Chapter 7

Bone Regeneration of the Cranio-maxillofacial and Dento-alveolar Skeletons in the Framework of Tissue Engineering

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Summary

Bone regeneration in the cranio-maxillofacial skeleton has undergone many advances over a short period of time. There is much activity in this area, where autogenous bone grafting still plays a significant role in clinical practice. Cranio-maxillofacial osseous reconstruction represents a very large potential market effecting many surgical specialties including, oral maxillofacial surgery, plastic surgery, otolaryngology, neurosurgery, general surgery and head and neck oncology. The area is also of vital interest to most specialties of dentistry including periodontics, orthodontics, endodontics, and even general dental practice. Indeed these combined specialties form the market basis for the development of many commercial products. Some have proven to be useful, others have been most disappointing. The future of tissue engineering in this particular anatomic area is not only bright, it is necessary. This chapter reviews the historical aspects of osseous reconstruction in this region, the efforts to minimize morbidity, and discusses new directions that the promise of tissue engineering may bring to this area.

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Introduction

Dento-alveolar bony defects are very common and pose a significant problem in dental treatment and rehabilitation. Reconstruction of dento-alveolar bony defects using minimally morbid techniques would greatly enhance the success and patient acceptance of this area of oral and maxillofacial reconstructive surgery. The potential market in the reconstruction of this area is great and includes virtually every dentist and dental specialist in some way. There are many patients who are just now discovering the fact that their jaws can be reconstructed with dental implants. Most of these patients require osseous reconstruction as well. This is the basis for the demand and market for dento-alveolar reconstruction. It is the reason why there have been so many reconstructive efforts in this relatively small anatomic area

However patient acceptance of such reconstructive procedures has been guarded at best. This is largely because both practitioners and patients alike have perceived these reconstructive methods as being rather invasive. Therefore the impetus of our research group has been the reduction of the morbidity associated with dento-alveolar and cranio-maxillofacial osseous reconstruction. The ultimate goal is to help increase patient acceptance and utilization of such techniques.

The reduction in morbidity could come from two approaches, either by the development of less invasive bone graft harvesting techniques or by the elimination of the bone graft donor sites by using a bone graft substitute or tissue engineering techniques. Even more attractive is the thought that hybrid grafts are now on the forefront. This means that tissue engineering principles and techniques can now take their rightful place in the armamentarium of the oral and maxillofacial surgeon who seeks to reconstruct the tooth bearing parts of the jaws using these novel techniques. In order to understand these emerging principles we must first understand bone as it pertains specifically to the dento-alveolar skeleton, the cells, the growth factors involved as well as the currently existing reconstructive options.

Structure, Function and Physiology of Bone

Bone is a specialized connective tissue with a mineralized extra-cellular matrix that functions to provide support, form and rigidity for the human skeleton and supplies a vast store of calcium necessary for calcium related homeostasis (1-6).

Embryologically, bone is formed by two separate developmental processes described as intra-membranous and endochondral ossification (7, 8). When ossification has occurred directly, it is classified as being intra-membranous in character. Embryonic mesenchymal cells with an abundant vascular supply develop loci of intracellular collagen deposition. Osteoblasts begin secreting osteoid into which calcium salts are deposited. Such direct bone formation is responsible for the genesis of the cranial vault, the facial skeleton and parts of the mandible, scapula and clavicle. Endochondral bone formation, involves a cartilaginous phase, where embryonic mesenchymal stem cells differentiate into a primitive hyaline cartilage. Blood vessels and bone forming units resorb the cartilage and replace it with osteoid while invading this matrix. Weight-bearing bones and those terminating in joints comprise most of this group of bones. In addition, most of the cranial base and a portion of the mandible are thought to have an endochondral origin (9). These embryologic origins should be kept in mind with future tissue engineering attempts.

Bone is composed of four cellular types; osteoblasts, osteocytes, osteoclasts and bone lining cells. Osteoblasts are cuboidal cells having a prominent Golgi apparatus and well-developed rough endoplasmic reticulum, a histological sign of protein production. These fully differentiated cells secrete both the type I collagen and the non-collagenous proteins of bone's organic matrix. They will also regulate the mineralization of this matrix. The osteocyte is thought to be a mature osteoblast that becomes trapped within the bone matrix. While their primary function is maintenance, they have demonstrated abilities to both synthesize and resorb bone (10). Bone lining cells are flat, fusiform cells that are found covering inactive bone surfaces. Little is known about the function of these cells; however they may be the precursors of osteoblasts. It is understood that certain cells (osteoprogenitor cells) are programmed to become bone cells and their origin is believed to lie with the primitive mesenchymal stem cells (11). Osteoclasts, unlike the other bone cells, which have local origins, arise from the fusion of mononuclear precursor cells originating in the

hematopoietic tissues. They function to resorb bone. Histologically, they have been characterized as having a ruffled border, where bone resorption is thought to occur. Coupling describes a process, which combines all of the above elements, whereby bone formation and resorption are maintained in balance (12). Once this balance is disrupted, excessive osteoclastic activity may lead to problems such as osteoporosis whereas increased osteoblastic activity may reflect bone growth, healing or pathological responses.

The architecture of bone is such that the outer shell of bone, referred to as cortical or compact bone, provides the mechanical support. It is composed of concentric sheets of collagen fibrils in the form of lamellar bone. The metabolic functions of bone are controlled by the centrally located cancellous, trabecular or spongy bone. In contrast to the densely packed fibrils of the cortical bone, the matrix of cancellous bone is loosely organized. Macroscopically, this bone appears as a honeycomb lattice in which hematopoietic elements are located. Bone is composed of 65 - 70% crystalline salts by weight, primarily in the form of hydroxyapatite, with the remaining 30 - 35% being composed of organic matrix. The organic matrix consists primarily of type I collagen (90 - 95%) interspersed with non-collagenous proteins such as osteopontin, osteocalcin, osteonectin, bone sialoprotein and various growth factors (13, 14).

The Unique Aspects of Alveolar Ridge Defects and Resorption

Alveolar bone is that specialized part of the cranio-maxillofacial skeleton that forms the primary support for the teeth. Alveolar bone is composed of bundles of bone which is built up in layers in a parallel orientation to the coronal-apical direction of the tooth. The anterior maxillary bone is less dense than mandibular bone but more dense than maxillary posterior bone (15).

Alveolar ridge defects and deformities can be the result of congenital maldevelopment, trauma, periodontal disease or surgical ablation, as in the case of tumor surgery. Resorption after tooth-loss

has been shown to follow a predictable pattern: the labial aspect of the alveolar crest is the principal site of resorption, which first reduces first in width and later in height (16-18).

