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Histological Analysis of an Implant Retrieved from a β-Tricalcium Phosphate Graft after 4 Years: A Case Study

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ABSTRACT: We describe the retrieval of a dental implant device that had been successfully osseointegrated for more than 4 years. After obtaining an informed patient consent, the device was retrieved for retreatment purposes from its position in a β-tricalcium phosphate (β-TCP) grafted sinus floor. The sinus floor augmentation, using β-TCP, had been performed in conjunction with the original implant placement, which in turn enabled the histological evaluation of specific regions of interest that were comprised of either grafted or native bone. Radiographs documented the rehabilitated area before and after grafting. The osteogenic events that occurred during the 4-yr-period depict the interplay of implant, synthetic graft material, and native bone in a dynamic process of osteogenesis, ongoing bone maturation, and remodeling that led to the development of haversian-like bone morphology. Two distinct areas were observed histologically, wherein osteointegration occurred uneventfully in both native bone and areas of grafted bone. Of particular interest was the presence of multiple remodeling sites of lamellar bone that could be seen between the plateaus—healing chambers—in which bone eventually evolved into a haversian cortical-like configuration.

KEY WORDS: β-tricalcium phosphate, retrieval, synthetic graft, resorption, dental implant, internal sinus lift, bone

I. INTRODUCTION

Bioactive ceramics have long been used as the basis for synthetic bone-grafting materials.[1,2] This versatile class of bone substitutes (alloplasts) provide manageable space holding, scaffolding, and a biowelcoming field, in which the process of osteogenesis unfolds in replacement and renewal of bone.[3] One such synthetic material is pure phase β-tricalcium phosphate (β-TCP), which is primarily used in dentistry for repair or augmentation of alveolar ridge defects.[4]

Other classes of grafting choices include autogenous, allogenic, and xenograft materials. Of these, autogenous grafts are the most osteogenic in that they possess both osteoinductive and osteoconductive potential as well as a broad range of bioactive agents.[5] The downside of such grafts include limited donor sites, morbidity associated with harvesting the graft, difficulty adapting the graft to recipient sites, and a somewhat unpredictable rate of resorption.[6] Concerns about potential transmission of infectious diseases arise with allografts and xenografts; thus, dentists are encouraged to use alloplastic, biocompatible, and synthetic grafts, which seem to have more advantages than drawbacks.[7,8]

Alloplasts are less osteogenic in that they are osteoconductive but not osteoinductive. On the other hand, these materials are abundantly available, potentially less expensive, avoid the morbidity associated with human donor sites, and do not carry the potential for transmission of disease or infection.[9] Additionally, as a synthetic substrate, their chemistry and manufacture can be increasingly manipulated to mimic the characteristics of natural bone. For example, efforts to modify their rate of resorption/degradation to align them with the behavior of natural bone remodeling (that takes from 4 to 6 mo)[10] include altering their crystallographic structure to produce the pure phase β-TCP. increasing their porosity,[11–14] changing the ratio of calcium to phosphate, and reducing the temperatures at which these ceramics are produced.[15]

A database of human retrievals of successfully functioning, plateau root form implants reports early (2 mo) and long-term (24 yr) osseointegration events.[16–18] In essence, plateau root form implants of different surface treatments, placed in different areas of either mandibular or maxillary alveoli, resulted in direct bone formation at the implant surface and healing chamber areas, where bone-to-implant contact and bone area fraction occupancy increased over time,[17] as did the bone's mechanical properties such as hardness and elastic modulus.[19] Although these findings have corroborated those of previous studies concerning the healing pathway of woven bone evolving toward a cortical-like lamellar bone with multiple remodeling sites, those studies involved osseointegration taking place in predominantly native bone.[20–22] The aim of this retrieval report is to present a human histological section depicting both native and grafted bone in the sinus area, interfacing in a successfully functioning implant.

II. CASE PRESENTATION

A 47-year-old male patient, who was living on the west coast of the USA at the time, had been in contact for several weeks with the Implant Dentistry Centre in Boston, MA, during which potential treatment plans were discussed. He expressed the desire to have as much treatment as possible during each visit in view of the logistics of travel. On March 8, 2012, he was presented for examination and possible treatment.

In the position of the maxillary right first molar, an osteotomy was prepared and an internal sinus lift performed, following manufacturer instructions.[23] At the time of implant placement, β-TCP graft material (SynthoGraft™, particle size 50–500 μm; Bicon, LLC; Boston, MA) was used to concomitantly perform a Summers' sinus augmentation.[24] Following graft placement using a two-stage procedure, a 5.0 × 6.0-mm Integra-CP (Bicon) SHORT® implant was inserted in the maxillary right first molar area. Implants were also placed in the areas of teeth 4, 6, 8, 10, 13, and 14. Postoperative instructions and pain medication were given, and a follow-up appointment was scheduled for the next day, after which the patient returned home.

