Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

BIOMATERIALS AND REGENERATIVE TECHNOLOGIES USED IN BONE REGENERATION IN THE CRANEOMAXILLO-FACIAL REGION. Consensus report of group 2 of the 15th European Workshop on Periodontology on Bone Regeneration

Journal:	*Journal of Clinical Periodontology*
Manuscript ID	Draft
Manuscript Type:	Supplement Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Sanz, Mariano; Universidad Complutense de Madrid, Periodoncia
Topic:	Treatment
Main Methodology:	Systematic Review
Keywords:	bone regeneration, biomaterials, barrier membrane, bioactive agents, cell therapies

BIOMATERIALS AND REGENERATIVE TECHNOLOGIES USED IN BONE REGENERATION IN THE CRANEOMAXILLO-FACIAL REGION. Consensus report of group 2 of the 15th European Workshop on Periodontology on Bone Regeneration

Sanz M[1], Dahlin C[2], Apatzidou D[3], Artzi Z[4], Bozic D[5], Calciolari E[6], De Bruyn H[7], Dommisch H[8], Donos N[6], Eickholz P[9], Ellingsen JE[10], Haugen HJ[11], Herrera D[1], Lambert F[12], Layrolle P[13], Montero E[1], Mustafa K[14], Omar O[2]and Schliephake H[15]

1. Department of Dental Clinical Specialties and ETEP Research Group, Faculty of Odontology, University Complutense of Madrid, Plaza Ramon y Cajal, E-28040 Madrid, Spain

2. Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Box 412, SE-405 30 Gothenburg, Sweden

3. Department of Preventive Dentistry, Periodontology and Implant Biology, School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

4. Department of Periodontology and Oral Implantology, School of Dental Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

5. Department of Periodontology, School of Dental Medicine, University of Zagreb, Gunduliceva 5, 10000 Zagreb, Croatia

6. Centre for Immunobiology & Regenerative Medicine & Centre for Oral Clinical Research, Institute of Dentistry, Barts & The London School of Medicine and Dentistry, Queen Mary University of London (QMUL), Turner Street E12AD, London, UK

7. Department Periodontology & Implantology. College of Dental Science, Radboud University Medical Center. Nijmegen, The Netherland

8. Department of Periodontology and Synoptic Dentistry Charité - Universitätsmedizin Berlin Assmannshauser Strasse 4-6, 14197 Berlin, Germany

9. Department of Periodontology, Johann Wolfgang Goethe-University, Frankfurt, Germany

10. Department of Prosthetics an Oral Function, Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo, PO Box 1109 Blindern, 0317 Oslo, Norway
11. Department of Biomaterials, Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo, PO Box 1109 Blindern, 0317 Oslo, Norway
12 Dental Biomaterials Research Unit (d-BRU), University of Liège (ULiège), Belgium. Dpt. of Periodontology and Oral Surgery, University of Liège (ULiège), Belgium
13 Inserm, U791, Laboratory for Osteoarticular and Dental Tissue Engineering, Faculty of Dental Surgery, University of Nantes, 1 Place Alexis Ricordeau, 44042 Nantes cedex 1, France
14. Department of Clinical Dentistry, Center for Clinical Dental Research, University of Bergen, Norway
15. Department of Oral and Maxillofacial Surgery, George-Augusta-University, Gottingen, Germany

Key Words
Bone regeneration, biomaterials, bone replacement graft, osteoconductive, osteoinductive, barrier membrane, guided bone regeneration, bio-absorbable, bioactive agent, cell therapies

Abstract
Background and Aims: to review the regenerative technologies used in bone regeneration: bone grafts, barrier membranes, bioactive factors and cell therapies
Material and Methods: four background review publications served to elaborate this consensus report
Results and Conclusions: Biomaterials used as bone grafts must meet specific requirements: biocompatibility, porosity, osteoconductivity, osteoinductivity, surface properties, biodegradability, mechanical properties, angiogenicity, handling and manufacturing processes. Currently used biomaterials have demonstrated advantages and limitations based on the fulfillment of these requirements. Similarly, membranes for guided bone regeneration (GBR) must fulfill specific properties and potential biological mechanisms to improve their clinical applicability. Pre-clinical and clinical studies have evaluated the added effect of Bone Morphogenetic Proteins (mainly BMP-2) and Autologous Platelet Concentrates (APCs) when used as

bioactive agents to enhance bone regeneration. Three main approaches using cell therapies to enhance bone regeneration have been evaluated: a) “minimally manipulated” whole tissue fractions; b) ex-vivo expanded “uncommitted” stem/progenitor cells, and c) ex-vivo expanded “committed” bone-/periosteum-derived cells. Based on the evidence from clinical trials, transplantation of cells, most commonly whole BMA or BMAC, in combination with biomaterial scaffolds has demonstrated an additional effect in sinus augmentation and horizontal ridge augmentation, and comparable bone regeneration to autogenous bone in alveolar cleft repair.