Alveolar bone is resorbed after tooth extraction or avulsion most rapidly during the first years. Non-traumatic loss of anterior maxillary teeth is followed by a progressive loss of bone mainly from the labial side. The magnitude of bone loss is estimated to be 40-60 % during the first 3 years following tooth-loss and then decreases to a 0.25 – 0.5 % annual loss rate thereafter (19, 20). In the deciduous paediatric dentition, loss of a retained second deciduous molar, which has no succedaneous permanent tooth to replace it, is also associated with bone loss. The rates of bone loss at these sites have manifested as alveolar ridge width decreases of 25% within 3 years after extraction of the retained primary molars, and this continues to diminish by a further 4% over the next 3 years (21). The cause for resorption of alveolar bone after tooth-loss has been assumed to be due to disuse atrophy, decreased blood supply, localized inflammation or unfavorable prosthesis pressure (20, 22).

Prevention of Alveolar Ridge Resorption

One strategy to deal with alveolar bone loss without resorting to a bone graft is to prevent its occurrence. A number of methods have been tried including the retention of tooth roots to help maintain the alveolus. These retained tooth roots can be used as abutments for overdentures for example and are effective at halting the process of alveolar ridge resorption (23). Malmgren *et al.* have introduced a method in which the alveolar ridge is preserved by removing the crown and filling the root of an ankylosed and infrapositioned tooth. The decoronated root is left in situ for slow resorption (24, 25). Other treatment alternatives to preserve alveolar bone without the use of bone grafts include autotransplantation of teeth (26) and orthodontic space closure (21). Simply adding a bone graft to alveolar bone and allowing it to function by loding it with a tissue bourne dental prosthesis such as a denture will only lead to continued resorption of the bone graft. The bone graft will ultimately be totally resorbed. The alveolar bone loss will then continue under the denture (27). This method of reconstruction with a bone graft and a tissue bourne dental

prosthesis should be regarded as only a temporary measure in today's world. Surgery and prosthodontics can be combined into a brilliant group effort to ensure the co-ordinated reconstruction of such demanding clinical situations. The placement of dental implants into alveolar bone or grafted alveolar bone has been shown to prevent further alveolar resorption, and this represents today's goal in reconstruction of the masticatory apparatus (28-32).

Methods to Augment Deficient Bone

The reconstructive options in the osseous reconstruction of the cranio-maxillofacial skeleton include autogenous bone grafts harvested from local or distant sources (33). Allogeneic bone from another individual may also be considered, as might xenogeneic bone from another species. Because the possibilities of immunogenic problems exist, such grafts were first treated with a freezing technique (34). Later other methods to deal with immunogenicity were developed (35). Alloplasts have also been developed to replace bone. In addition a number of surgical procedures have been designed to increase the amount of bone available locally without bone grafting (36-38). Bone reconstruction is best understood if the process of bone healing is first considered (39).

Osteoinduction

Osteoinduction describes a process whereby new bone is produced in an area where there was no bone before, where one tissue or its derivative causes another undifferentiated tissue to differentiate into bone. The phenomenon of osteoinduction was first described in the classic works of Urist (40-42). Bone matrix was shown to induce bone formation within muscle pouches of many species of animals. Later a specific extract from bone, a protein now referred to as Bone Morphogenetic Protein (BMP), was identified as that factor which caused the phenomenon (43, 44). Since then a

great deal of research has resulted in the discovery of a variety of entities having different effects on bone (45). These compounds may be classified as osteoinducers, osteopromoters or bioactive peptides (46).

Osteoconduction

Osteoconduction describes bone formation by the process of ingrowth of capillaries and osteoprogenitor cells from the recipient bed into, around and through a graft or bioimplant. Therefore the graft or bioimplant acts as a scaffold for new bone formation (35). Unlike osteoinduction, this process occurs in an already bone containing environment. Osteoconduction describes the facilitation of bone growth along a scaffold of autogenous, allogenic or alloplastic materials.

Local Procedures to Augment Existing Alveolar Bone

There are a number of techniques, which enable the surgeon to maximize the available bone in the cranio-maxillofacial skeleton without harvesting a bone graft. An appreciation of these existing techniques and strategies will help us understand the future application of tissue engineering to dento-alveolar and cranio-maxillofacial osseous reconstruction. These techniques serve to minimize reconstructive morbidity, as there is no graft donor site. Osteocondensation is one such technique. It can reshape the morphology of the alveolar bone of the maxilla for example, by compacting it in various directions using the condensing chisels or plungers. The procedure can establish a new contorur of the bone being condensed. This allows the clinician who is placing dental implants to more optimally house a dental implant, resulting in better primary stability in areas of poor bone quality. Orthopaedic surgeons have practiced osteocondensation since the early 1960s (47). The

major advantage of this technique is that an implant bed is created with either minimal drilling or no bone removal (48) and with osteotomes, which compress the bone. There are implants, which produce osteocondensation and are called press-fit fixtures (47, 49). In the cranio-maxillofacial skeleton, osteocondensation is best performed in the maxilla.

The major proponent of osteocondensation in oral and cranio-maxillofacial skeleton has been Summers who described a method to increase the width of alveolar bone and to facilitate sinus floor elevation, without opening the lateral sinus wall (50-53). The technique was further developed to include the use of D-shaped osteotomes and chisels which produced lateral widening of the alveolar ridge and osteocompression, increasing the density of cancellous bone (48, 54). The ridge expansion osteotomy is achievable using osteotomes which have concave tips and sharpened edges. The instruments are shaped to allow progressively larger osteotome tips to fit into the opening created by the previous osteotome. Instruments are sensitive to changes in bone texture and density and allow excellent tactile sensation for the surgeon (49). The minimum alveolar width necessary for lateral alveolar widening by compression is 2-3 mm assuming that spongious bone is found between cortical layers (50).

Alveolar ridges can also be widened using the crestal split technique using osteotomes and chisels to produce a “greenstick fracture” at the base of the alveolus. The remaining periosteum is left intact and attached to the bone. This pedicled buccal cortex is repositioned and a new implant bed is created without any drilling. Lateral widening by completely exposing the labial cortex has also been introduced (55). The major benefit of crestal widening is that it allows the thin alveolar bone to be utilized for implantation without grafting (37). Esthetics and implant positioning are improved and wider implants can also be used. The bone can be moulded to some extent due to its viscosity (48). Bone compression is achieved along with an increase in the density of trabeculations of the adjacent site (56). In addition the resulting gap can, if desired be covered by a nonresorbable membrane (57, 58) and filled with allogenic material (58). Interpositional autogenous bone grafts have been used to improve bony healing in the gap (59).

