Supporting Information (SI Appendix) for

In situ bone regeneration of large cranial defects using synthetic ceramic implants with a tailored composition and design

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1. SI Results

1.1. Promotion of bone regeneration in sheep cranial defects

*Macroscopic observations*

The macroscopic evaluation after 12 months showed restoration of the large cranial defects treated with the bioceramic (BioCer) implant versus the titanium (Ti). The BioCer implant resulted in complete repair of the overlying soft tissue, both on the skin and the dura (Fig. S1) sides. The tissue covering the BioCer implant on both sides (skin and dura) appeared similar to the surrounding native tissue overlying the recipient skull bone. In contrast, the Ti implants appeared to be incompletely covered by tissue, as judged by the observation of visible metal, e.g., as shown for the specimens facing the dura (Fig. S1).

*Histology*

The histological assessment of the sheep skull showed that both the Ti and BioCer implants were integrated into the recipient defect sites with no histological signs of adverse inflammatory reactions (Fig. S2). The Ti implants generally showed less bone than the BioCer implants (Fig. S2). Mature osteonal bone mainly extended from the native recipient bone into the peripheral interconnected pores of the Ti implants (Fig. S3, A-D). Few areas of immature bone (i.e., without remodeling) were sometimes detected centrally in the Ti implants (Fig. S3, E-H). On some occasions, osteoblast seams and mesenchymal-like cells were found in the Ti implants associated with newly formed immature bone (Fig. S3, B, H).

A major observation in the BioCer implants was the considerable amount of mature, well-vascularized, osteonal bone associated with the BioCer tiles and the filling of a substantial portion of the created defects (Fig. S2B). The osteonal bone enveloped and was in direct contact with the BioCer tiles, facing the recipient bone (Fig. S4, A-C) dura (Fig. S4D), skin (Fig. S4, E, F), and the neighboring tile centrally (Fig. S4, G, H). More bone appeared on the dura side.
than the skin side (Fig. S2B). Bone also formed internally within the BioCer material (Fig. S5). In the multiple voids and concavities of the partly degraded BioCer, morphological evidence of ongoing coupled bone formation and remodeling was demonstrated (Fig. S5). Multinucleated osteoclast-like cells were frequently observed in the immediate vicinity of the BioCer surfaces, indicative of ongoing material resorption (Fig. S5). Osteoblast seams were commonly detected in association with areas of new bone formation (Fig. S5). Blood vessels containing blood cells were commonly detected within the remodeling sites (Fig. S5).

At locations where the BioCer material coating of the Ti frame was resorbed, newly formed bone bridging the Ti and BioCer was observed (Fig. S6).

The skin and dura sides of the native recipient bone adjacent to the defect borders of Ti (Fig. S7, A, C, E) and BioCer (Fig. S7, B, D, F) implants showed periosteum and endosteum, respectively, containing blood vessels, bone-lining cells and progenitor cells. A major finding in the BioCer implant site was the regeneration of periosteum (Fig. S8A-D) and endosteum (Fig. S8, E-H) covering the bone formed on the BioCer on the skin and dura sides, respectively. The tissue revealed typical characteristics of the periosteum and endosteum, with thicknesses from 150–200 µm and a bilayered structure consisting of a cellular layer interfacing with the bone-BioCer side and a fibrous layer on the opposite side (Fig. S8). The cellular layer showed bone-lining cells, in close proximity to the bone surface, as well as several blood vessels. In contrast, the skin (Fig. S9, A, B) and dura (Fig. S9, C, D, G, H) sides of the Ti implants revealed fibrous encapsulation.

In the inter-tile regions of both implant types, vascularized loose connective tissue was detected (Fig. S10). Islets of newly formed woven bone were sometimes detected in the tissue within the inter-tile space for both implant types (Fig. S10).
**Histomorphometry**

The histomorphometric analysis demonstrated significantly higher BA% and BIC% values for the BioCer than the Ti, both in the total ROI and in the peripheral and central ROIs (Fig. S11, A-D). A significantly higher BA% was demonstrated for the BioCer implant on the dura side than the skin side in the total ROI (Fig. S12A) and the peripheral ROI (Fig. S12B). No difference in the BA% was found between the skin and dura sides in the central ROI of the BioCer implant (Fig. S12B). No differences between the skin and dura sides were detected for the Ti implant (Fig. S12, A, B). The BioCer implant revealed high and comparable BIC% values, irrespective of location (peripheral, central, skin, or dura) (Fig. S12, C, D).

**Microstructural analysis**

The BSE-SEM supported the histological observations of mature and well-remodeled bone in the peripheral regions of the Ti (Fig. S13) and BioCer (Fig. S14) implants. However, bone ingrowth in the peripheral regions of the Ti exhibited more spaces and separations from the Ti surface (Fig. S13). In contrast, bone union with the BioCer surface was demonstrated (Fig. S14). Several osteocyte lacunae and canaliculi were detected in bone at the interface with both the Ti and BioCer implants (Fig. S13 and S14).

**Raman spectroscopy**

Raman spectroscopy revealed that the mineral crystallinity in the central region of the BioCer and the peripheral region of the Ti was higher than that of the recipient native bone. On the other hand, the carbonate-to-phosphate and apatite-to-collagen ratios were higher in the recipient native bone than in all analyzed sites of the Ti (peripheral region) and BioCer (peripheral and central regions) implants.
Ultrastructural analysis

A distinct border between the bone and BioCer was often difficult to discern by ultrastructural analysis (Fig. S15, A, B). The morphology of the bone tissue showed a rod-like structure on the micron scale, indicating cut collagen fibers, assuming a parallel orientation to the surface of the BioCer material (Fig. S15C). Collagen striation, with the characteristic 67-nm periodicity, could be observed, indicating collagen filaments running in the plane of the image (Fig. S15D). The material side consisted of high-contrast grains in the micron range with more porous subsurface regions (Fig. S15, A, B). On the bone side, occasional holes in the range of 200 nm were observed, in agreement with osteocyte canaliculi (Fig. S15, A, C). The EDS results further supported chemical bonding between the bone and BioCer surface. The chemical composition, as judged by EDS, showed the continuity of calcium, phosphorous, oxygen and carbon across the interface. The bone tissue showed a higher carbon content than the BioCer, indicating the collagenous content, whereas the BioCer revealed higher levels of calcium and phosphorous.

1.2. Clinical retrieval

Histology

The histological evaluation of the retrieved implant showed that the implant components (BioCer tiles and Ti mesh) were well integrated with the surrounding tissue and bone, with no signs of adverse inflammation or fibrous encapsulation (Fig. S16, S17). Similar to the observations in the sheep skull, a considerable amount of mature, well-vascularized osteonal bone was revealed in association with the BioCer tiles in the large human skull defect, irrespective of the location within the defect (i.e., close or distant from the recipient defect boundaries) (Fig. S16, S17). At low magnification, bone was observed to be enveloping the BioCer tiles with varying degrees of extension into the inter-tile spaces (Fig. S16, S17). The
least amount of bone was found in the transitional zones of the implant (Fig. S16, S17E). Otherwise, bone appeared on all sides of the tiles: facing the skin, dura and inter-tile spaces (Fig. S16, S17-S22).

