Bone Regeneration in the Maxillofacial Region

Bone Regeneration in the Maxillofacial Region

Edited by

Randa Alfotawi

Cambridge
Scholars
Publishing

Bone Regeneration in the Maxillofacial Region

Edited by Randa Alfotawi

This book first published 2023

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-1702-0
ISBN (13): 978-1-5275-1702-8

TABLE OF CONTENTS

PREFACE

Over the years, scientists and surgeons have found it difficult to reconstruct maxillofacial continuity defects. The goal of maxillofacial reconstruction is to restore facial form, function, and complete restoration of occlusion and articulation. Revised surgical procedures and reconstruction methods increase patients' quality of life. This chapter will go over the innovative bone reconstruction techniques and confirm the right approach for continuity defect reconstruction. Each method's drawbacks will be outlined. The quest for more effective methods of bone regeneration employing biomaterials or biological tissues has been motivated by these drawbacks. The use of tissue engineering techniques has yielded effective instruments for long-lasting satisfactory outcomes, enabling individualized reconstruction and assisting with the natural regeneration process. This book will go into great detail on the upcoming developments in tissue-engineered reconstruction, which require interdisciplinary analysis to produce complicated tissue structures and provide clinically acceptable results for the majority of patients. This review book will also highlight relevant clinical developments and provide an outline of contemporary scientific ideas. The author will demonstrate in each chapter how bone augmentation and bone regeneration function at the cellular level, based on clinical or experimental methods. The final chapter will cover an update of several methods that can either physically or physiologically increase tissue perfusion. When implanted in vivo, the role of biomimetic scaffolds that provide a micro environmental and inductive extracellular stimulus can maintain the characteristics of the host cell. Before highlighting the clinical application of experimental methods, more development and improvement of these techniques is needed.

The book will provide a solid platform for researchers, knowledge for clinical evidence-based practice for surgeons, and a large repository of up-to-date literature for scientists.

CHAPTER 1

TECHNIQUES FOR MAXILLOFACIAL RECONSTRUCTION OF LARGE BONY DEFECTS

RANDA ALFOTAWI

Associate Professor and Consultant Oral and Maxillofacial Dept. Dental Faculty, King Saud University, P.O. Box 60169-15, Riyadh 11545, Saudi Arabia

1.1. Abstract

Reconstruction of critical size maxillofacial bone defect has proven to be a complex process due to functional and esthetic demand of this region. Over the years, scientists and surgeons have found it difficult to reconstruct maxillofacial continuity defects. Although various autogenous bone grafts and utilizing biomaterials are well known and routinely used for facial reconstruction. These methods are associated with morbidities, while the use of biomaterials alone are linked with a high failure rate. These reported limitations inspired the search for innovative techniques for bone bioengineering and switch to more reliable biomaterials. Tissue engineering approaches aim to provide powerful tool with long term satisfying results enabling customized reconstruction that support the natural healing process. This chapter will provide an overview of the currant clinical technique using autogenous bone grafts. Then highlight on the advances in tissue engineering which are essential to achieve reliable and satisfactory clinical outcomes.

1.2. Reconstruction Techniques for Large Bone Defects in the Maxillofacial Region

1.2.1. Free Bone Grafts

An autogenous bone graft is considered an efficient graft as it produces osteoconduction, osteoinduction, and osteogenesis. In osteoconduction, the

native bone uses the bone graft material as a scaffold for future bone development (Perciaccante and Jeffery, 2007). Osteoinduction entails the tonic osteoprogenitor cells evolving into osteoblasts that begin a new bone formation in the body (Perciaccante and Jeffery, 2007). Over the years, BMPs have become the most researched and studied type of osteoinductive cell mediators found in large concentrations in the cortical bone, making up 80% of the skeletal mass (Perciaccante and Jeffery, 2007). Vital osteoblasts from bone graft material secrete bone matrix (osteogenesis), which results in the growth of new bone in the body. Currently, the majority of surgeons favor using an anterior or posterior iliac crest corticocancellous block graft for jaw reconstruction (Goh et al., 2008). As the survival of the graft depends on revascularization from the recipient site, the success of these grafts is likely to be contingent on how the bone is secured (Goh et al., 2008). Revascularization, which is essential to the development of the new viable bone, is required for creeping substitution, or the gradual, nearly complete resorption of the graft with the concomitant deposition of the new bone (Goh et al., 2008). On the contrary, there are three significant disadvantages to using an autogenous free bone graft: poor osteointegration and excessive resorption when the defect is larger than 6 to 9 cm^3, a lack of blood supply to the graft and surrounding tissues because of infection and irradiation scarring and morbidity at the donor site (Kessler et al., 2005).

1.2.2. Particulate Cancellous Bone Marrow Graft (PCBM)

Long bones can be reconstructed using PCBM, as has been shown since 1944. When compared to cortical grafts, this form of grafting has been shown to have higher osteogenic potential and reduced rates of surgical complications (Rappaport, 1971). The PCBM graft is placed in a frame or crib to preserve its physical dimensions and offer mechanical stability due to the inherent lack of cohesiveness. Metal cribs made of stainless steel, tantalum, titanium and vitallium are widely used to convey PCBMs (Samman et al., 1999; Tideman and Lee, 2006; Tideman et al., 1998; Cheung et al., 1997). These metal trays have considerable drawbacks, particularly for individuals who will later receive radiotherapy. The intended local dosage distribution can be considerably changed by metallic implants. Additionally, it is more elastic than bone, which allows the tray to absorb most of the functional load and provide stress shielding (Goh et al., 2008). Dacron-coated polyurethane trays have been explored for the reconstruction of human mandibles to overcome the disadvantages of metal (Cheung et al., 1997; Schwartz et al., 1987).