Guided bone regeneration (GBR) has been used for minor augmentation procedures in the cranio-maxillofacial skeleton and prior to dental implant placement (36, 60-63). GBR is a technique in which bone growth is enhanced by preventing soft tissue ingrowth into the desired area and

utilizes either resorbable or nonresorbable membranes. Metallic membranes (63) or membranes supported by a titanium frame (63) have been tested and have been successful. An acellular dermal matrix has been used as a barrier membrane with demineralized freeze-dried bone allograft (64).

The use of membranes is a controversial issue in dental implantology and their use is certainly very technique-sensitive (65). The use of nonresorbable membranes requires a second operation for their removal (63). Resorbable membranes can be associated with inflammation (66). Intact periosteum, a split palatal or gingival flap are regarded by some as natural membranes and their use may obviate the need for a membrane (67). Nevertheless, good results with augmentation procedures using membranes have been presented (59, 62-64, 66).Vertical increase of a narrow alveolar crest has been shown to be possible with membranes (63, 64).

Distraction osteogenesis (DO) of the long bones in growing children has been used for decades to gradually lengthen osteotomized bones without a bone graft. The resulting distraction gap is initially filled with callus, which later matures into bone (68). DO has also been adapted to the maxillofacial area and special devices and implants are being developed for that purpose (37, 69).

The DO technique has also been adapted for limited augmentations of the alveolar crest prior to implantation. Some systems use hardware, which expands the jaw over time, and then is removed at the time of dental implant placement (69). Some have tried to utilize the implant itself as the distraction device (36, 70, 71). The daily rate of alveolar crest distraction ranges from 0.25-0.5 mm and is initiated from two days to one week after the primary osteotomy. DO is continued up to 30 days and the final gain will be between 4 and 7 mm (37, 72). In some cases overcorrection is recommended (37). However some local limitations due to the lack of stretching of the palatal tissues, may not allow the distracted segment to move exactly as planned. Appliances allowing three-dimensional DO have been introduced (67, 69). The benefits of DO are that donor site morbidity from harvesting of bone grafts and dehiscences of grafted bone are avoided (71). However, a second surgery to remove and perhaps replace hardware is needed if dental implant-based distraction is not used. While DO could eliminate a donor site and thereby limit morbidity, it is so labour intensive that the patient trades the morbidity of the bone graft donor site for the inconvenience of wearing and tolerating potentially cumbersome hardware for longer periods of time.

Autografts

At the present time, autogenous bone grafting is the gold standard by which all techniques of osseous reconstruction of the cranio-maxillofacial skeleton must be judged. Autogenous cancellous bone grafts produce the most successful and predictable results (73). Free bone grafts act mostly as scaffolds and are thus more osteoconductive than osteoinductive even though osteogenic activity may have remained in the spongious part of the graft (35). The major disadvantage of autogenous grafts is the need for a second surgical site and the morbidity resulting from harvesting. The source of autograft, however, is not limitless for the patient. A point may be reached in reconstruction where the donor site morbidity may exceed the discomfort of the presenting complaint. Moreover such potential discomfort is a serious reason for patients to avoid presenting themselves for reconstructive procedures.

There are essentially two forms of nonvascularized free autogenous bone grafts: cortical and cancellous (74-76). Buchardt has summarized the three essential differences between the two. Cancellous grafts are revascularized more rapidly and completely than cortical grafts. Creeping substitution of a cancellous graft initially involves an appositional bone formation phase, followed by a resorptive phase, whereas cortical grafts undergo a reverse creeping substitution process. Cancellous grafts tend to repair completely with time whereas cortical grafts remain as an admixture of necrotic and viable bone (35).

Cortical grafts are able to withstand mechanical forces earlier however, they take more time to revascularize. Cortical grafts are useful for filling defects where early mechanical loading is required (77). The cortical component can be incorporated into the fixation of the graft and can consequently be used in situations where bone is comminuted or where there are bony voids. In the cranio-maxillofacial skeleton these forms of grafts may also be used to onlay areas such as decreased vertical or horizontal alveolar ridges, to improve facial contours or they can be inlayed within bone to fill bony voids. Common sites for the harvesting of cortical grafts are the cranial vault, ribs and the medial or lateral table of the anterior aspect of the iliac crest, the posterior iliac crest as well as the mandibular symphysis (78-80).

Cancellous grafts have more widespread applications, are generally easier to manipulate and revascularize more rapidly (81). The most abundant source of cancellous bone is the anterior or posterior iliac crest. Cancellous bone imparts no mechanical strength so that when it is used to reconstruct large continuity defects additional stability and rigid fixation is required. In the cranio-maxillofacial skeleton these grafts are packed into bony defects such as alveolar clefts and maxillary sinus floor elevations (82). The Corticocancellous graft usually produces the best results by combining the attributes of both graft forms and can be place easily into an interpositional location (83, 84). These grafts allow for mechanical stabilization while at the same time providing for good revascularization. Others will particulate Corticocancellous bone creating a mixed graft which can be used for the restoration of continuity defects in the jaws (85-88).

Particulate bone grafts can be very advantageous. They can easily be harvested from intra oral sites using a specialy designed bone harvesting device or suction trap to collect the bone chips produced by drilling over the surface bone of a donor site (Fig. 1). The more morbidity of such particulate graft harvests is low and the patient acceptance is very good (33, 78-80). The particulate grafts have the distinct advantageous of being easily molded to the contours of most defects, as long as there is some underlying bony support. The volume available for harvesting an intra oral particulate graft is much less than that available with traditional extra oral harvesting techniques (33, 79). Their main limiting factor, however, is the lack of inherent stability of such grafts, unlike cortical or cortico-cancellous bone grafts. Particulate bone grafts are only as structurally stable as their underlying support from the already available alveolar bone for example. These particulate grafts can be packed around bony defects when dental implants are placed concurrently. Again the structural support for such grafts is also derived from the underlying bone and the stable dental implant fixtures themselves. Particulate bone grafts therefore have no structural integrity of their own, at the time of their placement (78-80).

Potential Autogenous Bone Graft Donor Sites

Autogenous bone grafts can be vascularized or non-vascularized. Vascularized bone grafts are much more complex to harvest and have a great deal of donor site morbidity associated with their use. Non-vascularized grafts are considerably simpler to harvest and use if they are placed into a well vascularized recipient bed (81).