In contrast to the observations in the sheep skull, relatively less bone appeared on the dura side of the BioCer tiles than on the skin side (Fig. S17, S18). Bone also appeared in the porosities and concavities of the BioCer (Fig. S19, S20, S21). The porosities and concavities of the BioCer implants demonstrated ongoing coupled bone formation and remodeling, with osteoclast-like cells associated with the BioCer surface, in conjunction with osteoblast seams forming new bone on the adjacent and opposite sides (Fig. S20, S21). Vascularization was evident in the areas of ongoing remodeling within the BioCer material (Fig. S19, S20, S21). Bone on the outer surfaces (skin, dura and inter-tile) (Fig. S17, S18) of the BioCer appeared more mature with more remodeling than bone detected in the porosities and concavities (Fig. S19). As observed in the sheep skull, regeneration of the periosteum and endosteum was found on the skin and dura sides of the tiles, respectively (Fig. S18, S22).

With respect to the Ti component of the implant, two major features were observed. First, bone was observed in direct contact with the coated Ti mesh within the tiles as well as in the inter-tile regions in the peripheral and central zones of the implant (Fig. S17, A, C). Second, bone was seldom detected in association with the Ti in the larger inter-tile spaces in the transitional zones (Fig. S17E). The remaining parts of the inter-tile spaces of the implant were occupied by relatively loose and well-vascularized connective tissue (Fig. S17, S19A).

**Histomorphometry**

The histomorphometric analysis revealed newly formed bone areas of 21%, 25%, and 17% in the tile ROI (Fig. S23A) and 30%, 50% and 23% in the inter-tile ROI (Fig. S23C) for the peripheral, central and transitional zones, respectively. For both the tile and inter-tile ROIs,
significantly higher BA% was found in the central zone of the implant than in the transitional zone (Fig. S23, A, C). Regarding the percentage of bone in contact with the BioCer (BIC%), generally high values were demonstrated (78–91%) for bone contact in the tile and inter-tile ROIs (Fig. S23, B, D). In the inter-tile ROI, comparable bone contact was found for the BioCer and Ti components in the peripheral and central zones (Fig. S23D). However, in the transitional zone, a significantly lower BIC% was found for the exposed Ti (2%) than for the BioCer in the transitional zone (88%) and the frame Ti in the peripheral (78%) and central (65%) zones (Fig. S23D). The percentage of bone growth into the inter-tile ROI was higher in the central zone (80%) than in the peripheral (60%) and transitional (40%) zones (Fig. S23E). The side-specific analysis of the tile ROI showed significantly higher BA% on the skin side of the implant than on the dura side in the central and transitional zones but not in the peripheral zone (Fig. S24A). A high BIC% was detected on both the skin and dura sides, irrespective of location (peripheral, central, and transitional zones) (Fig. S24B). The BIC% was significantly lower on the dura side than on the skin side in the transitional zone (Fig. S24B).

**Micro-computed tomography (micro-CT)**

The micro-CT evaluation of the human implant showed bone formation around and within the BioCer tiles in all analyzed peripheral, central and transitional zones. In general, bone was directly associated with the BioCer and was also observed along the Ti bars (Fig. S25, S26). Relatively less bone was found in the transitional zone (Fig. S25). Several tiles were bridged by bone in the central and peripheral zones (Fig. S26). The dura side showed a rougher appearance, indicating less bone than on the skin side, which had a smoother appearance (Fig. S26).

A qualitative assessment of a segmented micro-CT image and corresponding histological section showed that most of the bone could be discriminated by micro-CT (Fig. S27); however,
Ultrastructural analysis

TEM was performed using two samples from the central zone of the clinically retrieved implant. In one sample, the material side of the BioCer material-bone interface consisted of micron-sized high-contrast grains with a more porous subsurface region (Fig. S28, A, C). In the bone, the direction of the fibre bundles was generally parallel to the BioCer surface, as indicated by the circular and oval pattern in the bone (fibers cut perpendicular to the section plane) (Fig. S28A). At the immediate interface, the bone followed the contour of the surface, where collagen banding was occasionally visible (fibrils cut in the plane of the section) (Fig. S28, B, D). Union between the bone and BioCer material was demonstrated (Fig. S28A).

In another sample (Fig. S29), the high-contrast crystals were not observed; instead, the sample had a generally lower contrast yet porous appearance. The pores were frequently occupied by acicular apatite precipitates (Fig. S29, B, D). Bone demonstrated typical collagen banding (Fig. S29C) and had joined with the BioCer surface (Fig. S29A).

XRD analysis of the retrieved clinical implant

Quantitative analysis with Rietveld refinement (Fig. S30) on resin-embedded BioCer in the retrieved human samples revealed a relatively good fit between the observed and calculated patterns. Apatite and TCP were clearly the major crystalline phases, as indicated by relative peak intensity at 31.5, 31.8 and 32.6 ° 2θ (apatite), and 30.96, 27.7 and 25.6 ° 2θ (TCP), with distinct TCP peaks for β-TCP (Fig. S30b) and Mg-substituted TCP. It should be noted that the calculated pattern poorly fit the TCP peak at very low angles (5–18 ° 2θ), likely due to interference from the amorphous embedding material. Therefore, while the obtained refinement results (Table S2) are similar across the three human samples (e.g. calcium deficient
HA (CDHA) 71.9%, 65% and 71.7%) and display good fits (GOF 2.02-2.53), these values should be considered semi-quantitative, and do not reflect the relative weight percentage of amorphous phases, including amorphous calcium phosphate. Finally, while CDHA appeared to match the apatite peaks more closely than hydroxyapatite or substituted apatite, and refining patterns with multiple apatite phases did not improve the fit, the precise form(s) of apatite present could not be definitely determined by XRD as the samples were not perfectly crystalline.
2. SI Materials and Methods

