Mapping remodeling reversals with the aid of the scanning electron microscope

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The histologic research on craniofacial remodeling is briefly summarized. A new methodologic approach using the scanning electron microscope (SEM) and high-resolution replicas of craniofacial bone is evaluated. Two maxillae were chosen for illustrative purposes. The specimens were replicated and prepared for routine SEM examination. In addition, a grid was applied to the replicas so that the bone growth activity states could be mapped on a coordinate system. The topographic ('T') principle is introduced as a precedent for discriminating remodeling bone growth activity states with the SEM. These activity states in vivo specify characteristic microscopic surface topographies. The three distinctive surfaces are resorptive, depository, and resting, which are mapped on a coordinate system. Results obtained are similar to those of histologic studies. The primary advantage of the SEM/replica technique is that it does not damage the specimen. This feature will facilitate more extensive investigations of craniofacial remodeling. The time and financial investments for the SEM/replica technique are significantly less than for the histologic technique in the investigation of similar material. The SEM/replica technique, however, cannot be used to study nonexternal surfaces and evidence of remodeling contained within the bone cortex.

Key words: Scanning electron microscopy, 'T' principle, remodeling

Resorption from certain bone surfaces and concomitant deposition on other surfaces serves two functions: (1) to increase the size of the bone and (2) to remodel the bone so that its shape and proportions are maintained as the bone enlarges. The interfaces between fields of resorption and deposition delineate the remodeling activity and are termed remodeling reversals. The purpose of this study is to justify and direct attention to the utility of the scanning electron microscope (SEM) in mapping remodeling reversals of the craniofacial skeleton. This methodologic innovation is a departure from the corpus of remodeling research which, until now, has used the histologic technique. This research is summarized briefly below.

The role that remodeling has in craniofacial morphogenesis (CFM) has been examined by Enlow,1-3 Enlow and Harris,6 and Enlow and Bang.7 This literature has emphasized the concept of "cortical drift" (bone growth movement) and the "V" principle (gross morphologic bone restructuring, shaping, and enlargement) as the morphogenetic responses to remodeling. The morphogenetic role of remodeling as seen in the lateral headfilm has also been examined by Enlow6 and by Enlow, Kuroda, and Lewis.9

Limited material studies on the pattern and sequence of craniofacial remodeling changes have been performed by Mauser and associates10 on the prenatal skull and mandible and by Kurihara and colleagues11 during postnatal life for the maxilla and mandible. The former study found the prenatal "pattern" similar but not identical to that of the generalized postnatal "pattern." The latter study is the first to address (1) the timing of the onset of characteristic remodeling patterns and (2) their variability at different dental age categories, albeit for the mandible and maxilla only.

The research conducted by Enlow and his co-workers has shown the importance of remodeling in CFM. It has been shown, particularly, that bone and soft-tissue growth figure prominently in the displacement of bones and that remodeling is, to a large extent, a compensatory mechanism which maintains proper bone alignment and proportionate growth during displacement. With this in mind, the remodeling features of the craniofacial skeleton have been recognized as important indicators of craniofacial growth pattern.

Heretofore the research in this field has employed the histologic technique. The most critical limitation of this technique, is the necessity to cut specimens into many thin sections, thereby obliterating their gross morphology. With this problem in mind, I developed a
technique which could reproduce the histologic results without causing damage to the specimen. This technique involves the making of high-resolution replicas of subadult craniofacial bone which are then examined with the SEM for evidence of the characteristic microscopic surface topography of remodeling bone. Boyde foresaw this application some years ago, when he wrote: “The increasing availability of SEM time will lead to its application to studies of the growth patterns of bones and of bone pathologies, and the author confidently expects that the use of an SEM may displace existing techniques for determining bone surface activity states . . .” (p. 260).

**MATERIALS AND METHODS**

Two left maxillae were selected from archaeologic material excavated from a protohistoric Iroquoian site located in Kleinburg, Ontario, dated about A.D. 1,600. The dental eruption stage of each specimen is represented by the complete deciduous dentition before the alveolar eruption of the first permanent molar. Preservation of the skeletal material is excellent. As is usually the case with archaeologic material, soil acids and bacterial action have rapidly decomposed the nonmineralized collagen, leaving clean bone surfaces. Weathering has not been so acute, however, as to allow significant surface changes to occur. When postmortem changes do occur, they are easily identified with the SEM as abraded or etched surfaces which present an irregular topography or as having a morphology which is known to occur beneath the external cortices, such as trabecular bone (see Fig. 1, D and F which have been included to illustrate signs of mild etching). Occasionally microscopic remnants of periosteum do persist and are recognized with the SEM as randomly oriented collagen fiber bundles. In these cases I have applied cold solutions of sodium hypochlorite, which digests any residual collagen.