Introduction

This consensus report aims to describe the regenerative technologies currently used in bone regenerative interventions in the craniomaxillofacial region. The relevant scientific evidence that served to elaborate this report came from four background publications, being three narrative reviews using a systematic search approach and one systematic review, respectively:

1. Bone grafts: which is the ideal biomaterial? Haugen HJ, Lyngstadaas SP, Rossi, F and Perale G
2. Barrier membranes: more than the barrier effect? Omar O, Elgali I, Dahlin C and Thomsen P
3. The use of bioactive factors to enhance bone regeneration. A narrative review Donos N, Dereka X and Calciolari E
4. Cell therapy for orofacial bone regeneration: a systematic review and meta-analysis Shanbhag S, Suliman S, Pandis N, Stavropoulos A, Sanz M and Mustafa K

Biomaterials used as bone replacement grafts in regenerative interventions in the cariomaxillo-facial region

Biomaterials used as bone replacement grafts must meet specific requirements to achieve the goal of developing a new and healthy bone tissue formation:

- *Biocompatibility*. The interaction between the material and the tissues should not adversely affect the surrounding tissues, the intended healing result or the safety of the patient. (Williams, 2017). Ideally, biomaterials should be inherently bioactive in promoting the bone regeneration process (e.g. ion release, surface characteristics, etc.).

- *Porosity*. An adequate pore size, morphology and inter-connectivity is needed to allow for diffusion throughout the whole scaffold of bone cells, nutrients and exchange of waste products. It is important to distinguish between micro-porosity and macro-porosity (Hutmacher, 2000). Micro-porosity is defined as pores ≤ 10 μm o improve cell adhesion, to allow fluids and nutrients flow (permeability) and thus to enhance the bioactivity. Macro-porosity is defined as pores ≥ 100 μm to allow

for angiogenesis and to bone cell ingrowth, thus mimicking the porosity of trabecular bone, which has a mean value 250 μm, although it is highly variable. Inter-connectivity (the connection between pores) is also an important property to allow for permeability, vascularization and bone ingrowth.

- *Osteoconductivity/ Osteoinductivity*. All biomaterials used for bone regeneration should allow for bone growth directly in contact with the biomaterial surface from the surrounding bone (*osteoconduction*), but ideally it should also be able to promote *osteoinduction* (Albrektsson, 2001). An *osteoinductive* biomaterial should first be capable of recruiting mesenchymal-type osteoprogenitor cells. Secondly, it should be capable of transforming an undifferentiated mesenchymal cell into a mature, bone-forming osteoblast. Lastly, it should be capable of inducing in-growth of ectopic bone formation when implanted into extra-skeletal locations. This capacity may be related to its micro-porosity and surface properties.

- *Surface properties*. Surface topography at the nano and micro level as well as surface physico-chemistry are important characteristics for protein adsorption, extracellular matrix deposition, cell adhesion, differentiation, migration and finally bone formation.

- *Biodegradability.* The capacity of the biomaterial to bio-absorb during the tissue healing and remodeling process. The ideal bone graft substitute is expected to be fully replaced by bone, preferably at a predictable absorption rate, without losing tissue volume and without interfering with the healing and regeneration process. In case of biomaterials with a slow bio-absorbability rate, these should assure a process of new bone formation with sufficient volume in contact with the biomaterial.

- *Mechanical properties*. Compressive strength and elasticity should be high enough to absorb the load from the surrounding hard and soft tissues in non-contained defects. Ideally the compressive strength and elasticity of the biomaterial should be at least those of the natural bone at the site of regeneration. These mechanical properties are also influenced by pore morphology and size.

- *Angiogenicity.* The inherent biomaterial properties (porosity, surface, etc.) should promote angiogenesis and the appropriate vascularization of the graft volume.

- *Handling*. The biomaterial should be cohesive and dimensionally stable, easy for chairside use to adapt to the defect. When used in non-contained defects, it should allow for three-dimensional build up. Biomaterials for craniomaxillo-facial bone regeneration are usually available in the form of granules or blocks. Depending on the clinical needs, it is desirable to have a wide variability of sizes and forms, ranging from 0.1 mm-2.0 mm in the particulate form. For certain indications an injectable mode of application would be desired to fill the defect volume through its plasticity.
- *Manufacturing processes*. The biomaterial should be provided with certification or documentation of the appropriate manufacturing and sterilization processes and assure long shelf time and reduced production costs.

Which are the advantages and limitations of the currently used bone grafts for craniomaxillo-facial bone regeneration?

Autologous

Even though autologous bone is not a biomaterial per se, it is considered the gold standard graft material for bone regeneration and it has the following advantages: it contains the patient's own cells, growth factors and bio-molecules needed for osteogenesis, it has the highest degree of biological safety, biocompatibility, matched mechanical properties and scaffolding effect.

In regard to limitations, autologous grafts may need a second surgical site for its harvesting, which increases patient's morbidity, pain or discomfort and other complications related to increased surgical time and invasiveness. It has been reported that the resorption of these bone replacement grafts is higher, and their rate of resorption is not predictable. Depending on the source of the graft (cortical versus cancellous bone) the vascularization may be slowed, mainly in highly cortical bone grafts. It has also limitations in terms of volume availability, mainly when harvesting from intraoral sources and the resulting grafts, mainly in a block form may be difficult to adapt to the anatomy of the defect.

The application of particulate dentin has been recently suggested as another autologous source for minor ridge augmentation or socket site preservation. However, there is no clinical documentation to substantiate its clinical use.

Allogenic

There are different ways of processing allogenic bone replacement grafts (freeze dried, fresh frozen, etc.), which may change their biological properties. These allografts can be produced as particulate or blocks. In principle, the general advantage of this biomaterial is that it provides similar mechanical properties as the autologous bone and it may contain the collagenous matrix and proteins of natural bone, although it lacks viable cells. Similarly, handling properties are comparable to autologous bone, although the reduced surgical time needed for their implantation, in addition to their increased availability are clear advantages when compared with autologous bone.