2.1. Study design

The aim was to investigate whether critical-size cranial bone defects can be regenerated using 3D-printed cranial implants consisting of a Ti-reinforced BioCer without the systemic or local application of cells or the administration of growth factors. The hypothesis was that cranial defects in a large animal model and in humans can be repaired by an implant that has been tailored to promote bone regeneration and osseointegration in the entire defect. The study design consists of three parts. In the first controlled laboratory experiment, we established two large cranial defects (30 × 15 mm) in the parietal bone of 7 sheep. In each sheep, one defect received an experimental BioCer implant (test), whereas the other defect received a Ti implant (control). In the second controlled laboratory experiment, BioCer (test) and Ti (control) implants, equivalent to the skull implants, respectively, were implanted subcutaneously in another group of 6 animals. For both experiments, the implantation was performed under a pre-determined schedule, ensuring randomization between the right and left sides of the midline. The skeletal (skull) and non-skeletal (subcutaneous tissue) bone regeneration was investigated, qualitatively and quantitatively, after 12 and 3 months, respectively, using histology, histomorphometry, Raman spectroscopy, and scanning and transmission electron microscopies. A total of 13 animals were operated, with seven (n = 7 for each implant group) in the skull sites and six (n = 6 for each implant group) in the subcutaneous sites reaching the end-point. In the third part, a proof-of-principle that a large, hemi-craniecctomy (13.4 × 11 cm; 115 cm²) in a human can be restored with new bone, achieving a structure and composition similar to that of normal bone, was provided after the detailed investigation of a retrieved, customized BioCer implant 21 months post-surgery. No animals were excluded from the study, and no outliers were eliminated. Blinding of the surgical procedure and the different analyses was not possible due to the visible difference between the test and control implants.
2.2. Implant manufacturing

Experimental implants

The BioCer implant for the animal experiments consisted of calcium phosphate tiles, reinforced and interconnected by a Ti frame (Fig. 1A). Prior to fabrication of the BioCer implant, the Ti frame was additively manufactured by selective laser sintering of grade 23 Ti. Furthermore, a mould consisting of 6 rectangular cavities was designed and manufactured using computer-assisted design/computer-assisted manufacturing (CAD/CAM).

The BioCer material was prepared from a powder mixture of beta-tricalcium phosphate ($\beta$-TCP)/dicalcium pyrophosphate (Sigma-Aldrich, Steinheim, Germany) and monocalcium phosphate monohydrate (MCPM; Alfa Aesar, Thermo Fisher, Karlsruhe, Germany) and mixed with glycerol (1-3). The Ti frame was then positioned in the mould, and the BioCer paste was molded precisely over the Ti frame in the form of rectangular tiles (thickness, 6 mm; spacing between tiles, ~1 mm) (Fig. 1A). The BioCer implant was allowed to set overnight in sterile water. After removal from the mould, the implant was left in sterile water for 48 hours, in order to eliminate the glycerol.

The control implant (Fig. 1B) had a design and dimensions similar to those of the BioCer implant but was entirely additively manufactured from grade 23 Ti. The dimensions of the BioCer and Ti implants in the sheep skull and soft tissue were 30 × 15 mm and 18 mm × 18 mm, respectively. Apart from standard cleaning procedures (bead blasting and ultrasonic cleaning), no specific surface treatment was performed on the Ti implants. All implants were steam sterilized by autoclaving at 121 °C for 20 min.

Clinical implant

As for the experimental implants, the clinical implant was composed of calcium phosphate tiles reinforced and interconnected by a Ti frame (Fig. 1C). The frame was commercially pure grade 2 Ti that was laser-cut into sections specific to the geometry required for the calcium phosphate
tiles. The BioCer was precisely molded over each section of the Ti frame in the form of hexagonal tiles (thickness, 2–4 mm; spacing between tiles, 0.5–1 mm) (Fig. 1C). The cement used consisted of a powder mixture of β-TCP/dicalcium pyrophosphate and MCPM mixed with glycerol that was hardened in water, similar to that used for the experimental implants. The frame sections with BioCer tiles were welded together and customized manually to fit a 3D-printed model of the patient defect prior to steam sterilization at 121° for 20 min. The joints (transitional zone) between two adjacent mesh sections were not covered by the BioCer material and had a spacing of 1.5–2 mm with exposed Ti between two adjacent BioCer tiles. Implants were steam sterilized by autoclaving at 121°, for 20 min. All implants were produced by OssDsigin (OssDsigin AB, Uppsala, Sweden).

2.3. Material characterization

Phase composition

After autoclave sterilization, samples (n=10) of the BioCer implants were pulverized and dried powders were ground until a fine powder was achieved. The analysis was performed using an X-ray diffractometer (Aeris, Malvern Panalytical Ltd, UK) with a theta-theta (2θ) setup and Ni-filtered Cu-K irradiation. Diffraction patterns were collected using a beam knife between 2θ of 20°–45°, with a step of 0.011 and 11.5 s per step for β-CPP and β-TCP, and of 21.5 s for brushite, monetite and the CaP mixture. Rietveld refinements were applied to perform a quantitative phase composition analysis. High score Plus Software, Version 4.6a (4) was used for Rietveld refinement. Crystalline models were taken from the International Centre for Diffraction Data (ICDD) database based on literature references for beta-calcium pyrophosphate (β-CPP) (5), beta-tricalcium phosphate (β-TCP) (6), monocalcium phosphate monohydrate (MCPM) (7), brushite (8), monetite (9) and hydroxyapatite (from the inorganic crystal structures database, ICDD PDF# 01-089-6439). No other phases were identified in the
diffraction patterns. The complete result of the XRD analysis of the BioCer implant after autoclaving is provided in Table S1.

**Porosity**

The porosity of the BioCer implant was measured using Archimedes’ principle. The BioCer was dried in a vacuum oven at 60°C for 2 hours before being weighed to determine the dry weight (A). The samples were then immersed individually in deionized water for 24 hours at room temperature, transferred from the liquid and placed on cleanroom wipes. After the removal of surface droplets, each sample was weighed (wet weight (B)) and then weighed while immersed in water (immersed weight (C)). The apparent porosity was calculated using Eq. 1: $\rho(\%) = \frac{(B-A)}{(B-C)} \times 100$

**Specific surface area (SSA)**

The SSA was determined by nitrogen adsorption at 77 K according to the Brunauer–Emmett–Teller method (BET) in an ASAP 2020 system (Micromeritics Instrument Co., Norcross, GA, USA). The holder was filled with 0.7 g of sample powder.

**Density**

The skeletal density was measured using helium pycnometry (AccuPyc 1340; Micromeritics Instrument, Co.) in 10 runs.

**Microstructure**

The microstructure of the BioCer surface was analyzed using scanning electron microscopy (SEM; Merlin, Zeiss, Germany). The samples were dried under vacuum for 24 hours before analysis. Prior to observation, the samples were sputter-coated with Au/Pd for 60 seconds at a voltage of 2 kV and current of 20 mA using a Polaron SC7640 sputter-coater (Thermo VG Scientific, UK).
**Residual glycerol analysis**

The amount of glycerol diffusing from the BioCer implant was evaluated using an exhaustive extraction method. The BioCer implant was immersed in water at 37°C for 72 hours, while gently shaking at 10 rpm. Thereafter, the extraction solution was collected, and the same extraction procedure was repeated two additional times with fresh water for the same implant. Subsequently, the collected solutions, from the 3 consecutive extractions, were analyzed with high-performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD), using an Agilent 1100 HPLC system. A glycerol standard was prepared to determine the limit of detection of the instrument and a three-point calibration curve was established.

