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Biofunctionality of MBCP ceramic granules (TricOs™) plus fibrin sealant (Tisseel®) versus MBCP ceramic granules as a filler of large periprosthetic bone defects: an investigative ovine study

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Abstract We aimed to quantify bone colonization toward an untreated titanium implant with primary stability following filling of the defect with micromacroporous biphasic calcium phosphate (MBCP) granules (TricOs™) or MBCP granules mixed with fibrin sealant (Tisseel®). Medial arthrotomy was performed on the knees of 20 sheep to create a bone defect (16 mm deep; 10 mm diameter), followed by anchorage of a titanium screw. Defects were filled with TricOs or TricOs–Tisseel granules, a perforated MBCP washer, a titanium washer and titanium screw. Sheep were euthanized at 3, 6, 12 and 26 weeks. From Week 12 onwards, the percentage of bone in contact with the 8 mm anchorage part of the screw increased in both groups, confirming its primary stability. At 26 weeks, whereas bone colonization was similar in both groups, biodegradation of ceramic was more rapid in the TricOs–Tisseel group ($P = 0.0422$). The centripetal nature of bone colonization was evident. Bone contact with the titanium implant surface was negligible. In conclusion, the use of a model that reproduces a large metaphyseal bone defect around a titanium implant with primary stability, filled with a mixture of either TricOs ceramic granules or TricOs granules mixed with Tisseel fibrin sealant, suggests that the addition of fibrin to TricOs enhances bone filling surgical technology.

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1 Introduction

The United Nations has declared the first decade of the 21st Century as the "Bone and Joint Decade", in recognition of the increasing burden orthopedic conditions will have on world health as life expectancy increases [1]. Worldwide, it has been estimated that orthopedic surgeons currently perform around 2 million bone-grafting or bone repair procedures per year. In orthopedic surgery, the revision of hip replacement surgery is regarded as particularly challenging, given its complex nature and the additional risks to the patient over first-time hip replacement surgery. Assuming the revision procedure itself is successful, the main challenges are to obtain rapid bone colonization of the metaphyseal bone defect surrounding the metallic implant, followed by effective osteointegration. These steps are essential to achieve long-term fixation of the hip implant.

Given the well-documented limitations of autologous bone grafts in general, it is unsurprising that interest in effective bone substitutes is escalating. Several biomaterials have been developed to fill and reconstruct bone defects: natural coral, bovine porous demineralized bone, human demineralized bone matrix, bioactive glass ceramics and calcium phosphate ceramics. All of these materials are biocompatible and osteoconductive, guiding bone tissue from the edges toward the center of the defect.

As a consequence of the pioneering research of Jarcho, de Groot and Aoki in the early 1980s [2–4], synthetic hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) became commercially available as bone substitute materials for dental and medical applications [5]. In the late 1980s, the first basic research on biphasic calcium phosphate (BCP) with various proportions of HA/β-TCP was reported by Daculsi and co-workers [6–10], demonstrating that the bioactivity of these ceramics may be controlled by manipulating the HA/β-TCP ratios. Subsequently, focused studies on BCP by Daculsi and co-workers [11–13] led to the significant increase in the manufacture and use of commercial BCP bioceramics as bone substitute materials for orthopedic and dental applications [14–20]. Since the first human medical application was realized in Nantes hospital by Passuti et al. for spine arthrodesis [21], several other applications have been filed for spine or long-bone filling [11, 15, 22]. Today, BCPs are sold worldwide as bone graft or bone substitute materials for orthopedic, spinal, maxillo-facial and dental applications.

Pre-clinical studies have demonstrated the potential uses of BCP ceramic as scaffold for tissue engineering for bone regeneration, gene therapy, and drug delivery. More recently, the BCP granules concept has been applied in the development of a new generation of injectable and/or mouldable bone substitutes [23, 24]. BCP granules are combined with various polymers, biological (e.g. fibrin sealant—the combination of micro-macroporous BCP [TricOs^{TM}, Biomatlante, France] and fibrin sealant is indicated for use as a bone substitute biomaterial), or synthetic (e.g. hydrosoluble polymer—MBCP Gel$^{®}$ is an injectable, non-self-hardening biomaterial consisting of BCP granules combined with a hydrosoluble polymer) [25, 26], or calcium phosphate cement to improve macroporosity and greater osteoconduction MCPC$^{®}$ (a micro- and macroporous calcium phosphate cement containing BCP granules) [27, 28]. Micro-macroporous BCP (MBCP) ceramics have demonstrated bioactivity and osteoconductivity [10, 29, 30], but lack osteogenic properties for the regeneration of mineralized tissue into large bone defects. The addition of fibrin sealant to MBCP granules confers enhanced malleability, allowing easy implantation and moulding to the required shape.

