Injectable Bone Substitute Material on the Basis of β-TCP and Hyaluronan Achieves Complete Bone Regeneration While Undergoing Nearly Complete Degradation

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Purpose: In this prospective study, the regenerative potential and pathways of a new injectable bone substitute (IBS) material composed of beta-tricalcium phosphate (β-TCP) and hyaluronan were investigated for its application in alveolar bone regeneration within extraction sockets. Materials and Methods: The bone substitute material was implanted in 44 extraction sockets after removal of teeth not worth preserving in the maxillary and mandibular arches of 21 patients. Four months after augmentation, bone biopsy samples were harvested simultaneously with implant placement for histologic and histomorphometric analysis of tissue reaction and determination of tissue formation (newly formed bone, connective tissue, and remaining IBS) within the augmentation bed. Furthermore, the inserted bone-level implants (C-Tech Esthetic Line) were followed up clinically and radiologically for at least 1 year after prosthetic loading to determine the potential impact of tissue reaction to the IBS on implant stability and performance. Results: The histologic and histomorphometric analyses revealed a gentle tissue reaction with mainly mononuclear and only few multinucleated giant cells within the implantation bed. Histomorphometric analysis revealed mainly newly formed bone tissue (44.92% ± 5.16%) and connective tissue (52.49% ± 6.43%). Only a few remnants of the IBS (2.59% ± 2.05%) could be found. The IBS, with its easy application and fluidity, seemed to be suitable for three-dimensional stable defects such as the intact extraction socket. Conclusion: The IBS contributed to an osteoconductive tissue reaction while undergoing a time-controlled degradation. Clinical and radiological follow-up investigation of the implants inserted in the regenerated area revealed that the IBS contributed to a long-term stable implantation bed for dental implants. The appearance of the IBS can be described as a bulk that is formed within the augmentation bed and that promotes new bone formation through an osteoconductive procedure. Int J Oral Maxillofac Implants 2018;33:636–644. doi: 10.11607/jomi.6026

Keywords: β-TCP, C-Tech Implants, injectable bone substitute material, socket preservation, tissue engineering

In cases of atrophy of the alveolar crest or localized bone defects, bone substitute materials have been widely investigated in the past decades and serve as reliable alternatives to autologous bone transfer to enlarge the amount of bone prior to placement of dental implants.1 The use of autologous bone, harvested from different intra- or extraoral sites, was for a long time considered to be the gold standard for augmentation procedures, because of its osteoinductive, osteoconductive, and osteogenic potential.2 However, it is known that in certain indications (eg, the socket preservation), bone substitute materials perform equally. Furthermore, its use comes along with several disadvantages, such as the need for a second surgical site and the risk of donor site morbidity.3 Especially in smaller bony defects or established augmentation models, such as the socket preservation or the sinus augmentation procedure, the use of autologous bone transplants can be

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To achieve these goals, not only the peri-implant tissue conditions in combination with implant stability. Stable, healthy, and esthetic peri-implant hard and soft tissue stability of dental implants placed after socket preservation are of importance and therefore the burden to the patient.

In a previous in vivo study by the present authors’ group, IBS composed of beta tricalcium phosphate (β-TCP), cellulose, and hyaluronic acid was analyzed in the subcutaneous implantation model in Wistar rats over a period of 60 days. Implantation of pure solid β-TCP, injection of sodium chloride, and sham operation served as controls. By histologic and histomorphometric methodology, the cellular reaction, the inflammatory response, and the vascularization within the implantation bed were determined. The combination of β-TCP, cellulose, and hyaluronic acid generated a two-phasic bulk with an inner core of β-TCP granules and an outer core of an aqueous solution, which inhibited the premature ingrowth of connective tissue in the intergranular space within the first 30 days. Furthermore, and in contrast to the control groups, the outer structure induced the formation of multinucleated giant cells, which resulted in a higher vascularization of the implantation bed.4

In a further in vivo study, the β-TCP-based IBS material was investigated in a rabbit critical-size distal femoral condyle model for bone regeneration. Two defects were prepared, one filled with the IBS and the other used for control. Histologic and histomorphometric analysis after 1, 3, and 6 months revealed that bone tissue formation occurred through osteoconductive processes over time, starting from the defect borders to the center. The amount of bone formation in the experimental group was significantly higher than that found in the control group after 3 and 6 months (P < .05). After 6 months, the application of the IBS resulted in a restitutio ad integrum with nearly complete degradation of the bone substitute material.5

Besides tissue reaction to the IBS material, the potential impact of the IBS material on osseointegration and the hard and soft tissue stability of dental implants placed after socket preservation are of importance and interest to clinicians, physicians, and patients.

