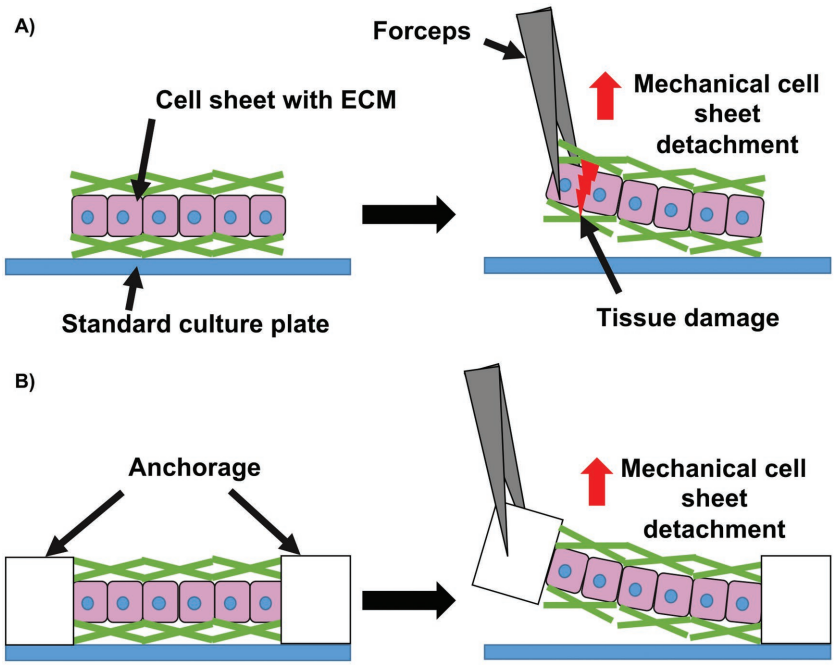

Figure 4. The self-assembly method relies on the capacity of mesenchymal cultured cells to secrete and assemble sufficient extracellular matrix (ECM) to generate a cell sheet in standard culture-treated plates. A) Cell sheets can be mechanically raised using forceps by catching the tissue directly, which causes tissue contraction, as well as matrix and cellular damages. B) To facilitate the manipulation of the generated cell sheet, an anchorage device can be inserted at the beginning of cell culture, which is then used to manipulate the tissue while preserving its integrity and preventing tissue contraction.

production of reconstructed osseous tissues featuring a robustly mineralized matrix.[112] This method allowed the production of ≈150 µm thick osseous tissues by stacking only three self-assembled sheets, a process that can be further customized. An intense osseous mineralization was observed in osteogenically differentiated tissues compared to non-osteogenically differentiated control tissues following alizarin red (calcium-containing mineral) and von Kossa (phosphate-containing mineral) staining, as well as using a specific fluorescent marker for hydroxyapatite (**Figure 5**A–I). Moreover, a significant amount of calcium was measured by the o-cresolphthalein-complexone method in osseous tissues compared to undifferentiated stromal tissues (Figure 5J). Research efforts have also been directed at prevascularizing these tissues, as we have already demonstrated the feasibility of generating a preformed network of capillaries within skin and adipose tissue substitutes.[121,122]

Another example of the applications of cell sheet technologies is the in vivo study conducted by Ueyama et al. to enhance maxillofacial bone regeneration in rats.[113] Rather than using an anchorage device to facilitate the detachment of the tissue, the osteogenic matrix cell sheets (OMCSs) were gently scraped together into a 2 mm diameter combined mass (**Figure 6**A,B). These OMCSs consisted of rat BM-MSCs cultured in the presence of dexamethasone and ascorbic acid without the use thermoresponsive culture plates, leading to sheets ≈30–50 µm thick (Table 3).[113,123] Thereafter, the OMCSs were packed into a rat mandibular symphysis defect, which is one of the common sites of human mandibular fractures (the most common being the mandibular angle) (Figure 6G–I). Maxillofacial bone regeneration was examined after 2, 4, and 8 weeks of implantation using micro-CT (Figure 6J,K). The authors showed that new bone formation area increased over time for the experimental group compared