Fibrin sealants are biological adhesives that mimic the final step of the coagulation cascade. The main components of the sealant are fibrinogen, plasmatic proteins and factor XIII on the one hand and thrombin, calcium chloride and an antifibrinolytic agent such as aprotinin on the other. The components are extracted from human plasma, except for aprotinin, which comes from bovine lungs, and calcium chloride, which is inorganic. Mixing fibrinogen and thrombin simulates the last stages of the natural coagulation cascade to form a structured fibrin clot similar to a physiological clot. This clot is naturally degraded by proteolytic enzymes from the fribrinolytic system, such as plasmin, within 10–14 days [31, 32]. High concentrations of these enzymes are present in response to tissue inflammation. As a result of their hemostatic and adhesive properties, fibrin sealants have been extensively used in most surgical specialties for over two decades [33, 34], where they are used to reduce intra-operative blood loss and post-operative bleeding.

In general, the combination of fibrin sealants and biomaterials confers multiple benefits. The physical properties of the composite are enhanced, with better mechanical resistance than in ceramic alone [35, 36]. Furthermore, initial stability of this bone filler may be achieved through its adhesion to the walls of the bone defect. A fibrin clot serves as the foundation of a complex provisional matrix to both initiate and support subsequent tissue repair [37]. Fibrin might also promote the development of blood vessels and the formation of highly vascularized granulation tissue [38]. Fibrin sealants are natural scaffolds for cell attachment and growth [39, 40], and the presence of several growth factors in the fibrin sealant may also have a positive effect during the initial stages of bone colonization [41].

The physical, chemical and biological properties of both bioceramics and fibrin selant will underpin the development of advanced bone substitutes. The ideal bone substitute should be biocompatible, biodegradable at the expense of bone growth, and mouldable, with adequate mechanical properties to fill and restore bone defects [42]. Towards this goal, we developed a specific combination of fibrin sealant (Tisseel, Baxter, Deerfield, IL, USA) and MBCP ceramic granules (TricOs). The use of this composite for maxillo-facial surgery and large bone defects in animal studies shows that as well as improving handling characteristics, the addition of fibrin provides a good matrix for bone formation and remodeling, with indications of osteogenic properties [12, 43–45].

The goals of our study were to quantify the kinetics of bone colonization toward the titanium implant with primary stability, following the filling of the bone defect with either TricOs alone (TricOs group) or TricOs granules mixed with fibrin sealant (TricOs–Tisseel group).

2 Materials and methods

2.1 Experimental animals, surgical procedure

Under general anesthesia, medial arthrotomy was performed on the knees of 20 female sheep aged 4–5 years and weighing 60–70 kg. All surgical procedures were performed under general anesthesia induced with intravenous diazepam, 1 mg/kg, and ketamine, 4 mg/kg,

followed by halothane. During surgery, animals received intravenous amoxicillin, 1 g. Animals were free to move in their stables and underwent weekly general health and functional evaluations. Analgesia was achieved by intramuscular injection of morphine (0.2 mg/kg every 4 h) and ketoprofen (3 mg/kg/day).

All animal handling and surgical procedures were conducted according to the European Community guidelines for the care and use of laboratory animals (DE 86/609/CEE). The study protocol was approved by the ethics committee of the National Veterinary School in Nantes, France.

2.2 Creation of defect and implantation of biomaterial

After opening of the medial joint capsule, a bone defect measuring 16 mm deep and 10 mm in diameter was created perpendicular to the medial femoral condyle, just cranial to the medial femoral epicondyle (Fig. 1). The depth was controlled by the use of a depth-stop on the drill bit. Using a drill sleeve, a second hole, 8 mm deep with a diameter of 2.5 mm, was centered within the 10 mm-diameter bone defect to allow bone anchorage of a titanium screw. The left and right bone defects were filled with TricOs–Tisseel granules or TricOs granules alone (diameter 1–2 mm), respectively. Before mixing TricOs granules with the fibrin sealant, the thrombin component was diluted from 500 to 50 IU/ml with sterile water. This dilution step was to slow down the fibrin polymerization process and obtain a mixture which remained malleable for up to 10 min, allowing easy implantation and molding [46]. The ratio of the mixture was 0.9 g of TricOs for one kit of Tisseel (1 ml). This was followed by insertion of a

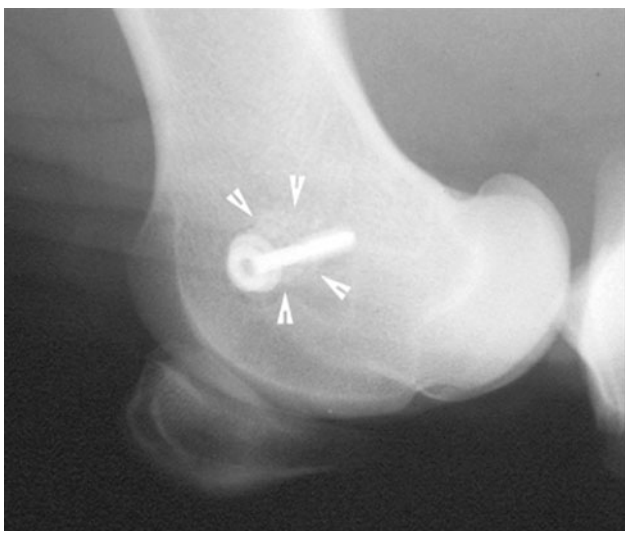

Fig. 1 Radiograph of a titanium screw implanted within the medial femoral condyle. Calcium phosphate biomaterials can be seen around the proximal part of the screw (*arrows*)