The ultimate goals in implant dentistry are long-term stable, healthy, and esthetic peri-implant hard and soft tissue conditions in combination with implant stability. To achieve these goals, not only the peri-implant tissue must meet special requirements, but the placed implant and therefore the implant system must also fulfill mechanical and constructional demands. In a previous study, a newly developed implant system (C-Tech Esthetic Line, C-Tech Implants) was investigated clinically and radiologically after a follow-up period of 2 years. In total, 50 implants were placed immediately after extraction of teeth not worth preserving in the anterior and premolar region. Two years after loading, none of the implants failed or presented an acute infection or peri-implantitis. All the implants presented a sufficient amount of healthy peri-implant keratinized soft tissue. The peri-implant bone level was stable, with only a minimum of marginal bone loss over 2 years. Therefore, it could be concluded that the observed bone-level implant system with a rough surface and a conical implant-abutment connection can maintain peri-implant hard and soft tissue health in immediately placed implants over a mean observation period of 2 years (unpublished data).

In the present prospective study, the ability of the IBS presented here was investigated on a clinical and histologic basis for the first time. After material augmentation in fresh extraction sockets of teeth not worth preserving, the extent of new bone formation, connective tissue, remaining IBS, and vascularization within the augmentation bed were analyzed. Furthermore, the clinical performance of the implant system and its ability to maintain peri-implant health and hard and soft tissue stability in the augmented region were analyzed after 1 year of loading.

**MATERIALS AND METHODS**

**Study Design in Humans**

In a group of 21 patients (11 female, 10 male) from the authors’ private dental clinic, the β-TCP-based IBS material was used for socket preservation after extraction of teeth not worth preserving in the maxillary and mandibular arches. Participating patients had an average age of 51.4 years (26–70 years).

The prospective study was approved by the Ethics Commission of the University of Frankfurt am Main and was carried out in accordance with the Fifth Revision of the World Medical Association Declaration of 2000 (version 2008) and the STROBE guidelines for observational studies. Prior to surgery, informed consent for the socket preservation procedure and the present study was obtained from all patients.

All participating patients presented with no suspicious general health and without contraindications against surgical interventions. Basic anamnestic data, including medical history and smoking habits, as well as an examination of the oral cavity, were recorded.
Socket preservations with the IBS were performed in teeth not worth preserving but without an acute infection. Only extraction sockets with intact vestibular lamella in the coronal part were included. After extraction, the extraction site was filled with the β-TCP, cellulose, and hyaluronic acid–based IBS and covered with a collagen matrix; wound adaptation was accomplished with tension-free single sutures. Postoperative medication consisted of antibacterial chlorhexidine 0.2% mouthrinse and ibuprofen 400 mg as an analgesic agent. In a second-stage surgery, 4 months after socket preservation, in total 44 dental implants (35 in the maxilla and 9 in the mandible) (Esthetic Line, C-Tech Implants) were inserted slightly subcrestally in the augmented regions. Simultaneously, 44 cylinder-shaped bone biopsy samples were taken from the augmented regions. In an average of 4.5 months (4–6 months) after implant placement, the implants were exposed by performing the roll flap technique, and a healing abutment was incorporated (Table 1).

Table 1 Information about Participating Patients and Dental Implants Placed

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<th>Gender (M/F)</th>
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F = female; M = male; Max = maxilla; Mand = mandible.
Clinical and Radiologic Follow-up Investigation

One year after prosthetic loading, implants were investigated clinically and radiologically according to previously published methods at the authors’ private dental clinics to investigate the influence of the tissue reaction and integration mechanisms of the augmented biomaterial on the clinical performance of the inserted implants. The following parameters were investigated: implant survival, ie, implants in situ; implant mobility, probing depths, and bleeding on probing (BOP) at four sites per implant; and presence of gingival recessions around the implants, which leads to exposure of the implant shoulder or implant threads. Presence of peri-implant osteolysis and marginal bone loss was analyzed by radiologic images.

