Cell-Based Bone Reconstruction Therapies—Principles of Clinical Approaches

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Cell-based bone tissue engineering is a rapidly evolving therapy option in bone reconstruction strategies. Some cell-driven approaches, especially the biophysical stimulation of the host cell population surrounded by the bone defect, are common treatment methods in maxillofacial surgery. Others, such as autologous cell implantation, have now gained acceptance for clinical trials. More advanced or complex therapeutical options (extracorporeal tissue engineering, stem cell use, genetic engineering) have been tested in preclinical investigations but have not reached the level of clinical use. Two different aspects are of special relevance in cell-based bone reconstruction therapies. The source of cells used to regenerate bone (discussed in detail in a complementary review in this issue of The International Journal of Oral and Maxillofacial Implants*) as well as the principal approach of a cell-driven bone regeneration therapy influence the outcome of such engineering strategies. All of the cell-driven repair strategies are under intensive investigation in an effort to provide surgeons with a limitless supply of tissue for bone repair and reconstruction in future procedures. An overview of the basic biological aspects as well as the inherent constraints of different cell-based approaches are given in this paper.* (More than 50 references.) Int J Oral Maxillofac Implants 2006;21:899–906

Key words: *bone reconstruction, bone tissue engineering, clinical treatment, stem cells*

The reconstruction of damaged or lost bone is a major clinical challenge today. Moreover, atrophy of the jaws in an aging population, accompanied by a considerable loss of bone, is of concern. Loss of maxillofacial bone structure is of special relevance, since implant-based masticatory rehabilitation relies on a sufficient amount of good-quality bone. Patients requiring maxillofacial reconstruction with bone grafts still experience significant morbidity from donor site procedures.[1–4] The repair of such bone defects still poses a significant problem for many clinicians. Cell-based bone reconstruction therapies may now offer new therapeutic opportunities for the repair of bone damaged by disease or injury. As a new option, tissue engineering represents a more biologically oriented approach to healing tissue defects of various oral and maxillofacial structures.[5,6] Efforts to regenerate lost maxillofacial bone through tissue engineering signify a transition from the historically biomaterial-based approaches in which mechanically stable, biocompatible materials were used to augment lost bone to a focus on cell-based devices (Fig 1). The repair of lost bone can be achieved by transplantation of cell-containing bone specimens (either a free transfer of bone tissue particles or bone block). In addition, the technique of flap surgery enables the surgeon to reconstruct bone with pedicled or free vascularized grafts. Transfer of cells, harvested and multiplicated ex vivo, is a promising alternative to the classical treatment options. The new approach combines biological properties of living cells and physical properties of specially designed materials in order to create artificial organs. Typically, scaffolds, bioactive factors, and cells are com-

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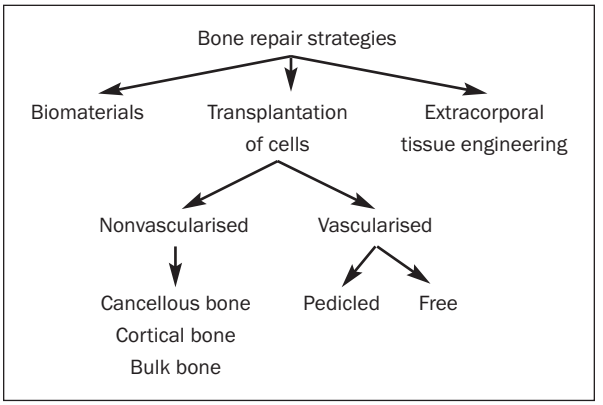

Table 1 Cell Repair Strategies

Augmentation of host cells	Cell transplant
• Cytokine stimulation	• Precursor cell transplantation
• Mechanical stimulation	• Determined cell transplantation
• Electrical field application	• Extracorporal tissue generation
• Membrane technique	
• Vector application	

Fig 1 Principles of bone repair approaches.

bined to create a surgically implantable product for tissue regeneration and functional restoration.[7,8] Historically, many attempts have been undertaken, with varying degrees of success, to restore bone defects using various biomaterials alone[9–13] or combined with bioactive cytokines, such as bone morphogenetic protein-7 (BMP-7), BMP-2, or BMP-2 mutants[14,15]; there are currently many efforts being made to establish cell-based strategies in bone tissue engineering. To critically assess the reports on cell-based maxillofacial bone reconstruction therapies, it is important to develop a conceptualized overview of the various aspects of cell-driven repair strategies.