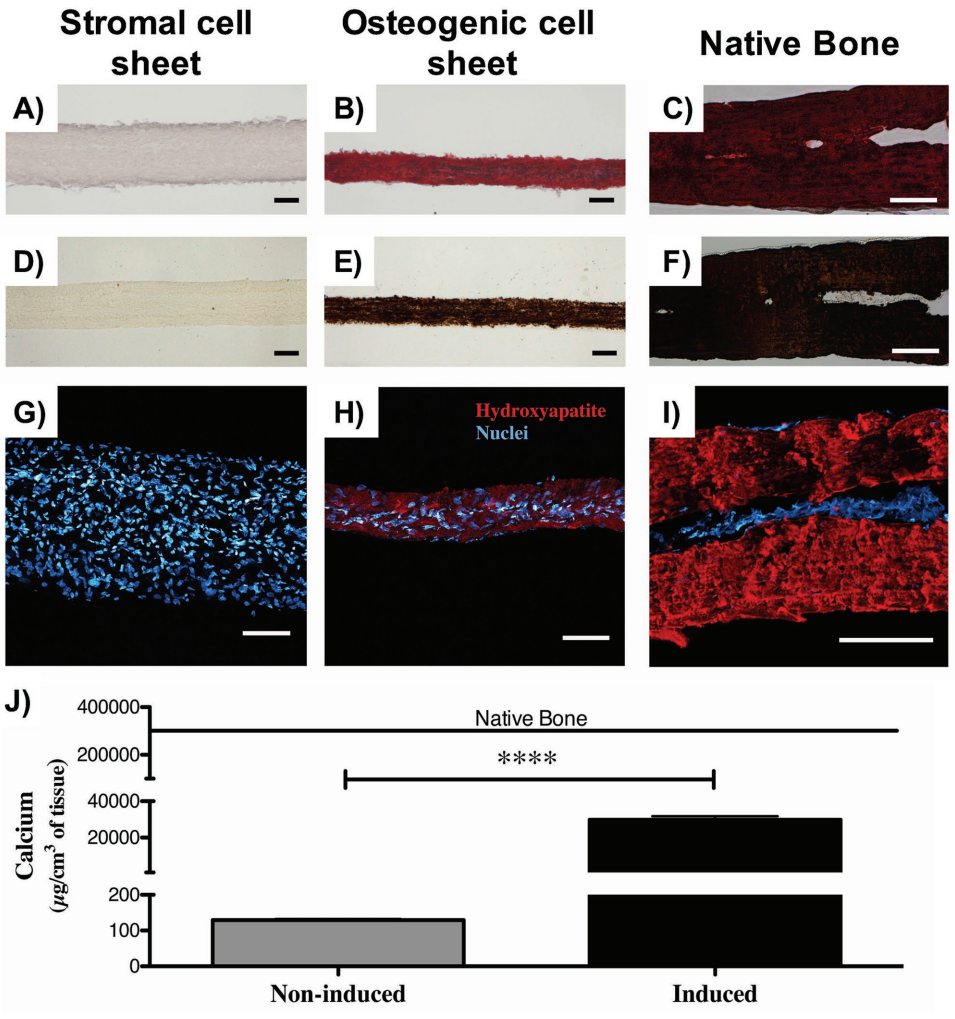

Figure 5. Robust in vitro bone mineralization is obtained when osteogenically differentiated human ASCs are cultured using the self-assembly method. Calcium and phosphate-containing mineralization was observed in reconstructed tissues following A–C) alizarin red and D–F) von Kossa staining, respectively, after 35 d of culture. G–I) Fluorescent staining for hydroxyapatite confirmed in vitro bone mineral formation (red) in osteogenically induced cell sheet compared to control. In all cases, native bone tissue was used as a reference ((C), (F), and (I)). Nuclei were stained in parallel with SYTOX green (blue). Scale bars, 100 µm. J) Quantification of calcium levels confirmed a significantly greater amount of calcium-containing mineral in osteogenic cell sheets (induced) compared to stromal cell sheets (noninduced). Calcium levels in native bone tissues were included as a reference (solid line). (****$P < 0.0001$). Adapted with permission.[112]

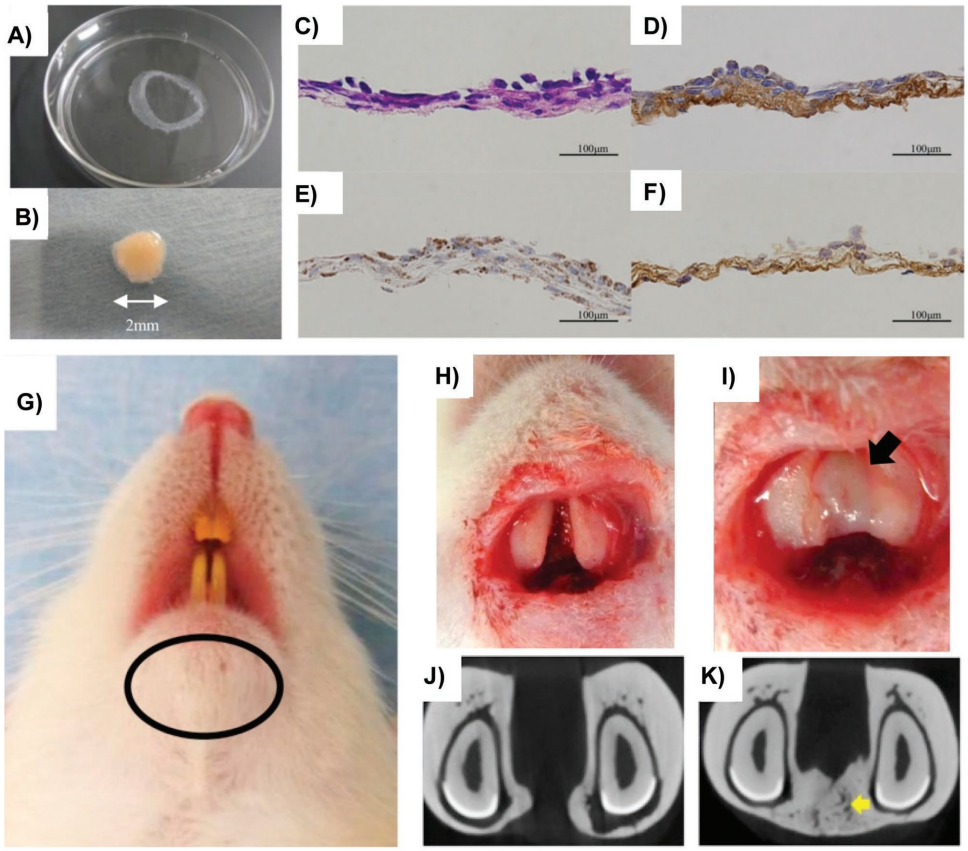

Figure 6. Osteogenic tissue mass derived from cell sheets enhances in vivo bone formation. A) Osteogenically differentiated cell sheets were detached from standard culture-treated plates and B) compacted into a 2 mm diameter mass. C) Histological appearance after hematoxylin and eosin (H&E) staining of the cell sheet before grafting. D) Positive immunohistochemical staining for type I collagen, E) osteopontin, and F) osteocalcin was observed in osteogenically induced cell sheets after 14 d of culture. Scale bars, 100 μm. G) A 10 mm circular incision was made in the skin over the inferior margin of the mandible (black circle) in healthy 15 week old syngeneic rats. H) The fibrous tissue occupying the mandibular symphysis was removed by curettage to create space for the graft. I) Cell sheet-derived masses were implanted into the mandibular symphysis (black arrow) without additional fixation. J,K) Intense bone formation is observed on micro-CT images when osteogenically differentiated cell sheets are packaged into the defect for four weeks compared to the nontreated group. Cracks and fissures were observed in the newly formed bone (yellow arrow). Adapted with permission.[113] Copyright 2016, Elsevier.

to the control group (without implantation), indicating OMCSs could have the potential to facilitate mandibular repair.[113]

As can be seen from these examples, while the osteogenic outcomes of cell sheet technologies can vary slightly between the approaches taken by different research groups, the results are promising for these cell-based reconstruction strategies featuring a natural matrix content, which can be used alone or in conjunction with exogenous biomaterials. In particular, the use of MSCs that can be induced in vitro toward osteogenic lineages has significant potential in the field of bone regeneration, as demonstrated by in vitro and preclinical investigations for maxillofacial and craniofacial repair.

7.2. Importance of Vascularization for Bone Healing

One of the challenges associated with bone tissue engineering is to produce substitutes that replicate the thickness, the volume, and the shape of the native bone defect.[124] Nutrient and oxygen supplies are also critical issues for the engineering of thick tissues, including bone substitutes.[125] Different in vitro techniques have been developed to overcome this obstacle.[126] For example, scaffold functionalization using pro-angiogenic factors, such as VEGF, has been performed in order to promote neovascularization via the release of these growth factors.[127,128] The addition of progenitors or mature ECs is another approach taking advantage of the ability of these cells to spontaneously assemble into capillary-like structures mimicking the microvascular system (prevascularization). The extent of in vitro capillary development is highly dependent on culture parameters such as cell density, cell alignment, the presence of endothelial growth factors, and the type of ECM.[129–131] This newly formed microvasculature can then spontaneously connect with the host's blood system within the first 10 d after implantation.[121,132–134] This process, called inosculation, ensures a faster functional vascularization of the constructs than relying only on neoangiogenesis from the host graft bed, thus leading to improved graft material survival.[135]

Co-culture techniques involving ECs are beneficial since cell–cell interactions between ECs and osteoblast cells play a critical role in the bone healing process. Indeed, Grellier et al.