Its biological safety due to possible disease transmission and potential unwanted immune reactions are clear disadvantages. Furthermore. the sources of donor material are heterogeneous, what may influence their biological activity and similarly, resorption rate are highly variable. Other drawbacks could be possible impairment to achieve vascularization of the grafted site. In the future its availability for clinical use may be reduced in light of the regulatory changes in Europe.

Xenogeneic

This is the biomaterial with the most documentation in the scientific literature for bone grafting in the cranio-maxillo-facial area. Its main advantages are that since it is derived from both natural cancellous and cortical bone its architecture and geometric structure resemble bone, although highly depending on the tissue source and manufacturing process. Its slow bio-absorbability may be a clinical advantage for preserving the augmented bone volume.

In regard to limitations, it lacks biological components thus limiting its biological activity. Similar to allogenic materials, its use implies a potential biological risk of disease transmission (*e.g.* prions and retroviruses) and/or immunogenic host tissue response, although these risks can be diminished through the manufacturing process (de-proteinization). In spite of this inherent risk, however, transmission of

bovine spongiform encephalitis (BSE) has not yet been reported associated with the implantation of this biomaterial.

Mechanical properties (brittleness) may vary depending on the source and manufacturing process. Since these biomaterials are mainly available for use in particulate form, they may have limitations in large defect regeneration interventions.

Synthetic Bio Ceramics

Calcium sulphate, calcium phosphate (CaP), bioactive glass and combinations are the most commonly used bio ceramics available at present**.** Their main advantage is the controlled manufacturing process which may assure biocompatibility, biodegradability and similarity in structure and inorganic composition to natural bone minerals. The most investigated CaP bone graft substitutes are hydroxyapatite (HA), β–tricalcium phosphate (β-TCP) and their combination, also called biphasic calcium phosphate (BCF). Bio ceramics have shown osteoinductive properties through the stimulation of inorganic matrix deposition, osteoblast differentiation, osteoblast growth and bone promotion. By modulating their chemical composition and sintering temperature their bioactivity and degradation time can be controlled to a certain extent.

Their main disadvantage is associated with their limited mechanical properties (load bearing resistance) and unpredictable bio-absorption rates. They are mainly delivered as particulates, which may limit their use in large bone defects.

Glass-based ceramics share the same problems of lower mechanical strength despite excellent material-bone interactions. Similarly, their degradation times can be unpredictable.

To improve their mechanical therapies (brittleness), bio ceramics have been mixed with polymers developing composite materials (Laurencin et al.,2014).

Synthetic Polymers

The most studied synthetic polymers used as biomaterials for bone tissue regeneration are aliphatic polyesters like poly (lactic-acid) (PLA), poly (ε-caprolactone) (PCL), and poly (glycolic-acid) (PGA) and their copolymers and derivatives. They share the advantage that their manufacture is controllable and

tunable in terms of adjusting their physiochemical structure, porosity and hence their biodegradability and shape, size and biomechanical properties can be customized.

Their most important limitation is that they have not demonstrated osteoconductivity and hence, their use as bone replacement grafts requires their combination with bio ceramics as composite materials or they can be functionalized, for example with different coatings. Their process of bio-absorbability usually leads to the release of acid compounds that may interfere with wound healing, although this limitation can be controlled by the manufacturing process as composite materials can be combined with bio ceramics. Furthermore, the bio absorbability of synthetic polymers is highly variable, which may impair their mechanical strength *in vivo*.

Composite bio-materials

Composite biomaterials are biomaterials generated by combining bio ceramics with polymers or xenogeneic biomaterials together with bio ceramics or polymers. Their properties will vary depending on their composition and manufacturing processes.

Other synthetic biomaterials

Granules made of titanium particles were marketed for use in bone regeneration, although their use is no longer available in the market.

Recommendations for Future Research

The future of cranio-maxillo-facial bone regeneration will probably entail the manufacturing of personalized biomaterials from 3-D digital data obtained from patients. Additive manufacturing (e.g. 3-D printing) of different biomaterials (e.g. bio ceramics) will allow rapid production of these customized scaffolds that will perfectly fit the bone defect anatomy. The addition of synthetic polymers in the design of composite biomaterials may mechanically reinforce these 3D constructed biomaterials. Similarly, the addition of cells (bio-printing) may add biological activity to the 3-D printed constructs

Future biomaterials should have optimized surface characteristics, pore size and interconnection. These characteristics will be adjusted to control of their bio-absorbability, promote osteoinduction and ensure ideal mechanical properties.

Biomimetic biomaterials should be developed at ambient temperatures by hydrolysis and precipitation of calcium deficient apatite, what will result in similar composition and crystallinity as natural bone. These biomaterials should be completely replaced by new bone through controlled processes of bio-absorbability and osteoinduction.

There is a need of standardized and validated pre-clinical models, with the use of small animal models for screening and large-animal models for comparing new biomaterials using established standards. In concordance with the ARRIVE guidelines to reduce animal research, there is a need for standardized pre-clinical models, such as *in silico* modeling and *ex-vivo* tissue engineering testing to reduce animal research.

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Membranes for Guided Bone Regeneration used in regenerative interventions in the cariomaxillo-facial region

This report addresses the scientific evidence on the effects of membranes used for guided bone regeneration (GBR), focusing on their properties and potential biological mechanisms, regardless of the clinical applicability.