Investigated Bone Substitute Material

The investigated IBS material is based on pure-phase β-TCP synthesized by a solid-state reaction as described previously. After its manufacturing, the generated tricalcium phosphate was crushed and sieved to a particle size of < 63 mm and sintered at about 1,000°C. Subsequently, the β-TCP was mixed with an organic substance containing methylcellulose and sodium hyaluronate (ie, a salt of hyaluronic acid). β-TCP and the aqueous polymer solution were mixed in a ratio of 70/30 wt%, resulting in a malleable and viscoplastic paste that was sterilized by high-pressure, saturated steam sterilization at 121°C for 15 minutes.

Investigated Dental Implants

In the present study, a newly developed implant system was investigated to determine its ability to maintain peri-implant hard and soft tissue stability and peri-implant health. The C-Tech Esthetic Line implant (EL, C-Tech Implants) combines several design features that have proven to have a positive impact on implant stability and peri-implant bone and soft tissue health. The bone-level implant design allows equi- or subcrestal setting and therefore prevents exposure of the implant through bone resorption, especially in the case of thin buccal bone and unfavorable gingiva biotypes. Implants consist of three threading profiles adapted to different bone structures along the depth of the implant, which promise high grades of primary stability. Furthermore, the implant has a grit-blasted and acid-etched surface topography to increase and accelerate bone apposition and osseointegration. The implant-abutment connection is designed in a morse-locking, conical connection with platform switching and an indexing hex.

Tissue Workup and Staining Procedures

The workup and staining methods of the clinical tissue samples were conducted using established methods. Briefly, after their harvesting, the tissue samples were fixed in 4% formalin, and then a tissue processor (TP1020, Leica) was used for the first step of the workup, ie, the treatment by an increasing alcohol series and xylool as well as a primary paraffin treatment. The samples were then embedded in paraaffin by an embedding station (Tissue Embedding Center EG1150, Leica) to produce consecutive histologic slides with a thickness of 2 to 4 µm by a rotation microtome (CUT 5062, SLEE Medical).

Histologic and Histomorphometric Analyses

The histologic analysis, which was conducted according to an established protocol, involved the following research parameters: fibrosis, hemorrhage, necrosis, vascularization; presence of granulocytes, lymphocytes, plasma cells, monocytes/macrophages, biomaterial-associated multinucleated giant cells (BMGCs); and the outcome of bone regeneration on the basis of the bone substitute. Therefore, a light microscope (Eclipse 80i, Nikon) was used, and the photomicrographs were prepared using an Axiocam 105 color digital camera connected to a computer running the ZEN 2 (blue edition) software (both: Carl Zeiss Microscopy GmbH).

The histomorphometric analysis was also conducted as previously published. Briefly, two slides of every tissue sample stained with Masson-Goldner and CD31 were digitized to generate “total scans” as the basis for the histomorphometric measurements, using a special scanning microscope (Eclipse Ni, Nikon) stocked with an automatic table (EK 14 mot, Merzhaus) and a DS-Fi1 digital camera (Nikon) connected to a computer running NIS Elements software (version 4, Nikon). This software was then used to measure both the amount of newly formed bone tissue and the vascularization (vessel number and area). Furthermore, the total area of the implant beds was measured to determine the different tissue amounts (newly formed bone tissue, remaining biomaterial, and connective tissue), in percent as well as the number of vessels per square millimeter (vessels/mm²), and the percent of vascularization.

Statistical Analysis

Prior to the study procedure, a sample size calculation was performed. According to the literature, a mean amount of newly formed bone of 30% to 40% after socket preservation was assumed. For the investigated bone substitute material, an increase of 5% and a standard deviation of 10% was expected. This led to a sample size of 40 patients that was necessary to prove the expected difference with a power of 80%. Data were statistically analyzed and graphed as mean ± standard deviations, using the GraphPad Prism 6.0c
software (GraphPad Software Inc). A statistical analysis of the different tissue amounts was conducted by analysis of variance (ANOVA), and differences were considered significant if $P$ values were less than .05 and highly significant if $P$ values were less than .01 or less than .001. Previous to the study, a sample size calculation was performed to achieve a power of 80% and a significance level of 0.95 ($P < .05$).