Wound dehiscence, tray exposure and postoperative infection have all been described as complications when using metal or Dacron trays (Kinoshita et al., 1997; Louis et al., 2004). Therefore, the development of resorbable alloplastic trays in the field of maxillofacial reconstruction has drawn the interest of numerous researchers to explore its viability before being used in clinical settings (Kinoshita et al., 1997; Louis et al., 2004). A freeze-dried allogenic mandibular tray filled with PCBM was utilized in clinical studies as an alternative to alloplastic cribs and demonstrated success in restoring form, shape and function in more than 80% of instances (Plotnikov and Sysoljatin, 1993; Lowlicht et al., 1990). The main constraint of this approach was the scarcity of allografts. Reconstruction techniques also include using the patient's own jaw after cleansing the diseased portion from the bone. After that, the bone is autoclaved or freeze-dried before being filled with PCBMs. Animal and human trials have indicated varying success rates with this technique (Simon et al., 2006; Lee et al., 2010a). Additionally, in both animal models and human clinical studies, the usage of plasma-rich protein and PCBMs has been evaluated for the restoration of mandibular continuity defects (Fennis et al., 2005; Simon et al., 2006; Marx et al., 1998).

The ability to establish an anatomic mandibular reconstruction with an acceptable height, symmetrical arch width and form, and providing adequate support for dental implants are the four presently listed benefits of PCBM grafts (Goh et al., 2008). Additionally, this form of graft has demonstrated its ability to treat severe mandibular defects (Lee et al., 2010a; Goh et al., 2008). Contrarily, the most prevalent drawbacks were wound dehiscence and graft resorption, both of which could result in graft loss (Lee et al., 2010a). In patients with malignant tumors, where scarring, fibrosis and lack of vascularity are serious issues with the covering soft tissues, the graft resorption is the main reason why PCBM grafts are not advised (Goh et al., 2008).

1.2.3. Pedicle Composite Grafts

This is a graft that is made of bone, muscle, or skin and is connected to the blood arteries that supply it (vein and artery). In the field of maxillofacial reconstruction, the use of pedicle bone and composite grafts has grown in popularity as an alternative to the problem of the vascularity of free bone grafts. Numerous methods have been advocated, and their practicality has been examined. Furthermore, effective clinical trials have also been reported (Green et al., 1981; Robertson, 1986; Tiwari, 1994). However, serious drawbacks with the use of various flaps were reported. The

sternocleidomastoid muscle-based pedicle composite flaps are not usually available or have insufficient vascularization. Additionally, the aesthetic outcomes of pedicle bone grafts are often not satisfactory and, finally, these techniques are linked with donor-site morbidity (Goh et al., 2008; Torroni, 2009).

1.2.4. Microvascular Free-Flap Transfer

The gold standard for mandibular reconstruction currently is microvascular free flaps since they often result in an acceptable functional reconstruction (He et al., 2007). In a prospective trial, free vascular bone grafts were used to reconstruct 82 irradiated mandibles. Twenty cases were followed up for ten years, which demonstrated a 90% success rate (Hidalgo and Pusic, 2002). However, it is challenging to shape microvascular bone grafts to repair complex, 3-dimensional orbito-maxillary defects (He et al., 2007). Additionally, the use of the iliac crest free vascular flap is constrained by its short vascular pedicle and the absence of segmental perforating arteries (Hartman et al., 2002). Contrarily, the fibula flap is said to have the widest use and can supply up to 30 cm of bone with optimal feeder vascular length and diameter (Hidalgo and Pusic, 2002). However, the use of this flap is restricted by the pre-existing peripheral vascular disease. The literature debates the fibula flap's inadequacy in height when combined with the dentate jaw as another flaw (Horiuchi et al., 1995).

1.2.5. Tissue Engineering and Regenerative Medicine

The term "tissue engineering" was first used to refer to a technique for in vitro production of biological tissue (Whitman et al., 1997). The development of technologies and surgical methods for the regeneration of tissue in vivo has recently been referred to as "regenerative medicine" (Lynch et al., 2008). The objective of tissue engineering is to use currently available information to create a product (tissue) that can mimic autogenous tissue and can replace any lost tissue at any moment. In comparison to traditional grafting approaches, tissue engineering and regenerative medicine aim to promote regeneration and, ideally, actual regeneration of tissue structure and function (Lynch et al., 2008) (Figure 1.1).

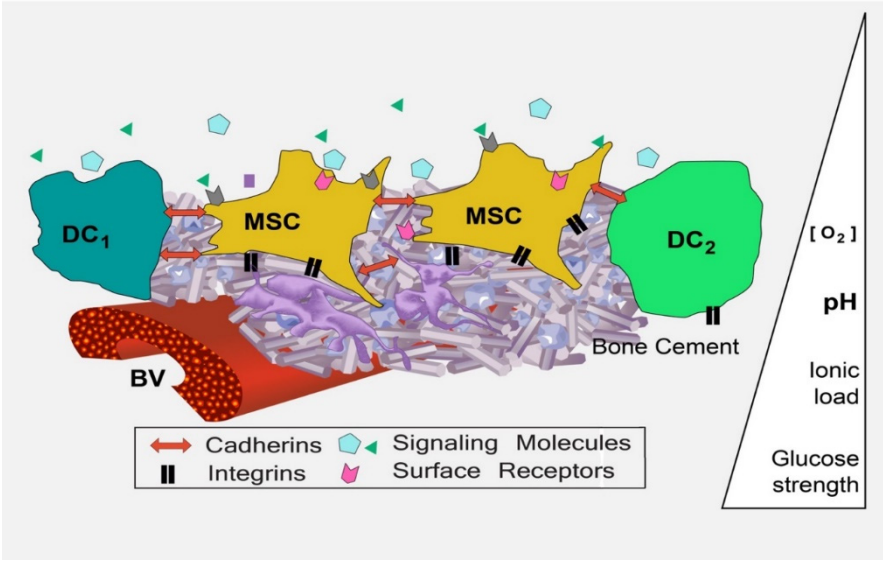

Figure 1.1. Habitat for mesenchymal stromal cells (MSCs). MSCs are depicted in their putative perivascular niche (BV, blood vessel), where they interact with (1) numerous other differentiated cells (DC1, DC2, etc.) via cell-adhesion molecules like cadherins, (2) extracellular matrix and cement deposited by the habitat cells via integrin receptors, and (3) signaling molecules including autocrine, paracrine, and endocrine factors. Another factor is O_2 tension, with MSCs in the bone marrow habitat linked to hypoxia, ionic load, and glucose strength (Modified from Kolf et al., 2007).