**2.4. Animal surgery**

The procedure was conducted in accordance with the OECD (10), European (11) and United States Food and Drug Administration (12) Good Laboratory Practice regulations, in adaptation to the ISO 10993 Standards Part 2 (13) and Part 6 (14). The study complied with the ARRIVE guidelines for animal studies. The animal experiment was approved by Ministry of National Education, Higher Education and Research (01139.2) (NAMSA, Chasse-sur-Rhône, France). A total of 13 adult female sheep (*Ovis aries*), skeletally mature, with an age range of 2–4 years and a weight of at least 60 kg, were operated (n=7 for skull bone defect implantation; n=6 for dorsum subcutaneous (s.c.) implantation). Before surgery, each sheep received a s.c. injection of the anti-inflammatory flunixin (Meflosyl®; Zoetis, Malakoff, France) and, as prophylactics, an intramuscular (i.m.) injection of amoxicillin (Duphamox LA®; Zoetis) and a s.c. injection of enrofloxacin (Baytril® 10%; Bayer SAS, Lyon, France). A pre-anaesthetic was administrated intravenously (i.v.) with a mixture of diazepam and butorphanol (Torbugesic®; Zoetis). Anaesthesia was induced with an i.v. injection of propofol (Propovet®; Axience SAS, Pantin, France) and maintained by isoflurane-O₂ inhalation (IsoFlo®; Axience) via a tracheal tube. During surgery, the arterial pressure, oxygen saturation, temperature and
electrocardiogram were monitored. Electrolyte solution was infused to maintain isovolumetric conditions. The surgical sites were clipped, scrubbed with povidone iodine and wiped with 70% isopropyl alcohol.

For the skull implantation, a half-circular skin incision was made from the orbital level to the occipital bone. The flap was elevated subperiosteally and retracted. Bilateral, rectangular defects (15 × 30 mm) were made in the parietal bone using a rotary craniotome perforator (Zyphr™ Disposable Cranial Perforator, Stryker Craniomaxillofacial, Kalamazoo, MI, USA) connected to a high-speed drilling unit (Integrated Power Console IPX1, Medtronic, Fort Worth, TX, USA). Two holes were made at the corners of the defect and joined using bone drills (F2/8TA23) and DuraGuard (AF02R; Midas Rex Legend, Medtronic). Care was taken to keep the dura intact. The procedure was performed under constant irrigation with saline. Thereafter, according to a pre-determined randomization protocol, each defect received either a BioCer (n=7) or Ti (n=7) implant. Each implant was stabilized to the parietal bone using 3 fixation screws. The soft tissues and skin were closed with resorbable (Vicryl® 2.0; Ethicon, Johnson & Johnson, Belgium) and non-resorbable (Prolene® 2.0; Ethicon) sutures, respectively, and surgical wound staples (Appose™ ULC Auto Suture™, Covidien, France).

For the subcutaneous implantations, incisions were made in the skin parallel to the vertebral column. In each incision, a pocket was created by blunt dissection in which either a BioCer (n=6) or Ti (n=6) implant was inserted and stabilized using non-resorbable sutures (Prolene® 3-0, Ethicon). Both, test (BioCer) and control (Ti) implants were placed in each animal and the incisions were closed using surgical staples.

Post-operatively, all surgical wounds were disinfected with oxytetracycline (Oxytetrin® spray; Intervet, France) and s.c. injections of buprenorphine were administered once daily for 2 days post-surgery. Anti-inflammatory medication (flunixin) was administered daily for 7 days, and antibiotics (amoxicillin and enrofloxacin) were given for 6 weeks post-surgery. The sheep were
fed daily on standard hay *ad libitum* and pelleted food. During the evaluation periods, the sheep were regularly observed for abnormal neuro-behavioral signs.

At 3 and 12 months, the sheep were sacrificed by an i.v. injection of a pentobarbital (sodium pentobarbital, CEVA Santé Animale, France). For the 3-month subcutaneous implantation, the implantation sites were exposed by surgical dissection. After gross examination, the implant and the surrounding subcutaneous tissues (including a margin of at least 5 mm) were harvested and fixed in 10% formalin. For the 12-month skull implantation, the implantation sites were exposed. After gross examination, the parietal bone containing the test and control implants was dissected and harvested using a bone saw and fixed in 10% formalin. In addition, one frontal skull bone biopsy from each sheep was harvested and fixed in 10% formalin.

2.5. Clinical retrieval

A 22-year-old male developed a subdural hematoma after a car accident and underwent decompressive hemicraniectomy. Cryopreserved autologous bone was reimplanted after 1.5 months and explanted 13 months later due to heavy resorption and a suspected infection. Five months later, the patient underwent cranioplasty with a BioCer implant. The size of the defect was 13.4 × 11 cm (115 cm²). Minor head trauma caused deformation of the implant, necessitating replacement 21 months post-operatively (Karolinska University Hospital, Sweden). The BioCer implant was retrieved, immediately fixed in 10% formalin and transported in a plastic container at room temperature over 5 hours to the Department of Biomaterials, University of Gothenburg, Sweden; Biobank 513. The sample was maintained in 2 l of formalin at room temperature for 2 weeks before processing. The retrieval and processing of the clinical BioCer implant was performed in accordance with the BRISQ guidelines. The study protocol was approved by the Regional Ethics Committee in Stockholm (Dnr 2017/251031). The patient provided signed informed consent. In brief, the informed consent consisted of clear information and full explanation to the patient regarding: publishing a report
on the retrieved implant in an international scientific journal; protection of the patient personal information and privacy; no cost or compensation; free choice not to participate and can withdraw at any time before submission, but not after. The patient signed specifically on every item in the consent.

2.6. Sample processing

After fixation, the retrieved implants with their associated tissues were processed for plastic embedding (15). The blocks were dehydrated in a graded series of ethanol and cleared in xylene followed by resin infiltration and polymerization. The clinically retrieved skull implant, sheep skull implants, sheep subcutaneous implants, and native bone biopsies were embedded in either LR White plastic resin (London Resin Co., Ltd., UK) or in polymethyl methacrylate (PMMA). Prior to further processing, the large block of the clinical implant was divided into smaller blocks for micro-computed tomography (micro-CT). Thereafter, the blocks were further dissected by sawing (EXAKT® Apparatebau GmbH & Co, Norderstedt, Germany) into multiple smaller specimens representing different zones of the implant (peripheral, central, and transitional zones; Fig. S16). Subsequently, all specimens, human and sheep, were bisected by sawing and grinding into two halves. One-half of each specimen was used to prepare ground sections (15–30 μm thick), stained with modified van Gieson's stain, toluidine blue, paragon, and/or basic fuchsin, for histological assessment and histomorphometry. The equivalent second halves were wet-polished using 400–4000 grit silicon carbide grinding paper and allocated for Raman spectroscopy, microstructural and ultrastructural analyses.