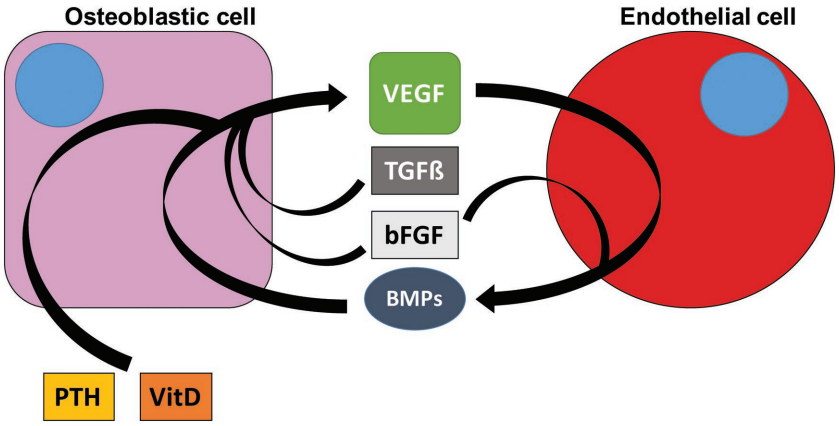

Figure 7. Paracrine cell–cell communication between osteoblasts and endothelial cells. Systemic hormone activity via parathormone (PTH) and vitamin D3 (VitD) enhances vascular endothelial growth factor (VEGF) secretion by the osteoblast. This soluble pro-angiogenic factor enhances endothelial cell proliferation and tubulogenesis. VEGF, as well as basic fibroblast growth factor (bFGF), stimulates the secretion of bone morphogenetic proteins (BMPs; such as BMP-2, 4, and 7) by endothelial cells. BMPs, as well as TGF-ß, will in turn promote osteoblastic cell proliferation, differentiation, and VEGF secretion. All these factors activate specific signaling pathways via surface receptors and stimulate the expression of important proangiogenic and osteogenic factors.

demonstrated the importance of paracrine cell–cell communication by the enhancement of osteogenesis as well as endothelial cell tubulogenesis when ECs are co-cultured with osteoblastic cells (**Figure 7**).[136] Some publications suggested that ECs can stimulate the secretion of BMPs, such as BMP-2, 4 and 7, and bFGF by osteoblast cells via the secretion of VEGF-A.[137,138] While bFGF activates the proliferation of ECs,[139] it will also control osteoblastic differentiation of osteoprogenitor cells.[140] Additionally, parathyroid hormone (PTH)-activated vitamin D3 secretion has the potential to enhance VEGF secretion by the osteoblast.[141] This osteoblast-secreted VEGF will, in return, stimulate endothelial cell proliferation, tubulogenesis, as well as BMP secretion.[142] Following a positive loop of regulation, EC-secreted BMPs will promote osteoblastic cell proliferation, osteogenesis, and VEGF secretion.[143,144] Consequently, tissue-engineered substitutes that integrate, in vitro, a co-culture of ECs with osteoblastic cells may be superior candidates for bone tissue engineering, as assessed using various reconstruction strategies.[96,129,145–149]

For example, in the context of cell sheet engineering and EC co-culture, Pirraco et al. developed a model of bone-like cell sheets with a thickness of 50 µm and devoid of exogenous biomaterials using thermo-responsive technology and osteogenically differentiated rat BM-MSCs (**Figure 8**A and Table 3).[114] In their model, human umbilical vein endothelial cells (hUVECs) were seeded onto the developing constructs after 21 d of culture (Figure 8B). Colonies of hUVECs that formed during the first 3 d of co-culture later developed into capillary-like structures after stacking the osseous sheet tissues. Then, prevascularized bone-like sheets, as well as those lacking hUVECs, were subcutaneously implanted on the dorsal flap of nude mice for 7 d. When osteogenically induced cell sheets were co-cultured with hUVECs, the degree of osteogenic differentiation as evaluated by quantitative real-time (RT) polymerase chain reaction (PCR) and the level of calcium as measured by the o-cresolphthalein-complexone method were elevated compared to controls (without hUVECs) (Figure 8C,D). Following immunohistochemical staining of the explanted tissue for human CD31 and examination of the vessels for erythrocyte presence, the authors suggested the existence of perfused blood vessels 7 d post-implantation (Figure 8E,F).[114] This potential to connect with the host's vasculature suggests better graft survival. The positive impact of ECs incorporated into osseous cell sheet tissues to promote in vivo ectopic bone formation has also been seen in different models[150,151] and warrants further investigation.

Chen et al. also studied the impact of the co-culture of human ECs and self-assembled human cell sheets produced without reliance on thermo-responsive culture dishes.[115] After deriving osteogenically differentiated cell sheets (ODCSs) from human BM-MSCs cultured for 14 d, varying densities of hUVECs were seeded onto ODCSs to initiate vascular network development (**Figure 9**A). Analyses of the prevascularized ODCSs showed better alignment and migration of hUVECs compared to undifferentiated cell sheets (UDCS) co-cultured with hUVECs (Figure 9B–G). Moreover, their results suggest that a density of 50 000 cells cm^{-2} is an optimal seeding concentration of hUVECs to promote network formation in vitro. These encouraging results indicated the potential to construct self-assembled human bone-like tissue co-cultured with ECs for bone repair without using thermo-responsive surfaces.

In summary, the use of several sources of MSCs from different species has been highlighted with a common osteogenic potential and ability to produce cell-derived sheets that can be co-cultured with ECs (Table 3). Many in vitro and in vivo studies have now provided essential information to support the potential of cell sheet technologies to promote the repair of bone defects.[110–115] The future will inform us on how autogenous bone tissues devoid of exogenous scaffolds using human MSCs could perform when used in a clinical setting.

8. Perspectives on the Clinical Use of Bone Substitutes

Over the past decades, bone tissue engineering has gone through a constant evolution with significant transitions. Due to frequent need for bone substitutes in maxillofacial surgery, the demand for academic researchers and private companies to develop innovative bone-like materials has greatly increased. There are several commercially available bone substitutes, each with their own advantages and limitations regarding their biocompatibility, immunogenicity, osteogenicity, osteoconductivity, osteoinduction, and osseointegration characteristics, as already discussed in this review.

Human ASCs and other stem cell sources not only provide a suitable option for the development of bone-like tissues but can