Three-dimensional biomaterial scaffolds were first used in tissue engineering to promote regeneration and replace autografts. For usage in orthopedic surgery and oral surgical procedures, many matrices, including allogenic, xenogenic, and synthetic graft materials, have been marketed. These matrices work by providing a base for cells to advance from the edges of wounds (osteoconduction), finally resulting in the regeneration of the defect. The main drawback of employing scaffolds alone is the absence of osteoinductive and osteogenic characteristics (Lynch et al., 2008). Moreover, some types of allografts may not integrate with the surrounding tissue effectively due to the rate of resorption being out of pace with new bone growth (Lee et al., 2002). The development of BMP marked a significant defining moment in the field of bone reconstruction. BMP encourages osteoinduction, which causes osteoprogenitor cells to generate bone and attracts mesenchymal stem cells to the location of the defect as a

result (Herford et al., 2007) (further details about this study in section 3.8). The use of BMP yielded positive outcomes at the level of preclinical animal investigations (Chao et al., 2006; Ayoub et al., 2007; Miguez et al., 2011). A few instances of the effective reconstruction of the craniofacial region utilizing BMP in clinical studies have also been published (Abu-Serriah et al., 2005; Herford et al., 2007). Utilizing BMP and scaffolds, two prospective, longitudinal controlled clinical trials were conducted (Herford and Cicciù, 2010; Herford et al., 2007). The results of both investigations led the author to the conclusion that BMP-2 is a viable alternative to traditional methods (Herford et al., 2011). On the other hand, the author came to the conclusion that BMP could not take the role of autogenous bone grafts in significant bone defects due to a reported failure rate of 13.9%.

In the same context, Ferretti and Ripamonti (2002) used naturally produced BMP from human cortical bone chips to assess bone regeneration in mandibular critical continuity deficits in humans. Osteogenesis in patients receiving autologous bone grafts was compared with this. To create the construct for the experimental group, the latter was combined with a demineralized bovine bone matrix. In comparison to the autogenous bone graft, the BMP graft's morphometric analysis data revealed significantly active osteogenesis. The author concluded that the study was a successful initiation to this new approach of regeneration of critical mandibular defects through the extraction of BMP from the natural milieu of bovine bone matrix, which eliminated the need to harvest autologous bone, even though this study recorded a failure to induce bone in four of the constructs. Engineered grafts utilizing BMP have a bright future; however, it is not yet known how this biochemical will affect diverse tissue cells over the long-term or whether it will have an oncogenic effect (Goh et al., 2008). Another limiting issue is the high expense associated with using the currently available BMPs, which prevent their widespread adoption (Goh et al., 2008). The use of prefabricated bioengineered bone flaps is another aspect of bone tissue engineering. Sections 3.8 and 4.5 detail in-depth explanations of the various techniques documented in the literature.

Funding: NA

Conflicts of Interest: The author declares no conflicts of interest.

References

(Abu-Serriah et al., 2005) Abu-Serriah, M., A. Kontaxis, A. Ayoub, J. Harrison, E. Odell, and J. Barbenel. 2005. Mechanical evaluation of mandibular defects reconstructed using osteogenic protein-1 (rhOP-1) in a sheep model: A critical analysis. *International Journal of Oral and Maxillofacial Surgery* 34: 287–93.

(Ayoub et al., 2007) Ayoub, A., S. R. R. Challa, M. Abu-Serriah, J. McMahon, K. Moos, S. Creanor, and E. Odell. 2007. Use of a composite pedicled muscle flap and rhBMP-7 for mandibular reconstruction. *International Journal of Oral and Maxillofacial Surgery* 36: 1183–92.

(Chao et al., 2006) Chao, Mimi; Tom Donovan, Cirilio Sotelo, and Michael H. Carstens. 2006. In situ osteogenesis of hemimandible with rhBMP-2 in a 9-year-old boy: Osteoinduction via stem cell concentration. *Journal of Craniofacial Surgery* 17: 405–12.

(Cheung et al., 1997) Cheung, L. K., N. Samman, T. W. Chow, R. K. F. Clark, and H. Tideman. 1997. A bone graft condensing syringe system for maxillofacial reconstructive surgery. *British Journal of Oral and Maxillofacial Surgery* 35: 267–70.

(Fennis et al., 2005) Fennis, J. P. M., P. J. W. Stoelinga, and J. A. Jansen. 2005. Reconstruction of the mandible with an autogenous irradiated cortical scaffold, autogenous corticocancellous bone graft and autogenous platelet-rich-plasma: An animal experiment. *International Journal of Oral and Maxillofacial Surgery* 34: 158–66.

(Ferretti and Ripamonti, 2002) Ferretti, Carlo, and Ugo Ripamonti. 2002. Human segmental mandibular defects treated with naturally derived bone morphogenetic proteins. *Journal of Craniofacial Surgery* 13: 434–44.

(Goh et al., 2008) Goh, Bee Tin, Shermin Lee, Henk Tideman, and Paul J. W. Stoelinga. 2008. Mandibular reconstruction in adults: A review. *International Journal of Oral and Maxillofacial Surgery* 37: 597–605

(Green et al., 1981) Green, Michael F., John R. Gibson, J. Richard Bryson, and Eric Thomson. 1981. A one-stage correction of mandibular defects using a split sternum pectoralis major myocutaneous transfer. *British Journal of Plastic Surgery* 34: 11–16

(Hartman et al., 2002) Hartman, Ed H. M., Paul H. M. Spauwen, and John A. Jansen. 2002. Donor-site complications in vascularized bone flap surgery. *Journal of Investigative Surgery* 15: 185–97

(He et al., 2007) He, Yue, Zhi-yuan Zhang, Han-guang Zhu, Weiliu Qiu, Xinquan Jiang, and Wei Guo. 2007. Experimental study on reconstruction of segmental mandible defects using tissue-engineered bone combined bone marrow stromal cells with three-dimensional tricalcium phosphate. *Journal of Craniofacial Surgery* 18: 800–5.

(Herford and Cicciù, 2010) Herford, Alan S., and Marco Cicciù. 2010. Recombinant human bone morphogenetic protein type 2 jaw reconstruction in patients affected by giant cell tumour. *Journal of Craniofacial Surgery* 21: 1970–75.

(Herford et al., 2011) Herford, Alan S., Enrico Stoffella, and Rahul Tandon. 2011. Reconstruction of mandibular defects using bone morphogenic protein: Can growth factors replace the need for autologous bone grafts? A systematic review of the literature. *Plastic Surgery International* 2011: 165824.