Both intra-oral and extra-oral bony donor sites have been used successfully as sources of non-vascularized autogenous bone for grafting of maxillofacial defects (81). The volume of bone graft required determines the choice of the donor site.

If the defect is small, often local, intra-oral sources can be used (89). Intra-oral sites are often preferred since they allow harvesting of bone from the area adjacent to the reconstruction. A second distant surgical site and the extra-oral scar can be avoided. Intra-oral harvesting can mostly be performed on an outpatient basis under local anaesthesia. These intra-oral sites can include mandibular symphysis, mandibular ramus and retromolar area, coronoid process, maxillary tuberosity, maxillary torus palatinus or mandibular tori, if they are present, and the zygomatic bone using a specially designed bone collector or suction trap (90, 91) (Fig. 1). However the volume of bone available in intra-oral sites may be insufficient for moderate to large defects (33).

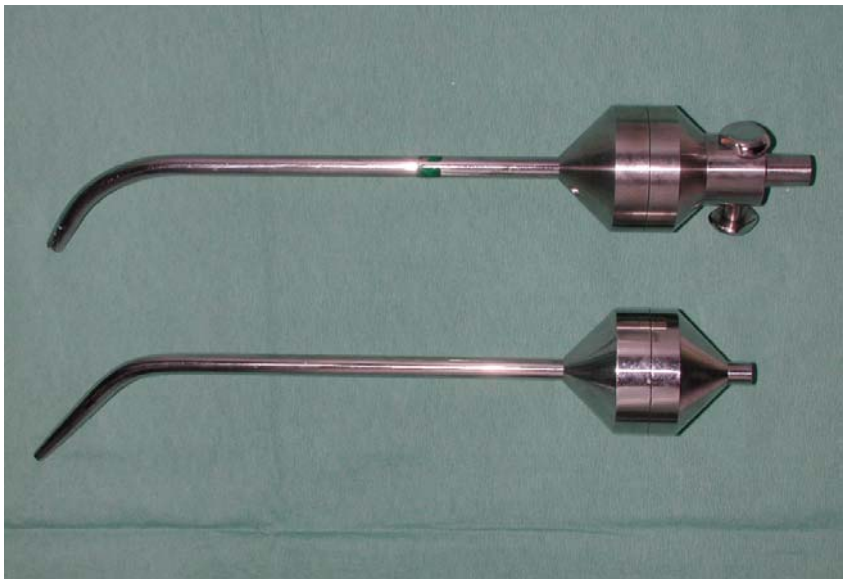
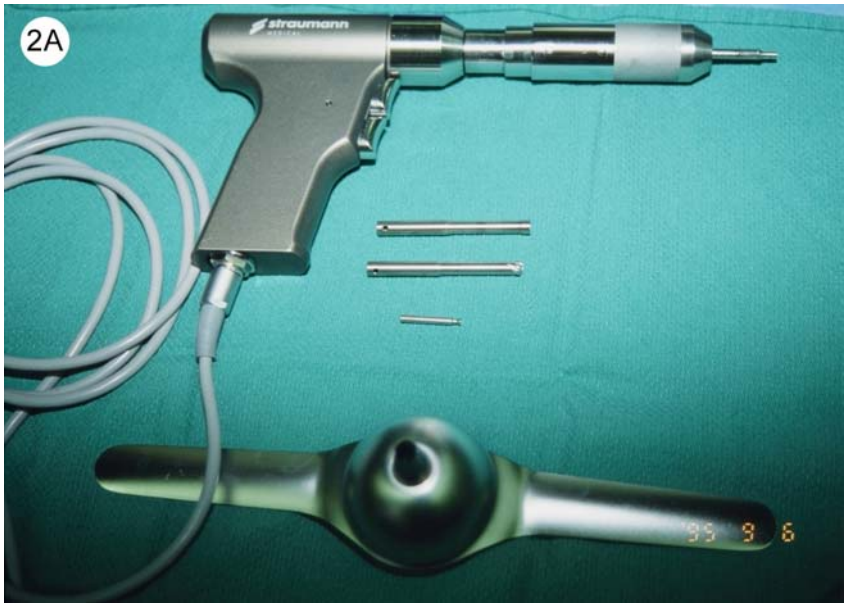


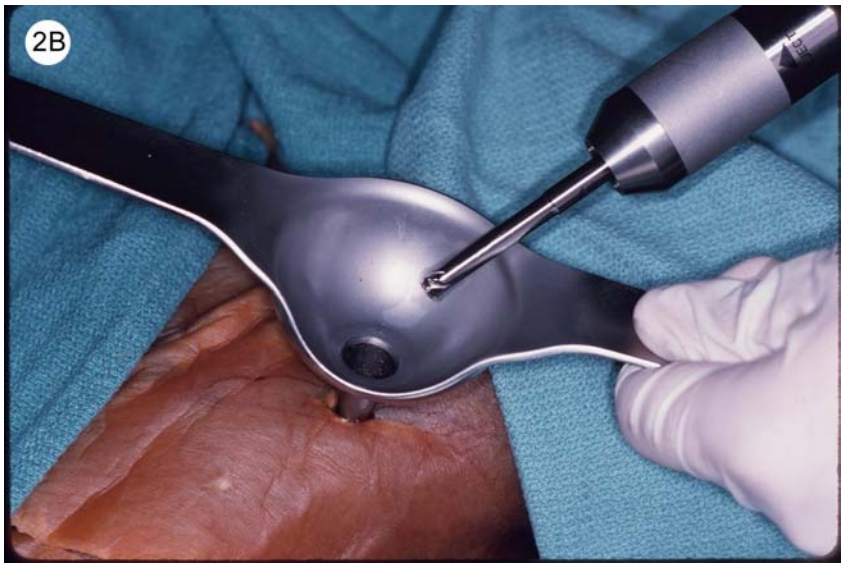
Fig. 1: A specially designed bone collector used to harvest intra-oral cortical bone grafts of membranous origin, such as from the zygomatic bone. This collector is used as a suction trap. The surface of the donor site is drilled or trephined with a series of burrs producing a fine dust or slurry of bone. This is suctioned into the bone trap. Great care is taken during an intra oral harvest to avoid suctioning saliva and dental plaque or other tooth debris into the harvested bone particles. The suction trap has two control features to avoid this potential harvesting problem.

When a greater volume of bone is required, extra-oral sources are usually employed. These may include the anterior or posterior iliac crest, the calvarium, the rib and the proximal tibia (77, 78, 92) (Fig. 2 a, Fig. 2b).