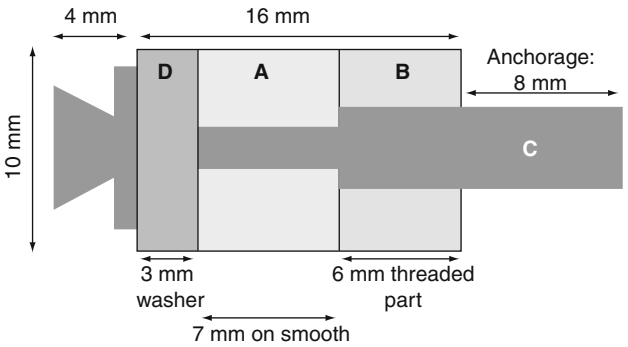

Fig. 2 Schematic representation of the bone defect. (*A*) TricOs granules around smooth core of the screw; (*B*) TricOs granules around the threaded core of the screw; (*C*) bone anchorage of the screw; and (*D*) MBCP washer

perforated MBCP washer (10 mm in diameter and 3 mm thick) (Biomatlante, France) obturating the bone defect to prevent migration of TricOs granules. The MBCP washer was held in place by the insertion of a titanium washer and a titanium spongious screw (4 mm diameter; 28 mm in length; Strycker SA, France). This screw was anchored in the 8 mm-deep hole within the core defect (Fig. 2). MBCP washer and TricOs granules were humidified with NaCl physiologic solution immediately prior to surgical implantation or mixture preparation. Synovial capsule, subcutaneous tissue and skin were routinely closed using interrupted mattress suturing (Safil®; Aesculap Division, B Braun, Tuttlingen, Germany).

2.3 Sample harvesting, histological and histomorphometrical evaluations

Five sheep were euthanized by intravenous pentobarbital after each pre-defined study period of 3, 6, 12, and 26 weeks. Distal femoral metaphysis samples (i.e. bone defect containing biomaterials and the titanium screw) were isolated by transverse microtome section, so that 1 cm of peripheral bone was sourced from the outer limit of the bone defect. Harvested samples were immersed for 3 weeks in formaldehyde solution. Following this fixation step, the samples were embedded in methylmetacrylate and then sectioned transversally to the long axis of the screw, with a diamond-saw microtome (Leica SP 1600, Leica Microsystems, Wetzlar, Germany) to obtain 100 µm sections. Bone sections were examined at two standardized levels from the head of the screw: Level A biomaterials around the smooth core of the screw; and Level C bone anchorage of the screw (Fig. 2). The analyst was blinded to the treatment group of each specimen for all evaluations performed.

Qualitative histological analysis: Three 100-µm bone sections per specimen harvested from each of the two

levels (A and C) were examined by light microscopy (Olympus BH2, Olympus Optical Co., Tokyo, Japan) under polarized light or by cyanin-solochrom staining.

Global analysis: After sectioning, the surface of the residual block opposite the screw's head (one per specimen) was observed via a scanning electron microscope (6300, Jeol, Japan) in back-scattered electron mode (×20, 15 kV). Using a semi-automatic image analysis program (Quantimet Q500, Leica Microsystems, Wetzlar, Germany), the percentages of bone and ceramic in the bone defect were calculated at Level A (Fig. 2).

Centripetal analysis: Using the same back-scattered electron microscopy images obtained at Level A for global analysis (above), the kinetics of bone colonization were assessed (Quantimet Q500, Leica Microsystems, Wetzlar, Germany). This evaluation was performed using an original model that calculates the percentage of bone and bioceramic in four concentric bands of 0.86 mm width (i.e. 30 pixels). This involved four iterative transformations by erosion of the surface of the postoperative bone defect up to 0.31 mm from the surface of the titanium screw.

Bone contact: At Levels A and C (Fig. 2), we also calculated the percentage of bone contact with the titanium screw and the percentage of bone around the screw on three concentric bands of 300 µm, obtained by iterative transformations by dilatation of the shape of the screw.

2.4 Statistical analysis

For the global, centripetal and bone contact analyses we used a non-parametric test (Kruskal–Wallis) to detect any differences. If significant differences were found, we performed an analysis of variance (ANOVA). A 2 × 2 comparison was performed with Fisher's PLSD post hoc test.

3 Results

No lameness, postoperative complications, or surgical model device dislocations were observed during the 26-week study period.

3.1 Validity of model

3.1.1 Reproducibility of mechanical condition

At Level C (the 8 mm anchorage portion of the screw), the mean percentage of bone in contact with the titanium implant increased from 12 weeks onward (Fig. 3; $P = 0.0007$ and 0.0003 at 12 weeks versus 26 weeks for TricOs and TricOs–Tisseel groups, respectively). These results confirm the primary stability of the screw, considered to be an essential prerequisite for secondary osteointegration. We observed a similar level of bone contact in both groups.