(Herford et al., 2007) Herford, Alan S., Philip J. Boyne, Rick Rawson, and Roland P. Williams. 2007. Bone morphogenetic protein-induced regeneration of the premaxillary cleft. *Journal of Oral and Maxillofacial Surgery* 65: 2136–41.

(Hidalgo and Pusic, 2002) Hidalgo, David A., and Andrea L. Pusic. 2002. Free-flap mandibular reconstruction: A 10-year follow-up study. *Plastic and Reconstructive Surgery* 110: 438–49.

(Horiuchi et al., 1995) Horiuchi, Katsuhiro, Akinobu Hattori, Ikuhisa Inada, Toyohiko Kamibayashi, Masahito Sugimura, Hiroshi Yajima, and Susumu Tamai. 1995. Mandibular reconstruction using a double-barrel fibular graft. *Microsurgery* 16: 248.

(Kessler et al., 2005) Kessler, P., M. Thorwarth, A. Bloch-Birkholz, E. Nkenke, and F. W. Neukam 2005. Harvesting of bone from the iliac crest—Comparison of the anterior and posterior sites. *British Journal of Oral and Maxillofacial Surgery* 43: 51–56.

(Kinoshita et al., 1997) Kinoshita, Yukihiko, Masaru Kobayashi, Takehiro Hidaka, and Yoshito Ikada. 1997. Reconstruction of mandibular continuity defects in dogs using poly(L-lactide) mesh and autogenic particulate

cancellous bone and marrow: Preliminary report. *Journal of Oral and Maxillofacial Surgery* 55: 718–23.

(Kolf et al., 2007) Kolf, Catherine M., Elizabeth Cho, and Rocky S. Tuan. 2007. Mesenchymal stromal cells—Biology of adult mesenchymal stem cells: Regulation of niche, self-renewal and differentiation. *Arthritis Research and Therapy* 9: 204.

(Lee et al., 2002) Lee, Gregory H., Joseph G. Khoury, John-Erik Bell, and Joseph A. Buckwalter. 2002. Adverse reactions to OsteoSet bone graft substitute, the incidence in a consecutive series. *The Iowa Orthopedic Journal* 22: 35–38.

(Lee et al., 2010) Lee, Jun, Hark-Mo Sung, Jae-Deog Jang, Young-Woo Park, Seung-Ki Min, and Eun-Cheol Kim. 2010. Successful reconstruction of 15-cm segmental defects by bone marrow stem cells and resected autogenous bone graft in central hemangioma. *Journal of Oral and Maxillofacial Surgery* 68: 188–94.

(Louis et al., 2004) Louis, Patrick, Jon Holmes, and Rui Fernandes. 2004. Resorbable mesh as a containment system in reconstruction of the atrophic mandible fracture. *Journal of Oral and Maxillofacial Surgery* 62: 719–23.

(Lowlicht et al., 1990) Lowlicht, Roger A., Mark D. Delacure, and Clarence T. Sasaki. 1990. Allogeneic (homograft) reconstruction of the mandible. *Laryngoscope* 100: 837–43.

(Lynch et al., 2008) Lynch, S., R. E. Marx, M. Nevins, and L. Wisner-Lynch. 2008. *Introduction. Tissue engineering application in oral and maxillofacial surgery and periodontics*, 2nd ed. Chicago: Quintessence Publishing.

(Marx et al., 1998) Marx, Robert E., Eric R. Carlson, Ralph M. Eichstaedt, Steven R. Schimmele, James E. Strauss, and Karen R. Georgeff. 1998. Platelet-rich plasma—Growth-factor enhancement for bone grafts. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* 85: 638–46.

(Miguez et al., 2011) Miguez, P. A., M. Terajima, H. Nagaoka, Y. Mochida, and M. Yamauchi. 2011. Role of glycosaminoglycans of biglycan in BMP-2 signaling. *Biochemical and Biophysical Research Communications* 405: 262–66.

(Perciaccante and Jeffery, 2007) Perciaccante, V., and J. Jeffery. 2007. Oral and maxillofacial reconstruction. In *Oral and Maxillofacial Secrets*, 2nd ed. Edited by O. AbuBake and K. Benson. Philiadephia: Elsevier, pp. 389–403.

(Plotnikov and Sysoljatin, 1993) Plotnikov, Nickolaj A., and Pavel G. Sysoljatin. 1993. Mandibular primary osteoplasty using orthotopic allografts. *Journal of Cranio-Maxillofacial Surgery* 21: 43–49.

(Rappaport, 1971) Rappaport, I. 1971. The particulate graft in tumor surgery. *The American Journal of Surgery* 122: 748–55.

(Robertson, 1986) Robertson, G. Andrew. 1986. The role of sternum in osteomyocutaneous reconstruction of major mandibular defects. *American Journal of Surgery* 152: 367–70.

(Samman et al., 1999) Samman, N., W. K. Luck, L. K. Cheung, L. K. Cheung, H. Tideman, and R. K. F. Clark. 1999. Custom-made titanium mandibular reconstruction tray. *Australian Dental Journal* 44: 195–99.

(Schwartz et al., 1987) Schwartz, Harry C., Donald L. Leake, and Arturo Pizzoferrato. 1987. Evaluation of autologous cancellous bone grafting of mandibular discontinuity defects in dogs using dacron-urethan prosthesis. *Journal of Oral and Maxillofacial Surgery* 45: 143–48.

(Simon et al., 2006) Simon, E. N. M., M. A. W. Merkx, F. M. Shubi, B. M. Kalyanyama, and P. J. W. Stoelinga. 2006. Reconstruction of the mandible after ablative surgery for the treatment of aggressive, benign odontogenic tumours in Tanzania: A preliminary study. *International Journal of Oral and Maxillofacial Surgery* 35: 421–26.

(Tideman and Lee, 2006) Tideman, H., and S. Lee. 2006. The TL endoprosthesis for mandibular reconstruction—A metallic yet biological approach. *Asian Journal of Oral and Maxillofacial Surgery* 16: 5–8.

(Tideman et al., 1998) Tideman, H., N. Samman, and L. K. Cheung. 1998. Functional reconstruction of the mandible: A modified titanium mesh system. *International Journal of Oral and Maxillofacial Surgery* 27: 339–45.