In fact specially designed devices have been developed to minimize the morbidity at the second surgical site, made necessary by the harvesting of such grafts. The motorized trephine shown in Figure 2a consists of a pre-cutter, an internal bone forcep, and a trephine that is capable of ejecting the harvested cancellous bone core from the anterior iliac crest. This motorized trephine can be used through a small, 1 cm stab incision over the anterior iliac crest. Up to 7 cores of bone measuring 4.1 mm in diameter by 30 mm in length can be harvested from each anterior iliac crest (Fig. 2b). The intervening bone between the harvested bone cores can also be removed, doubling the size of the harvest. The harvested cores (Fig. 2c) appear to be well trabeculated in the histologic section that is shown. The grafts can be seen to be quite cellular, containing many osteogenic elements. This is

one of the main advantages of such an autogenous bone graft. The morbidity of this technique is much lower compared to traditional open anterior iliac crest harvesting techniques. Open procedures generally require inpatient hospital admission of patients; the closed trephine approach is routinely performed in day surgery, as an outpatient procedure without hospital admission (78, 80).





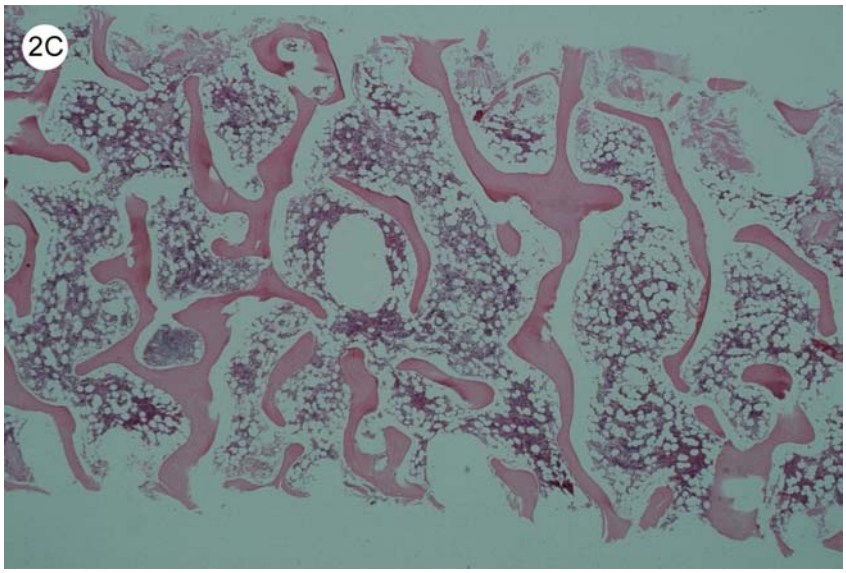


Fig. 2: Minimizing the morbidity of extra-oral bone graft harvesting using a percutaneous power-driven trephine to procure bone graft material from the anterior iliac crest.

In figure 2a, the components of the motorized trephine are shown. The device consists of a motorized drilling unit, an internal forceps, a bone pre-cutter and a trephine. The device easily ejects the cores of cancellous bone which it can easily harvest from the anterior iliac crest.

In figure 2b a small 1 cm stab incision has been made and the trephine engages the anterior iliac crest through this simple percutaneous approach. A funnel or propeller shaped retractor is shown keeping the soft tissues away. In figure 2c there is a photomicrograph of a bone core harvested from the anterior iliac crest. Note the well trabeculated nature of this cancellous bone graft, and its great cellularity.

Allografts

Allogeneic bone is non-vital osseous tissue taken from one individual and transferred to another individual of the same species. There are three forms of allogeneic bone: fresh frozen, freeze-dried and demineralized bone matrix (DBM). Fresh frozen bone is rarely used today for the purposes of bony reconstruction in the cranio-maxillofacial skeleton because of concerns related to the transmission of viral diseases (35). The risk of transmitting HIV with a properly screened demineralized freeze-dried bone allograft has been calculated to be 1 in 2.8 billion (93). Bone

harvested from a patient who died from AIDS related disease was tested for the p24 core protein and reverse transcriptase and found to be positive. When this same bone was processed to make DBM, no evidence of either was found (94). It is therefore assumed that the process to make DBM eliminates or inactivates the p24 core protein and reverse transcriptase.

Freeze-dried allogeneic bone is processed to remove the moisture from the bone. This results in an implant with mechanical strength that can be used to onlay areas or as a crib to retain autogenous bone (81). This implant, while osteoconductive, has no osteogenic or osteoinductive capabilities and consequently requires a source of osteocompetent cells. Therefore freeze-dried allogeneic implants are usually placed in conjunction with autogeneic grafts when reconstructing the cranio-maxillofacial skeleton.

By demineralizing the freeze-dried bone to create DBM, the implant loses its mechanical strength but may retain some osteoinductive properties (95-97). Removal of the mineral component from the bone matrix may expose native proteins, such as bone morphogenetic protein (BMP). The potential osteoinductive capabilities of DBM make it a valuable tool for the surgeon.

Recent advances have seen DBM incorporated into various carriers such as collagen or selected polymers (98-100). These forms are either sponge-like or gel/putty-like in consistency. Putties are simple to apply and are well retained within the recipient tissue bed. These products could potentially be used in the treatment of periodontal infrabony defects, extraction sites to prevent ridge resorption, alveolar ridge reconstruction, bone reconstruction associated with dental implant placement, bone reconstruction associated with dental implant complications and cysts or bony defects of the jaws (101, 102, 103, 104, 105, 106, 107, 108,109). If larger volumes of bone are required, such as in maxillary sinus augmentation prior to dental implant placement, then DBM may be used as a bone graft expander to reduce the volume of bone graft required to fill an osseous defect (110-112). This reduced graft volume may allow the use of an intra-oral harvest site. While reducing patient morbidity by avoiding an extra-oral donor site, the major disadvantage of this technique is the cost of the DBM material.

Xenografts

Xenogeneic bone grafts consist of skeletal tissue that is harvested from one species and transferred to the recipient site of another species (113, 114). These grafts can be derived from mammalian bones and coral exoskeletons. Bovine derived bone has been commonly used (115, 116), even though other sources are such as porcine or murine bone are available. Xenogeneic bone was popular in the 1960's but fell into disfavour due to reports of patients developing autoimmune diseases following bovine bone transplants (35, 117). The re-introduction of these products in the 1990's comes after the development of methods to deproteinate bone particles (118). This processing reduces the antigenicity making these implants more tolerable to host tissues (119). The result is that the organic component of bone, referred to at the beginning of this chapter, is almost completely removed.