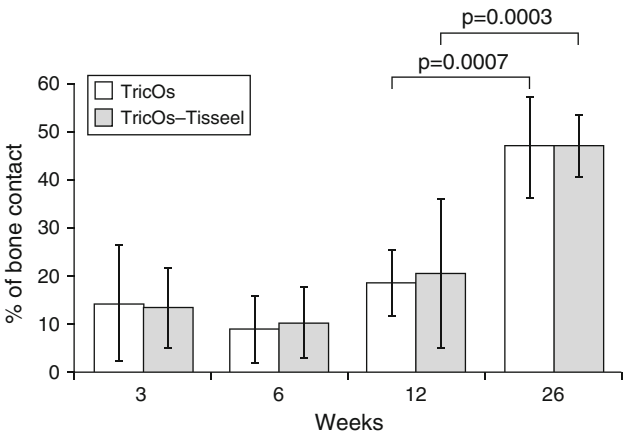

Fig. 3 Mean [SD] percentage of bone contact with the titanium implant at Level C (bone anchorage of the screw) in the two groups (TricOs alone or TricOs–Tisseel)

3.1.2 Homogeneity of defect size

As illustrated in Fig. 4, the size of the defect at Level A was homogeneous for both types of biomaterials tested (TricOs vs. TricOs–Tisseel) over the 26 weeks of the study (around the smooth core of the screw). In contrast, the heterogeneity of the bone defect size observed at Level B (around the threaded core of the screw) indicates that this measurement is too heterogeneous to be of value (data not shown).

3.1.3 Quantity and distribution of the ceramic granules within the bone defect

At 3 weeks, global analysis showed that the amount of ceramic at Level A was similar in both biomaterial groups

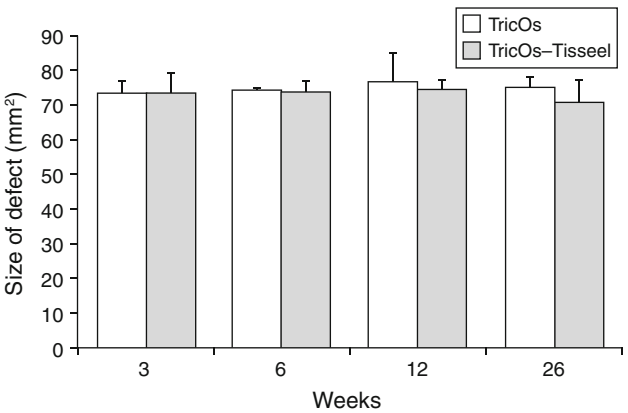

Fig. 4 Mean [SD] size (mm^2) of bone defect at Level A in the two groups (TricOs alone or TricOs–Tisseel)

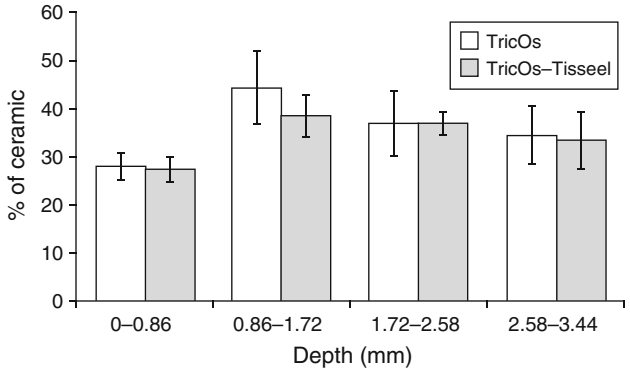

Fig. 5 Mean percentage [SD] of ceramic distribution along the depth of the titanium implant at Level A in the two groups (TricOs alone or TricOs–Tisseel), at 3 weeks. A depth of 0 mm represents the outer limit of the bone

(TricOs vs. TricOs–Tisseel). Furthermore, as illustrated in Fig. 5, there was little difference in the distribution of ceramic along the depth of the bone defect toward the screw.

3.2 Bone colonization and ceramic biodegradation in the bone defect

3.2.1 Global analysis

As shown in Fig. 6, the two biomaterial groups demonstrated similar rates of bone colonization of the defect, with a rapid colonization phase up to 12 weeks, followed by a period of stabilization. In comparison, the biodegradation of the ceramic tended to be more rapid for the TricOs–Tisseel group compared with the TricOs group, with the difference becoming more evident at Week 26 ($P = 0.042$) (Fig. 7).