(Tiwari, 1994) Tiwari, Rammohan. 1994. Mandibular reconstruction with conventional bone graft. In *Excision and Reconstruction of Head and Neck Cancer*. Edited by D. Soutar and R. Tiwari. Edinburgh: Churchill Livingston, pp. 59–85.

(Torroni, 2009) Torroni, Andrea. 2009. Engineered bone grafts and bone flaps for maxillofacial defects: State of the art. *Journal of Oral and Maxillofacial Surgery* 67: 1121–27.

(Whitman et al., 1997) Whitman, Dean H., Ronald L. Berry, and David M. Green. 1997. Platelet gel: An autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *Journal of Oral and Maxillofacial Surgery* 55: 1294–99.

CHAPTER 2

ROLE OF STROMAL CELLS IN BONE REGENERATION

RANDA ALFOTAWI

Associate Professor and Consultant Oral and Maxillofacial Dept. Dental Faculty, King Saud University, P.O. Box 60169-15, Riyadh 11545, Saudi Arabia

2.1. Abstract

The principle of regenerative medicine is based on a combination of biological and/or synthetic materials with the addition of viable cells. The ability of mesenchymal stromal cells (MSCs) to develop into a variety of mesenchymal tissues, such as muscle, bone and cartilage, makes them an excellent choice. The potential for osteogenic differentiation of MSCs in combination with various scaffolds and signaling factors for bone repair has been extensively studied. MSCs were utilized in a significant number of preclinical and clinical research, and they have important characteristics such as multipotent qualities, ease of cell isolation and a rapid rate of proliferation (Markham et al., 2002). The nature, biology, prospective uses of molecular biology, cell harvesting, culture and the typical variability of the cell population of MSCs will all be highlighted in this chapter. The orthodox and unorthodox plasticity of marrow stromal cells, as well as their clinical use, is also discussed. Data from the literature are used to explain and support the osteogenic potency of MSCs from various sources or regions in addition to examining the clinical use of stem cell treatment and its limits, including its ethical dilemma.

2.2. Biology of Mesenchymal Stromal Cells (MSCs)

The phrase “mesenchymal stem cells” refers to the plastic-adherent cells that have been isolated from bone marrow, adipose tissue, and other tissues that have the ability to differentiate into several types in vitro (Horwitz et

al., 2005). Based on the work of Friedenstein et al. (1968, 1974), Owen (1985) recommended the use of the term "mesenchymal stromal cell" instead; nevertheless, Caplan (1991) is credited with popularizing the phrase. Pereira et al. (1995, 1998) were against mentioning stem cell identification when publishing their preclinical experiments. Clarifying the terminology for these crucial cells was advised by the International Society for Cellular Therapy's (ISCT) Mesenchymal and Tissue Stem Cell Committee. The board's aim was to promote the adoption of standard terminology that is scientifically accurate in order to ease information sharing with the general public. The ISCT urged the scientific community to use mesenchymal stem cells' standard nomenclature in all written and spoken communications. Horwitz et al. (2005) recommended the use of the abbreviation MSCs for the cell population of plastic-adherent cells and the unfractionated plastic-adherent cells in their review.

Horwitz et al.'s argument is backed by a number of factors, including the use of the acronym MSC in the literature for at least two decades, which has preserved the consistency of the scientific language and the electronic literature databases and lessened confusion. Second, the available information does not permit classification of the unfractionated plastic-adherent marrow cells as stem cells; as a result, the term "multipotent mesenchymal stromal cell" is proposed to describe these distinct characteristics without demonstrating homogeneity or stem cell activity. Third, it is untrue that the entire population of human marrow cells identified for plastic adherence are stem cells; nonetheless, a fraction of these cells may contain mesenchymal stem cells. Fourth, the potential for differentiation remains unknown, and MSCs may differentiate into tissue-originating cells in more than one embryonic germ layer. Finally, the cells appear to be present in situ inside the supporting stromal compartment of resident tissues; therefore, the unfractionated population, regardless of origin, may be accurately named stromal cells (Horwitz et al., 2005).

Another term for mesenchymal stem cells found in the literature was Multipotent Adult Progenitor Cells (MAPCs). Cells co-purified with mesenchymal stem cells were referred to by the term MAPCs. In actuality, the phrase referred to differentiated cells at the single cell level, as well as cells with visceral mesoderm, neuroectoderm, and endoderm features that could be achieved in vitro (Jiang et al., 2002). Progenitor cells, as defined by Ballas et al. (2002), are cells that exist between stem cells and fully differentiated cell types. Furthermore, the term MPCs was frequently used in rheumatology publications. The name referred to mesenchymal progenitor cells with a strong proliferative potential and the ability to

develop into many mesenchymal lineages, including bone and cartilage (Jones et al., 2002).

Mesenchymal stem cells were defined as skeletal stem cells by one group of researchers in 2010: their presence in bone marrow may be directly identified as a particular type of mural cell/pericyte, and cells located in the wall of sinusoids, which are known as adventitial reticular cells (Bianco et al., 2010). The cells can self-renew in vivo and may be characterized by the expression of CD146. Furthermore, these cells can give rise to skeletal tissues but not to skeletal muscle or any other mesodermal (heart muscle) or non-mesodermal-derived tissues. Skeletal cells from bone marrow should have distinct angiopoietic and hematopoietic niche-related capabilities (Bianco et al., 2010).

In conclusion, the term multipotent MSCs appears to be the best scientifically appropriate description for the plastic-adherent population without implying unsubstantiated biological or therapeutic potential.

2.3. Bone Marrow Stromal Cells: Nature, Biology, and Potential Applications

MSCs are defined in vitro as quickly adhering cells that are clonogenic and capable of long-term proliferation (Bianco et al., 2001). Stromal cells in bone marrow are the progenitors of skeletal tissue components such as bone, cartilage, hematopoiesis-supporting stroma, and adipocytes. Furthermore, they may be experimentally encouraged to differentiate, potentially generating neuronal and myogenic cells. As such, they represent an essential model of post-natal non-hematopoietic stem cells and are a readily available source of potential therapeutic application (Bianco et al., 2001). Before undertaking any research on MSCs, important properties relating to the nature, biology, and possible applications of MSCs must be understood.