2.7. Analytical procedures

Micro-computed tomography (micro-CT)

Smaller blocks, containing peripheral, central or transitional tiles, of the clinical implant were subjected to micro-CT analysis (SkyScan 1172 scanner, Bruker microCT, Kontich, Belgium). Depending on the size of the blocks, the resolution was set to have the entire sample within the
field of view throughout a full 360° rotation. All analyses were carried out with a source voltage of 100 kV and a 40-µm Cu and 0.5-mm Al filter. For the central and peripheral zones, the scanner was operating with a 2 × 2 binning, while for the transitional zone, 4 × 4 binning was used, giving a voxel resolution of 9.9 µm, 11.08 µm and 19.79 µm, respectively. The reconstruction was conducted in NRecon software (Version 1.7.0.4; Bruker microCT). The data sets were aligned in DataViewer (Version 1.5.2.4; Bruker microCT). Visualization was achieved with CTvox (Version 3.2) and CTVol (2.3.2.0 - Version 2.0) softwares, and the data analysis was performed using CTAn software (1.16.9.0 - Version 1.16), all from Bruker microCT. The slices were oriented to match corresponding histological sections to correlate with the greyscales representing the bone. A qualitative assessment throughout the volume was performed to evaluate the bone formation pattern around the implant with respect to the dura and skin sides and to evaluate the degree of bridging between the tiles.

**Histology and histomorphometry**

All histomorphometric analyses (clinical and sheep implants) were performed in an optical microscope (Nikon Eclipse E600; Nikon, Ltd., Tokyo, Japan) equipped with imaging and analytical software (NIS-Elements; Nikon, Ltd., Tokyo, Japan). The histomorphometric parameters (bone area percentage (BA%) and bone-to-implant contact percentage (BIC%)) were determined using a counting procedure. Software grids were designed depending on the size of the implants/sections of the different sample types (i.e. clinically retrieved implant and the sheep skull and soft tissue implants). The grid consisted of 4-6 rectangular area (8000 µm × 3000 µm each) for clinical implant sections; 6 rectangular area (5000 µm × 3000 µm each) for sheep skull implant sections and 36 rectangular area (1500 µm × 2000 µm each) for sheep soft tissue implant sections. The grids were superimposed over the tissue and the histomorphometric parameters were counted and calculated.
For the clinical implant, a total of 17 sections were judged suitable and assessed histomorphometrically using a ×10 objective (Fig. S16). Each section consisted of 2–5 tiles located in (i) the peripheral zone (proximal to recipient defect borders; n=26 tiles), (ii) the central zone (distant from the recipient defect borders; n=14 tiles), (iii) and the transitional zone (tiles adjacent to exposed Ti and with large inter-tile space; n=10 tiles). In each group, the analysis was performed in two different ROIs: one was the area occupied by each tile with the surrounding tissue and bone (tile ROI); and the other was the inter-tile region, which included two adjacent tile borders and their surrounding bone and tissue in the inter-tile area (inter-tile ROI). For the tile ROI, the histomorphometric evaluation comprised the percentages of bone area (BA%) and bone-to-implant contact (BIC%). For the inter-tile ROI, the BA%, BIC% and bone growth % were determined. The BA% was determined by calculating the area occupied by bone in relation to the total area of the ROI (bone area/total ROI area × 100). The BIC% was determined by calculating the length of bone in direct contact with the material (BioCer and/or Ti) in relation to the available circumference of the material in the ROI (bone length in contact with material surface/total length of material circumference × 100). The bone growth % was calculated as the percentage of the furthest distance the bone could be detected from the surfaces of two adjacent tiles in relation to the total distance between the two surfaces (maximum bone growth distance between two tiles/maximum distance between two tiles × 100).

For the sheep skull implants, the histomorphometric evaluation was performed using a ×10 objective. The implant with the surrounding tissue was superimposed by a software grid that allowed division of the defect site into peripheral and central ROIs. For BA%, the area occupied by the newly formed bone was determined in each ROI, and the percentage was then calculated in relation to the respective ROI area (i.e., peripheral and central). For the BIC%, the distance of bone in direct contact with the materials (BioCer or Ti) was calculated in each ROI, and the
BIC% was then calculated in relation to the available circumference of the material in the respective ROI. Furthermore, the total BA% and the total BIC% were calculated in relation to the total area and the total circumference of the material, respectively, in the entire grid area. For the subcutaneous implants, the analysis was performed using a ×4 objective. The implant with the surrounding tissue was superimposed by a software grid consisting of 36 rectangular areas. The grid was subdivided into 3 zones, each consisting of 12 rectangular areas: the skin zone (the upper 1/3 of the implant facing the skin side); the middle zone (the middle 1/3 of the implant); and the muscle zone (the lower 1/3 of the implant facing the muscle side). The prevalence of bone was scored first for the total number of animals (x out of 6) and second for each zone of the grid for each animal (x out of 12), from which the median values were obtained. The scoring was recorded as follows: - = No detection of bone in any of the 12 rectangles; + = bone detected in 1-4 out of 12 rectangles; ++ = bone detected in 5-8 out of 12 rectangles; and +++ = bone detected in 9-12 out of 12 rectangles.

**Microstructural analysis**

Resin-embedded and polished half-blocks were evaluated using low-vacuum SEM (Quanta 200 environmental SEM, FEI Company, The Netherlands) operated in the backscattered electron (BSE) mode at an accelerating voltage of 20 kV, water vapor pressure of 1.0 Torr, and working distance of 10 ± 0.1 mm.

**Raman spectroscopy**

The composition of implant-associated bone was evaluated using a confocal Raman microscope (WITec alpha300 R, Ulm, Germany) equipped with a 532-nm laser. The laser was focused on the surface of polished samples using a ×100 objective with a numerical aperture of 0.9. Spectra were collected in the 300–1800 cm⁻¹ spectral range behind a 600-mm⁻¹ grating. Background fluorescence subtraction was performed using WITEC Control FOUR software. Curve fitting, using mixed Gaussian and Lorentzian functions, and integral area quantification...
was performed using MagicPlot (www.magicplot.com). The Raman metrics investigated included mineral crystallinity, taken as the reciprocal of the full-width at half-maximum (1/FWHM) of the $\nu_1$ PO$_4^{3-}$ (16), the carbonate-to-phosphate ratio ($\nu_1$ CO$_3^{2-}$/$\nu_1$ PO$_4^{3-}$) (17), and the apatite-to-collagen ratio, also referred to as the mineral-to-matrix ratio ($\nu_2$ PO$_4^{3-}$/amide III) (18, 19).