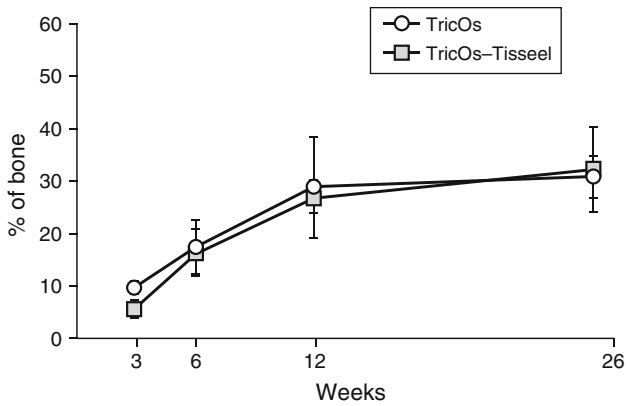

Fig. 6 Mean percentage [SD] of bone colonization within the defect at Level A in the two groups (TricOs alone or TricOs–Tisseel)

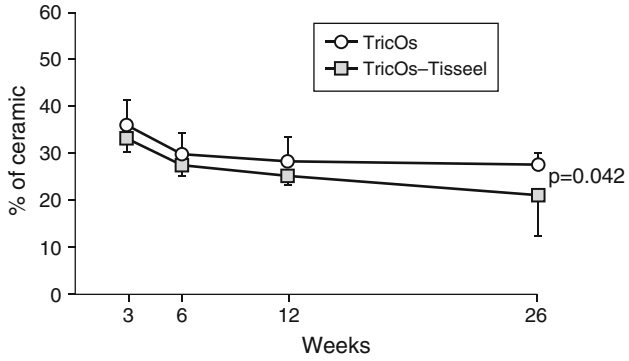

Fig. 7 Mean percentage [SD] of biodegradation of the ceramic at Level A in the two groups (TricOs alone or TricOs–Tisseel)

3.2.2 Centripetal analysis

At 3 weeks, centripetal analysis revealed similar bone colonization in TricOs and TricOs–Tisseel groups with a trend toward faster peripheral bone colonization in the TricOs group (Fig. 8a). In both groups, the majority of bone colonization occurred in the periphery of the defect. We observed far less bone colonization within the center of the defect. Hence at 3 weeks, the centripetal nature of bone colonization was clearly evident.

The pattern of centripetal bone colonization was still evident at 6 weeks, with slightly less colonization apparent in the TricOs–Tisseel group at a level deeper than 1.72 mm (Fig. 8b).

The patterns observed at 6 weeks were more pronounced at 12 weeks (Fig. 8c). The TricOs–Tisseel group showed more bone in the periphery and less in the center of the defect, compared with the TricOs group. The statistically non-significant centripetal tendency was no longer evident for the TricOs group; bone colonization in this group showed approximate uniformity across all depths from the defect.

At 26 weeks, bone colonization was globally at the same level as 12 weeks (Fig. 8d). The pattern of colonization in the two groups appeared to be similar, with a statistically non-significant tendency toward more bone in the periphery than in the center of the defect noted in the TricOs–Tisseel group.

There was no evidence of different rates of ceramic biodegradation at different defect depths. Ceramic biodegradation appeared uniform throughout the defect at each time-point in both groups.

As shown in Fig. 9, bone contact with the titanium screw at Level A was negligible in both groups, with the highest value being observed in the TricOs group at 26 weeks (7%). Further analysis revealed that there was minimal bone colonization in direct contact with the surface of the titanium implant, even at a depth of 300 μm from the screw interface (data not shown).