At the moment, the use of autologous cells in maxillofacial bone defect reconstruction can be considered to be the "gold standard." There are 2 classic ways to repair bone defects using autologous cells: one utilizes mechanisms of defect repair through enhancement of the local host cell population, while the other is based on transplantation of grafted cells (Table 1).

ENHANCEMENT OF LOCAL CELL POPULATIONS

Augmentation of the host cell population can be used effectively to improve the healing of bone lesions. The ability to repair or reconstruct lost bone structure with this technique depends on the condition of the defect site. If the soft and hard tissues of the defect site are healthy, expansion of host cells is often successful. In circumstances of impaired defect conditions, such as wound infection, tissue necrosis, or irritation, however, cellular augmentation of the local bone cell population will probably fail. Four different strategies have been introduced in maxillofacial surgery:

1. Application of cytokines or vectors at the defect site
2. Application of mechanical loads
3. Exposure to electromagnetic fields
4. The use of separating membranes

All of these techniques have been acknowledged to be effective in regenerating maxillofacial bone.[16–19] A main advantage of augmenting the local cell population is that the regenerate tissue can be expected to have the same biological properties as the lost tissue. It should be considered that the condition of the host cells regulates the biological outcome of bone regeneration to a great extent. Augmentation of the host cells can be considered to be a predictive measure of a cell-based reconstruction therapy only in cases where the defect site hosts healthy cells. With regard to these strategies, it is important to consider that different healing environments (eg, bone types) require different repair tissues. For example, a mandibular bone deficiency primarily requires cortical bone to be formed, whereas maxillary alveolar crest augmentation primarily requires membranous (cancellous) bone.

Additionally, it can be assumed that different stimuli exert diverse effects on these 2 healing processes. Alternatively, the same factors may be involved but have different outcomes because of differences in the biophysical environments or host cell populations. From a clinical point of view, it is therefore likely that there will not be a single best strategy or stimulus; in the end, the "best strategy" may be determined by the individual patient situation.[20]

Transplantation of Cells or Cell-containing Tissues

When cell-based bone reconstruction is performed using a cellular implantation or a cell-containing tissue transplantation, various technical aspects must be considered. The harvesting and culture technique, the implantation procedure, and immunological aspects have a major impact on the clinical outcome, especially when ex vivo approaches are under consideration. In order to gain well-defined ex vivo multiplicated cells, it is important to adjust and optimize the various aspects of cell biology. The techniques used to harvest cells, the methods used to isolate and possibly

enrich cells, the standard conditions used for cell maintenance and cultivation, and the methods used to screen the cells differ for various cell sources.

Various donor sites allow the grafting of different types of cells for later insertion in defect bony sites. The harvesting of autologous cell sources can be performed using different grafting procedures, depending on the desired cell source. The various grafting procedures are accompanied by donor site morbidities of varying severity. Autologous grafting procedures theoretically range from minimally invasive (eg, obtaining peripheral blood to gain hematopoetic stem cells) to major open surgical approaches. Various surgical methods allow mature bone cells to be gained (eg, harvesting of periosteum pieces, bone marrow, and removal of cancellous or cortical bone). Endochondral bone from the ilium, tibia, or rib can be harvested as well as membranous bone from the facial skeleton. Animal studies indicate that membranous bone-based cells are less prone to resorption effects compared to endochondral bone.[21] After removal of the tissue specimens, cells are extracted using different techniques, and isolated cells are subsequently cultured. Cultured cells can be used as a sole therapeutic agent, or cells can be coaxed with scaffold materials prior to transplantation into the defect site.