This inorganic bone matrix then has the structure of bone making it osteoconductive without the osteoinductive abilities imparted by the organic elements. Eventually xenogenic bone should be replaced by host tissue, which would make it useful for defect or extraction site filling in the alveolus prior to dental implant placement or prosthetic rehabilitation (120-126). Resorption of bovine derived bone has been observed in animals studies (127) but not consistently in human clinical trials (125, 126, 128). Since the material is usually a powder it may require some form of retentive structure such as a membrane to keep the xenograft in the desired location (129-132). While bovine xenografts may reduce morbidity by eliminating the donor site, their disadvantage is the concern with the possibility of future bovine spongiform encephalopathy due to potential slow virus transmission in bovine-derived products (133, 134).

One interesting xenogeneic transplant, Biocoral, is derived directly from the exoskeletons of corals from the Group Madrepora of the genus *acropora* (135). These corals are harvested from the relatively unpolluted waters of the reefs off New Caledonia, a point of importance since corals from contaminated waters can contain petrochemical impurities. Both solid blocks and particulated implants fashioned from this material are composed largely of calcium carbonate and are osteoconductive. They are simultaneously incorporated into the human bony skeleton and replaced by human bone. The enzyme carbonic anhydrase, liberated by osteoclasts is responsible for the breakdown of this material. The time for total replacement of this implant by bone in the human

craniofacial skeleton is approximately 18 months (136). Since the use of coral-derived granules gives rise to bone with the material's eventual replacement, it could decrease morbidity by avoiding a bone graft harvest donor site (137).

Synthetic Bone Substitutes

Alloplastic bone substitutes are synthetic substances that have been processed for clinical use in osseous regeneration. There are three types of alloplastic substances in clinical use today: hydroxyapatite, other ceramics and polymers.

Hydroxyapatite (HA) is a ceramic. HA can be divided into two groups depending upon its ability to resorb (138, 139, 140, 141). Some refer to the internal pore size as a means of differentiating between various types of hydroxyapatite (142-144). The porous form of HA allows rapid fibrovascular tissue ingrowth, which may stabilize the graft and help resist micromotion (145, 146). HA can be machined to many shapes or consistencies (147-149). HA has several potential clinical applications including the filling of bony defects, the retention of alveolar ridge form following tooth extraction and as a bone expander when combined with autogenous bone during ridge augmentation and sinus grafting procedures (150-154). Although the use of HA can eliminate donor site morbidity, the tendency for granular migration and incomplete resorption has become a long-term problem (155-158).

Apart from HA, there are three other types of ceramics: tricalcium phosphate (TCP), bioglasses, and calcium sulphate (159, 160, 161, 162, 163). TCP is a similar to HA being a calcium phosphate with a different stoichiometric profile (164, 165). TCP has been formulated into pastes, particles or blocks, which have demonstrated an ability to be biocompatible and biodegradable (166). Clinically the one disadvantage with TCP is its unpredictable rate of bioresorption. Its degradation has not always been associated with concomitant deposition of bone (167, 168). Two products (Norian SRS®, Norian Corporation, Cupertino, California, USA and Bone Source®, Leibinger, Dallas, Texas, USA) have

been used for the repair of cranial vault defects. Calcium salts are mixed with water to form a paste having an isothermic setting reaction and placed into the defect. Early versions of these materials tended to be easily washed out of the wound by haemorrhage. The materials tend to fracture and are resorbed unevenly in cranial vault defect studies (169).

Bioactive glasses are silico-phosphate chains that been used in dentistry as restorative materials such as glass ionomer cement. These materials have the ability to chemically bond with bone (170). Bioactive glasses may have osteoinductive properties and have been tested in animal trials (171). Bioactive glasses have been used in the treatment of periodontal bony defects (172, 173). In order to preserve the form of the alveolar ridge after tooth-loss, bioactive glass root replicates have been introduced (67). While these are able to preserve the crestal width and height of the alveolus, they may impair the later placement of dental implants due to incomplete resorption

Polymers by their nature can be fashioned in seemingly endless configurations (152, 174, 175). Combinations of polyglycolic acid (PGA) and polylactic acid (PLA) have been successfully used in the form of bioresorbable sutures for many years (176) and more recently as bioresorbable fixation materials (177, 178). Giant cell reactions presented as a problem with earlier combinations of this material (179). As with bioglasses, root replicates have been introduced to preserve the form of the alveolar ridge after tooth-loss. These are made of PLA (180). The ability of PLA implants to preserve the crestal width and height is an advantage. Unfortunately because of incomplete resorption they may impair the later placement of dental implants (180). The future of bone regeneration could lie with this class of synthetic materials (85). These materials could be better utilized once their ability to resorb at variable rates, over set periods of time is better understood and an appreciation for their compatibility with the emerging bioactive agents is developed. The ideal would be a completely synthetic bioimplant, which is predictably degradable and is innately osteocompetent (85). Such synthetic materials could also play a very important role in tissue engineering (181), serving as bioactive scaffolds.

One important advantage related to all xenogenic and allogenic materials is that they could potentially be used as bone graft expanders by mixing them with autogenous bone chips. This

mixing could decrease the volume of autogenous bone graft needed, which in turn could convert an extra-oral harvesting procedure to an intra-oral harvesting procedure potentially reducing donor site morbidity (33, 128).

Osteoactive Agents

An osteoactive agent is any material which has the ability to stimulate the deposition of bone (85). The phenomenon of osteoinduction was first described in the works of Urist and co-workers in (40, 95, 182). Bone matrix was shown to induce bone formation when implanted within muscle pouches of a number of different species of animals. Urist's group identified a specific extract from bone, a protein now referred to as Bone Morphogenetic Protein (BMP), as that factor which caused the phenomenon (41-43). Since then, many other entities have been found with a variety of effects on bone (44). These may be classified as osteoinducers, osteopromotors or bioactive peptides (45).

The compounds in the first two categories are growth factors, a group of complex proteins of approximately 6 to 45 kilo Daltons which function to regulate normal physiological processes and biological activities such as receptor signaling, DNA synthesis, and cell proliferation (183, 184). Growth factors that are referred to as cytokines have a lymphocytic origin, being nonantibody proteins released by one cell population on contact with a specific antigen and act as intracellular mediators. Other growth factors are described as morphogens. These are diffusible substances in embryonic tissues that influence the evolution and development of form, shape or growth. Still other growth factors are mitogens. They induce blast transformation by regulating DNA, RNA and protein synthesis (185).