Ideally, a new developed membrane should pass the cascades of evaluations from *in vitro* to clinical testing until being approved as medical devices according to current ISO standards and specifications. In addition, the new European Medical Device Regulation (EU-MDR) for implantable medical devices requires confirmation of the product claims through prospective clinical studies.

Beside their inherent barrier effect, membranes for guided bone regeneration should have certain properties:

- *Biocompatibility.* The biomaterial shall perform with an appropriate tissue response. Hence, the interaction between the material and the tissues should not adversely affect the surrounding tissues, the intended healing result or the safety of the patient. (Williams, 2017).
- *Biological activity.* There is increasing evidence that membranes not only function due to their occlusive properties, but they actively promote bone regeneration within the osseous defect below the membrane. Specifically, this biological activity

may include recruitment of cells, angiogenesis, bone formation and bone remodeling leading to bone fill of the defect with mature bone. There is experimental evidence that collagen membranes allow inward migration of cells that express and secrete osteogenic and angiogenic factors. It is not yet established whether similar biological processes are shared by membranes of different composition.

- *Porosity / occlusive properties.* A defined porosity or a certain degree of barrier effect of the membrane are not prerequisites for their use in guided bone regeneration, although these properties may affect the regenerative outcomes. There is a wide variability in the pore size and degree of permeability in the commercially available membranes for GBR, ranging from: micro-porosity (5 – 20 um), which may limit the passage of cells, but allows the passage of chemicals, biomolecules, viruses; moderate porosity (non-resorbable materials ≤100 μm) that also allows the passage of bacteria, cells and tissue integration/migration (tissue integration occurs ≥30 – 40 μm); macro-porosity (non-resorbable materials >100 μm) which allows unrestricted passage of chemicals, biomolecules, viruses, bacteria, cells and allows tissue integration and migration. The pore size can increase during the process of membrane degradation within the tissue, which may in turn influence its bioactivity, passage of nutrient and cells and the ingrowth of nutrients and soft and hard tissue cells. The optimal membrane porosity has not been defined yet.

- *Mechanical properties.* The ideal GBR membrane should be sufficiently rigid for an adequate space-making capacity and able to withstand the pressure of the overlying soft tissues during function in order to prevent its collapse. At the same time. It should possess certain degree of plasticity and elasticity to be easily contoured and adapted to the anatomy of the defect. In situations where the membrane does not possess the required mechanical properties, it should be combined with a bone replacement biomaterial/graft that serves as a scaffold, to attain the desire volume of regenerated bone.

- *Integration with the tissues.* The integration of the membrane with the adjacent connective tissues is essential for optimal primary wound closure and healing. In fact, lack of integration and membrane exposure is associated with inferior

regenerative outcomes. There is evidence that mobility of the membrane and lack of hydrophilicity will impair connective tissue integration and bone formation. In case of non-resorbable membranes, the degree of tissue integration should be coupled with an easy and atraumatic removal. There is lack of information on the optimal degree of membrane tissue integration during healing.

- *Exposure tolerance.* Membrane exposure and its subsequent bacterial contamination may hamper the regenerative outcomes irrespectively whether the membrane is biodegradable or non-resorbable. In case of exposure, the exposed membrane should be kept in situ and continue to function during the during the regenerative process, although in case of overt infections, its removal should be considered. When combined with biomaterials, the incidence of infections might increase.

- *Biodegradability.* Systematic reviews have shown comparable clinical outcomes between resorbable and non-resorbable membranes. However, there is clear evidence that the membrane must retain its function for a certain amount of time to achieve a predictable regenerative outcome. In fact, the longer the membrane maintains its function the greater the maturity of the bone is, although in spite of 30 years of GBR research, the ideal membrane bio absorption time has not yet been established. Moreover, the inflammatory response elicited by the degradation of the membrane should not adversely affect the regenerative outcome.

What are advantages and limitations of currently available membranes used in GBR?

PTFE and modifications

Due to its synthetic nature, PTFE membranes have the advantage of not eliciting any immunological reaction and being resistant to breakdown by the host tissues. Compared with biodegradable membranes, they have superior space making capability, mainly when these membranes have titanium reinforcement, which makes them the ideal membranes for vertical bone regeneration. Their main limitation is the increased frequency of membrane exposure with a subsequent risk for bacterial contamination and infection, in fact superior regenerative outcomes with these membranes are associated with a closed uneventful healing. Other limitation is the

difficulty in their removal due to their soft tissue integration. Moreover, the cost of PTFE membranes is higher compared to biodegradable membranes.

Synthetic polymers

The main advantages of polymeric membranes are their manageability, process ability, tuned biodegradation, and drug-encapsulating ability. However, their degradation might elicit a strong inflammatory response, leading to resorption of the regenerated bone. The resorption rate of these types of membranes is largely dependent on the type of polymer used.