2.4. Heterogeneity of the MSC Population

The population of MSCs consistently demonstrates a diverse nature. These colonies forming unite (CFU-Fs) are seen to exhibit a variety of colony sizes, which correspond to diverse growth rates, and various cell morphologies (from spindle-shaped to huge flat cells) (Gronthos et al., 2001). Numerous researches were carried out to assess the heterogeneity and multipotency of these colonies, and some of these studies discovered that some colonies were extremely positive for alkaline phosphatase (ALP).

A third variety was positive in the center and negative on the periphery, while the others were all negative for the same test (Friedenstein et al., 1982). According to a study by Herbertson and Aubin (1997), some colonies can produce calcium/phosphate nodules that can be recognized using alizarin red or Von Kossa staining. Furthermore, oil red staining revealed that certain colonies accumulated fat. Alcian blue staining revealed that certain colonies develop cartilage (Berry et al., 1992). Additionally, they experimented with the in vivo transplantation of heterogeneous MSC colonies and discovered that some of the colonies were capable of fully regenerating bone or any other mesenchymal organ in which the bone cells, myelosupportive stroma, and adipocytes were of clonal and donor origin, whereas hematopoiesis and the vasculature were of recipient origin (Kuznetsov et al., 1997). These findings outlined the original CFU-Fs, from which the clonal strains were derived, as stem cells. Nevertheless, research revealed that not all of these clonogenic cells were multipotent stem cells.

A larger proportion of cells that respond with the hematopoietic cell surface markers CD34 and CD14 were found in bone marrow (Smiler et al., 2008). Because of the evidence that bone marrow MSCs promote the proliferation of hematopoietic stem cells into osteoclast progenitors, megakaryocytes, monocyte/macrophages and myeloid progenitor cells, long-term in vitro culture techniques, such as Dexter cultures, were developed. Additionally, the presence of MSCs was indicated by the significantly increased reaction to CD105 in bone marrow aspirates. Kaiser et al. (2007) established a different theory to explain how a relatively small population of MSCs originated from CD34 and CD45 positive cells. Hematopoietic cells were better able to adhere to stromal layers when CD34 was expressed, and, thus, this adherence is crucial for processes like hematopoietic stem cell homing to the bone marrow. Therefore, it's plausible that CD34 functions as a homing molecule on both MSCs and hematopoietic cells (Healy et al., 1995).

According to Satomura et al. (2000), the multipotency of a chosen clone in vivo was not reflected in the behavior of these MSC colonies as in in vitro growth media. The optimal strategy that was suggested to guarantee homogenous cell colonies was to divide the cells into various colonies, each of which has a set of shared traits and characteristics. For both theoretical and practical reasons, it would be crucial to be able to identify the fraction of marrow stromal cells that have the greatest capacity for reproduction and differentiation. A number of laboratories have created monoclonal antibodies employing MSCs as an immunogen to discover one or more markers appropriate for stromal cell preparation, identification and sorting

(Bruder and Caplan, 1989; Joyner et al., 1997; Gindraux et al., 2007; Kaiser et al., 2007). The best course of action for stromal stem cells' phenotypic fingerprinting would be to continuously alter their response to both in vitro and in vivo micro environments. It has been demonstrated via considerable research that the MSC populations as a whole share many, but not all, of the characteristics of fibroblast cells. These include the expression of the matrix proteins, some myofibroblastic cell markers (smooth muscle actin), and some endothelial cell features, such as endoglin (CD105) and MUC-18 (Gronthos et al., 2000; Gronthos and Simmons, 1996).

Numerous studies used immuno-detection and immuno-magnetic techniques during direct enrichment (isolation) to compare the MSCs from mouse, rat and human bone marrow in order to explore the native phenotyping of these cells. They showed that the CD49-positive cell population contains all rodent multipotential CFU-Fs (Gindraux et al., 2007). Because of this, the CD49 protein is a conserved marker that enables the direct enrichment of bone marrow MSCs from different mammalian species (Gindraux et al., 2007).

The ISCT established the basic requirements for MSCs in 2005; (a) MSCs must exhibit adherent growth characteristics under standard culture conditions, (b) MSCs must express CD105, CD73 and CD90 as well as CD45, CD34, CD14, or CD11b, CD79a, or CD19, and the HLA-DR surface molecule, (c) MSCs can undergo in vitro cell differentiation to produce cells such as bone cells, fat cells and cartilage cells (Horwitz et al., 2005). Furthermore, despite the fact that researchers mention a number of distinct MSC indicators, the exact markers of MSCs and multipotency have not yet been identified because culture-expanded MSCs may lack some of these markers while still being multipotent (Jones and McGonagle, 2008). Since MSCs can differentiate into three different types of connective tissue cells—adipocytes, osteocytes and chondrocytes—the researchers used this knowledge to describe MSCs (Kaiser et al., 2007).

Nonetheless, it is unclear, whether a population of highly purified or cloned MSCs is required for the craniofacial structure engineering (Mao et al., 2006). First, the mesenchymal and hematopoietic stem cells are present in the stromal cell populations that form the native craniofacial structure including the mandibular joint. Second, stem cell delivery systems that utilize porous biomimetic scaffolds are likely to experience host cell invasion and stem cell homing (Christopherson et al., 2004; Hidalgo and Frenette, 2005).

2.5. Orthodox Plasticity of Marrow Stromal Cells

The cell plasticity of bone marrow stromal cells both in in vivo and in vitro environments is one of their key characteristics. Because this adaptability still exists within the context of the organ from which the cells were derived, it is known as orthodox. Data from the literature has demonstrated the reversible nature of the differentiation of a number of cell types, which were previously thought to be the culminations of discrete pathways/lineages. It has been revealed that clonal strains of marrow adipocytes are capable of being driven to an osteogenic differentiation and forming true bone in vivo (Bennett et al., 1991; Beresford et al., 1992). Furthermore, it has been demonstrated by Bianco et al. (1988) and Weiss (1981) that marrow reticular cells can develop into adipocytes in vivo. According to several studies, fully differentiated chondrocytes can proliferate in culture and then transition to an osteogenic phenotype (Galotto et al., 1994; Gentili et al., 1993). The fundamental relevance of our findings was the physiological need for connective tissue cell plasticity; namely, to adapt to diverse tissues that live adjacent to one another during organ development (Alfotawi et al., 2019).