Bone Morphogenetic Protein

Bone morphogenetic protein (BMP) has been shown to have osteoinductive properties (186, 187). It is recognized to be part of a larger family of growth factors referred to as the TGF- β superfamily (188) with a 30-40% homology in amino acid sequence with other members in the family. BMP acts as an extracellular molecule that can be classified as a morphogen as its action recapitulates embryonic bone formation. The identifying pattern of the BMP subfamily is their seven conserved cysteine residues in the carboxy-terminal portion of the protein and this is where the unique activity of BMP's is thought to reside (188).

Bovine & porcine sources were used in much of the original work attempting to purify the BMP molecule, a protein less than 50 kilo Daltons in size (189-193) and a number of recombinant human forms of BMP (rhBMP) have been derived. Interestingly the amount of human rhBMP necessary to produce bone induction *in vivo* is more than ten times higher than that of highly purified native bone extracted BMP (194). This difference was also demonstrated between human BMP derived from human bone matrix and human rhBMP (195), suggesting that native BMP is a combination of different BMP's or represents a synergy between them (193). This has revived interest in xenogenic derived native BMP's (196). Although concern regarding the immunigenicity of interspecies BMP has been raised in the literature, moose-derivered BMP showed strong osteoinductive capacity and weak immunogenicity in a sheep study (197).

Large and small animals have been used to study the influence of BMP on bone regeneration (198-201). Critical sized osseous defects are defined as bony defects of a specific size, which will not heal spontaneously with bone tissue alone (202-204). Defects larger than the critical size will not fill in with bone alone but may contain fibrous scar tissue. BMP has demonstrated the ability to heal many different varieties of critical sized defects including cranial vault defects, long bone defects and mandibular continuity defects (202, 204-206) without the addition of a bone graft.

One of the challenges in the use of BMP is in its delivery to a site of action. As a morphogen BMP is rapidly absorbed into the surrounding tissues dissipating its effectiveness. Many different carrier

vehicles have been used to deliver BMP including other noncollagenous proteins, DBM, collagen, HA, PLA and or PGA combinations, calcium carbonate, calcium sulphates and fibrin glue (207-214). More recently biodegradable gels, collagen sponges impregnated with BMP and silica glass have been used as carriers (215, 216, 217, 218, 219). DBM has been shown to contain BMP and may be used as a bone graft substitute with predictable healing in critical sized rabbit calvarial defects (169, 220) and has been used successfully in a human mandibular defect in vivo with native human BMP, a poloxamer carrier and bank bone (220, 221). Further success has been reported more recently with different types of BMP (222) and the reconstruction of mandibular defects and the treatment of pathologic fractures of the mandible with BMP as well (223).

One problem with the use of BMP's in general has been the regulation of their effects. BMPs are currently being used in "super-physiologic" concentrations. The resulting tissue effects are occasionally overwhelming when viewed from a clinical point of view. The reaction of the soft tissues with notable edema, erythema and inflammation is most remarkable (222). The effects of BMP must therefore be regulated. One substance which may hold some promise as a BMP regulator is the serum protein fetuin. There is increasing evidence that fetuin may serve as one regulator of BMP's effects (222, 224).

Transforming Growth Factor

The proteins in the family of transforming growth factor β (TGF-β) should be considered as osteopromotors, agents, which enhance bone healing. TGF- β is found in the same supergene family as BMP. TGF- β has been shown to participate in all phases of bone healing (225). During the initial inflammatory phase TGF- β is released from platelets and stimulates mesenchymal cell proliferation. It is chemotatic for bone forming cells, stimulating angiogenesis and limiting osteoclastic activity at the revascularization phase. Once bone healing enters osteogenesis then TGF- β increases osteoblast mitoses, regulating osteoblast function and increasing bone matrix synthesis, inhibiting type II

collagen but promoting type I collagen. Finally, during remodelling it assists in bone cell turn-over (222-229).

While less work has been undertaken to explore the applications of TGF- β than with BMP's as an adjunct to bone healing, TGF- β may be more effective than BMP in those situations where enhanced bone healing is preferred to bone induction (85). Moreover, combinations of BMP and TGF- β, may enhance the osteoinductivity of an implant while, at the same time, making it osteopromotive. As with BMP, carrier vehicles for the delivery of TGF- β are under development.

Platelet-Derived Growth Factor

Platelet derived growth factor (PDGF) is angiogenic and is known to stimulate the reproduction and chemotaxis of connective tissue cells, matrix deposition (230-233). These properties are all crucial to bone healing.

Insulin-like growth factor (IGF) has demonstrated a capacity to increase bone cell mitoses and increase the deposition of matrix. PDGF and IGF have shown an ability to work together during the reparative stages of bone healing. PGDF-IGF impregnated devices have proven to increase bone healing in defects associated with dental implants and teeth (234, 235, 236).

Platelets are known to contain a number of different growth factors of which TGF- β, and PDGF are two. As platelets degranulate they release these factors which may play a role in initiating graft healing. Platelet rich plasma (PRP) is one potential source of concentrated platelets that could be used in bone regeneration (237-239). A single unit of freshly harvested autologous blood is centrifuged at 5,600 rpm to separate the platelet poor plasma from the erythrocytes and the buffy coat (platelets and leukocytes). Once platelet poor plasma is removed, the specimen is further centrifuged at 2,400 rpm to separate the packed red blood cells from the PRP. The remaining PRP contains 500,000 to 1,000,000 platelets, which are mixed with a thrombin/calcium chloride (1,000units/10%) solution to form a gel (238). This gel can then be used in conjunction with bone

regeneration materials such as HA or DBM as a source of autogeneic growth factors (237). When used in combination with autogenous bone, PRP is reported to increase the maturation rate of a bone graft up to 2 fold and also increase the bone density of the graft (32, 238).

Other Bioactive Molecules

The last category of bioactive molecules is the polypeptide group. They may act as osteoinducers or osteoenhancers. Two short amino acids chain peptides that have demonstrated a bone activity are known as P-15 and OSA-117MV. The P-15 polypeptide was designed to take advantage of a conformational arrangement known as the "beta bend", which was found to have an influence on bone induction and growth when utilized in some *in vitro* studies (240, 241). The OSA molecule is even smaller than P-15 and was discovered in relation to the treatment of osteoporosis where OSA's effect is concentrated in areas of high stress. Researches have started to explore the local effects of this peptide and initial reports (85) suggest that it may enhance the osteoinductive effect of demineralized bone matrix.