Naturally derived membranes:

Collagen (non-crosslinked)

Collagen-based membranes are the most commonly used naturally derived membranes for GBR and their degradation does not exert any potential deleterious effect to the tissues. Their use has not been associated with relevant adverse effects since collagen is the principal component of connective tissues, playing important role in tissue structural support and in cell matrix communication. Their main limitation is their lack of rigidity, which limits their space making capabilities and requires their combination with a scaffold, Yet, collagen membranes can be used alone for alveolar bone defects which do not require extra fixation and stability such as bone dehiscence and fenestration defects. Moreover, as their degradation is fast (approximately four weeks) they may not meet the duration of time required for optimal tissue regeneration

Chemically modified collagen

In order to slow down the bio-absorption process of collagen membranes, a number of different methods of physical/chemical cross-linking have been developed, which may also enhance the membrane mechanical properties. Although chemical cross-linking has resulted in improvement of collagen stability, release of chemicals residues (e.g. amides or aldehydes) has been associated with severe inflammation at the implantation site. Generally, the predictability of the collagen membrane not only depends on the origin of the collagen material but also its preparation and manufacturing process (de-cellularization, sterilization, and method of cross-linking).

Chitosan, Alginate

Their material properties include biocompatibility, biodegradability, low immunogenicity, and a bacteriostatic effect. Experimental results indicate similar regenerative outcomes when compared with collagen membranes. Despite the experimental studies, their clinical use for GBR has not been documented.

Metals

Titanium is a commonly used material in dentistry. Among its properties are biocompatibility, high strength and rigidity for space maintenance, low density and weight, the ability to withstand high temperatures, and resistance to corrosion. The use of titanium for GBR was inspired from a successful outcome of using a titanium mesh for reconstruction of maxillofacial defects. Titanium mesh alone or with bone substitutes is a procedure for localized alveolar ridge augmentation prior to, or simultaneously with, implant placement. Occlusive titanium and micro-perforated titanium membranes have also been introduced and used for treatment of peri-implant bone defects and ridge augmentation. Limited experimental data exists on CoCr membrane for bone augmentation. Their limitations include difficulties in their removal due to connective tissue integration, mainly associated with the titanium mesh. Conversely, lack of tissue integration has been reported with the use of solid titanium materials.

Which is the role of the exogenous administration of biological cues to the membrane?

The use of growth factors and/or cell therapies have provided promising experimental results when used in combination with GBR, mainly in combination with resorbable membranes. The evidence of efficacy in clinical trials is, however, lacking and these strategies may be hampered by financial and regulatory constraints as well as for the potential adverse risks associated with these therapies.

Recommendations for Future Research

The bone promoting environments in the membrane and defect compartment during GBR can likely be optimized by several strategies targeting both material aspects and host-tissue responses.

The membrane, the main component of GBR, can be improved depending on the functional requirements and the involved biological mechanism. These modifications may include: (i) optimizing the physicochemical and mechanical properties, e.g., the porosity, structure, thickness, rigidity and plasticity; (ii) incorporating biological factors and synthetic bioactive materials (iii) incorporating antibacterial agents and antibiotics.

From scientific, developmental and clinical perspectives, new developments in tissue engineering and drug delivery may enhance the barrier concept associated with GBR and expand the clinical opportunities for bone regeneration in the future.

Bioactive factors used in regenerative interventions in the cariomaxillo-facial region to enhance bone regeneration

Bioactive agents or factors are defined as natural mediators of tissue repair capable of eliciting a response from a living tissue, organism or cell. The majority of pre-clinical and clinical studies on bone regeneration have focused on Bone Morphogenetic Proteins (mainly BMP-2) and Autologous Platelet Concentrates (APCs). Less evidence is available for other growth factors (mainly Platelet Derived Growth Factor PDGF-BB, Fibroblast Growth Factor FGF-2 and Vascular Endothelial Growth Factor VEGF) and amelogenins. The combination of different bioactive factors has also been proposed, with the aim to reduce the dosages of each factor (and associated side effects) and, at the same time, promote synergistic effects. While significant literature has documented the use of bioactive factors (mainly amelogenins, FGF-2, PDGF-BB and APCs) for the regeneration of periodontal intrabony defects, it is outside the remit of this consensus to comment on periodontal regeneration.

What are advantages of the use of bioactive factors in bone regeneration?

The consensus was based on a critical review assessing the outcomes of the use of bioactive substances in preclinical models and clinical applications. The preclinical bone regeneration models included ridge/socket preservation, alveolar ridge augmentation (horizontal and vertical), regeneration of bone defects at the moment of implant placement, sinus augmentation and regeneration of critical/sub-critical

bone defects. The clinical evidence is based only on RCTs, CCT and Case Series (> 5 cases) that included histological and/or radiographical assessment of bone regeneration for ridge preservation, ridge augmentation, regeneration of bone defects at the moment of implant placement and sinus augmentation.

Bone morphogenetic protein (rh-BMP-2)

Overall, pre-clinical studies suggest that rhBMP-2 (at different dosages) significantly promotes, either directly or indirectly, bone regeneration in critical and sub-critical size bone defects and *de novo* bone formation regardless of the carrier adopted. rhBMP-2 enhances ridge augmentation in chronic and combined defects and promotes ridge preservation. Conflicting results have been reported regarding the benefits in peri-implant circumferential defects and sinus augmentation. In challenging ridge augmentation or peri-implant defects the combination with a space providing material is recommended.

As a carrier, the Absorbable Collagen Sponge (ACS) can be successfully used, with or without space-providing materials. For clinical application, an absorbable collagen sponge carrier (ACS) impregnated with rhBMP-2 was approved by the Food and Drug Administration for ridge preservation and sinus augmentation. Therefore, most of the clinical studies have employed BMP-2/ACS, although a combination of rhBMP-2 with different grafts has also been suggested. Clinical studies suggest 1.50 mg/ml as the optimal dosage for ridge preservation and a range between 1.05 and 4.2 mg/ml for ridge augmentation purposes, while in some studies on sinus augmentation high supra-physiological doses of up to 48mg of BMP-2 per subject have been reported.