The implants were uncovered 4.5 mo later, in July 2012. Final restorations were completed in September 2012. In August 2016, after more than 4 yr of function, the patient adamantly decided to seek nonmetallic implants and requested that all of his present titanium implants be removed. The implants were removed, and the patient received a temporary restoration. The implant explanted from the maxillary right first molar position (#3) was of particular interest for histological analysis, because it had been successfully functioning and osseointegrated in both natural and synthetic bone.

A. Retrieval Sample Preparation Methods

Following explantation, the specimen was fixed in a 10% buffered formalin solution for 24 hr, washed in tap water for another 24 hr, and gradually dehydrated in a series of 70% to 100% ethanol solutions. After dehydration, the sample was embedded in a methacrylate-based resin (Technovit 9100; Kulzer GmbH; Wehrheim, Germany), according to manufacturer instructions. Aiming at the center of the implant along its long axis, a block was then cut with a precision diamond saw (Isomet 2000; Buehler Ltd.; Lake Bluff, IL), glued to acrylic slides with an acrylate-based resin, and allowed to set for 24 hr before grinding and polishing. The sections were then reduced to a final thickness of ~100 μm by a series of SiC abrasive papers (600, 800, and 1200; Buehler Ltd.) on a grinding/polishing machine (Metaserv 3000I; Buehler, Lake Bluff, IL) under constant irrigation.[25]

The sections were stained with Stevenel's Blue and van Gieson's picro fuchsin (SVG) to differentiate between bone and soft tissue. SVG stains mineralized tissue red-orange and soft tissue blue-green; this helped during the analysis process, when the first stained sections were scanned into an automated microscope system (Aperio Technologies; Vista, CA).

To determine histological regions of interest (ROIs) along the implant interface with either native or grafted bone, we outlined the original sinus floor with a dotted line in the preoperative dental radiograph (Fig. 1[A]). In addition, we drew a sloped line on the histologic specimen and radiograph of the implant in place (Fig. 1[B] and [C]) that corresponded to the approximate inclination of the maxillary sinus floor. Because these lines were drawn at an angulation that was similar to the original sinus floor, we established a type of borderline between native bone and new bone that was formed as the graft material resorbed. In Fig. 1, β-TCP graft material appears above the line, and native bone appears below it. This in turn created an opportunity to easily compare native bone and graft-related new bone that participated in the process of osteogenesis.

In Fig. 1(C), histological ROIs are indicated with squares (labeled 1–3) in the histological section. Each ROI is further magnified to show greater detail of the implant interface with host bone, and newly formed bone. The lines and boxes were applied with presentation software (Microsoft® PowerPoint for Mac, version 16.25).

B. Results

Retrieval micrographs show that native bone and newly formed bone that was associated with the graft were osseointegrated with the dental implant, with no discernable difference between original host bone and new bone that was produced by the remodeling and graft resorption (Fig. 1[C]).

Figure 2(A)–(C) are magnifications of ROIs 1–3, respectively. ROI 1 and 2 show the distal and

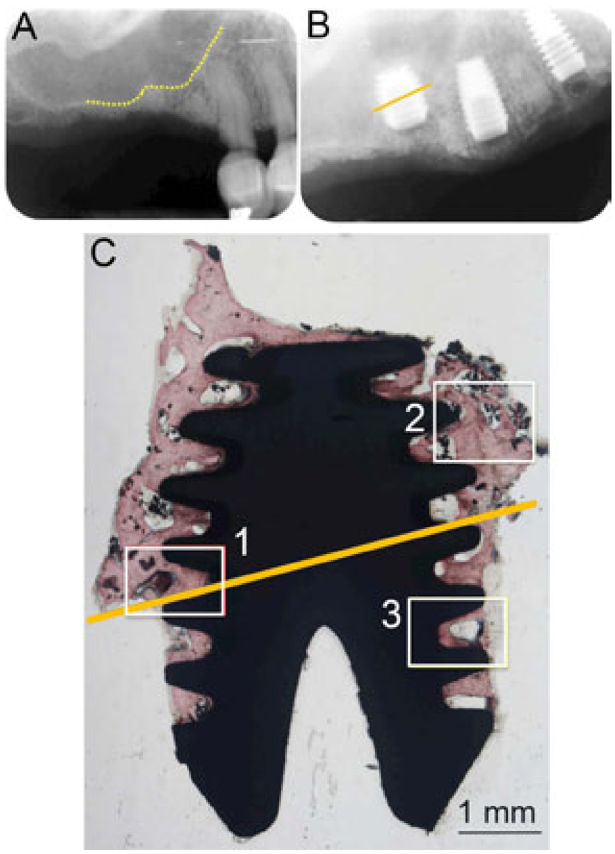

FIG. 1: (A) Preoperative dental radiograph of implant site. Dotted line traces preoperative floor of pneumatized sinus. (B) Postimplant radiograph. Straight line indicates orientation of preoperative floor of pneumatized sinus. (C) Low-magnification micrograph of explanted specimen. In this histological section, the line shown in (B) is superposed onto its corresponding position at the sinus floor and delineates β-TCP material (above line: boxed ROIs 1 and 2) from native bone below (boxed ROI 3).