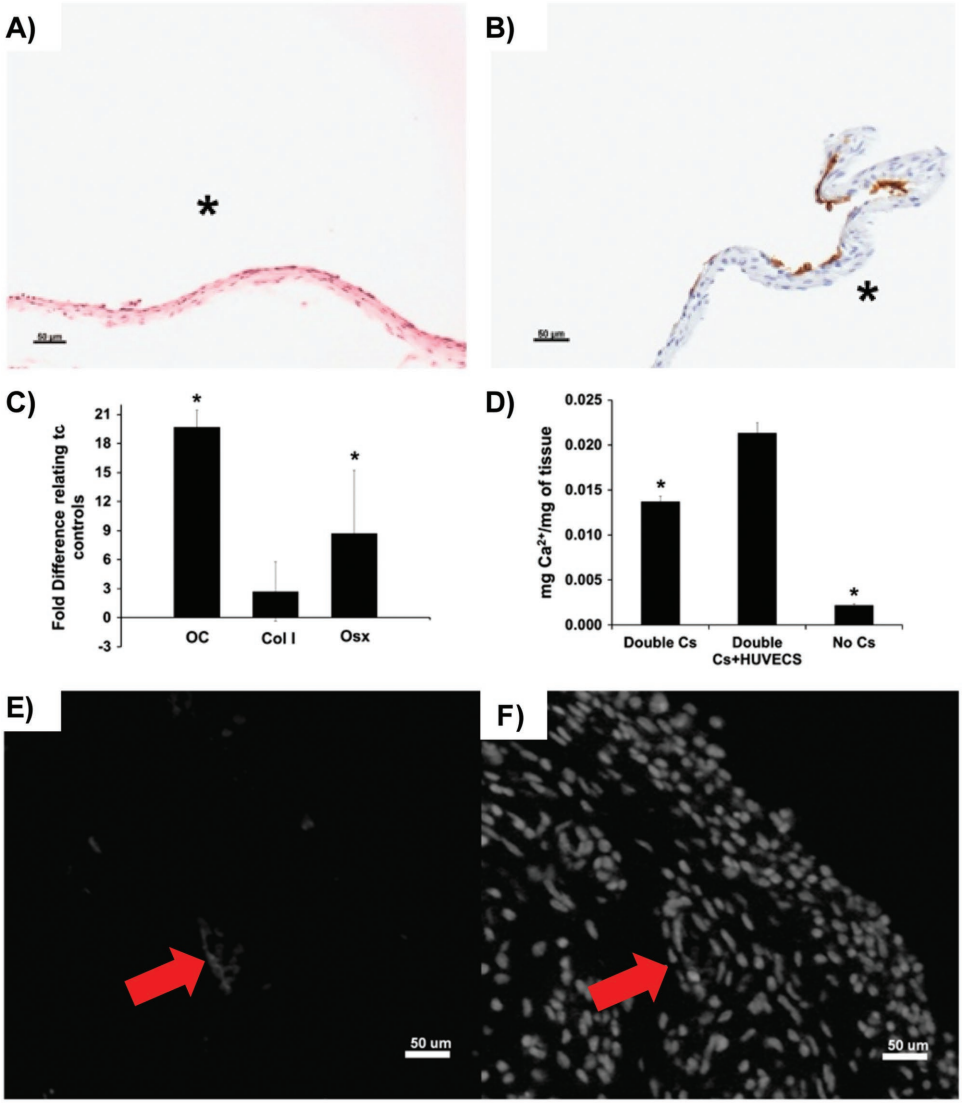

Figure 8. In vitro EC co-culture enhances osteogenic differentiation. A) Osteogenic cell sheets (Cs) obtained with thermo-responsive technology were stained with H&E and B) immunostained for human CD31. Scale bars, 50 µm. Asterisks (*) indicate basal side of the cell sheets. C) Quantitative RT-PCR analysis of osteocalcin (OC: Bglap) and osterix (Osx: Sp7) mouse genes revealed elevated osteogenic potential in the co-cultured cell sheet constructs compared to control (*$P < 0.05$). D) Calcium content was quantified using the o-cresolphthalein-complexone method and values were normalized by tissue mass. The greatest levels of calcium were found in co-cultured tissues compared to control (*$P < 0.05$). E,F) Tissues grafted for 7 d were immunopositive for human CD31 (red arrows). F) Implants were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Scale bars, 50 µm. Modified and reproduced with permission.[114] Copyright 2014, Nature Publishing Group.

also be used in conjunction with endothelial cells to create in vitro prevascularized 3D constructs.[96,147] Such constructs, with adequate distribution of nutrients and oxygen throughout the developing tissue, have the potential to support the development of more voluminous tissues with improved host integration and long-term survival.[126] Future studies designed to evaluate the clinical applicability of prevascularized cell-based tissues are warranted given their advantages in repair settings, such as enhanced survival and rapid integration. This will require the evaluation of parameters such as important regulators of in vitro tubulogenesis and the interconnectivity of the capillary network of the developing tissue in relation to in vitro mineralization.

When specifically considering the recent advances in bone tissue engineering using cell sheet technologies, these substitutes are currently limited to small-scale tissue development. While this may restrict their immediate use in large bone defects or sites presenting significant impact forces, initial studies suggest they have the potential for multiple future clinical applications in maxillofacial reconstruction. These applications include re-establishing adequate bone volume at tooth extraction sites or other sites of small volume loss, or providing structural support for subsequent implant procedures. The natural cell and matrix composition of these constructs can provide for a rapid integration into host tissue via the action of local osteoblasts while reducing immunological reactions. Moreover, in small

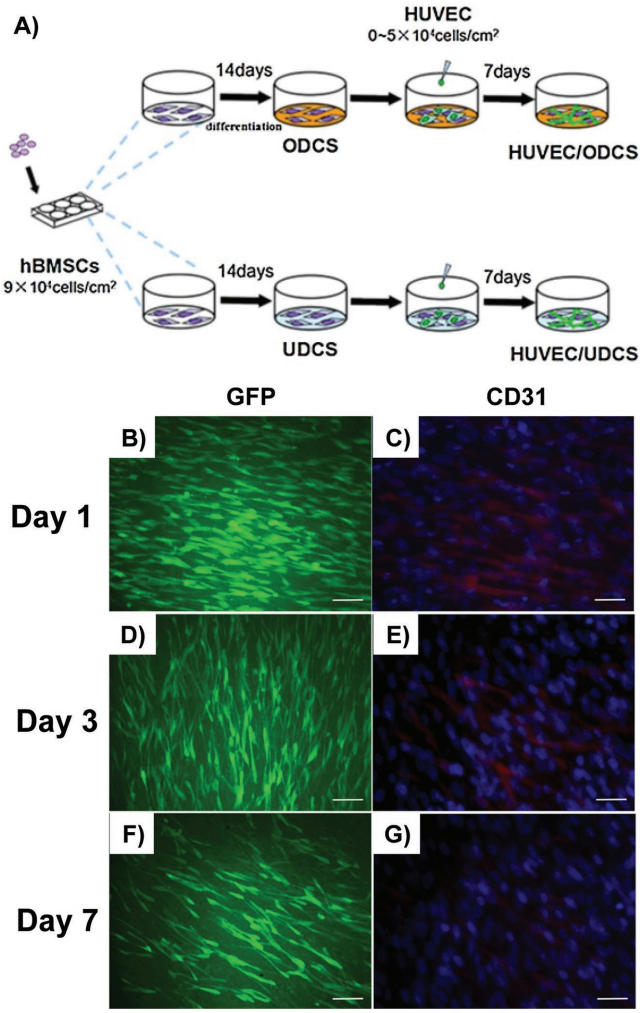

Figure 9. The co-culture of osteogenically differentiated human cell sheet and endothelial cells promote in vitro EC tubule-like reorganization. A) Schematic of the cell sheet engineering method used to produce osteogenically differentiated cell sheets (ODCS) and undifferentiated cell sheets (UDCS) prevascularized with hUVECs. B–G) Immunofluorescent imaging of green fluorescent protein (GFP)-labeled hUVECs and CD31 showing endothelial cell alignment when hUVECs were seeded into hBM-MSCs cell sheet for 7 d. Fluorescent images show that hUVECs aligned randomly (green: GFP; red: human CD31, Alexa Fluor 594 antibody; blue: DAPI). Scale bars for (B), (D), and (F); 50 µm; for (C), (E), and (G): 20 µm. Reproduced with permission.[115] Copyright 2016, Elsevier.

volume defects, it is likely that these constructs would not be plagued by complications associated with internal fixation such as infection, implant migration, and loosening.