The external surfaces were replicated with CutterSil Light, a precision silicone impression material, from the frontomaxillary suture, coursing inferiorly to involve the entire anterior face of the maxilla and posterolaterally toward the maxillary tuberosity and the posterior facing surface adjacent to the anterior fibers of the temporalis muscle. After polymerization of the impression material was complete, the replicas were separated from their specimens and cut with a razor blade into sizes suitable for the SEM chamber.

The cut portions from each replica were pressed into lumps of soft dental molding wax, and walls were built up on all sides so that the replicated surface formed the floor of the “replica boat.” The replica/wax contact was sealed by application of a hot metal probe to the contact, melting a fine layer of wax against the replica edges. Positive replicas were produced by pouring a high-resolution epoxy resin into the “replica boat.” The epoxy resin was allowed to cure at room temperature for 24 hours and then was separated from the wax and negative replica.

To facilitate the mapping procedure, household Fiberglas screening material was cut into small pieces, one side was very delicately wiped with Permatbond 910 adhesive, and, with the glue side toward the positive replica, carefully pressed onto the surface so that contact was made everywhere. After several minutes the periphery was trimmed of excess screening. The replica was prepared by mounting it on an SEM stub with silver conduction paint and then coated with gold palladium to a thickness of 10 nanometers.

It should be noted that there are a number of methods for applying the grid to the positive replica. I have also engraved grids with a scoring tool made from the tips of razor blades fixed at 1 mm. intervals. This is best done by scoring the replica after the gold palladium is applied and is particularly suited for soft-cure epoxy resins. A very fine-lined grid is the result, but it is a difficult and time-consuming method for surfaces which are not flat. A grid overlay made from single strands of very fine wire or silk could be made, but this is also very time consuming. Better means of applying a grid will likely be developed, but at this time I have found the Fiberglas screening material to present the best compromise between fiber thickness (310 microns) and hole width (1,240 microns) and length (1,340 microns). (Thinner screen fibers are commercially available, but they come with hole dimensions so small as to make the mapping procedure exceedingly tedious.) In addition, the Fiberglas screening is available in most large hardware stores, is very inexpensive, and takes only a couple of minutes to apply.

The main purpose for applying a grid is to facilitate the mapping procedure. It allows easy, systematic scanning along the grid lines in alignment with the X and Y axes of the SEM. In addition, some convention to ensure that the SEM operator is efficiently guided through the grid system without getting off track is needed. This requires that a detailed representation of the replica and grid be available for reference while one

*Ciba-Geigy XP-3294, Mississauga, Ontario.
†Root Wire, Ltd., Brampton, Ontario.
‡Permabond International Corporation, Englewood, N. J.
Fig. 1. A, The grid as it appears with the SEM. B, Resorption lacunae on compact bone. C, A transition zone from well-defined resorption lacunae at top, a resting resorption surface at lower middle to a neutral surface at bottom. D, An instance in which the remodeling reversal is sharply defined along the meeting edge of two planes—resorption at right and neutral bone at left (etched). E, Resorption interpreted to be proceeding rapidly, or differently, over woven bone. F, A depository surface (etched). Note incomplete mineralization of large collagen fiber bundles (waisted appearance) running from bottom to top.
is operating the SEM. For small specimens, a drawing of the replica on graph paper is adequate if several distinctive features of the specimen are registered. Another technique, however, particularly necessary for large replicas, is to photograph them after they have been fully prepared and make 8 x 10 inch enlargements. The distinctive features within the holes are readily identified between the photograph and replica at low magnification. With a sheet of acetate tracing paper clipped over the photograph, the surface characteristics of the bone can be noted over each hole.

Each prepared replica was observed in the SEM at an accelerating voltage of 10 kv. with the X and Y axes of the specimen grid in alignment with the SEM’s own X and Y axes. (An International Scientific Instruments, Inc., 60 SEM, Santa Clara, Calif., was used in this study.) Upon viewing each replica, it was briefly scanned to locate distinguishable features on the prepared coordinate system or photograph. The replica was then situated to begin systematic scanning, in either the X or Y direction, from a convenient coordinate location.