Harvesting and Culture of Precursor Cells

Bone marrow-derived precursor cells are commonly gained through a marrow aspiration procedure. Common locations for harvesting bone marrow are the iliac crest and the sternum.[22–26] Unfractioned fresh autologous or syngeneic bone marrow was used in initial attempts to create tissue-engineered bone.[27–29] Because bone marrow is known to contain osteogenic precursor cells, its use was perceived as likely to facilitate bone regeneration. Principally, cells were harvested for these investigations through marrow aspiration, expanded in culture, and then reimplanted. Studies using this method have indicated that mouse marrow fibroblastic cells gained through the aspiration procedure and implanted locally or injected systemically homed to bony sites and persisted there, thus participating in the regenerative processes as demonstrated for mature periosteal cells.[30,31] However, it is uncertain what kind of cell source is responsible for this finding. Various investigations used different methods to selectively isolate and enrich defined cell sources gained through the surgical harvesting procedure.[32,33] There was special focus on gaining selectively stem cells. Isolation of mesenchymal stem cells (MSCs) commonly has been performed by density gradient centrifugation and subsequent cell culturing techniques. Different approaches have been developed to allow MSCs to be cultured and expanded in numbers without their undergoing differentiation.[34,35] The phenotype of the cells appeared to be stable throughout the culture period, without loss of osteogenic, chondrogenic, or adipogenic potential.[36] With regard to clinical approaches to reconstructing bone using "defined" stem cells, no unique phenotype has yet been identified that permits the reproducible isolation of MSC precursors with predictable developmental potential. The isolation of stromal cells is dependent on their ability to adhere to plastic as well as their "selective" expansion potential. Human bone marrow progenitor cells, for example, have been shown to be isolated and enriched using surface markers.[37,38] As a number of markers are expressed in MSCs, some of these have been used to selectively gain subpopulations of more determined cells. Not only have human MPCs derived from bone marrow been reported to maintain their differentiation capacity into the osteogenic lineages for more than 40 cell doublings,[39] but purified MPCs also undergo a development characterized by the transient induction of alkaline phosphatase (ALP), expression of bone matrix protein mRNAs, and deposition of calcium when cultured in the presence of dexamethasone and ascorbic acid or "selectively" enriched through phenotypic markers.[36] Although it has been suggested that MPCs are able to form mineral-like foci (indicative of an osteoprogenitor phenotype[40,41]), it has not been resolved whether this mineral formation resembles the mineral formation present in bone tissue. Despite the success that has been obtained using bone marrow–derived stem cells in clinical situations, one biological consideration limits its widespread application. From a clinical point of view, it is frequently not possible to obtain sufficient amounts of bone marrow with the requisite number of osteoprogenitor cells using marrow aspiration. In addition, the age-related decrease in bone marrow components, accompanied by a partial loss of precursor cells,[42,43] is a frequent clinical limitation to obtaining sufficient numbers of stem cells. As mentioned before, the outcome of the in vitro use of bone marrow explants is critically dependent on the transfer of sufficient numbers of these progenitors. Therefore, the use of marrow-derived stem cells may be least applicable in those situations where it is most needed. In this respect it has been shown that osteoprogenitors represent approximately 0.001% of the nucleated cells in healthy adult marrow,[35,44] which is indicative of the practical problems in gaining "pure" stem cell sources by the aforementioned harvesting and cell culture strategies. Therefore, improvements in all aspects of stem cell use are necessary to further select, expand, and administer the progenitor marrow cell fraction in order to get clinically relevant numbers of osteogenic or chondrogenic stem cells.

When considering cell implantation strategies, it is important to consider not only the cell source and harvesting technique but also the culture conditions in which the cell destiny will be decided during the multiplication phase. Cell performance is to a great extent regulated by the conditions of the culture milieu. Standard conditions for the expansion of cells have been established in a number of experimental studies.[23,24,39] Once such cells have been isolated from the tissue, a number of parameters influence the expression of the osteoblastic phenotype in cell culture, most importantly the culture medium, culture time, number of passages, and compounds present. The presence of ascorbic acid, β-glycerophosphate, and dexamethasone influence the expression of the osteoblastic phenotype in a differentiated manner. β-glycerophosphate, for example, induces phenotypic matrix maturation by enabling the formation of mineral in osteoblastlike cell cultures. Dexamethasone—as an additional factor—is described as inducing cell differentiation but has a negative effect on cell proliferation, which is indicative of a reciprocal and functionally coupled relationship between proliferation and differentiation. Additional conditions include cell density as well as the presence of serum (in most preclinical studies fetal bovine serum, in clinical studies autologous serum). Cells have been grown directly—that is, unmanipulated after collection—or, more often, after density gradient separation. As cell density is known to be a critical factor affecting the growth of cells, attempts were made to enrich cells above a critical cell density using distinct culture techniques. From a clinical point of view, it is imperative that cells be cultivated in either an autologous or an artificially, chemically defined medium. A minimal requirement is therefore that during in vitro culture the medium be devoid of animal or nonautologous human products. The ideal culture milieu for cell differentiation in vitro should therefore be chemically defined, and it should either be serum-free or utilize synthetic serum replacements or autologous serum.[45,46] The possible supplementation of specific recombinant cytokines and growth factors may enhance the growth and differentiation of cells, especially when a serum-free medium is in use.[47] To date, autologous serum can be considered the ideal source of nutritional and stimulatory support of cells when cells are used in immunocompetent animals or in clinical trials. Multiplication of cells in an autologous serum-based medium is therefore used in most clinical bone reconstruction therapies. Therefore, it is convenient to select suitable experimental conditions for cultivation. As with stem cell cultivation, the culture conditions should be well defined in order to standardize the product.