Given the natural structure and organization of tissues developed via the cell sheet approach, essential cell–cell and cell-ECM communication, as well as functional characteristics, can be better preserved. However, the studies described different models of cell sheets with various thicknesses and culture times. Relevant parameters such as the length of culture time, quickness of use, tunability, or mechanical properties will have to be thoroughly considered for future clinical translations.

9. Conclusion

Bone reconstruction involves a complex process of healing. Historically, human BM-MSCs were the first stem cell type utilized for bone tissue engineering; however, further research has highlighted the potential of other unique stem cell sources for osseous tissue development. Most notably, human ASCs are suitable candidates for bone repair considering their accessibility, their osteoblastic-lineage potential, and their pro-angiogenic secretome. In summary, the use of cell sheet technologies for the development of fully autologous bone-like substitutes that can readily be vascularized is promising for maxillofacial reconstructive surgery. As investigations such as those cited in this review demonstrate, the potential utility of natural scaffolding materials is gaining considerable attention. These and future studies will be important sources of information for researchers interested in cell-based methods that incorporate natural, biomimetic ECM as a scaffold for developing osseous tissue. Finally, these strategies are an interesting alternative, while the value of their ultimate application will rely on their ability to replicate the native properties of healthy bone tissue and withstand the rigors of commercial bone substitute evaluation in clinical trials.

Acknowledgements

The authors would like to acknowledge the contributions of the LOEX research center, the Quebec network for cell and tissue therapies – ThéCell (a thematic network funded by the FRQS), as well as the *Soeur Mallet* student scholarship to F. Kawecki.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

bone substitutes, cell sheet, prevascularization, stem cells, tissue engineering

Received: August 1, 2017
Revised: October 2, 2017
Published online: December 27, 2017

[1] H. Shegarfi, O. Reikeras, *J. Orthop. Surg.* **2009**, *17*, 206.
[2] A. Van Heest, M. Swiontkowski, *Lancet* **1999**, *353*, SI28.
[3] B. T. Smith, J. Shum, M. Wong, A. G. Mikos, S. Young, *Adv. Exp. Med. Biol.* **2015**, *881*, 57.
[4] S. N. Khan, E. Tomin, J. M. Lane, *Orthop. Clin. North Am.* **2000**, *31*, 389.
[5] N. D. Futran, *Facial Plast. Surg. Clin. North Am.* **2009**, *17*, 239.
[6] D. A. Pedersen, G. L. Wehby, J. C. Murray, K. Christensen, *PLoS One* **2016**, *11*, e0156261.
[7] V. Rana, S. Shafi, A. Agarwal, *Natl. J. Maxillofac. Surg.* **2010**, *1*, 91.
[8] R. Tse, *Semin. Plast. Surg.* **2012**, *26*, 145.
[9] C. Firat, Y. Geyik, *J. Craniofac. Surg.* **2013**, *24*, 1322.
[10] J. W. Tuckett, A. Lynham, G. A. Lee, M. Perry, U. Harrington, *Surgeon* **2014**, *12*, 106.