**RESULTS**

**Clinical Results**

According to the study protocol, 44 implants were inserted in 21 patients after a 4-month mean healing period of the augmentation procedure and investigated clinically at least 1 year after prosthetic loading. The aim of the follow-up investigation was to determine the potential influence of the tissue reaction to the IBS material on implant osseointegration and long-term hard and soft tissue stability. One year after loading, all 44 implants were in situ and suitable for prosthetic rehabilitation. This results in an implant survival rate of 100%. All implants were restored with fixed prosthetics. Analysis of the recorded radiologic images showed no osseous peri-implant defects, and the peri-implant bone level of all implants reached the implant shoulder. With only a marginal bone loss of 1 mm in four implants and 0.5 mm in another four implants, the mean marginal bone loss calculated was 0.136 mm after 1 year of prosthetic loading (Table 2). Probing depths and the BOP index were evaluated to determine the potential impact of the IBS material on the peri-implant soft tissue health. The peri-implant sulcus was measured with a calibrated blunt periodontal probe. Besides the depths of the peri-implant pocket, it was observed whether bleeding on probing occurred. The mean probing depth was 2.56 mm with the maximum pocket depth of 4 mm. In 18.75% of the probing sites and 36.4% of the implants, BOP was observed, mostly in sites with probing depths of 3 or 4 mm. Recession of the gingiva around the implants was observed on six implants (13.6%). In further clinical investigation, these implants have also shown the presence of BOP (Table 2).

Figures 1a to 1c show socket preservation in patient 5 after extraction of a tooth not worth preserving because of a root fracture.

**Histologic Analysis**

The histologic analysis showed that relatively high amounts of newly formed bone tissue were found within the implantation areas of the IBS material (Fig 2a). Interestingly, similar amounts of connective tissue were detected, and only very low amounts of the remaining bone substitute material were visible (Fig 2a). The analysis at higher magnifications revealed that only a few of the $\beta$-TCP particles of the IBS were detectable and mostly embedded within the newly formed bone matrix (Fig 2b). Within the connective tissue, small amounts of the pasty material parts (asterisks) were found that promote the ingrowth of new bone tissue (blue arrows) (Figs 2c and 2d). Furthermore, the bone substitute material surface was mostly colonized with mononuclear cells (Fig 2d) that seem to be mainly osteoblasts, because the detection of the CD68 antigen showed that none of these cells were macrophages (data not shown).

In addition, the histological analysis showed that a relatively high vascularization was detected within the former implant beds of the IBS material (Fig 3a). Within the connective tissue surrounding the newly formed bone tissue, only small remnants of the bone substitute were found (Figs 3b and 3c). Within these spots, the $\beta$-TCP particles did not seem to elicit an inflammatory tissue reaction; only single mononuclear cells were found (Figs 3b and 3c). Furthermore, few remnants of the pasty material component were observed in the connective tissue (Fig 3c). The tissue reaction related to these components of the IBS material consisted of mainly mononuclear cells and only some single multinucleated giant cells (Fig 3c).

**Histomorphometric Measurements**

The histomorphometric measurements showed that similar amounts of newly formed bone tissue (44.92% ± 5.16%) and connective tissue (52.49% ± 6.43%) were found in the implantation beds of the IBS (Fig 4). Only the amount of the remaining bone substitute material (2.59% ± 2.05%) was significantly lower ($P < .001$) compared to both the other amounts (Fig 4).

The analysis of the implant bed vascularization showed that 10.22 ± 2.14 vessels/mm² were found in the implant beds of the bone substitute, which accounted for an area of 0.58% ± 0.14% of the total implant area.