In the clinical implant samples, the composition of new bone interfacing the BioCer implant was determined in the inter-tile ROI in the peripheral and central zones of the implant. A native human skull bone biopsy obtained at the time of retrieval was included as a reference control. In the sheep skull implants, BioCer and Ti implants were analyzed in the peripheral and central ROIs. Furthermore, native sheep skull bone sites were included as controls. In the subcutaneous implants, the composition of new bone interfacing the BioCer implant in inter-tile sites was analyzed.

The composition of the BioCer material itself was also analyzed, both prior to implantation and after retrieval, with emphasis of possible material transformation into apatite after implantation. The material analysis was performed using a confocal Raman microscope (Renishaw inVia™ Qontor®) equipped with a 633 nm laser. The laser was focused down on to sample surface using a $\times$100 objective with a numerical aperture of 0.9. Spectra were collected in the 330–1500 cm$^{-1}$ spectral range behind an 1800 mm$^{-1}$ grating. Background fluorescence subtraction was performed using Renishaw WiRE 5 software.

**Ultrastructural analysis**

Selected resin-embedded specimens of the human and sheep skull implants were used for ultrastructural analysis. The specimens were sputter-coated with a 10-nm-thick layer of Au/Pd. An *in situ* lift-out technique (20) was used for the sample preparation using an FEI Versa 3D FIB-SEM system (FEI company, Eindhoven, The Netherlands). In brief, the region of interest was protected by a 1-µm-thick layer of Pt deposited with ion assistance (area, 30 × 5 µm).
Rough milling was performed using a high-current Ga ion beam. The lamella was cut and transferred to a transmission electron microscopy (TEM) grid for final thinning using a decreasing beam current down to 100 nA, resulting in a ≈100-nm-thick lamella. In total, two samples from the central zone of the retrieved human implant were prepared, and one sample from the central ROI of one of the sheep skull implants was prepared. The TEM samples were evaluated using a Tecnai T20 LaB$_6$ TEM system (FEI company, Eindhoven, The Netherlands) operating at 200 kV. Imaging was performed in bright-field TEM and high-angle annular dark-field scanning mode (HAADF-STEM). Chemical analysis was performed using a nanoprobe in scanning mode by energy dispersive X-ray spectroscopy (EDS) along a line across the interfacial zone with an acquisition time of 10–20 seconds for each spectrum.

**XRD analysis of the retrieved clinical implant**

Approximately 50 mg of BioCer was obtained from resin-embedded human skull implant (n = 3) using a trephine and finely ground in an agate mortar. X-ray diffractograms were obtained using a Bruker D8 Advance (Lynx-Eye detector, CuK$_{\alpha}$), over 3–60 degrees, 5500 steps, and 0.3–1 s dwell time. Crystalline phases were identified using the following reference patterns from the International Centre for Diffraction Data database (ICDD): monetite (DCPA: 04-009-3755), calcium deficient hydroxyapatite (CDHA: 00-046-0905), hydroxyapatite (HAp: 01-074-0565), substituted hydroxyapatite (HAp-O: 04-011-1880), beta-tricalcium phosphate (β-TCP: 04-008-8714), Mg-substituted TCP (TCP-Mg: 01-076-8366) and beta-calcium pyrophosphate (β-CPP: 04-009-3876). PROFEX (v4.1.0) software was used for Rietveld refinement, with all detected phases refined simultaneously for: lattice parameters (a, b, c), Lorentzian size broadening (B1), scale factor (GEWICH), and preferred orientation (spharm). Since HAp apatite peaks were imperfectly fit, CDHA and substituted apatite were also run and compared alone, or in combination. Each refinement was run from 5–60 °2θ, including a simulated amorphous peak, with maximal intensity at 18 °2θ, to model the background from
the embedding resin. Preliminary analysis of each sample revealed that microstrain (K2) and particle size dispersion were not significant contributors to the pattern fit.

2.8. Statistics

All specimens were coded, processed and analyzed systematically according to the study design. Due to the gross difference between the test (BioCer) and control (Ti) implants, complete blinding of the examiner was not possible. The examiner of the human skull implant was blinded with respect to the regions of interests (i.e. periphery, center and transition). All obtained data were processed without transformation, and no outliers were removed. The quantitative histomorphometric data were tested for normality using the Shapiro–Wilk test, which revealed non-normal distributions; hence, non-parametric statistical comparisons were executed. Based on the experimental design, histomorphometric comparisons of the sheep skull and subcutaneous implants (BioCer vs. Ti) were analyzed using paired Friedman and/or Wilcoxon signed-rank tests. Histomorphometric comparisons of the peripheral, central, and transitional zones of the clinical implant were analyzed using non-paired Kruskal–Wallis and Mann–Whitney U tests. For Raman spectroscopy, non-paired Kruskal–Wallis and Mann–Whitney U tests were used. All statistical analyses were performed in SPSS (v. 23; IBM Corporation). The histomorphometric variables (BA%, BIC% and bone growth%) are presented as the mean ± SE. In addition, the individual data values for these variables are presented in dot plots in the Supporting Information. The bone prevalence in soft tissue is presented as the median and range. The Raman spectroscopy variables are presented as the mean ± SD. All reported p-values are two-sided, and p-values of < 0.05 were considered statistically significant. The individual p-values for the statistically significant differences are provided in the respective graphs/tables.
Fig. S1. Macroscopic evaluation after 12 months in sheep skull. Photographs showing the bottom view (dura side) of the bioceramic (BioCer) and titanium (Ti) at 12 months after implantation. The black arrows indicate visible areas of the Ti implant.
Fig. S2. Survey light micrographs of titanium and bioceramic implants in sheep cranial defects. Survey light micrographs of toluidine blue-stained sections of titanium (Ti) (A) and bioceramic (BioCer) (B) implants after 12 months in sheep cranial defects. EO = endosteum;
FC = fibrous capsulation; NOB = new osteonal bone; PO = periosteum; RB = native recipient bone.
Fig. S3. Light micrographs of titanium implant in sheep cranial defect. Light micrographs of toluidine blue-stained sections of a titanium (Ti) implant after 12 months in a sheep cranial defect. The micrographs show bone formation in the peripheral (A-D) and central (E-H)
regions of the defect. BV = blood vessel; CT = connective tissue; MLC = mesenchymal-like cells; NB = new bone; NOB = new osteonal bone; NWB = new woven bone; Ob = osteoblast; OCL = osteoclast-like cell; Ot = osteocyte.
Fig. S4. Light micrographs of bioceramic implant in sheep cranial defect. Light micrographs of toluidine blue-stained sections of a bioceramic (BioCer) implant after 12 months in a sheep cranial defect. The micrographs show bone formation in the peripheral
region (A, B, C), on the dura side (D), on the skin side (E, F) and in the central region (G, H).