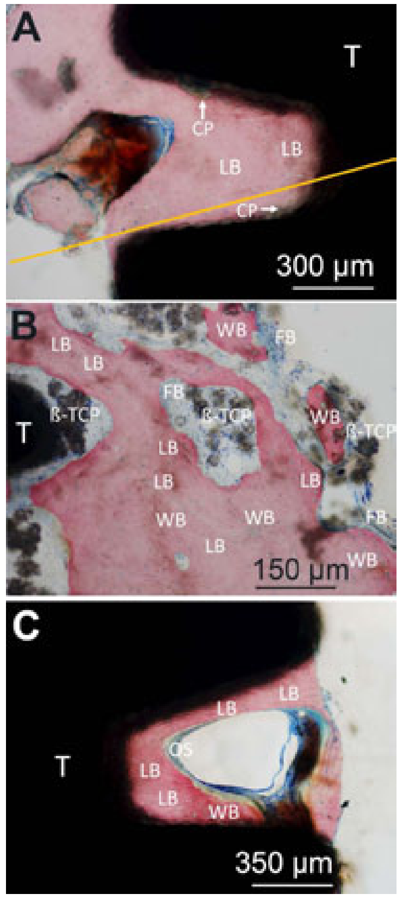

FIG. 2: Magnifications of ROIs 1–3 shown in (A)–(C), respectively. Remnants of β-TCP particles are visible mostly in ROI 2 but can also be seen in ROI 1, where woven bone has evolved to more organized lamellar bone. Regardless of the presence of β-TCP particles, bone regions within plateaus depict intimate contact with implant surface, with some areas of calcium phosphate still present after 4 yr of clinical function. β-TCP, β-Tricalcium phosphate; CP, calcium phosphate; FB, fibroblast; LB, lamellar bone; T, titanium; WB, woven bone.

mesial aspect, respectively, of the area of the implant between plateaus (Fig. 2[A] and [B]). The calcium phosphate coating of the implant can be observed to be in intimate contact with bone. Other observations include a variety of cells and osteonic structures, findings that corroborate the fact that osseointegration was successfully achieved and maintained between implant surface and grafting material. In ROI 2 (Fig. 2[A]), which corresponds to the apical portion of the implant, some residual graft particles can still be seen undergoing the process of being replaced by woven bone, whereas most of bone has already evolved into lamellar bone with multiple osteonic structures. ROI 3 (Fig. 2[C]) depicts native bone in intimate contact with implant. Lamellar bone presents a haversian-like structure, with multiple osteons showing several remodeling cycles.

III. DISCUSSION

In successful or failed cases, human retrieval analysis is an invaluable tool for assessing host-to-biomaterial interaction.[17,19] The presently retrieved implant was successfully functioning and retrieved from β-TCP grafted bone (that was used during a sinus lift procedure) due to patient-demanded retreatment purposes. Most of the grafted material had been resorbed and replaced by organized lamellar bone with evident osteonic structures, minerals, and vascular contents. Only a few particles of the β-TCP particles remained at the apical portion of the implant that had been inserted within the lifted sinus. The implant design creates large healing chambers between the plateaus that are rapidly filled by blood immediately after placement[26–28] resulting in direct contact of blood osteogenic cells with the implant surface, graft material particles, and instrumented bone. This process also promoted the formation of a fibrin-rich network that not only connected the different materials but in turn stimulated the migration of osteogenic cells, leading to rapid woven bone formation.[29–31] Over time, woven bone was replaced by lamellar bone with its cortical-like structures—many of which were haversian-like—as reported in other human retrieval studies.[21,22]

The patient's demand for removing successfully functioning implants and replacing with a metal-free implant was respected, but we also explained that long-term evidence for metal-free implant use was unavailable in the literature, making such an implant choice questionable for daily practice applications.[32,33] Given that allergy rates for titanium are very rare (0.6%), as observed in 1500 consecutive patients,[34] titanium has been the material of choice for many years for dental implantology.[35] In the case at hand, the implant retrieved from both grafted and host bone provided an unusual opportunity to conduct a histological analysis. It enabled documentation of an uneventful process of intimate bone-to-implant contact as well as the opportunity to observe several modeling/remodeling cycles within healing chambers between implant plateaus.

Although β-TCP is a well-documented biomaterial, the histological analysis of this retrieved sample corroborates its osteoconductive, cell-mediated, and osteogenic process (as previously reported in animal studies).[16] Although bioactive, the graft material is immunologically inert, showing no evidence of inflammatory foreign body reaction. The graft provided and maintained sufficient space, and the volume of new bone was shown to be progressively occupying that space. Evidence of scaffolding was provided by osteoblastic cells that were seen as arrayed on the graft particles.

IV. CONCLUSIONS

The retrieval of a successfully functioning implant that was anchored partially in native bone and partially in β-TCP biomaterial—used for a sinus lift procedure—provided the unique opportunity to observe intimate bone-to-implant contact, a dynamic remodeling process that replaced most of the graft material, and eventual formation of a cortical-like bone structure.

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