Based on 3 RCTs, rhBMP-2/ACS combined with osteoconductive grafts and/or a titanium mesh for ridge augmentation is comparable to autologous bone and titanium mesh or Deproteinized Bovine Bone Mineral based on radiographic/histological outcomes. In a recent RCT of 4 months duration, the use of autologous block grafts was superior in terms of amounts of mineralized tissue when compared to DBBM block grafts loaded with BMP-2. 3 RCTs have used BMP-2 combined with ACS or other carriers for ridge preservation. The evidence of using rhBMP-2 in regeneration of bone defects following implant placement is scarce. The results of the studies using rhBMP-2 as a graft material in sinus floor augmentation are conflicting.

Amongst the available bioactive factors BMP-2 is supported with the highest evidence, albeit heterogeneous. The existing RCTs suggest that there is a similar beneficial effect of rhBMP-2/ACS compared to commercially available bone grafting materials for socket preservation and ridge augmentation. Currently, this material has not been approved in Europe for clinical use in oro-maxillofacial applications.

Other growth factors (PDGF-BB, FGF-2, VEGF)

Direct administration of PDGF-BB, FGF-2 and VEGF with different carriers and indirect administration has been studied in pre-clinical studies with demonstration of enhanced bone regeneration when used with GBR. The available evidence for their use for ridge preservation is not robust enough to draw conclusions and make recommendations

Studies in chronic alveolar defects have shown that rhPDGF-BB combined with block or particulate grafts can significantly promote ridge augmentation. It is unclear whether the addition of a barrier membrane has an impact on the final regeneration outcome. The pre-clinical evidence that rhFGF-2 associated with different synthetic biomaterials can promote ridge augmentation is still not robust. There is limited evidence from experimental studies suggesting that rhPDGF-BB alone or combined with IGF-1 or bone grafts might enhance the regeneration of peri-implant defects. There is insufficient evidence on the use of growth factors other than BMP-2 for sinus augmentation.

rhPDGF-BB and rhGDF-5 are the growth factors, apart from rhBMPs, which have been evaluated in clinical studies for bone regeneration when combined with different bone replacement grafts. The available studies have used 0.5 mL PDGF-BB at concentration of 0.3 mg/ml, and between 500 µg to 500 mg rhGDF-5 per gram of β-TCP. There is limited evidence on the efficacy of rhPDGF-BB for ridge preservation and ridge augmentation. RCTs are needed to clarify whether rhPDGF-B combined with different grafting materials can promote post-extraction ridge preservation and the horizontal and vertical regeneration of alveolar defects. No controlled studies have investigated the use of rhPDGF-BB for the treatment of peri-implant defects and there are only limited studies reporting on the use of rhPDGF-BB or rhGDF-5 for sinus augmentation. Therefore, no robust conclusions could be drawn.

Enamel matrix derivatives (EMD)

Although various fractions of EMD have shown osteoinductive properties, only few pre-clinical studies have investigated the use of amelogenins (EMD) for bone regeneration. EMD has limited effect in enhancing bone formation and does not offer significant advantages over the use of a membrane or a bone graft or the combination of both.

The available clinical evidence does not support the use of EMD in sinus augmentation, ridge augmentation, ridge preservation or bone defects following implant placement.

Autologous platelet concentrates (APCs)

APCs are intended to enhance bone regeneration by triggering the natural healing process with a supplement of highly concentrated bioactive factors. One of the main challenges in reviewing the scientific evidence is the heterogeneity of the APC preparation protocols and accuracy in the use of the terminology.

Some pre-clinical studies have shown that Platelet Rich Plasma (PRP) or Platelet Rich Fibrin (PRF) compared to spontaneous healing, fibrin glue derived from Platelet Poor Plasma, collagen sponge or hydrogel improve the amount and quality of regeneration of experimentally induced bone defects. However, there is no robust evidence supporting the addition of these APCs to bone grafts or for *de novo* bone formation.

The evidence on the potential of Plasma Rich in Growth Factors (PRGF) to promote bone regeneration in experimentally induced bone defects is limited. Regarding the use of all APCs, there is insufficient evidence for ridge/socket preservation, regeneration of bone defects after implant placement and sinus augmentation. The limited pre-clinical studies on the use of PRP for ridge augmentation suggest that, while autologous bone remains the gold standard, the combination of PRP, cells and different bone substitutes could be a promising alternative.

Clinical research has shown that the combined therapy of APCs with bone grafts and/or cells offer promising results for ridge augmentation procedures. More RCTs are needed to clarify which of the APCs is superior. For ridge preservation

procedures, APCs may accelerate clinical healing, soft tissue epithelialization and reduce postoperative pain, but there is insufficient and contrasting evidence of a significant effect on hard tissue regeneration. The clinical effect of APCs on defects after implant placement has been studied in a limited number of investigations but the available evidence does not allow for robust conclusions. Conflicting outcomes in terms of bone formation and implant stability emerged from the available studies on the use of APCs for sinus augmentation, with no clear benefits of one APC over the other.

Which are the limitations of the use of growth factors for bone regeneration?