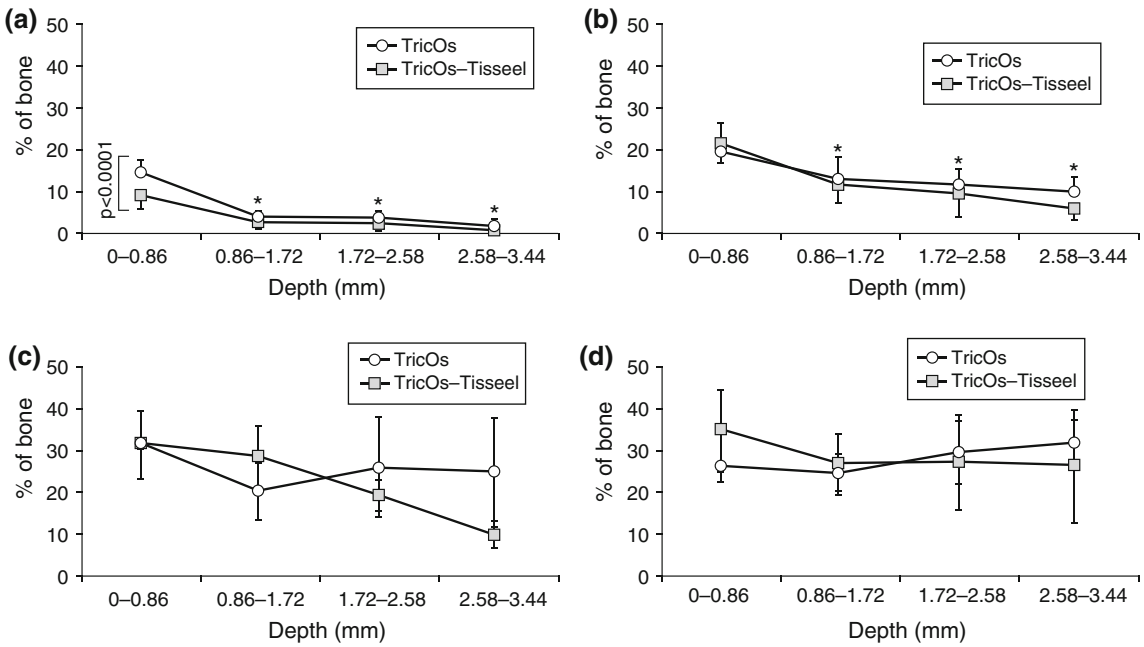

Fig. 8 Mean [SD] percentage of bone colonization toward the titanium implant in the two groups (TricOs alone or TricOs–Tisseel) at Level A (**a**) at 3 weeks; (**b**) at 6 weeks; (**c**) at 12 weeks; (**d**) at 26 weeks. A depth of 0 mm represents the outer limit of the bone defect. * $P < 0.05$ versus bone colonization at 0–0.86 mm in each treatment group

3.3 Qualitative histological analysis

Observation of the ceramic granules under light microscopy revealed atypical signs of biodegradation. Firstly, a 'moth-eaten' appearance was evident in the TricOs–Tisseel samples compared with the TricOs samples. Secondly, the TricOs samples had a low level of cells in the soft tissue surrounding the bone and ceramic. In contrast, the TricOs–Tisseel samples appeared to be rich in cells. Thirdly, observation under polarized light indicated that the soft tissue of the TricOs–Tisseel samples was not fibrous, as encountered in fibrosis, but had the appearance of a non-mineralized bone collagenic matrix, with signs of Haversian organization, at 12 weeks onwards.

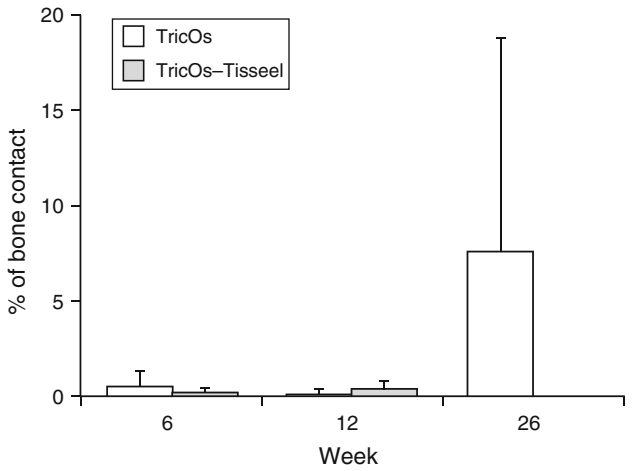

Fig. 9 Mean [SD] percentage of bone contact with the titanium implant at Level A over time in the two groups (TricOs alone or TricOs–Tisseel)

As shown in Fig. 10A, in the TricOs group there was evidence of bone trabeculae formation emerging from the host bone toward the titanium surface. Bone bridge grew from the host bone, to the surface of some BCP granules by osteoconduction and progressively to other granules until reaching the metal surface. A similar process was observed in the TricOs–Tisseel group, but generally the grains were smaller, largely resorbed, and in addition some bone trabeculae formed into the intergranule spaces independently of the BCP granules surface (Fig. 10B). This suggests that formation of bone trabeculae may be promoted by the provisional extracellular matrix formed by the fibrin sealant.

4 Discussion

The combination of fibrin-based biomaterials with other biomaterials provides possibilities for tissue regeneration approaches, either to enhance biological activity or to improve the biomechanical properties of the composite matrix. It has been shown that a fibrin matrix enhances the osteogenic properties of ceramic biomaterials including osteointegration, bone remodeling, and new bone formation [12].