The greatest advantage of using the SEM to examine high-resolution replicas is its depth of field, which presents views that can be appreciated in three dimensions. This usually permits efficient scanning and surface identification. This method is effective because the cells responsible for the different remodeling activities have different formative effects on the bone surface that they directly overlie. This fact has recently been gaining validation and support from several authors who have investigated this relationship with the SEM. In the form of a principle, the topographic ("T") principle states simply: The activity states of cortical bone during growth and remodeling are distinguishable by their characteristic microscopic surface topographies. In general, the three distinctive surfaces are (1) resorptive, characterized by osteoclasts which break down bone and leave impressions of resorption lacunae; (2) depository, characterized by osteoblasts over an array of partially mineralized collagen fiber bundles and forming osteocytic lacunae; (3) and resting (or neutral), characterized by cells performing no bone growth activity over a relatively smooth bone surface composed of completely mineralized collagen fiber bundles (Fig. 1).

Once each replica was viewed and the microscopic surface identifications were mapped on their respective coordinate systems, the grids were placed in their proper linear sequence. The result is a map of the surface extent of the remodeling activity (Figs. 2 and 3).

**RESULTS**

The result of the mapping procedure is a coordinate representation of the bone remodeling activity at a single moment in time. In general, most grid hole surfaces predominated in one activity state, but some surfaces were divisible while others appeared to represent a mixed topography. Figs. 2 and 3 illustrate the surface...
Fig. 4. Anterior (a) and posterolateral (b) views of specimen 1. Boundaries of remodeling areas are indicated by dashed lines. This is a generalized representation of the remodeling activity illustrated in Fig. 2. Closed circles indicate resorption; open squares illustrate deposition; and triangles illustrate mixed remodeling activity. Areas not identified are resting bone. The posteriormost aspects of the posterolateral views were not evaluated.

Fig. 5. Specimen 2. (For details, see legends for Fig. 3 and Fig. 4).

identifications in two dimensional form without regard to gross morphologic features. The over-all impression is that the resorptive, depository, and resting surfaces occur in fields. This suggests, as it should, that remodeling activities are coordinated during growth.

Variations in the surface extent of resorptive and depository fields are associated with gross morphologic
variations in the bone itself. It is important, therefore, that each specimen be represented in visual perspective, so that this relationship can be appreciated. Fig. 4 illustrates the remodeling activity on specimen 1. On the anterior surface of the bone, resorptive areas surround the relatively more anterior column (in the coronal plane) of depository bone running from the lateral aspect of the nasal aperture inferolaterally to the alveolar process. This resorption is associated with the regressed appearance in these areas and the concave sway extending down and inferior to the zygomatic process.

The remodeling activity on the posterior aspect of the maxilla is not clearly defined. Remnants of resorptive activity, however, suggest their possible role in developing the concave surface.

The frontal process is predominantly depository. This activity may be responsible for the relatively anterior profile of the process. The resorption taking place at its superior edge is likely a response to anterior growth of the maxilla. This resorption indicates a compensatory response to maintain its relationship with the frontal bone at nasion. Fig. 5 illustrates the remodeling activity on specimen 2. Resorption surrounding the canine eminence is associated with the sloping profile beneath the nasal aperture and the canine fossa. The large concavity in the infraorbital region, although neutral in its observed "activity," shows evidence of recent resorption (resorption lacunae not well defined) on many of the grid hole surfaces.

The resorptive activity on the posterior aspect of the maxilla coincides with the medial situation of the alveolar process relative to the laterally projecting zygomatic process. In both specimens the lateralmost aspect of the zygomatic process is associated with a small depository field.

The resorptive character of the frontal process is possibly related to the vertical depression running along its length. The depository superior portion may indicate an anterior growth response at nasion to shift forward the posteriorly receding profile in this area.

**DISCUSSION**

This is principally a methodologic article for which two maxillae have been selected to demonstrate the utility of the "T" principle and the SEM in craniofacial remodeling research. Boyd and Jones are explicit with respect to this research potential: "... scanning electron microscopes (SEM) are built to look at surfaces which are of prime interest in understanding the development of the complex three-dimensional structure of enamel, dentin, cementum, bone, and cartilage" (p. 243).

My intention here is not to add to the body of research detailing facial remodeling but to introduce an efficacious technique and justification for using it. In this regard, the results compare favorably with the histologic study by Kurihara and associates for maxillae of similar dental age (their Stage III). Small notable resorptive fields on the frontal process and posterior aspect, however, which are not illustrated in previous work, may simply be unique population or individual variants. These differences may even point out a possible sampling error in the limited histologic samples investigated.