When evaluating the application of growth factors for bone regeneration, there is lack of a clear understanding of their mechanism of action, the ideal dosage, frequency and mode of administration and the delivery system. Furthermore, there is a clear need for developing standardized protocols for controlling their release and clearance at the application site. In general, despite some encouraging results, the available evidence does not support the use of bioactive factors as a routine alternative to the currently used bone regenerative interventions in the caniomaxillo-facial area.

High dosages of rhBMP-2 have been associated with side effects, including long lasting oedema formation, as well as mucosal erythema, osteoclast-mediated bone resorption and inappropriate adipogenesis. Combinations of rhBMP-2 with other growth factors (preclinical studies) have been suggested to reduce these dosage-related complications of rhBMP-2.

Moreover, a concern that could be raised on the use of rhBMP-2 is the development of immunological factors, such as anti-rhBMP-2 and anti-bovine collagen type I (6% and 20% incidence, respectively, as per FDA report on rhBMP-2/ACS).

Recommendations for Future Research

Well-designed and adequately powered RCTs showing clinical and histological outcomes of bioactive agents are required to clarify their potential and actual need in regenerative dentistry.

In the future, studies need to be designed to overcome the heterogeneity currently present in the literature concerning biological agent's dosage, formulation, concomitant biomaterials used, types of defects, methods of investigation and follow-up periods.

Research efforts should be also directed towards the development of delivery systems enabling controlled spatio-temporal delivery of single or combination of bioactive factors. The ultimate aim should be mimicking the synergistic wound healing activity of the combinational release profiles of growth factors and extracellular matrix components that occurs in physiological wound healing.

Cell therapies used in regenerative interventions in the cariomaxillo-facial region to enhance bone regeneration

This consensus report is based on the review of the evidence from pre-clinical and clinical studies on the use of cell therapies for cranio-maxillofacial bone regeneration. Three main approaches using cell therapies have been evaluated: a) *"minimally manipulated"* whole tissue fractions; b) *ex-vivo* expanded "*uncommitted*" stem/progenitor cells, and c) *ex-vivo* expanded "*committed*" bone-/periosteum-derived cells. Minimally manipulated whole tissue fractions, preserve the physiological microenvironment or 'niche' of multiple cell types in their natural ratios; these mainly include bone marrow aspirates – either whole (BMA) or concentrated (BMAC), adipose stromal vascular fractions (A-SVF), and tissue "micrografts". The major limitation of this approach is that mesenchymal stem (and progenitor) cells (MSCs) represent a very limited fraction of the implanted cells.

Ex vivo expansion strategies exponentially increase the number of cells of a specific phenotype, i.e., *uncommitted* or *committed*, available for transplantation. The most commonly used source of *uncommitted* MSCs is the bone marrow (BMSCs), but more recently less invasive sources such as adipose tissue (ASCs) and dental tissues, have been tested. Sources of *committed* cells are the periosteum and cancellous bone/marrow of the alveolar bone itself. The major limitation of *ex vivo* expansion strategies is the need for highly sophisticated laboratories according to Good

Manufacturing Practices (GMP), thereby significantly increasing the cost of this therapy.

What is the effect of cell therapies (either whole tissues or ex vivo expanded cells) added to biomaterials or autologous bone, or combinations thereof, when compared with biomaterials or autologous bone alone, or combinations thereof, for the different clinical indications in the craniomaxillo-facial area?

Clinical studies have evaluated cell therapies, mainly BMA and BMAC, in combination with biomaterials or autologous bone, or combinations thereof vs. relevant controls in various indications (e.g. sinus augmentation, horizontal ridge augmentation, alveolar cleft defects) and at various observation times. The clinical evidence is mostly based on randomized (sinus and ridge augmentation) and non-randomized controlled trials (alveolar cleft repair).

Specifically:

a) in sinus augmentation, significantly more bone regeneration was observed after cell therapy in 1 meta-analysis of histomorphometric results (6 studies, vs. scaffolds, 6 months) and in 1 meta-analysis of micro-computed tomography (μ-CT) results (3 studies, vs. scaffolds, 4-7 months), while in 1 meta-analysis of histomorphometric results no benefit was observed (12 studies, vs. scaffolds, 3-4 months). Based on a meta-regression analysis of histomorphometric data from 15 studies, there were no differences between the various cell therapy strategies, i.e. whole tissues vs. expanded *uncommitted* cells vs. expanded *committed* cells, in terms of the amount of bone regeneration.

b) in horizontal ridge augmentation, significantly more bone regeneration was observed after cell therapy in 1 meta-analysis of histomorphometric results (3 studies, *vs*. scaffolds; 1 study, *vs*. scaffolds + autogenous bone, 4-6 months).

c) in alveolar cleft defects, 1 meta-analysis failed to show a benefit of cell therapy over autogenous bone, as evaluated with CT (3 studies, 6 months).

d) limited clinical evidence suggests that the ‘conditioned medium’ or ‘secretome’ from MSCs may promote bone regeneration in sinus augmentation [1 study (4 patients), *vs*. scaffold, 6 months].

The above observations are in general supported by the results of meta-analyses of pre-clinical *in vivo* studies in large animals, which have mainly evaluated *ex vivo* expanded "uncommitted" cells, mainly BMSCs, in combination with biomaterials vs. relevant controls in various models (e.g. sinus augmentation, critical size defects, alveolar cleft defects) and platforms (e.g. dogs, pigs, sheep/goats) at various observation times.