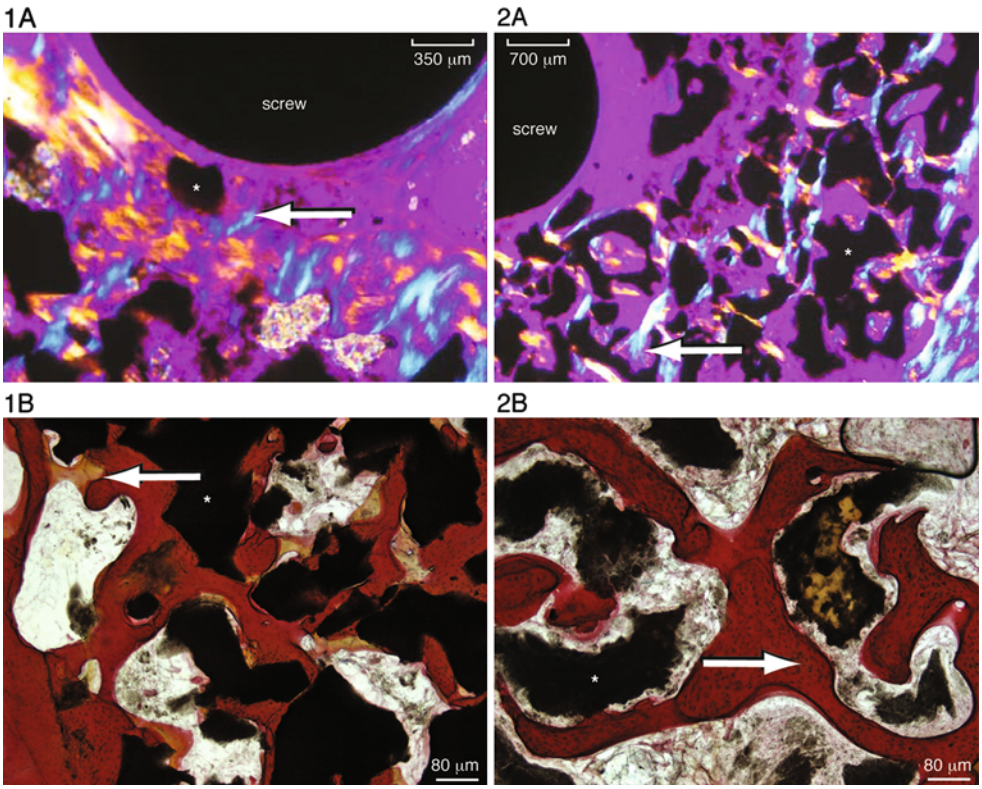

Fig. 10 Polarized light micrographs of bone defect sections at Level A after 26 weeks of screw implantation in **1A** the TricOs group; and **2A** the TricOs–Tisseel group. Cyanin-solochrom stained micrographs of bone defect sections at Level A after 26 weeks in **1B** the TricOs group and **2B** the TricOs–Tisseel group. TricOs granules (*); Bone trabeculae (*arrows*)

The influence of fibrin sealant on the composite has been investigated in a number of experimental studies. Kania et al. showed the important role played by fibrin sealant in the healing of bone defects [47]. In a study on rabbits, femoral bone defects were filled with coral granules alone or with a mixture of fibrin sealant and coral granules, or were left empty. At 1 month post-operation, osteogenesis increased by 46% with fibrin sealant–coral particles compared to coral alone. At 6 months, the cavities filled with the composite showed mature bone similar to that of adult bone in non-operated animals.

The positive effects of the combination of fibrin sealant with ceramic biomaterials have essentially been observed in clinical studies [12, 43–45]. Contrary to experimental animal studies, no immunologic reactions to fibrin components have been reported in humans. The fibrin sealant–biomaterial composite has a positive impact on handling and adhesion to the walls of the bone defects. These physical and biological properties provide clear benefits for surgeons. Unfortunately, the limited number of biopsies performed makes it difficult to investigate the osteointegration of the composite in clinical indications. A comparison with results in experimental animal models is therefore subject to bias and requires further clinical studies.

Several clinical studies in support of the efficacy of TricOs combined with Tisseel for bone filling have been published, e.g. as a substitute for MBCP wedges in patients undergoing high tibial osteotomy of valgisation [48]; for the filling of benign bone defects [49]; and for maxillary sinus floor augmentation before dental implantation [45]. The specific combination of MBCP and fibrin sealant offers several advantages. Fibrin sealant acts as a polymerizing matrix, thereby significantly improving the handling properties of MBCP. In addition, fibrin sealant fills the empty spaces between and around the MBCP granules and promotes their adherence to the surrounding tissues. The thrombin component of fibrin sealant seems to enhance the osteoconductive effect of MBCP, whilst the fibrin itself seems to have an osteopromoting effect [50]. These biological effects may be explained by a stimulatory effect of the fibrin matrix on osteoblastic precursor cells and mesenchymal cells to enhance osseous extracellular formation. In our results (Fig. 10B) we observed that some bone trabeculae were formed into the inter-granule spaces independently of the BCP granule surface. This suggests that the formation of trabeculae may be promoted by the extracellular matrix formed between residual granules, independent of the TricOs osteoconductive. Also, the fibrin network matrix is established to promote angiogenesis and

vascular development; this is another important property of fibrin-based biomaterials in the context of bone regeneration [37].

Currently, there are no published data on the use of a mouldable bone substitute for revision surgery, a rapidly-growing field due to the aging of the population. Such a surgical approach would require a synergy of prosthesis, highly osteoconductive/osteogenic biomaterials and surgical technique to secure and maintain the metallic implant in place. To establish validity of our sheep model to replicate a large metaphyseal bone defect, we examined the reproducibility of the mechanical condition, homogeneity of defect size and consistency of ceramic granules within the bone defect. The primary stability of the metallic implant is considered essential for its secondary osteointegration. The percentage of bone in contact with the titanium screw increased from Week 12 onwards, confirming the model's primary stability.