[11] C. C. Black, R. R. Addante, C. A. Mohila, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2010**, *110*, 585.
[12] B. Manjunatha, G. Kumar, *J. Oral Maxillofac. Pathol.* **2013**, *17*, 71.
[13] M. Rotellini, G. Maggiore, M. Trovati, M. S. Saraceno, A. Franchi, *J. Oral Maxillofac. Res.* **2016**, *7*, e5.
[14] Y. Azuma, K. Kaji, R. Katogi, S. Takeshita, A. Kudo, *J. Biol. Chem.* **2000**, *275*, 4858.
[15] B. Zhao, S. N. Grimes, S. Li, X. Hu, L. B. Ivashkiv, *J. Exp. Med.* **2012**, *209*, 319.
[16] U. H. Lerner, *J. Dent. Res.* **2006**, *85*, 584.
[17] M. Taberner-Vallverdú, M. Nazir, M. Á. Sánchez-Garcés, C. Gay-Escoda, *Med. Oral Patol. Oral Cir. Bucal* **2015**, *20*, 633.
[18] C. L. Cardoso, M. T. V. Rodrigues, O. F. Júnior, G. P. Garlet, P. S. P. de Carvalho, *J. Oral Maxillofac. Surg.* **2010**, *68*, 1922.
[19] C. Myeroff, M. Archdeacon, *J. Bone Jt. Surgery-American* **2011**, *93*, 2227.
[20] J. R. Jinkins, *Atlas of Neuroradiologic Embryology, Anatomy, and Variants*, Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, USA **2000**.
[21] B. H. Campbell, *A Textbook of Head and Neck Anatomy*, Wiley Subscription Services, Inc., A Wiley Company, Hoboken, New-Jersey, USA **1990**.
[22] T. W. Sadler, J. Langman, *Langman's Medical Embryology*, Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, USA **2013**.
[23] J. D. Currey, *Bones: Struct. Mech.*, Princeton University Press **2002**.
[24] C. Schmidt, A. A. Ignatius, L. E. Claes, *J. Biomed. Mater. Res.* **2001**, *54*, 209.
[25] S. G. Pneumaticos, G. K. Triantafyllopoulos, E. K. Basdra, A. G. Papavassiliou, *J. Cell. Mol. Med.* **2010**, *14*, 2561.
[26] S. T. Moore, J. M. Katz, R. M. Zhukauskas, R. M. Hernandez, C. S. Lewis, P. R. Supronowicz, E. Gill, S. M. Grover, N. S. Long, R. R. Cobb, *J. Biomater. Appl.* **2011**, *26*, 151.
[27] J. Wilson-Hench, *Prog. Biomed. Eng.* **1987**, *4*, 29.
[28] E. García-Gareta, M. J. Coathup, G. W. Blunn, *Bone* **2015**, *81*, 112.
[29] T. Albrektsson, *Color Atlas and Text of Dental Maxillofacial Implantology*, Mosby, St Louis, Missouri, USA **1995**, p. 9.
[30] R. Z. LeGeros, R. G. Craig, *J. Bone Miner. Res.* **1993**, *8* (Suppl. 2), S583.
[31] S. Sittitavornwong, R. Gutta, *Oral Maxillofac. Surg. Clin. North Am.* **2010**, *22*, 317.
[32] L. B. Moura, P. H. de A. Carvalho, C. B. Xavier, L. K. Post, M. A. Torriani, M. Santagata, O. L. Chagas Júnior, *Int. J. Oral Maxillofac. Surg.* **2016**, *45*, 1388.
[33] J. D. Conway, *Orthop. Clin. North Am.* **2010**, *41*, 75.
[34] D. A. Hidalgo, A. Rekow, *Plast. Reconstr. Surg.* **1995**, *96*, 586.
[35] N. F. Jones, S. Monstrey, B. A. Gambier, *Plast. Reconstr. Surg.* **1996**, *97*, 707.
[36] N. P. Boyne, A. L. Lambrianides, C. Pollard, *ANZ J. Surg.* **2002**, *72*, 156.
[37] E. M. Younger, M. W. Chapman, *J. Orthop. Trauma* **1989**, *3*, 192.
[38] B. Al-Nawas, E. Schiegnitz, *Eur. J. Oral Implantol.* **2014**, *7* (Suppl. 2), S219.
[39] R. J. Lomas, H. L. Gillan, J. B. Matthews, E. Ingham, J. N. Kearney, *Biomaterials* **2001**, *22*, 913.
[40] D. P. Callan, S. L. Salkeld, N. Scarborough, *Implant Dent.* **2000**, *9*, 36.
[41] J. Wiltfang, O. Zernial, E. Behrens, A. Schlegel, P. H. Warnke, S. T. Becker, *Clin. Implant Dent. Relat. Res.* **2012**, *14*, 421.
[42] Y.-K. Kim, P.-Y. Yun, H.-J. Lee, J.-Y. Ahn, S.-G. Kim, *Implant Dent.* **2011**, *20*, 267.
[43] J. J. Kuttenberger, N. Hardt, *J. Craniomaxillofac. Surg.* **2001**, *29*, 75.
[44] O. Adamopoulos, T. Papadopoulos, *J. Mater. Sci. Mater. Med.* **2007**, *18*, 1587.
[45] I. Niechajev, *Aesthetic Plast. Surg.* **2012**, *36*, 917.
[46] E. Gómez, M. Martín, J. Arias, F. Carceller, *J. Oral Maxillofac. Surg.* **2005**, *63*, 8.
[47] T. Nakajima, Y. Yoshimura, Y. Nakanishi, T. Kanno, H. Sano, Y. Kamei, *J. Craniomaxillofac. Surg.* **1995**, *23*, 64.
[48] L. F. Rodella, G. Favero, M. Labanca, *Int. J. Biomed. Sci.* **2011**, *7*, 81.
[49] C. H. F. Hämmerle, R. E. Jung, D. Yaman, N. P. Lang, *Clin. Oral Implants Res.* **2007**, *19*, 19.
[50] I. A. Urban, S. A. Jovanovic, J. L. Lozada, *Int. J. Oral Maxillofac. Implants* **2009**, *24*, 502.
[51] K. A. Selvig, R. E. Nilveus, L. Fitzmorris, B. Kersten, S. S. Khorsandi, *J. Periodontol.* **1990**, *61*, 515.
[52] S. Verardi, M. Simion, *Pract. Proced. Aesthet. Dent.* **2007**, *19*, 111.
[53] R. L. Van Swol, R. Ellinger, J. Pfeifer, N. E. Barton, N. Blumenthal, *J. Periodontol.* **1993**, *64*, 622.
[54] N. Miller, J. Penaud, B. Foliguet, H. Membre, P. Ambrosini, M. Plombas, *J. Clin. Periodontol.* **1996**, *23*, 1051.
[55] A. J. Asikainen, M. Pelto, J. Noponen, M. Kellomäki, H. Pihlajamäki, C. Lindqvist, R. Suuronen, *J. Mater. Sci. Mater. Med.* **2008**, *19*, 53.
[56] G. Fabbri, M. Brennan, M. Manfredi, G. Ban, *Int. J. Periodontics Restorative Dent.* **2009**, *29*, 543.
[57] S. Boruah, D. L. Subit, G. R. Paskoff, B. S. Shender, J. R. Crandall, R. S. Salzar, *J. Mech. Behav. Biomed. Mater.* **2017**, *65*, 688.
[58] Y. Hu, Y. Shanjani, E. Toyserkani, M. Grynpas, R. Wang, R. Pilliar, *J. Biomed. Mater. Res., Part B.: Appl. Biomater.* **2014**, *102*, 274.
[59] M. Mravic, B. Péault, A. W. James, *Biomed. Res. Int.* **2014**, *2014*, 5.
[60] K. Anselme, *Biomaterials* **2000**, *21*, 667.
[61] K. J. Burg, S. Porter, J. F. Kellam, *Biomaterials* **2000**, *21*, 2347.
[62] P. Habibovic, K. de Groot, *J. Tissue Eng. Regen. Med.* **2007**, *1*, 25.
[63] R. Spin-Neto, A. Stavropoulos, F. L. Coletti, R. S. Faeda, L. A. V. D. Pereira, E. Marcantonio, *Clin. Oral Implants Res.* **2014**, *25*, 226.
[64] V. Orti, P. Bousquet, P. Tramini, C. Gaitan, B. Mertens, F. Cuisinier, *J. Periodontal Implant Sci.* **2016**, *46*, 291.
[65] C. J. Snyder, J. A. Bleedorn, J. W. Soukup, *J. Vet. Dent.* **2016**, *33*, 160.
[66] V. Olivier, N. Faucheux, P. Hardouin, *Drug Discovery Today* **2004**, *9*, 803.
[67] U. Edlund, S. Dånmark, A.-C. Albertsson, *Biomacromolecules* **2008**, *9*, 901.
[68] M. C. Teven, S. Fisher, G. A. Ameer, T.-C. He, R. R. Reid, *Ann. Maxillofac. Surg.* **2015**, *5*, 4.
[69] V. Tollemar, Z. J. Collier, M. K. Mohammed, M. J. Lee, G. A. Ameer, R. R. Reid, *Genes Dis.* **2016**, *3*, 56.
[70] R. Langer, J. Vacanti, *Science* **1993**, *260*, 920.
[71] C. R. M. Black, V. Goriainov, D. Gibbs, J. Kanczler, R. S. Tare, R. O. C. Oreffo, *Curr. Mol. Biol. Rep.* **2015**, *1*, 132.
[72] J. R. Porter, T. T. Ruckh, K. C. Popat, *Biotechnol. Prog.* **2009**, *25*, 1539.
[73] A. R. Amini, C. T. Laurencin, S. P. Nukavarapu, *Crit. Rev. Biomed. Eng.* **2012**, *40*, 363.
[74] A. J. Friedenstein, R. K. Chailakhjan, K. S. Lalykina, *Cell Prolif.* **1970**, *3*, 393.
[75] A. J. Friedenstein, *Haematol. Blood Transfus.* **1980**, *25*, 19.
[76] C.-S. Lin, Z.-C. Xin, J. Dai, T. F. Lue, *Histol. Histopathol.* **2013**, *28*, 1109.
[77] M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, R. Deans, A. Keating, D. Prockop, E. Horwitz, *Cytotherapy* **2006**, *8*, 315.
[78] P. Bourin, B. A. Bunnell, L. Casteilla, M. Dominici, A. J. Katz, K. L. March, H. Redl, J. P. Rubin, K. Yoshimura, J. M. Gimble, *Cytotherapy* **2013**, *15*, 641.