Harvesting of Determined Cells

As the use of determined osteoblastlike cells, unlike the use of stem cells, does not raise legal issues, and there are no problems of immune rejection, determined bone cells are at present the most important cell source in clinical cell-based bone reconstruction strategies. Cultures containing determined "osteoblastic" or "osteoblastlike" cells have been established from different cell populations in the lineage of osteogenic cells[48] (osteoprogenitor cells, lining cells, osteoblasts, and osteocytes). They can be derived from several anatomic sites using different explant procedures. Bone cell populations may be derived from the cortical or cancellous bone, bone marrow, periosteum, and in some instances, from other tissues. Isolation of determined cells embedded in calcified or noncalcified tissue structures can be performed with a variety of techniques, including mechanical disruption, explant outgrowth, and enzyme digestion.[49] Digestive or outgrowth measures are commonly used procedures to gain cells. Outgrowth of bonelike cells can be achieved by culturing periosteum pieces or bone explants. Cells located within the periosteum and bone can differentiate into fibroblastic, osteogenic, or reticular cells.[50–54] Periosteal-derived mesenchymal precursor cells, like bone marrow cells, generate progenitor cells committed to 1 or more cell lines with an apparent degree of plasticity and interconversion.[55–61] In culture, expanded bone cells were shown to retain their ability to heal bone defects after being reimplanted and to induce osteogenic tissue.[62]

It remains uncertain whether cortical or spongy bone, gained by different postsurgical processing techniques, is a better choice of material[62–64] to grow cells in culture. It has been suggested that particulate culturing is superior to bone chip culturing[65,66] because when particle size decreases, the total amount of surface area of the tissue specimens in the culture dish increases, which would increase the amount of living cells released. Springer and associates[67] demonstrated in an experimental study that bone chips obtained from trabecular bone provided a higher number of cells than those from cortical bone. Surprisingly, they found that processing spongy bone graft in the bone mill to created bone particulate resulted in lower absolute amounts of osteoblastlike cells, whereas the use of the bone mill with cortical bone had a smaller impact on the number of cells counted. The authors speculated that the use of a bone mill or rotating instruments could reduce the amount of bone cells supplied.

In contrast to the use of outgrowth measures, Ecarot-Charrier and colleagues[68] were the first to present a method for isolating osteoblasts from newborn mouse calvaria using digestive enzymes in solution.

As it has been demonstrated that isolated osteoblasts gained through this method retain their unique properties in culture, tissue digestion has become a common method of cell harvesting for in vitro purposes.

IMMUNOLOGIC ASPECTS

Immunologic reactions against the transplanted cells and the host's response to the carrier material are critical after transplantation of cells.[69] Implant rejection can be avoided or at least diminished in clinical practice by using autologous or isogeneic cells as the principal cell source. Cells derived from an autologous source can be rendered immunogenic if they are genetically modified[70] or extensively cultured in vitro[71] prior to their transplantation. If allogeneic or xenogeneic cells are incorporated into the device, the prevention of immune rejection by immune suppressive treatment, the induction of tolerance in the host, or immuno-modulation of the graft become important clinical issues.[72] The use of allogeneic or xenogeneic cells has the principal clinical advantages of avoiding the need for cell harvesting from the patient and time delay between initial cell explantation and final implantation, but immunologic concerns limit the clinical use of these materials today. Various strategies focusing on immunosuppressive therapies or downregulation of immunogenity of cells are under intensive investigation. Microencapsulation, for example, is a clinically applied method to prevent immune reaction against allogeneic or xenogeneic cells.[73]

Genetic Engineering

Various genetic engineering strategies are closely related to cell-based tissue engineering strategies.[74–76] The strategies include local or systemic delivery approaches in vivo, especially the transfection of cells ex vivo.[77,78]