Intuitively, bone defect size is an important determinant of reproducibility in any model of bone repair, with homogeneity of size around the metallic implant being essential. In our model this parameter was tightly controlled and reproducible over the 26 weeks of the study at Level A (the smooth core of the screw). It is well accepted that bioactivity of ceramic bone substitutes is determined by their osteoconductive properties. Consequently, any heterogeneity in the quantity or distribution of ceramic granules within the bone defect may have a significant impact on bone colonization. Ideally this would be evaluated in the immediate post-operative period to avoid interference with eventual degradation of the ceramic. The earliest timepoint at which global analysis data were collected was Week 3. Whereas this may be regarded as a limitation of our study, it is reasonable to hypothesize that biodegradation will be minimal at 3 weeks, as supported by our assessments.

In our model, the amount of ceramic at Level A was similar with both biomaterials (TricOs group vs. TricOs–Tisseel group), suggesting that the addition of fibrin to TricOs granules does not alter the total amount of ceramic present in the defect. However, this does not provide any information about the distribution of ceramic throughout the defect. We observed few differences in the distribution of ceramic along the depth of the bone defect. This validates the comparison between the different levels of 'centripetal analysis'.

In summary, based on the above findings, we believed that our experimental model was sufficiently valid to justify its further use for the evaluation of resorption and bone ingrowth as a reproducible model for associations between bone substitutes and metal implants. Hence we proceeded to compare the kinetics of bone colonization and ceramic biodegradation between the TricOs and TricOs–Tisseel groups. Histomorphometrical analyses indicated similar rates of bone colonization in defects in both biomaterial groups, with rapid rates observed up to Week 12, followed by a slower period of stabilization. In contrast, a difference in the rate of ceramic biodegradation was observed between the two groups, with the TricOs–Tisseel group experiencing more rapid degradation, confirming resorption of the ceramic.

Bone kinetics evaluation revealed that at 3 weeks, the centripetal nature of bone colonization was evident, i.e. colonization at the periphery but not at the center of the defect, in both groups. At 12 weeks, a difference between the two biomaterial groups was noted, with the TricOs–Tisseel group showing more bone in the periphery and less in the center than the TricOs group. This difference was due to the time delay in permeability until the resorption of fibrin was achieved. This difference persisted at 26 weeks, but was less marked due to the appearance of physiological bone remodeling with longer times following metal implant. In terms of ceramic biodegradation, there was no evidence of a difference between groups at this timepoint. Degradation appeared uniform throughout the defect at each timepoint, in both groups.

Bone contact with the titanium screw at Level A was negligible in both groups, reaching a peak of 7% in the TricOs group at 26 weeks. Further analysis indicated that there was very little bone even at a distance of 300 μm from the interface. This suggests that TricOs granules, alone or in association with Tisseel, are slow to promote osteointegration of a titanium screw surrounded by a large bone defect. These data demonstrate that several weeks do not allow adequate bone ingrowth for the stabilization of a metal implant (e.g. screw, dental implant or stem prosthesis). Initial mechanical stability is required. To promote bone ingrowth directly to metal surfaces it was necessary to select prostheses with osteoconductive surface treatment. In contrast to these findings, light microscopy indicated non-mineralized bone matrix, with signs of Haversian organization, in the TricOs–Tisseel samples. Coupled with the absence of bone contact with the screw's surface in the TricOs–Tisseel group, this suggests that the addition of fibrin to TricOs granules may slow bone colonization toward the metallic implant, but without impeding bone mineralization. Indeed, polarized light microscopy indicated that the addition of fibrin sealant to TricOs promotes bone trabeculae formation independently of the TricOs scaffold osteoconductive property.

The results of the second part of our study concur with those of Le Guehennec et al. [43], in their study examining the effect of adding fibrin sealant to MBCP in a rabbit femoral model of defect filling. In the Le Guehennec study, newly formed bone developed at a small distance from the surface of the ceramic when fibrin sealant was added to

MBCP. Within the fibrin network, a small delay in deep bone colonization was observed. Furthermore, two different bone apposition processes were identified. Without fibrin, bone rested directly on the surface of the ceramic granules, denoting osteoconduction. In contrast, the addition of fibrin sealant seemed to modify this standard osteoconduction phenomenon, in that the newly-formed bone appeared to grow at a distance from the surface of the granules, on the fibrillar network. This could be considered osteoinductive, promoting osteogenic cell differentiation from mesenchymal stem cells.

Arnaud et al. [51] also observed modified osteoconduction when fibrin sealant was added to coral granules. It is postulated that the addition of fibrin sealant may modify the surface properties of the ceramic granules, changing the interaction between the granules and the solution, leading to modified osteoconduction [43].

In conclusion, the use of a model that reproduces a large metaphyseal bone defect around a titanium implant with primary stability, filled with a mixture of either TricOs ceramic granules or TricOs granules mixed with Tisseel fibrin sealant, suggests that the addition of fibrin to TricOs enhances bone filling surgical technology. To our knowledge, this is the first study of its kind to utilize a large bone defect model. Further studies are needed to explain the precise effects observed, and to accumulate a solid body of evidence to support the study of large bone defects as an emerging field that will help advance new osteoarticular surgical technology.

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