[79] A. I. Caplan, *J. Orthop. Res.* **1991**, *9*, 641.
[80] H. Ohgushi, M. Okumura, S. Tamai, E. C. Shors, A. I. Caplan, *J. Biomed. Mater. Res.* **1990**, *24*, 1563.
[81] J. Goshima, V. M. Goldberg, A. I. Caplan, *Clin. Orthop. Relat. Res.* **1991**, *269*, 274.
[82] S. E. Haynesworth, J. Goshima, V. M. Goldberg, A. I. Caplan, *Bone* **1992**, *13*, 81.
[83] S. E. Haynesworth, M. A. Baber, A. I. Caplan, *J. Cell. Physiol.* **1996**, *166*, 585.
[84] A. I. Caplan, S. P. Bruder, *Trends Mol. Med.* **2001**, *7*, 259.
[85] J. Doorn, J. van de Peppel, J. P. T. M. van Leeuwen, N. Groen, C. A. van Blitterswijk, J. de Boer, *Biomaterials* **2011**, *32*, 6089.
[86] L. Chen, E. E. Tredget, P. Y. G. Wu, Y. Wu, *PLoS One* **2008**, *3*, e1886.
[87] M. B. Murphy, K. Moncivais, A. I. Caplan, *Exp. Mol. Med.* **2013**, *45*, e54.
[88] G. Siegel, R. Schäfer, F. Dazzi, *Transplantation* **2009**, *87*, S45.
[89] N. Li, H. Sarojini, J. An, E. Wang, *J. Neurochem.* **2010**, *112*, 1527.
[90] A. Cselenyák, E. Pankotai, E. M. Horváth, L. Kiss, Z. Lacza, *BMC Cell Biol.* **2010**, *11*, 29.
[91] S.-Y. Kim, J.-H. Lee, H. J. Kim, M. K. Park, J. W. Huh, J. Y. Ro, Y.-M. Oh, S.-D. Lee, Y.-S. Lee, *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2012**, *302*, L891.
[92] C. S. Mitsiades, N. Mitsiades, V. Poulaki, R. Schlossman, M. Akiyama, D. Chauhan, T. Hideshima, S. P. Treon, N. C. Munshi, P. G. Richardson, K. C. Anderson, *Oncogene* **2002**, *21*, 5673.
[93] M. Mirotsou, Z. Zhang, A. Deb, L. Zhang, M. Gnecchi, N. Noiseux, H. Mu, A. Pachori, V. Dzau, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 1643.
[94] P. A. Zuk, M. Zhu, H. Mizuno, J. Huang, J. W. Futrell, A. J. Katz, P. Benhaim, H. P. Lorenz, M. H. Hedrick, *Tissue Eng.* **2001**, *7*, 211.
[95] B. M. Strem, K. C. Hicok, M. Zhu, I. Wulur, Z. Alfonso, R. E. Schreiber, J. K. Fraser, M. H. Hedrick, *Keio J. Med.* **2005**, *54*, 132.
[96] M. Barba, G. Di Taranto, W. Lattanzi, *Expert Opin. Biol. Ther.* **2017**, *17*, 677.
[97] S. K. Kapur, A. J. Katz, *Biochimie* **2013**, *95*, 2222.
[98] J. Rehman, D. Traktuev, J. Li, S. Merfeld-Clauss, C. J. Temm-Grove, J. E. Bovenkerk, C. L. Pell, B. H. Johnstone, R. V. Considine, K. L. March, *Circulation* **2004**, *109*, 1292.
[99] D. L. Hutton, R. Kondragunta, E. M. Moore, B. P. Hung, X. Jia, W. L. Grayson, *PLoS One* **2014**, *9*, e107199.
[100] J. Y. Oh, M. K. Kim, M. S. Shin, H. J. Lee, J. H. Ko, W. R. Wee, J. H. Lee, *Stem Cells* **2008**, *26*, 1047.
[101] K.-I. Kim, S. Park, G.-I. Im, *Biomaterials* **2014**, *35*, 4792.
[102] A. J. Salgado, R. L. Gonçalves, N. J. Sousa, J. M. Gimble, *Curr. Stem Cell Res. Ther.* **2010**, *5*, 103.
[103] T. Kushida, M. Inaba, K. Ikebukuro, N. Ichioka, T. Esumi, H. Oyaizu, T. Yoshimura, T. Nagahama, K. Nakamura, T. Ito, H. Hisha, K. Sugiura, R. Yasumizu, H. Iida, S. Ikehara, *Stem Cells* **2002**, *20*, 155.
[104] M. T. Chung, A. S. Zimmermann, K. J. Paik, S. D. Morrison, J. S. Hyun, D. D. Lo, A. McArdle, D. T. Montoro, G. G. Walmsley, K. Senarath-Yapa, M. Sorkin, R. Rennert, H.-H. Chen, A. S. Chung, D. Vistnes, G. C. Gurtner, M. T. Longaker, D. C. Wan, *Stem Cells Transl. Med.* **2013**, *2*, 808.
[105] M. F. Pittenger, A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, M. A. Moorman, D. W. Simonetti, S. Craig, D. R. Marshak, *Science* **1999**, *284*, 143.
[106] S. Lendeckel, A. Jödicke, P. Christophis, K. Heidinger, J. Wolff, J. K. Fraser, M. H. Hedrick, L. Berthold, H.-P. Howaldt, *J. Craniomaxillofac. Surg.* **2004**, *32*, 370.
[107] G. K. Sándor, J. Numminen, J. Wolff, T. Thesleff, A. Miettinen, V. J. Tuovinen, B. Mannerström, M. Patrikoski, R. Seppänen, S. Miettinen, M. Rautiainen, J. Öhman, *Stem Cells Transl. Med.* **2014**, *3*, 530.
[108] T. Thesleff, K. Lehtimäki, T. Niskakangas, S. Huovinen, B. Mannerström, S. Miettinen, R. Seppänen-Kaijansinkko, J. Öhman, *Stem Cells Transl. Med.* **2017**, *6*, 1576.
[109] T. Owaki, T. Shimizu, M. Yamato, T. Okano, *Biotechnol. J.* **2014**, *9*, 904.
[110] N. Kaibuchi, T. Iwata, M. Yamato, T. Okano, T. Ando, *Acta Biomater.* **2016**, *42*, 400.
[111] Q. Xie, Z. Wang, Y. Huang, X. Bi, H. Zhou, M. Lin, Z. Yu, Y. Wang, N. Ni, J. Sun, S. Wu, Z. You, C. Guo, H. Sun, Y. Wang, P. Gu, X. Fan, *Biomaterials* **2015**, *66*, 67.
[112] T. Galbraith, W. P. Clafshenkel, F. Kawecki, C. Blanckaert, B. Labbé, M. Fortin, F. A. Auger, J. Fradette, *Adv. Healthcare Mater.* **2017**, *6*, 1600889.
[113] Y. Ueyama, T. Yagyuu, M. Maeda, M. Imada, M. Akahane, K. Kawate, Y. Tanaka, T. Kirita, *Arch. Oral Biol.* **2016**, *72*, 138.
[114] R. P. Pirraco, T. Iwata, T. Yoshida, A. P. Marques, M. Yamato, R. L. Reis, T. Okano, *Lab. Invest.* **2014**, *94*, 663.
[115] J. Chen, D. Zhang, Q. Li, D. Yang, Z. Fan, D. Ma, L. Ren, *Tissue Cell* **2016**, *48*, 442.
[116] N. Yamada, T. Okano, H. Sakai, F. Karikusa, Y. Sawasaki, Y. Sakurai, *Die Makromol. Chem., Rapid Commun.* **1990**, *11*, 571.
[117] J. Kobayashi, T. Okano, in *Biomaterials Surface Science*, (Eds: A. Tauber, J. F. Mano, J. C. Rodríguez-Cabello), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany **2013**, pp. 491–510.
[118] J. M. Davidson, P. A. LuValle, O. Zoia, D. Quaglino, M. Giro, *J. Biol. Chem.* **1997**, *272*, 345.
[119] J. Geesin, *Exp. Cell Res.* **1993**, *206*, 283.
[120] D. Chan, S. R. Lamande, W. G. Cole, J. F. Bateman, *Biochem. J.* **1990**, *269*, 175.
[121] P.-L. Tremblay, V. Hudon, F. Berthod, L. Germain, F. A. Auger, *Am. J. Transplant* **2005**, *5*, 1002.
[122] K. Aubin, C. Vincent, M. Proulx, D. Mayrand, J. Fradette, *Acta Biomater.* **2015**, *11*, 333.
[123] M. Akahane, A. Nakamura, H. Ohgushi, H. Shigematsu, Y. Dohi, Y. Takakura, *J. Tissue Eng. Regen. Med.* **2008**, *2*, 196.
[124] R. M. Nerem, A. Sambanis, *Tissue Eng.* **1995**, *1*, 3.
[125] A. J. Salgado, O. P. Coutinho, R. L. Reis, *Macromol. Biosci.* **2004**, *4*, 743.
[126] M. Lovett, K. Lee, A. Edwards, D. L. Kaplan, *Tissue Eng., Part B* **2009**, *15*, 353.
[127] A. H. Zisch, M. P. Lutolf, M. Ehrbar, G. P. Raeber, S. C. Rizzi, N. Davies, H. Schmökel, D. Bezuidenhout, V. Djonov, P. Zilla, J. A. Hubbell, *FASEB J.* **2003**, *17*, 2260.
[128] S. M. Bauer, R. J. Bauer, Z.-J. Liu, H. Chen, L. Goldstein, O. C. Velazquez, *J. Vasc. Surg.* **2005**, *41*, 699.
[129] M. W. Laschke, M. D. Menger, *Biotechnol. Adv.* **2016**, *34*, 112.
[130] J. Rouwkema, P. E. Westerweel, J. de Boer, M. C. Verhaar, C. A. van Blitterswijk, *Tissue Eng., Part A* **2009**, *15*, 2015.
[131] R. E. Ayata, S. Chabaud, M. Auger, R. Pouliot, *Biomed Res. Int.* **2015**, *2015*, 9.
[132] W. Chen, W. Thein-Han, M. D. Weir, Q. Chen, H. H. K. Xu, *Dent. Mater.* **2014**, *30*, 535.
[133] J. E. Nör, M. C. Peters, J. B. Christensen, M. M. Sutorik, S. Linn, M. K. Khan, C. L. Addison, D. J. Mooney, P. J. Polverini, *Lab. Invest.* **2001**, *81*, 453.
[134] M. C. Peters, P. J. Polverini, D. J. Mooney, *J. Biomed. Mater. Res.* **2002**, *60*, 668.
[135] J. Rouwkema, N. C. Rivron, C. A. van Blitterswijk, *Trends Biotechnol.* **2008**, *26*, 434.
[136] M. Grellier, L. Bordenave, J. Amédée, *Trends Biotechnol.* **2009**, *27*, 562.
[137] H. Gerber, *Trends Cardiovasc. Med.* **2000**, *10*, 223.
[138] U. Mayr-Wohlfart, J. Waltenberger, H. Hausser, S. Kessler, K.-P. Günther, C. Dehio, W. Puhl, R. E. Brenner, *Bone* **2002**, *30*, 472.