Stem Cells and Hybrid Grafts as Applied Tissue Engineering

The area of tissue engineering has brought to the forefront, the possibilities of hybrids of biomaterials seeded with osteocompetent cells to be used as an implant. The hybrid could consist of a porous matrix, on which bone marrow cells could grow (242).

The use of bone marrow as the source of cells is logical as bone marrow contains stem cells which have the potential to differentiate along various pathways and lines, including the direction of bone producing osteocompetent cells (243, 244, 245, 246, 247). Seeding a porous matrix with bone

marrow cells could enhance the osteogenic potential of the matrix as a hybrid. Another possibility is the tissue culturing of bone marrow cells to further expand their numbers (242). Bone marrow derived cells are responsive to the influence of dexamethasone and 1, 25 dihydroxycholecalciferol (242, 248) and can be influenced to differentiate in the direction of bone cells. Human bone marrow cells have been reported to adhere to porous coral matrices (242) and to matrices made of HA and TCP (249, 250, 167). Osseous cells could be colonized onto or combined with such matrices, producing hybrid grafts. The source of bone cells could be suction trap harvested (Fig. 1) cortical membranous bones rather than stem cells (Fig. 3) (91). In the case of suction trap harvested bone cells, future hybrid grafts for the same individual could be made at the time of harvesting, or from the same harvested but stored froze cells at a later date (251-253) (Fig. 4). The development of such hybrids, culturing and storage methods may be the way of the future and could also diminish donor site morbidity by the total elimination of the donor site.

While many of these particular concepts were regarded a visionary a few years ago, they have now reached clinical reality, in planned phased clinical trials (253). As this chapter in surgical history is re-written, over and over again, there will be frequent additions to this exciting area of knowledge.

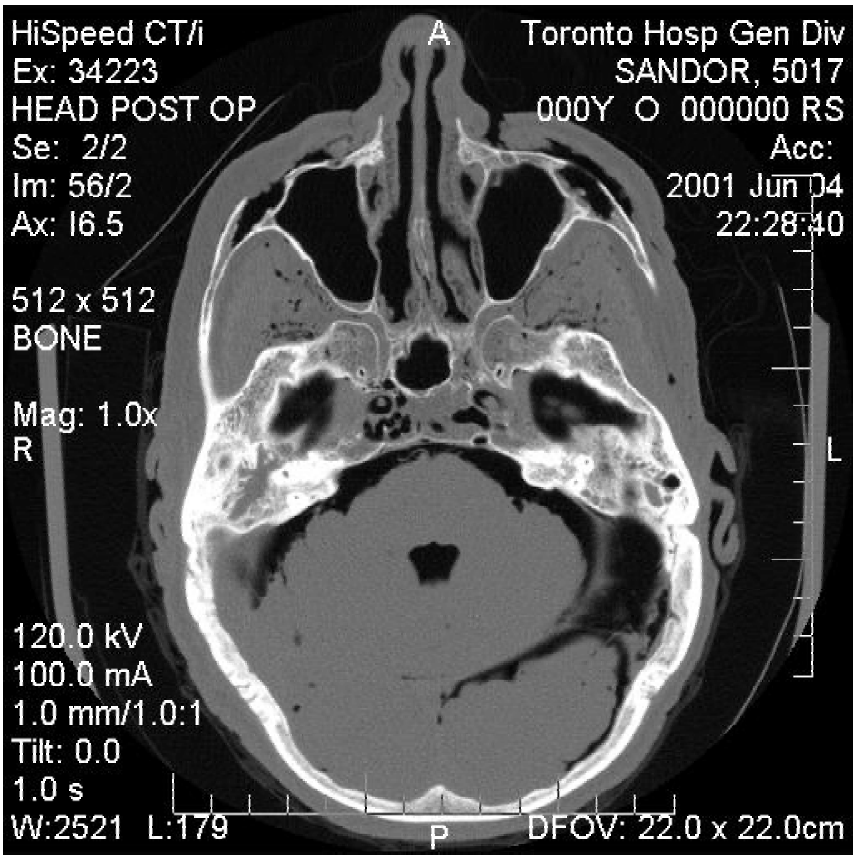


Fig. 3: Bilateral zygomatic bone graft harvest sites are visible as the bony defects on this CT-scan. The harvested sites are visible as the bony defects of the anterior zygoma donor site. Because the soft tissue covering this part of the cranio-maxillofacial skeleton is thick, there is no deformity visible extra orally in a patient who has undergone such a procedure.

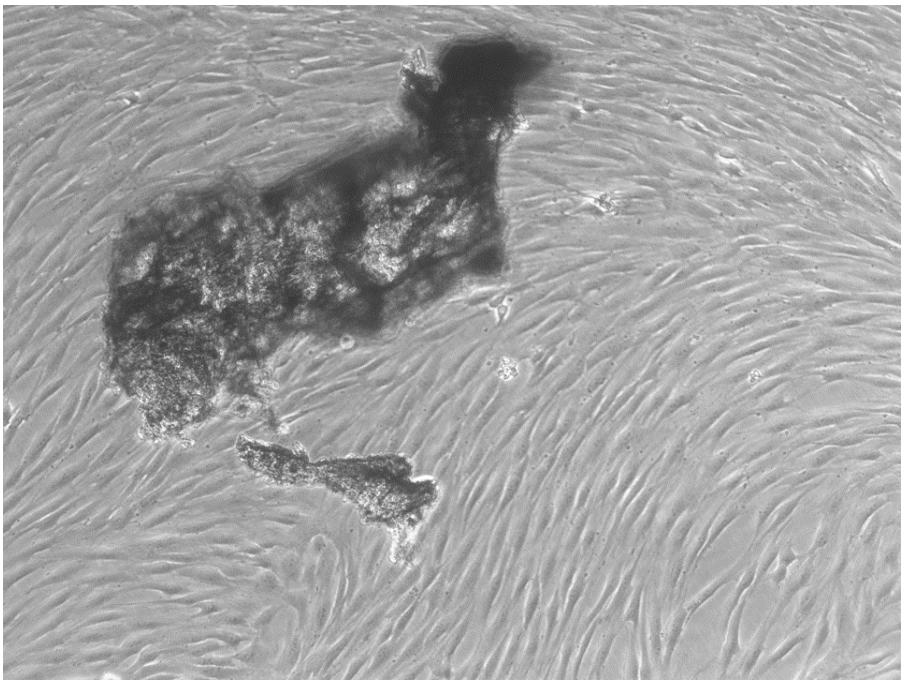


Fig. 4: A 21 day cell culture of osteocytes harvested from human cortical bone of membranous origin, the zygomatic bone. Note confluence of the cells.

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