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Biological factors involved in alveolar bone regeneration

Consensus report of Working Group 1 of the 15th European Workshop on Periodontology on Bone Regeneration

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Abstract

Background and Aims: to describe the biology of alveolar bone regeneration.

Material and Methods: Four comprehensive reviews were performed on: 1) mesenchymal cells and differentiation factors leading to bone formation; 2) the critical interplay between bone resorbing and formative cells; 3) the role of osteoimmunology in the formation and maintenance of alveolar bone; and 4) the self-regenerative capacity following bone injury or tooth extraction were prepared prior to the workshop.

Results and Conclusions: This summary information adds to the fuller understanding of the alveolar bone regenerative response with implications to reconstructive procedures for patient oral rehabilitation. The group collectively formulated and addressed critical questions based on each of the reviews in this consensus report to advance the field. The report concludes with identified areas of future research.

Conflict of interest and source of funding statement

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Preamble:

The remit of Group I was to describe the biology of alveolar bone regeneration. The focus was made on the molecular and cellular processes of intramembranous bone regeneration of the alveolus following injury (such as subsequent to tooth extraction) or diseases (occurring around teeth or dental implants). The interface of the periodontal ligament and cementum as a part of periodontal regeneration was not addressed. However, with respect to bone regeneration, it may include both the alveolar bone and/or the alveolar bone proper in the case of tooth-supporting bone regeneration. The group considered the bone regenerative process in systemically healthy individuals contrasted with compromised wound healing affected at the local or systemic levels. *Bone regeneration* was defined as the regrowth or reconstitution of a lost or damaged bone to restore its former architecture and function, while *bone remodeling* was considered as the physiologic remodeling of bone that takes place in a biologically coupled system of activation, resorption, and formation (Broggini et al., 2007).

The evidence focused on *in vitro* and *in vivo* models of bone regeneration to better understand the biological basis of alveolar bone regeneration. The group identified early stage, preclinical *in vivo* models as well as those with a closer translation to the human clinical situation. Human studies available for evaluation were few.

The report was based on four comprehensive reviews on: 1) mesenchymal cells and differentiation factors leading to bone formation (Bartold et al., 2019); 2) the critical interplay between bone resorbing and formative cells (Lerner et al., 2019); 3) the role of osteoimmunology in the formation and maintenance of alveolar bone (Gruber, 2019); and 4) the self-regenerative capacity following bone injury or tooth extraction (Sculean et al., 2019). These works add to the fuller understanding of the alveolar bone regenerative response with implications to reconstructive procedures for patient rehabilitation. The group collectively formulated and addressed critical questions based on each of the reviews in this consensus report. The group also identified areas of future research.

Q1. What are the critical biological phases characterizing bone regeneration?

Alveolar bone regeneration follows a temporal series of events (Bastian et al., 2011; Stegen et al., 2015; Araujo et al., 2015):

- Hemostasis and establishment of the blood coagulum
- inflammatory phase
- angiogenesis: cellular recruitment and capillary ingrowth
- mesenchymal cell recruitment, provisional non-mineralized matrix deposition followed by interactive processes involving mineralization, bone-forming cell differentiation, and finally bone formation -
  - role of growth and differentiation factors
  - processes of woven and lamellar bone formation
- remodeling of newly formed bone; coupling of osteoclasts and osteoblasts which continues throughout life

Other critical events identified at the molecular and cellular levels need to be explored before definite conclusions defining the sequence of events involved in bone regeneration can be made.

Q2. What biologic/growth factors are involved in the bone regeneration process?

Growth & differentiation factors / signaling molecules are well documented in pre-clinical in vivo models (Table 2; Bartold et al. 2019) but less well characterized for humans.