[139] J. H. W. Distler, A. Hirth, M. Kurowska-Stolarska, R. E. Gay, S. Gay, O. Distler, *Q. J. Nucl. Med.* **2003**, *47*, 149.
[140] P. J. Marie, *Gene* **2003**, *316*, 23.
[141] D. S. Wang, M. Miura, H. Demura, K. Sato, *Endocrinology* **1997**, *138*, 2953.
[142] P. J. Bouletreau, S. M. Warren, J. A. Spector, Z. M. Peled, R. P. Gerrets, J. A. Greenwald, M. T. Longaker, *Plast. Reconstr. Surg.* **2002**, *109*, 2384.
[143] G. Schmidmaier, P. Schwabe, B. Wildemann, N. P. Haas, *Injury* **2007**, *38* (Suppl. 4), S35.
[144] A. C. Carreira, F. H. Lojudice, E. Halcsik, R. D. Navarro, M. C. Sogayar, J. M. Granjeiro, *J. Dent. Res.* **2014**, *93*, 335.
[145] C. Correia, W. Grayson, R. Eton, J. M. Gimble, R. A. Sousa, R. L. Reis, G. Vunjak-Novakovic, *J. Tissue Eng. Regen. Med.* **2014**, *8*, 629.
[146] C. Correia, W. L. Grayson, M. Park, D. Hutton, B. Zhou, X. E. Guo, L. Niklason, R. A. Sousa, R. L. Reis, G. Vunjak-Novakovic, *PLoS One* **2011**, *6*, e28352.
[147] Y. Liu, J. K. Y. Chan, S.-H. Teoh, *J. Tissue Eng. Regen. Med.* **2015**, *9*, 85.
[148] B. M. Roux, M.-H. Cheng, E. M. Brey, *J. Cell. Mol. Med.* **2015**, *19*, 903.
[149] L. H. Nguyen, N. Annabi, M. Nikkhah, H. Bae, L. Binan, S. Park, Y. Kang, Y. Yang, A. Khademhosseini, *Tissue Eng., Part B* **2012**, *18*, 363.
[150] H. Liu, W. Zhou, N. Ren, Z. Feng, Y. Dong, S. Bai, Y. Jiao, Z. Wang, Y. Zhao, *Sci. Rep.* **2017**, *7*, 3038.
[151] R. M. Eman, H. A. W. Meijer, F. C. Öner, W. J. A. Dhert, J. Alblas, *Tissue Eng., Part A* **2016**, *22*, 253.