**DISCUSSION**

This prospective study reports the histologic, histomorphometric, and clinical analysis of an injectable $\beta$-TCP-based bone substitute material for socket preservation. Clinically, the IBS composed of $\beta$-TCP, methylcellulose, and sodium hyaluronate was convincing by its ease of handling and easy application technique. The defect geometry of an extraction socket, which can be compared to a cylinder-shaped defect, gives the IBS enough stability to stay in place and avoids the fluid IBS from dissolving prematurely. A slight
Table 2  Results from Clinical Follow-up Investigation of Placed Dental Implants

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<th>Patient</th>
<th>Location (FDI numbering system)</th>
<th>Implant loss or implant mobility</th>
<th>Probing depths (mm) at four sites (MB, DB, MO, DO)</th>
<th>Bleeding on probing at four sites (MB, DB, MO, DO)</th>
<th>Gingival recessions</th>
<th>Peri-implant osteolysis</th>
<th>Marginal bone loss (mm)</th>
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+ = present, – = absent; MB = mesiobuccal; DB = distobuccal; MO = mesio-occlusal; DO = disto-occlusal; Max = maxilla; Mand = mandible.
Fig 1  Socket preservation in patient 5 after extraction of a tooth because of a root fracture. After extrac-

tion (a), the socket was filled with the injectable β-TCP–based bone substitute material and a collagen

membrane (b), and achieved wound closure (c).

Fig 2  Representative images of the regenerative characteristics of the IBS. (a) Overview of a tissue

sample from the mandible. Within the augmentation area (AA) of the IBS, high amounts of lamellar, newly

formed bone (NB) were observable beside well-vascularized connective tissue (CT). RB = residual bone,

Masson-Goldner stain, ×100 magnification, scale bar = 500 µm. (b) Only single remnants of the β-TCP

particles (black arrows) of the IBS were observed integrated with the newly formed bone tissue (NB),

whereas no signs of the pasty components were found (CT = connective tissue). Masson-Goldner stain,

×400 magnification, scale bar = 50 µm. (c and d) The pasty component (asterisks) of the IBS allowed a

guided bone growth (blue arrows) starting from the surrounding bone tissue (B). Movat Pentachrome stain;

(c) ×100 magnification, scale bar = 100 µm; (d) ×400 magnification, scale bar = 50 µm.

Fig 3  Inflammatory tissue reaction to the components of the IBS. (a) In general, the connective tissue of

the implant bed of the IBS showed a high level of vascularization (red arrows) and most often no or minor

signs of inflammatory influences. Masson-Goldner stain, ×100 magnification, scale bar = 50 µm. (b and c)

Within the connective tissue of the implant beds of the IBS, single spots were found containing both β-TCP

particles (black arrows) and pasty material components (asterisk in c). While the β-TCP particles (black ar-

rows) that were not embedded in newly formed bone tissue (B) were covered only by mononuclear cells (blue

arrows in b), the pasty component (asterisk in c) induced a granulation tissue within mostly mononuclear

cells (purple arrows in c) and a few multinucleated giant cells (purple arrowheads in c). B = bone tissue,

CT = connective tissue. Movat Pentachrome stain, ×400 magnification, scale bars = 50 µm.
overfilling of the socket proved advantageous, as the application of the collagen membrane and the wound closure formed the IBS.

At the reopening after the socket preservation mean healing period of 4 months, the augmentation bed showed in all cases a profound osteogenesis with a stably regenerated mature bone, which allowed placement of dental implants in all cases. The implant placement procedure was not impaired by the socket preservation procedure, because the implants could be placed with a high primary stability comparable to natural bone and without fracture or other adverse effects.

The results of the clinical and radiologic follow-up investigation of the bone-level implants 1 year after implant placement confirmed the clinical impressions at implant placement. All implants were in situ, stable, and useful for prosthodontic rehabilitation. None of the implants presented any signs of peri-implant infection or hard or soft tissue deficits.

The histologic and histomorphometric analyses of the bone biopsy samples extracted during implant placement revealed a comparable large amount of newly formed bone and connective tissue and only a few remnants of remaining IBS. The newly formed bone appeared in a trabecular structure and was in direct contact with the few remaining β-TCP particles. The degradation of the majority of the applied IBS seems to be promoted by newly formed vessels within the augmentation bed that promote the supply of mostly mononuclear cells, which covered the bone substitute surface. Only a few multinucleated giant cells could be found within the augmentation bed and on the surface of the β-TCP particles.