Cell plasticity would also aid in the maintenance of the organ from where they originated. According to previous research, certain cells were controlled by important elements depending on their pathway or lineage. Core binding factor 1 (cbfa1), also known as RUNX2, which is frequently seen in MSCs from bone marrow and retained during differentiation towards other cell types, is an example of a gene influencing the osteogenic commitment of the cells (Satomura et al., 2000). There may be other alternative paths of differentiation even though the presence of cbfa1 is indisputable evidence that cells are committed to osteogenesis (Bianco and Robey, 2000). As an example of the latter, freshly harvested MSCs isolated as stromal cells and sorted by Stro-1 have demonstrated negative for α1 cbfa1, and positive for endothelial markers CD34 and CD45 (Bianco et al., 2001).

In conclusion, since all cell types (chondrocytes, osteoblasts, reticular cells, and adipocytes) are present in a single skeletal segment, these cells should express as a marker of osteogenic commitment. These cells can switch to one another within the same family in certain situations since they are simultaneously present at various stages of organogenesis (Bianco et al., 2001).

2.6. Unorthodox Plasticity of Marrow Stromal Cells

According to previous studies, progenitor cells may be present in various tissues and have the capacity to develop into cells unrelated to the tissue of origin (Ferrari et al., 1998; Woodbury et al., 2000). Ferrari et al. (1998) established that bone marrow contains myogenic progenitors with the same potential for systemic transplantation as brain stem cells. Bjornson et al. (1999) investigated the potential of transforming neural stem cells into hematopoietic cells in irradiated mice. Furthermore, Lagasse et al. (2000) investigated the possibility of bone marrow producing hepatocytes. Mezey et al. (2000) looked at the feasibility of using bone marrow to generate brain cells. MSCs can differentiate not only into mesenchymal lineage cells, but also into endothelium and endoderm lineage cells (Reyes et al., 2001, 2002; Schwartz et al., 2002; Oswald et al. 2004). If stromal cells in the bone marrow can form liver, brain and muscle, then there must be safeguards in place to prevent undesirable tissue or organ growth within the marrow itself. As a result, a signal system for tissue maintenance must exist and be utilized by differentiated cells (Bianco et al., 2001).

One key aspect of stem cells is that their plasticity, self-renewal and multipotency are constrained to specific times and events in vivo but considerably less ex-vivo. MSCs can develop significantly in culture, but after skeletal growth has ceased the majority of the cells will probably stop proliferating in the body. This is a defense strategy to maintain stem cell numbers while avoiding DNA deterioration. Only a small number of cells, possibly in reaction to diseases or injury, are involved in bone turnover. On the contrary, embryonic stem cells (mother cells) only continue to be pluripotent and capable of self-renewal within the embryo for a relatively brief period of time until totipotent cells can only be found in the germ line (Bianco et al., 2001).

2.7. Transplantation and Transplantability of Marrow Stromal Cells

The gold standard for determining the potential for differentiation of marrow stromal cells has been in vivo transplantation under controlled conditions (Bianco et al., 2001). Studies on the transplantability of MSCs revealed that ectopic transplantation resulted in the development of the hematopoietic microenvironment (HME) and hematopoietic tissue (blood) at the ectopic recipient sites (Bianco et al., 2001). The local transplantation of MSCs for therapeutic purposes has been the subject of several preclinical

trials, and they demonstrate effective reconstruction of large bony defects (Goshima et al., 1991; Kon et al., 2000). The results of this study have, however, shown that the optimal ex-vivo expansion conditions, the composition and structure of the ideal carrier, as well as the quantity of cells necessary for the regeneration of a volume of bone, have not been identified.

Based on the findings of the marrow stromal cells' unorthodox plasticity, it would be able to reconstruct neural tissues using them after an unorthodox transplant. Furthermore, it might be useful to deliver the necessary gene products to unorthodox locations, such as the central nervous system. In fact, this method might do away with the requirement for the challenging process of harvesting brain stem cells (Bianco et al., 2001).

The fact that MSCs are immune-modulating cells and do not exhibit major histocompatibility complex class II (MHC-II) is another crucial aspect of their phenotype. These characteristics appear to be the same for bone and adipogenic MSCs and are maintained even after osteogenic stimulation in vitro. This implies that MSC implantation from an allogenic source would be feasible; for instance, in the context of tissue engineering (Niemeyer et al., 2007). A unique interaction between blood monocytes and MSCs produced from bone marrow was also described by Groh et al. (2005). This interaction inhibits T-lymphocyte activation, and transforming growth-factor-released (TGF) cytokines from MSCs were responsible for this inhibition.

2.8. Microenvironment and Stem Cell Properties In Vitro and In Vivo

The microenvironment is a compartment in which the cell component survives for an extended period of time and is constantly exposed to the extracellular matrix. The microenvironment is involved in both the support and the preservation of MSCs in either a differentiated or undifferentiated state. The cells can be affected by a variety of parameters, including O_2 tension, glucose concentration, growth hormones and the physiochemical quality of the environment including pH, ionic strength and load (e.g., Ca^{2+} concentration). Their functions are essential for the preservation of stem cell characteristics and the potential for reprogramming (Figure 1.1, Chapter 1).

The microenvironment allegedly controls how bone MSCs behave (Bianchi et al., 2001). The growth medium and environment are crucial in determining the fate of MSC subpopulations when they are grown in vitro. As an illustration, careful management of cells in vitro during culture can prevent

cells from reaching a stage of terminal differentiation that may compromise their capacity to proliferate and differentiate after being delivered in vivo. Research on the effects of growth factors on MSCs is substantial (Gronthos and Simmons, 1995; Rodan et al., 1989). Contrary to other growth factors examined in the same study, it was shown that fibroblast growth factor-2 (FGF-2) demonstrated a stronger potential to increase the proliferation of these cells (Bianchi et al., 2001; Martin et al., 1997). Dexamethasone can efficiently stimulate these cells' osteogenic capacity. Remarkably, adding FGF-2 to dexamethasone-treated cultures increased the expression of the osteogenic phenotype (Bianchi et al., 2001). Furthermore, Gronthos and Simmon (1996) found that in the absence of serum in the culture media, the presence of growth factors is essential for cell proliferation.