The primary advantage of this methodologic approach is its harmless nature. Because only surfaces are examined, it is not necessary to section or otherwise alter specimens in any way. This method will therefore facilitate remodeling studies of rare specimens which may not be histologically sectioned. Also, data collection may extend to large research collections heretofore unavailable for histologic work. The nondestructive advantage will therefore permit remodeling research on many individuals so that age and population differences are more thoroughly understood and the range of variability of remodeling patterns more fully appreciated.

Forthcoming correlative histologic/SEM studies will prove very informative and help justify the method employed here. It should be noted, however, that application of the "T" principle and SEM/replica technique underpin a valid approach which generates its own type of interpretable results. The observations made here point out the sensitivity of the method for discriminating specific bone growth activity states as well as gradations (resting resorptive and intermediate depository stages) and mixed resorptive, depository, and resting surfaces.

A fuller understanding and appreciation of remodeling microscopic surface topographies will come as a result of this research. Much of the prior SEM research has been performed on experimentally manipulated bone growth surfaces and pathologic bone. Although this SEM literature provides an excellent catalogue of bone growth activity state descriptions, observations of bone remodeling as a consequence of growth and development sometimes exhibit surfaces at variance with those often reported in the experimental literature. For example, Fig. 1 illustrates several resorptive surfaces which reflect apparent differences in activity and bone type being resorbed.

In addition to the nondestructive advantage of the new method, there are other time and cost advantages over the histologic approach. It is estimated that a minimum of 3 months would be necessary to prepare one maxilla completely with the histologic technique. The cumulative time involved in fixing specimens (24 hours), decalcification and x-ray verification (about 3
months for best results), processing (12 hours if the processing is automated), microtome sectioning (1 to 2 days), drying, staining and coverslipping, etc. (2 days) clearly amounts to a substantial investment. Assuming that at least 300 slides were produced at an average of $1.00 per slide (if the investigator is doing his/her own work), then the financial investment is also rather large.

The time and cost investments are markedly different when investigating the same material with the SEM. The cumulative time involved in preparation and making of the negative mold (10 minutes), preparation of the epoxy resin replicas (20 minutes), curing of the epoxy resin (24 hours), SEM stub preparation and coating (1 hour), and SEM analysis (5 hours of actual digital read-out filament time plus an hour for specimen changes, etc.) is clearly less than that of the histologic method. Indeed, if the investigator is doing his/her own work, it is a relatively simple matter to prepare the replicas in the morning, allow them to cure till the next day and do the SEM stub preparation, coating, and analysis that afternoon unless photographs are required. Furthermore, a liberal estimate of $3.00 for materials (plus photographic expenses) and $100.00 SEM filament time (based on a user charge of $20.00 per hour) is much less than that for similar histologic work. (SEM rates vary widely, and it is best to take advantage of subsidized institutional rates.)

There are obvious disadvantages with the SEM/replica technique, however, which require consideration. First, unless the element being studied is disarticulated from all other bones of the craniofacial complex, it is not possible to examine resorption/deposition fields which characterize the contralateral aspects of many bones. For this reason, a comprehensive assessment of the growth mechanisms affecting any one bone is very difficult when this technique is used. It is also not possible to investigate the pattern of remodeling reversals in large diploë and sinuses.

Second, application of the "T" principle is limited to the study of remodeling patterns at the moment the bone (or the surface of bone examined) died. Histologic sections expose the column of bone between endosteal and periosteal cortices, which can provide information on past growth and remodeling. The SEM/replica technique, on the other hand, examines surface activity in these fields which characterize the contralateral aspects of many bones. For this reason, a comprehensive assessment of the growth mechanisms affecting any one bone is very difficult when this technique is used. It is also not possible to investigate the pattern of remodeling reversals in large diploë and sinuses.

Second approach taken here. This study was generously supported by the Ontario Heritage Foundation.

REFERENCES


ERRATUM

In the abstract of "A Study of Class II, Division 1 Malocclusions Treated by the Andresen Appliance" by A. M. Cohen, which appears on page 568 of the November, 1981, issue of the AMERICAN JOURNAL OF ORTHODONTICS, the last two sentences should read as follows: "He blames this largely on lack of cooperation and attendance. The reviewer associates this percentage with the minimal financial involvement by patients under the British Health Scheme."