Concluding the clinical and the histologic results, the appearance of the IBS could be described as a bulk that is formed within the augmentation bed and promotes new bone formation through an osteoconductive procedure. Simultaneously, the β-TCP particles seem to be nearly completely degraded by mostly mononuclear cells that are provided by a vessel-rich granulation tissue. The pasty material component seemed to be integrated with the connective tissue that presented the largest fraction within the investigated biopsy samples.

Regarding the timeline of the tissue reaction after socket preservation, it should be noted that the biopsy samples were taken after a mean integration period of 4 months after the augmentation procedure, which is rather early compared to recommendations of other bone substitute materials. However, already after 4 months of integration, a relatively large fraction of newly formed bone (44.92% ± 5.16%) and a comparable small fraction of remaining IBS (2.59% ± 0.20%) were found. Further, all implants could be placed with a high rate of primary stability. Thus, there seems to be a consequent osteoconduction process in correlation with degradation of the β-TCP component, without a premature breakdown of the augmented IBS bulk. Histologically and clinically, no adverse reactions were found.

When applying and analyzing an IBS material like the IBS investigated in this study, it must be questioned how this biomaterial can be interpreted in correlation with bone substitute materials of different origin and composition that require different handling.

Xenogeneic bone substitute materials, such as the well-investigated and frequently used Bio-Oss (Geistlich Biomaterials), usually made from bovine demineralized bone matrix, are known to support osteoconduction while remaining within the implantation bed for a comparably long period after augmentation. Similar to the tissue reaction to the present study’s IBS, the tissue reaction to this bone substitute material consists mainly of mononuclear cells and only a few multinucleated giant cells. Both bone substitute material classes present almost contrary characteristics (stability versus degradation and solid versus injectable), and both materials have their justification and specific indications. While the IBS with its easy application and fluidity seems to be suitable for three-dimensional stable defects such as the intact extraction socket that avoids dissolution, the solid bone substitute materials seem to be advantageous in geometrically more complex defects, in which the bone substitute material has a space holder function, such as in sinus augmentation procedures.

The present results from clinical, histologic, and histomorphometric analysis of IBS present an example for translational research. The previous histologic results from implantation of IBS in the subcutaneous tissue of Wistar rats for 60 days revealed that implantation of IBS generates a two-phasic bulk with an inner core of β-TCP granules and an outer core of an aqueous solution, which inhibited the premature ingrowth of connective tissue in the intergranular space within the first
30 days. After this promising result, IBS was investigat-
ed in a rabbit critical-size distal femoral condyle model, in which bone tissue formation occurred through os-
teoconductive processes over time, resulting in a resti-
tutio ad integrum with nearly complete degradation of the bone substitute material. Further completing the research on this IBS, the present clinical study was performed, which could confirm the results from small and larger animal studies. The bone substitute mate-
rial contributed to new bone formation and a stable surrounding for dental implants with a favorable and gentle tissue reaction and almost complete biomate-
der degradation.

Overall, this study highlights that understanding of physicochemical material characteristics leads to the correct clinical application of biomaterials. Consequently, materials such as the present study's IBS can be used for defects that are surrounded by the patient's own bone tissue, while materials that persist longer in their augmented region might be more use-
ful for more complex defects when the biomaterial will serve as a mosaic-like structure to rebuild lost bone tis-

tue. To what extent materials that undergo a complete degradation like the present material will also undergo a volume decrease over time needs further elucidation. Further analysis and especially long-term analysis of augmented regions with synthetic bone substitute materials are necessary to answer these questions.

CONCLUSIONS

The regenerative capacity of an IBS material composed of β-TCP and hyaluronan was investigated histologi-
cally and histomorphometrically 4 months after the socket preservation procedure. Furthermore, dental implants were followed up clinically after at least 1 year of prosthetic loading. The histologic analysis revealed a mild tissue reaction with mainly mononuclear cells within the augmentation bed and only a few remnants of the applied IBS material. The histomorphometric analysis showed 44.92% ± 5.16% newly formed bone tissue, 52.49% ± 6.43% connective tissue, and only 2.59% ± 2.05% remnants of the IBS. The IBS was convic

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