Local gene therapy in bone healing can be considered a form of cell-driven repair strategy.[79,80] Two approaches can be distinguished: first, vectors can be applied directly to/introduced directly into the host tissue (in vivo),[81–84] and secondly, cells harvested from the patient can be genetically modified by DNA transfection in vitro. For the ex vivo tissue engineering approach, genetically altered cells are then attached to a carrier (gene activated matrices) and reimplanted. Ex vivo gene delivery can be considered a more defined method of cell-based bone engineering than vector application to the host site.[85] This technique is safer than the in vivo approach because gene transfer and manipulation occur outside the body under controlled in vitro conditions. An additional advantage of this method is that nearly all cells can be transfected; this is very hard to achieve in vivo. Experimental studies have shown that for bone tissue engineering gene therapy is effective in maxillofacial bone reconstruction. The effectiveness of gene therapy has been demonstrated by in vitro and in vivo studies. Although gene delivery methods have been successful in promotion of bone healing in experiments with animal models,[86–88] clinical studies are currently not available, since such trials are hindered by legal and ethical issues.

Clinical Aspects

The evaluation of a multitude of animal experimental studies indicates that cell-based bone regeneration strategies are able to accelerate bone healing.[89–91] It was recently demonstrated that even mandibular defects can be regenerated under distinct circumstances by the use of cell transplantation technology.[92] The first clinical investigations on cell transplantation studies have therefore been performed in the maxillofacial area.[93] A special region of interest is the development of tissue engineering strategies to optimize implant osseointegration.[94] The challenge in the tissue engineering of alveolar bone surrounding dental implants can be achieved by noncellular or cellular measures. Altering the implant surface structure,[95] coating implant surfaces with biomolecules or genes, or application of targeted and sustained delivery systems of growth-promoting molecules at the osteotomy site can be considered as noncellular approaches.[96] In contrast, peri-implant bone reconstruction by placement of tissue-engineered bone using mature or premature bone cells (MSCs), often in combination with cytokines, is a cellular approach.[97–100] Both therapeutic options are used in implant dentistry. The aim is to shift from merely achieving successful osseointegration to achieving final restorative outcomes that mimic natural dentition and their surrounding oral tissues. As optimal esthetics of implants requires their placement in a position approximating that of the natural teeth they replace, tissue engineering strategies may help to improve esthetics outcomes in implant dentistry.[101] In order to regenerate orofacial structures to an extent that the artificially generated tissue mimics the naturally occurring tissue structure, improvements have to be made in all fields of engineering. Attention has mainly been paid to biological and material-based strategies. In material science, improvements have been made in the implementation of new materials (naturally occurring extracellular matrices, hydrogels, new synthetic materials) and new technologies (nanotechnology, solid free-form fabrication).[95,102–104] The fabrication of cell-containing complex scaffolds containing factors (proteins, genes) that can be released to a predeter-

mined extent enables researchers to create an artificial bonelike tissue.[105,106]

From a clinical point of view, clinicians should be aware that cell-based maxillofacial bone reconstruction therapies have unique features, as they differ in different anatomic locations. For example, bony regeneration is often observed after a sinus lift procedure in the maxillary sinus region, even in the absence of implanted biomaterial, whereas reconstruction is more difficult to achieve in mandibular bone. As this technique is only at the beginning of clinical use, no detailed information can be given on the effectiveness of ex vivo cell-based approaches. A critical evaluation of outcome measures on well-designed, well-conducted studies has to be performed before this treatment can be considered a standard measure to regenerate maxillofacial bone.

CONCLUSION

Cell-based bone tissue engineering is a rapidly evolving therapy option in bone reconstruction.[107] Some cell-driven approaches (most of them covered by the broader US definition of tissue engineering) have gained acceptance for routine clinical work in maxillofacial surgery, whereas other strategies (eg, cell implantation) have reached the level of first clinical trials. More advanced or complex therapeutic options (extracorporeal tissue engineering, genetic engineering) may afford future surgeons a limitless supply of autologous tissue for bone repair and reconstruction. Recent studies are directed to the engineering of hybrid organ structures by advanced tissue engineering concepts.[108] Knowledge of the biological, engineering, and clinical challenges as well as their inherent constraints will presumably improve the outcome of bone reconstruction therapies in the near future.

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