BV = blood vessel; CT = connective tissue; NB = new bone; NOB = new osteonal bone; NWB = new woven bone; Ob = osteoblast; Ot = osteocyte; PO = periosteum; RB = native recipient bone.
Fig. S5. Light micrographs of bioceramic implant in sheep cranial defect. Light micrographs of toluidine blue-stained sections of a bioceramic (BioCer) implant after 12 months in a sheep cranial defect. The micrographs show ongoing coupled bone formation and...
remodeling within BioCer porosities and concavities. BV = blood vessel; NB = new bone; NWB = new woven bone; Ob = osteoblast; OCL = osteoclast-like cell; Ot = osteocyte.
Fig. S6. Light micrographs of bioceramic implant in sheep cranial defect. Light micrographs of toluidine blue-stained sections of a bioceramic (BioCer) implant after 12 months in a sheep cranial defect. The micrographs show bone bridging between the partly degraded BioCer and the Ti component of the implant. BV = blood vessel; NB = new bone; NOB = new osteonal bone; Ob = osteoblast; Ot = osteocyte.
Fig. S7. Light micrographs of native recipient bone adjacent to cranial defect in sheep.

Light micrographs of toluidine blue-stained sections showing the endosteum of the native recipient bone immediately adjacent to defects treated with either titanium (A, C, E) or bioceramic (B, D, F) implants for 12 months. BV = blood vessel; EO = endosteum; LC = lining cells; MLC = mesenchymal stem cells; Ot = osteocyte; RB = native recipient bone.
Fig. S8. Light micrographs of bioceramic implant in sheep cranial defect. Light micrographs of toluidine blue-stained sections showing the periosteum and endosteum covering the bone formed around the bioceramic (BioCer) implant on the skin (A-D) and dura
(E-H) sides, respectively. BV = blood vessel; EO = endosteum; LC = lining cells; MLC = mesenchymal stem cells; NOB = new osteonal bone; Ot = osteocyte; PO = periosteum.
Fig. S9. Light micrographs of titanium implant in sheep cranial defect. Light micrographs of toluidine blue-stained sections showing fibrous encapsulation of the titanium (Ti) implant on the skin (A, B) and dura (C, D, G, H) sides. Fibrous capsulation was not evident in relation
the Ti in the inter-tile region (E, F). BV = blood vessel; FC = fibrous capsulation; MLC = mesenchymal stem cells.
Fig. S10. Light micrographs of titanium and bioceramic implants in sheep cranial defects.