Major growth and differentiation factors identified to date include:

- **Bone–derived Growth Factors & Differentiation Factors**

  Bone Morphogenetic Proteins (BMPs)

  - BMP-2
  - BMP-7

  Growth Differentiation Factors (GDFs)

  Platelet-Derived Growth Factor (PDGF)

  Fibroblast Growth Factors (FGFs)

  - aFGF
  - FGF-2

Transforming Growth Factor-β (TGF-β)

Insulin-like Growth Factors (IGFs)

IGF-1

IGF-2

Vascular Endothelial Cell Growth Factor (VEGF)

Skeletal Growth Factor (SGF)

Parathyroid Hormone-related Peptide (PTHrP)

- **Bone Growth and Regeneration Signaling Pathways**

TGF-β Family Signaling

FGF Signaling

Wnt Signaling

Hh Signaling

- **Bone Growth and Regeneration Families of Transcription Factors**

Homeobox Gene Family of Transcription Factors

Dlx Homeobox Gene Family

Homeobox Gene Family

Hox Homeobox Gene Family

Paired Box (Pax) Homeobox Gene Family

LIM Homeobox Gene Family (Lhx)

Paired-Like (Pitx) Homeobox Gene Family

Runx Transcription Factors

SRY-Related HMG-Box Family of Transcription Factors

bHLH Family of Transcription Factors

Twist

D proteins

The myogenic regulatory factors (MRFs)

Snail Family of Transcription Factors

Smad Transcription Factors

β-Catenin/LEF/TCF Transcription Factors

Gli Transcription Factors

Forkhead Family of Transcription Factors

Q3. What is the role of mesenchymal stem cells, their niche and extracellular matrix in bone regeneration?

Mesenchymal stem cells provide the reservoir for new bone forming cells.

Niches associated with the alveolar bone (e.g., marrow and periosteal locales) provide potential sources and environment of MSC for bone regeneration and include blood, perivascular source, cells lining the wall of bone defect and periosteum. These provide a source of pluripotent stem cells capable of differentiating and initiating tissue regeneration.

Critical to tissue regeneration is the production of a new extracellular matrix that provides the environment for subsequent cell differentiation and neo-ossification. Thus, the role of the extracellular matrix is to provide a platform for the initiation of tissue-specific regeneration. Fibrous and non-fibrous elements of the extracellular matrix provide a number of critical functions central to tissue regeneration and include:

- a reservoir of growth and differentiation factors that can be released in well-controlled spatial and temporal sequences;
- induction of angiogenesis;
- homing signals for mesenchymal stem cells;
- bioactive space maintaining matrix for cell differentiation and,
- an environment of both osteoinduction and osteoconduction

Q4. What coupling factors regulate bone remodeling?

Coupling between bone resorption and bone formation refers to the process in which osteoclastic bone resorption is linked to the differentiation of osteoblasts and their bone-forming activity. This process is mediated by factors released from the bone matrix during bone resorption, i.e., soluble and membrane products of the osteoclasts and signals from osteocytes and osteoblasts. Osteoclast-derived factors include BMP6, WNT 10b, CT-1 and S-1-P; matrix-derived factors include BMPs, TGF-, IGF-1, FGFs, EGFRs and its ligands as well as miRNAs. Osteocyte/osteoblast-derived factors include sclerostin, Dickkopf-1, WNT-1, RANKL/OPG, PTHrp. Combined osteocyte/osteoblast and osteoclast factors include semaphorins, ephrins and ephrin receptors.

Q5. What coupling factors involved in bone remodeling have regenerative potential for clinical use?

BMP-2 and BMP-7 are in clinical use and BMP-5, -6, -9 exhibit osteogenic properties. Currently, the most studied signaling pathway associated with bone regeneration is the WNT system. Neutralizing antibodies to sclerostin have been demonstrated to increase bone mass in phase III studies. Other factors with potential for regeneration are described in detail in reports from Group 2.

Q6. What is the role of inflammation and its resolution in the process of bone regeneration?