It has been reported that when other components in the microenvironment of the marrow are eliminated, cell proliferation rates decrease, which may be responsible for a complex network of soluble and cell-contact signals to bone MSCs (Bianchi et al., 2001). Similarly, Castano-Izquierdo et al. (2007) discovered that the in vitro culture period of MSCs is crucial for their ability to regenerate bone when implanted into an orthotopic site. Their findings were consistent with previous research, which also found that scaffolds seeded with MSCs and pre-cultured in osteogenic media for a short period of time (six to seven days) had the maximum osteoinductivity (Van Den Dolder et al., 2002; Sikavitsas et al., 2003).

Finding a suitable environment is crucial for cell survival in vivo. Ex-vivo cultured cells are chosen based on how well they adhere to plastic. Therefore, it is not unexpected that more than 90% of MSCs that are injected into the bloodstream are unable to find an appropriate niche and perish. Therefore, in order for the cell to maintain a self-sustaining microenvironment for more than four months, a scaffold must be used (Bianchi et al., 2001). A scaffold gives the cells a place to cluster and control the substrate to develop a local habitat where they can survive for an extended period of time. Evidently, neither cell survival nor transgenic expression are influenced by the chemical makeup of the matrix.

2.9. The Osteogenic Potency of MSCs from Different Sources or Regions

The ability of MSCs to produce at least three cell lineages, including osteogenic, chondrogenic, and adipogenic, is well established. MSCs may also be the source of several lineages, including myogenic, neurogenic, and

tenogenic. It really is interesting to note that MSCs from long bones and MSCs from the mandible differ from one another. In comparison to iliac-crest-derived marrow cells from the same individuals, human mandibular or maxillary marrow stromal cells show higher cell proliferation, prolonged senescence, and better expression of osteoblastic markers (Akintoye et al., 2006).

This implies that the stromal cells in the marrow of the long bone and the mandible have different roles, levels of differentiation, and osteogenic potential. More colonies develop in mandible MSC cultures, indicating a higher CFU population (Aghaloo et al., 2010). Mandibular and long-bone MSCs both underwent osteoblast differentiation. Mandible MSCs, on the other hand, showed increased mineralization, ALP activity, and osteoblast gene expression. Importantly, when compared to long-bone` MSCs, mandible` MSCs generated 70% larger bone nodules with three times as much mineralized bone after being implanted into nude mice (Aghaloo et al., 2010). Data from the literature also suggests that mandible MSCs have greater osteogenic potential to stimulate bone formation in vivo and in vitro than long-bone MSCs (Akintoye et al., 2006; Leucht et al., 2008; Aghaloo et al., 2010).

This discrepancy may have arisen as a result of the influence of the developmental origin of the organ. The jaws are different from the axial and appendicular skeletons but similar to other craniofacial bones. According to Chai and Maxson (2006), the mandible develops from neural crest cells in the neuroectoderm germ layer rather than the mesoderm and undergoes intramembranous ossification as opposed to endochondral ossification (Karaplis, 2002). In particular, the mandible is predominantly created by intramembranous ossification, with endochondral components being added later by secondary cartilage at its proximal end. In addition, as compared to lengthy bones, the maxilla and mandible display several homeostatic processes. The mandible loses much less bone than the proximal tibia when exposed to external stressors, such as ovariectomy and starvation (Mavropoulos et al., 2007). Conversely, Cherubism (Ueki et al., 2001), syndrome of the hyperparathyroid-jaw tumor (Simonds et al., 2002), and bisphosphonate-related osteonecrosis of the jaws (BRONJ) (Ruggiero et al., 2004), all displayed a distinct hemostatic mechanism than the damaged long bone in the same patient. This is supported by variations in mechanical loading of the mandible during mastication (Mavropoulos et al., 2004). In fact, the forces exerted during walking are nearly half of those experienced by the alveolar bone during mastication (Knoell, 1977; Daegling and Hylander, 1997).

In the last decade, research into MSC-like cells in certain tissues has revealed a range of stem cells in every organ and tissue of the body (Baksh et al., 2004; Porada et al., 2006; Kolf et al., 2007). Dental pulp stem cells, stem cells from exfoliated deciduous teeth, stem cells from the periodontal ligament, stem cells from the apical papilla, and dental follicle progenitor cells are the five different types of human dental stem/progenitor cells that have been isolated and characterized so far. These cells have the ability to self-renew and have shown strong odontogenic differentiation potential. However, they can also develop other cell lineages that are comparable to bone marrow MSCs but have a different level of potency (Huang et al., 2009).

The periosteal-derived human MSCs have a good osteogenic potential in a three-dimensional collagen scaffold, according to a study by Ryu et al. (2011) on the osteogenic differentiation of human MSCs. The potential of deciduous stem cells to create an in vivo graft between a child and parent without leading to an allogenic graft rejection was more significant (Yamada et al., 2011). In contrast, although they are not identical, MSCs obtained from adipose tissue and umbilical cord blood resemble bone marrow-derived MSCs in terms of morphology and immunophenotype (Kern et al., 2006). In a previous study, MSCs from bone, adipose tissue, and the umbilical cord were examined to see how they behaved in terms of the number of colonies they created and their rate of proliferation. The study demonstrated that umbilical cord-derived MSCs of all sources produced the fewest colonies and had the maximum capacity for proliferation, whereas adipose tissue-derived MSCs form the most colonies and bone marrow MSCs have the lowest (Mareschi et al., 2001; Ballen et al., 2008).

Human adipose-derived stromal cells (ADSCs) have been the topic of an earlier clinical investigation (Halvorsen et al., 2000). The cells were used in a rat model to restore the lost bone in critical size calvaria lesions (Rhee et al., 2011). Variable success rates have been documented in some clinical papers emphasizing the use of ASCs in the reconstruction of the skull, maxilla, and mandible (Lendeckel et al., 2004; Kulakov et al., 2008; Mesimaki et al., 2009). There is currently a dearth of information describing the processes by which ASCs affect an osseous deformity. It is not known whether ASCs serve as effective ‘factories’ to generate strong pro-osteogenic cytokines or if they directly form bone.

Muscle-derived stem cells (MDSCs) have been investigated to test their osteogenic potential. It has been found that MDSCs participated in endochondral bone regeneration during cranial defect healing (Gao et al.,