Light micrographs of toluidine blue-stained sections showing vascularity and woven bone formation in the inter-tile region of the titanium (Ti) (A, C, E, G) and bioceramic (BioCer) (B,
D, F, H) implants after 12 months in sheep cranial defects. BV = blood vessel; LCT = loose connective tissue; MLC = mesenchymal stem cells; NOB = new osteonal bone; NWB = new woven bone; white arrows = BioCer particulates.
Fig. S11. Histomorphometry of titanium and bioceramic implants in sheep cranial defects. Dot plots of data from individual animals (n=7) showing the percentages of bone area (A and C) and bone-implant contact (B and D). The measurements were performed in the total (A and B) as well as in the peripheral and central (C and D) ROIs of the titanium (Ti) and bioceramic (BioCer) implants at 12 months after implantation. Significant differences (p < 0.05) are indicated by asterisks.
Fig. S12. Histomorphometry of titanium and bioceramic implants in sheep cranial defects. The column graphs show the percentages of bone area (A and B) and bone-implant contact (C and D) (mean ± SE). The measurements were performed in the total (A and C) as well as in the peripheral and central (B and D) ROIs of the titanium (Ti) and bioceramic (BioCer) implants at 12 months after implantation. Significant differences (p < 0.05) are indicated by asterisks.
Fig. S13. Electron microscopy images of implant in sheep cranial defect. Backscattered electron scanning electron microscopy (BSE-SEM) overview micrographs of the peripheral regions of a titanium (Ti) implant after 12 months in a sheep cranial defect. NOB = new osteonal bone; Ot.Lc = osteocyte lacuna.
Fig. S14. Electron microscopy images of bioceramic implant in sheep cranial defect. Backscattered electron scanning electron microscopy (BSE-SEM) overview micrographs of the peripheral regions of a bioceramic (BioCer) implant after 12 months in a sheep cranial defect. NOB = new osteonal bone; Ot.Lc = osteocyte lacuna.
Fig. S15. Electron microscopy images of bioceramic implant in sheep cranial defect. Transmission electron micrographs of the interface between new bone (NB) and the BioCer surface. Intimate contact between bone and bioceramic (BioCer) is observed (A and B). Multiple canaliculi are observed as round holes with a size of 200 nm, both close to and far from the surface (B and C). Collagen banding is observed in the bone (D). The insert black boxes (b and c, d) in (A) indicate the regions presented in the corresponding higher-magnification images (B, C, D), respectively. HAADF-STEM (A, B, C) and bright-field TEM (D).
Fig. S16. Computed tomography scan and histological sections of clinical implant. A computed tomography scan of the clinical implant in the patient’s skull prior to retrieval. The color coding shows the tiles in the respective zones of the implant subjected to different
analyses. The dotted lines indicate the site of the histological sections obtained and embedded in LR White resin after retrieval. Red = peripheral zone tiles (P 1-9); green = central zone tiles (C 1-3); yellow = transitional zone tiles (T 1-5).
Fig. S17. Light micrographs of clinical bioceramic implant in human cranial defect. Light micrographs of toluidine blue-stained sections of the bioceramic (BioCer) implant after 21 months in a human cranial defect. The micrographs show the new osteonal bone in the interstitial spaces in the peripheral (A, B), central (C, D), and transitional (E, F) zones of the implant.
BV = blood vessel; EO = endosteum; NOB = new osteonal bone; PO = periosteum; Ot = osteocyte; Ti = titanium.
Fig. S18. Light micrographs of clinical bioceramic implant in human cranial defect. Light micrographs of toluidine blue-stained sections of the bioceramic (BioCer) implant after 21 months in a human cranial defect. The micrographs show the new osteonal bone on the skin (A, C, E) and dura (B, D, F) sides of tiles in the peripheral (A, B), central (C, D), and transitional (E, F) zones of the implant. BV = blood vessel; EO = endosteum; NB = new bone; NOB = new osteonal bone; PO = periosteum.
Fig. S19. Light micrographs of clinical bioceramic implant in human cranial defect. Light micrographs of toluidine blue-stained sections of the bioceramic (BioCer) implant after 21 months in a human cranial defect. The micrographs show ongoing coupled bone formation and remodeling within BioCer porosities and concavities of tiles in the peripheral (A, B), central
(C, D), and transitional (E, F) zones of the implant. BV = blood vessel; LCT = loose connective tissue; NB = new bone; NOB = new osteonal bone; Ob = osteoblast; OCL = osteoclast-like cell; Ot = osteocyte.
Fig. S20. Light micrographs of clinical bioceramic implant in human cranial defect. Light micrographs of toluidine blue-stained sections of the bioceramic (BioCer) implant after 21
months in a human cranial defect. The survey micrograph (A) shows ongoing coupled bone formation and remodeling in an inter-tile region. The insert black boxes (b, c, d) in (A) indicate the regions presented in the corresponding higher-magnification images (B, C, D), respectively. The areas of remodeling are encountered in the new bone filling the inter-tile space (A, C) as well as at the interface between the bone and the BioCer (A, B) and titanium (Ti) (A, D). BV = blood vessel; NOB = new osteonal bone; Ob = osteoblast; OCL = osteoclast-like cell; Ot = osteocyte.
Fig. S21. Light micrographs of clinical bioceramic implant in human cranial defect. Light micrographs of basic fuchsin-stained sections of the bioceramic (BioCer) implant after 21 months in a human cranial defect. The survey micrograph (A) shows ongoing coupled bone formation and remodeling in a BioCer tile. The insert black boxes (b, c) in (A) indicate the regions presented in the corresponding higher-magnification images (B, C), respectively. BV = blood vessel; NOB = new osteonal bone; Ob = osteoblast; OCL = osteoclast-like cell; Ot = osteocyte; Ti = titanium.
Fig. S22. Light micrographs of clinical bioceramic implant in human cranial defect. Light micrographs of toluidine blue-stained sections showing the periosteum and endosteum covering the bone formed around the bioceramic (BioCer) implant on the skin (A, B) and dura (C, D) sides, respectively. BV = blood vessel; EO = endosteum; LC = lining cells; NOB = new osteonal bone; Ot = osteocyte; PO = periosteum.
Fig. S23. Histomorphometry of clinical bioceramic implant in human cranial defect. Dot plots of the histomorphometric measurements showing the percentages of bone area (A) and bone-implant contact (B) in the tile ROI as well as the bone area (C), bone-implant contact (D)
and bone growth (E) in the inter-tile ROI in the peripheral, central and transitional zones of the clinical bioceramic implant retrieved after 21 months in a human cranial defect. Significant differences ($p < 0.05$) between the different zones are indicated by asterisks. BioCer = bioceramic; Ti = titanium.
Fig. S24. Histomorphometry of clinical bioceramic implant in human cranial defect. The column graphs show the percentages of bone area (A) and bone-implant contact (B) in the tile ROI in the peripheral, central and transitional zones after 21 months in a human cranial defect (mean ± SE). Significant differences (p < 0.05) between the dura and skin sides in the same zone are indicated by asterisks. Significant differences (p < 0.05) between the different zones with respect to the dura and skin are indicated by small letters. BioCer = bioceramic.
Fig. S25. Micro-computed tomography images of clinical bioceramic implant in human cranial defect. Micro-computed tomography images of selected parts of the blocks in the central, transitional and peripheral zones, corresponding to color-coded zones on the computed tomography scan.
Fig. S26. Micro-computed tomography images of clinical bioceramic implant in human cranial defect. Different views of the central block. The dura side shows less bone than the skin side, as indicated by the rougher surface in the volume rendering and the red areas in the surface rendering of the segmented data set. In the center, a maximum-intensity projection shows the extensive bridging between the tiles.
Fig. S27. Histological and corresponding micro-computed tomography images of clinical bioceramic implant in human cranial defect. Histological section (A) and the corresponding micro-computed tomography segmentations (B, C) from the human skull implant (tiles in the central zone).
Fig. S28. Electron microscopy images of clinical bioceramic implant in human cranial defect. Transmission electron micrographs of the interface between new bone (NB) and the bioceramic (BioCer) surface in the central zone (Sample 1). Intimate contact between the bone and BioCer is observed (A, B and D). A more porous subsurface is observed (C). The insert black boxes (b, c, and d) in (A) indicate the regions presented in the corresponding higher-magnification images (B, C, D), respectively.
Fig. S29. Electron microscopy images of clinical bioceramic implant in human cranial defect. Transmission electron micrographs of the interface between new bone (NB) and the bioceramic (BioCer) surface (Sample 2). Intimate contact between the bone and BioCer is observed; however, at this site, the dense grains are not visible (A, B and D). The porous region of the implant shows precipitated apatite (B and D). The collagen banding perpendicular to the implant indicates the direction of the fibre bundles (parallel to the surface) in the bone (C). The insert black boxes (b, c, and d) in (A) indicate the regions presented in the corresponding higher-magnification images (B, C, D), respectively.
Fig. S30: Phase analysis using X-ray diffraction. A: BioCer in the human skull site (individual samples are labelled a, b, and c). B: Rietveld refinement and phase identification.
**Table S1:** Phase composition analysis using X-ray diffraction (XRD) of the BioCer implant after manufacturing and autoclave sterilization (n=10).

| Sample (#) | Brushite | Monetite | β-TCP | HA    | β- CPP |
|------------|----------|----------|-------|-------|--------|
| #1         | 0.00     | 84.90    | 8.30  | 0.10  | 6.70   |
| #2         | 0.00     | 81.60    | 10.70 | 0.10  | 7.60   |
| #3         | 0.00     | 85.30    | 8.10  | 0.10  | 6.50   |
| #4         | 0.00     | 85.40    | 7.90  | 0.20  | 6.50   |
| #5         | 0.10     | 86.00    | 6.90  | 0.10  | 6.90   |
| #6         | 0.10     | 84.40    | 8.60  | 0.20  | 6.70   |
| #7         | 0.10     | 85.00    | 8.10  | 0.10  | 6.70   |
| #8         | 0.00     | 84.70    | 8.60  | 0.10  | 6.60   |
| #9         | 0.00     | 85.70    | 7.50  | 0.00  | 6.80   |
| #10        | 0.10     | 84.40    | 8.70  | 0.10  | 6.70   |

**Mean (± SD)**  
0.04 (0.05)  84.74 (1.22)  8.34 (1.00)  0.11 (0.06)  6.77 (0.32)
Table S2: Phase composition analysis using X-ray diffraction (XRD) of the BioCer clinical implant after 21 months of implantation (n=3).

| Sample (#) | Brushite | Monetite | β-TCP* | Calcium deficient HA | β-CPP |
|------------|----------|----------|--------|----------------------|-------|
| #1         | 0.00     | 1.55%    | 16.14% | 71.87%               | 10.44%|
| #2         | 0.00     | 2.51%    | 27.62% | 65.02%               | 4.85% |
| #3         | 0.00     | 3.86%    | 20.29% | 71.67%               | 4.18% |

Mean (± SD) 0.00 (0.00) 2.64 (1.16) 10.68 (5.74) 69.52 (3.90) 6.49 (3.44)

* β-TCP values represent the combined amounts (weight percentage) of β-TCP (04-008-8714) and magnesium substituted β-TCP (01-076-8366).
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