There is a large body of data from preclinical models supporting the general concept that inflammation is an important component of bone regeneration. Data needs to be interpreted carefully as fracture and osteotomy defect models were utilized involving long bones and genetically distinct murine models. However, genetic ablation of cyclooxygenase-2 (COX-2) in rodents treated with COX-2-selective non-steroidal anti-inflammatory drugs led to impaired fracture healing (Simon et al., 2002) that could be rescued by activation of prostaglandin E_2 receptor subtype 4 (Xie et al., 2009). Mice lacking the 5-lipoxygenase gene (Manigrasso and O'Connor, 2010)and systemic inhibition of 5-lipoxygenase were associated with increased bone regeneration. In addition, TNF-α receptor-deficient animals and systemic administration of anti-TNF led to impaired fracture healing. Application of low concentrations of TNF-α promotes fracture repair. Moreover, IL6 and IL17A knockout animals display impaired fracture healing.

There is emerging evidence from pre-clinical *in vivo* studies in small and large animals that pro-resolving lipid mediators such as RvE1 and LxA_4 have positive modulatory effects on bone regeneration, beyond their inflammation-resolving properties. These appear to be receptor-mediated (ERV1 and BLT-1) and reduce osteoclast differentiation and activation, whilst at the same time promoting osteoblast-mediated healing. Presence of RvD1 in the acute phase of the inflammatory response to an implanted biomaterial had a positive role in subsequent bone tissue repair (Vasconcelos et al., 2018).

Q7. What is the role of different macrophage phenotypes, in particular osteomacs, in bone regeneration?

Preclinical models support a critical role for macrophages in bone regeneration. Macrophage depletion by Fas-induced apoptosis in mice or clodronate liposome delivery showed impaired intramembranous osteotomy defects and endochondral bone regeneration in fracture models. Depletion of CD169 expressing macrophages ("Osteomacs") led to impaired intramembranous and endochondral ossification (Batoon et al., 2017).

Q8. What is the role of lymphocytes in bone regeneration?

The majority of studies reviewed investigated the role of T and B-lymphocytes in bone regeneration using fracture models. T and B-lymphocytes infiltrate the fracture callus and participate in bone remodeling. Bone remodeling is accelerated in RAG1 knockout mice, which do not possess mature B and T lymphocytes. Similarly, others found RAG1 knockout mice to have a larger but lower density callus compared to controls (Nam et al., 2012). Depletion of CD8 T cells in a murine osteotomy model resulted in enhanced fracture regeneration, whereas a transfer of CD8 (+) T cells impaired the healing process (Reinke et al., 2013). In animals deficient in γδ T cells bone regeneration was inhibited. Absence of B-cells in mice does not compromise bone formation in a tibial injury model (Raggatt et al., 2013). It appears therefore that heterogeneity exists in T-cell behavior, with some T-cell populations influencing osteolysis, whereas others (γδ T cells) are associated with enhanced bone formation.

Q9. What is the role played by osteoclasts in bone regeneration?

Bone resorption occurs as an important stage in the regeneration process (Vasak et al., 2014). The molecular mechanisms underpinning this process may be initiated by the release of induction signals for osteoclastogenesis by apoptotic osteocytes and subsequent resorption of necrotic elements of the alveolar bone (Chen et al., 2018, Cha et al., 2015).. In contrast to bone remodelling, bone formation within osteotomy sites or micro-cracks is not a coupled process and can arise independently of bone resorption. Knowledge of the role played by osteoclasts in bone regeneration is derived from studies employing bisphosphonates and RANKL activity blockade. Bisphosphonate administration, as well as RANKL-blockade using Denosumab increased fracture callus volume with a retained trabecular bone structure in rodents (Amanat et al., 2007, Gerstenfeld et al., 2009). Moreover, Bisphosphonate use and RANKL activity blockade also increased bone formation in osteotomy defects (Bernhardsson et al., 2015) and supported early bone formation around implants (Aspenberg, 2009). The available literature supports the contention that early bone formation does not appear to require osteoclasts, but bone maturation, requires bone remodelling and thus the coupling of osteoclast to osteoblast function.