Based on the data from the clinical studies, the following conclusions may be derived:

1. Transplantation of cells, most commonly whole BMA or BMAC, in combination with biomaterial scaffolds results in superior bone regeneration compared to implantation of scaffolds alone in sinus augmentation and horizontal ridge augmentation, and comparable bone regeneration to autogenous bone in alveolar cleft repair.
2. Based on studies of sinus augmentation, no superiority of *ex vivo* expanded cells, either *uncommitted* or *committed*, over whole tissue fractions (BMA/C or A-SVF) was observed. However, the appropriateness of sinus augmentation as a model to test cell therapies and detect clinically relevant benefits may be questioned, owing to the "self-healing" capacity in this site (Duan, 2017).
3. The analyzed studies, both clinical and pre-clinical, showed a wide range of effect sizes and prediction intervals, suggesting a high degree of heterogeneity, and emphasizing the need for well-designed future studies to ascertain the true effect of cell therapies.

Is osteogenic pre-differentiation in *ex vivo* expansion strategies beneficial?

Based on limited evidence from pre-clinical *in vivo* and uncontrolled clinical studies, use of pre-differentiated BMSCs has not demonstrated a significant added effect in terms of enhancing bone regeneration compared with using undifferentiated BMSCs, while osteogenic pre-differentiation of ASCs or the addition of osteoinductive factors, e.g., BMP-2, seemed to enhance bone regeneration. However, no included studies in the review (pre-clinical or clinical) directly compared pre-differentiated cells *vs*. undifferentiated cells, either BMSCs or ASCs.

Based on this limited evidence:

a) In BMSCs, osteogenic pre-differentiation may not show any additional beneficial effect.

b) In ASCs, additional osteogenic stimulation, via pre-differentiation or addition of osteoinductive factors, e.g., BMP-2, may be beneficial.

Recommendations for Future Research

The relatively large effect sizes in favor of cell therapy observed in pre-clinical *in vivo* studies are diminished in clinical trials, suggesting a gap in translation and the need for better pre-clinical models.

Vascularization remains a key challenge in cell therapy, especially in large defects, since an inadequate vascularization in the internal parts of the cell-scaffold construct can impair cell survival and thereby compromise clinical outcomes. Promising pre-clinical research has been conducted in the area of 'pre-vascularized' bone constructs but remains to be translated clinically.

The potential of using allogeneic cells as an "off-the-shelf" therapy has been tested with favorable results in a limited number of pre-clinical studies. However, the possibilities of immune reactions associated with using allogeneic human cells are still unclear and require further investigation (Kiernan, 2018).

The potential use of 'cell-free' strategies, which exploit the paracrine or trophic effects of MSC-secretomes to promote regeneration, should be explored. Similarly, alternative mechanisms of MSC activity, for example, via "empowerment" of host cells and modulation of immune cells in the context of bone regeneration should be investigated.

There is a need to evaluate the cost-effectiveness of cell therapy in comparison to current standards of care. Moreover, there is a need of well-designed studies to evaluate the efficacy/cost-effectiveness of different cell therapy strategies, i.e., whole tissues vs. expanded *uncommitted* cells vs. expanded *committed* cells.

Acknowledgements:

The authors acknowledge the contribution of the representative from the Osteology Foundation, Benjamin Muller and the sponsor (Geistlich Pharma), Niklaus Steifel for their contribution during the workshop.

References:

Albrektsson, T. & Johansson, C. (2001) Osteoinduction, osteoconduction and osseointegration. European Spine Journal 10 Suppl 2, S96-101. doi:10.1007/s005860100282.

Donos, N., Dereka, X., and Calciolari, E. (2019). The use of bioactive factors to enhance bone regeneration. A narrative review Journal of Clinical Periodontology, Spec Issue

Duan, D.H., Fu, J.H., Qi, W., Du, Y., Pan, J., Wang, H.L. (2017) Graft-Free Maxillary Sinus Floor Elevation: A Systematic Review and Meta-Analysis. Journal of Periodontology, 88**,** 550-564.

Haugen, H.J., Lyngstadaas, S.P., Rossi, F., and Perale, G, (2019). Bone grafts: which is the ideal biomaterial? Journal of Clinical Periodontology, Spec Issue

Hutmacher, D. W. (2000) Scaffolds in tissue engineering bone and cartilage. Biomaterials, 21, 2529-2543. doi:10.1016/S0142-9612(00)00121-6.

Kiernan, C.H., Wolvius, E.B., Brama, P.A.J., Farrell, E. (2018). The Immune Response to Allogeneic Differentiated Mesenchymal Stem Cells in the Context of Bone Tissue Engineering. Part B Reviews, 24, 75-83.

Omar O., Elgali. I, Dahlin, C., and Thomsen, P. (2019). Barrier membranes: more than the barrier effect? Journal of Clinical Periodontology, Spec Issue

Shanbhag, S., Suliman, S., Pandis, N., Stavropoulos, A., Sanz, M., and Mustafa K (2019) Cell therapy for orofacial bone regeneration: a systematic review and meta-analysis. Journal of Clinical Periodontology, Spec Issue

Wang, Y, Chen, X, Cao, W, Shi, Y. (2014) Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nature Inmmunology 15**,** 1009-16.

Williams, D. F. (2017) Biocompatibility Pathways: Biomaterials-Induced Sterile Inflammation, Mechano-transduction, and Principles of Biocompatibility Control. ACS Biomaterials Science & Engineering 3, 2-35.