Q10. Does bone regeneration in alveolar extraction sites in animals reflect the clinical situation in humans?

The sequential phases of regeneration after tooth extraction appear to be similar among rodents, canines, non-human primates, and humans. However, bone remodeling in general takes longer time in humans as compared to the other species.

Q11. Does the morphology and location of the defect affect the regenerative capacity?

The available data indicate that defect morphology (e.g., number of bony walls and defect dimensions, i.e. depth, width, volume), location (e.g., extraction sockets, periapical, symphysis or ramus donor sites), and closed or open healing environment substantially influence regeneration of bone defects. Data indicate that the cells responsible for bone regeneration originate from the surrounding bony walls and periosteum. Blood supply, wound stability, and availability of cells are influenced by defect morphology and location.

Q12. What is the regenerative capacity of cystic defects or intra-oral bone graft donor sites?

Defects following peri-apical surgery or cystectomy possess a substantial self-regenerative capacity and largely heal with bone in the vast majority of cases without the use of any adjunct measures. The strong intrinsic potential for regeneration of bone defects after periapical surgery or cystectomy is most likely due to their favorable morphology and location. At bone graft donor sites such as the mandibular symphysis or ramus, repair of the defects following bone block harvesting is generally incomplete. There are no data to draw conclusions on the size of the defect that is critical for complete regeneration in cystic or intra-oral bone graft donor sites.

Future research

Future research efforts will need to target both stem cells and biologics through well-controlled clinical trials based on the *in vitro* and preclinical studies published to date. Combining cell-based therapies with controlled temporal delivery of regulatory molecules, using tissue engineering approaches, offers many exciting prospects for bone regeneration. It is not until we understand the process of formation that regeneration will become an achievable and predictable clinical endpoint for managing disease and trauma. This will certainly be the case for bone regeneration.

For cell and biological therapies, manipulation of extracellular matrix to enhance regenerative outcomes will be of value and include: identify stem cell niches and the influence that different niches have on the ultimate phenotype of stem cell differentiation; understanding mechanisms of cell-cell and cell-matrix communication through the secretome; explore lab-on-a-chip technologies as matrix modulation models that may substitute for preclinical in vivo studies.

Pre-clinical models to study molecular mechanisms of bone regeneration will be developed. These will include models testing the role of single mediators or pathways using technology such as point mutations, gene deletion, or gene over-expression. In addition, the field will benefit from molecules with possible future therapeutic use such as antibodies, inhibitors or small RNAs. A major challenge with several of these agents lies in the delivery and targeting to the site as well as the management of potential off-target side-effects.

Macrophages demonstrate significant plasticity in model systems, and respond to various environmental cues and other molecular signals that influence differentiation into either type-1 (M1), or type-2 (M2) cells. The association of the M1 phenotype with pro-inflammatory responses and the M2 class with anti-inflammatory and/or pro-resolving activities is rather simplistic and requires further research. Emerging evidence indicates that induction of the M2 phenotype is associated with decreased expression of RANKL and a reduced number of osteoclasts (Zhuang et al 2018). However, the role of M1 and M2 cells in bone regeneration requires further research. In addition, the use of cytokines, chemokines, transcription factors and micro-RNAs to influence a shift in the balance of M1 and M2 macrophages for bone regeneration is worthy of investigation.

More information is needed on modifying factors that affect regeneration of bone (such as epigenetic influences, aging (inflammaging), smoking, drugs, and systemic conditions. The role of the gut and oral microbiomes in bone regeneration remains to be explored. Potential avenues need to account for interactions between the microbiome and the osteoimmune response in order to determine specific biological pathways.

More information is needed on the influence of defect morphology, location, closed or open healing environment, on bone regeneration in extraction sites, cystic and intra-oral bone graft donor sites.

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