A comparative study of tissue-engineered constructs from Acropora and Porites coral in a large animal bone defect model

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Objectives
To compare the therapeutic potential of tissue-engineered constructs (TECs) combining mesenchymal stem cells (MSCs) and coral granules from either Acropora or Porites to repair large bone defects.

Materials and Methods
Bone marrow-derived, autologous MSCs were seeded on Acropora or Porites coral granules in a perfusion bioreactor. Acropora-TECs (n = 7), Porites-TECs (n = 6) and bone autografts (n = 2) were then implanted into 25 mm long metatarsal diaphyseal defects in sheep. Bimonthly radiographic follow-up was completed until killing four months post-operatively. Explants were subsequently processed for microCT and histology to assess bone formation and coral bioresorption. Statistical analyses comprised Mann-Whitney, t-test and Kruskal–Wallis tests. Data were expressed as mean and standard deviation.

Results
A two-fold increase of newly formed bone volume was observed for Acropora-TECs when compared with Porites-TECs (14 ± 1089 mm³ versus 782 ± 507 mm³; p = 0.09). Bone union was consistent with autograft (1960 ± 518 mm³). The kinetics of bioresorption and bioresorption rates at four months were different for Acropora-TECs and Porites-TECs (81% ± 5% versus 94% ± 6%; p = 0.04). In comparing the defects that healed with those that did not, we observed that, when major bioresorption of coral at two months occurs and a scaffold material bioresorption rate superior to 90% at four months is achieved, bone nonunion consistently occurred using coral-based TECs.

Discussion
Bone regeneration in critical-size defects could be obtained with full bioresorption of the scaffold using coral-based TECs in a large animal model. The superior performance of Acropora-TECs brings us closer to a clinical application, probably because of more suitable bioresorption kinetics. However, nonunion still occurred in nearly half of the bone defects.

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Article focus
- Bone regeneration using tissue-engineered constructs (TECs) combining mesenchymal stem cells (MSCs) and coral granules to repair large bone defects in preclinical animal model
- Coral genera and modified scaffold architecture influence scaffold resorption kinetics
- Premature resorption of the scaffold leads to healing failure
- Scaffold resorption kinetics is not the only determining factor to achieve bone regeneration
- Acropora coral is a more relevant scaffold than Porites for TEC-mediated bone regeneration of large segmental bone defects in large animal models

Key messages
- Bone regeneration in critical-size defects could be obtained with full bioresorption of the scaffold using coral-based TECs in a large animal model
**Introduction**

In critical-sized segmental bone defects (CSD) resulting from trauma, tumour or osteomyelitis, endogenous mechanisms are insufficient to achieve bone repair, prompting the need for bone replacement. Although bone autograft is the most effective therapy, it has several limitations: aside from the donor-site morbidity, the available quantities are limited and the technique is unsuccessful in defects exceeding 60 mm in length.\(^1\) Given these limitations, alternative strategies including tissue-engineered constructs (TECs), associating mesenchymal stem cells (MSCs) and porous scaffolds have been developed to treat CSDs. Despite enthusiasm at the prospect of treating bone disorders using TECs, a TEC that can be scaled up for clinical use has not yet been developed. Central to this remain key issues including MSC viability post-implantation and suitable scaffold selection.

Biocompatibility, mechanical properties and pore size have often been described as critical specifications for the ideal scaffold. However, there is no consensus as to which scaffold is optimal for this application. Tricalcium phosphate, hydroxyapatite, polymer and coral-containing scaffolds have been used with encouraging results in large animal CSDs.\(^2,10\) These studies also identified scaffold bioresorbability and its kinetics as a critical feature for achieving bone regeneration.

Coral exoskeletons from either the *Porites* or *Acropora* genus are attractive scaffold candidates for TECs because of their mechanical properties, proven biocompatibility and bioresorbability, as well as their capability to act as a delivery system for MSCs.\(^1,13\) In fact, both the *Porites* - and *Acropora*-TECs placed in clinically relevant CSDs exhibited superior osteogenic ability than that of the control scaffolds without cells, and were able to match the osteogenic ability of autografts in 10% to 20% of the animals.\(^6,8\) However, the bone-forming capacity and scaffold bioresorbability of *Porites* - and *Acropora*-TECs cannot be compared based on these studies because in these studies, TECs were prepared using different granule numbers, cell expansion protocols and densities.\(^7,8\) Hence, it remains unclear whether *Porites* or *Acropora* exoskeleton is the most suitable to act as a scaffold for TECs.

These considerations and the desire to select a scaffold that allows its gradual replacement by newly formed bone provided the impetus for the present study. Therefore, we sought to compare the bone-forming capacity and scaffold bioresorbability of *Porites* - and *Acropora*-TECs in a CSD in a sheep model. We used a validated preclinical model that permitted the exploitation of bones at four months post-implantation\(^14\) in order to appreciate scaffold bioresorption kinetics.

**Materials and Methods**

**Animals.** A total of 15 healthy, two-year-old, Pré-Alpes sheep (60 kg) were obtained from a licensed vendor (INRA, Jouy-en-Josas, France) and raised in accordance with European laws (Directive 24.11.1986.86/609/CEE). Animal housing and care were carried out using procedures described in previous publications by members of our team.\(^6,7\) All procedures were performed in compliance with legislation concerning animal experimentation and approved by the Ethical Committee.

**TEC preparation.** Autologous MSCs were isolated from bone marrow harvested from the sheep iliac crest and amplified as previously described until the second passage (supplementary material 1).\(^8\) Coral cubes (3x3x3 mm\(^3\)) from either *Acropora* or *Porites* (Biocoral France, Saint-Gonnery, France) were used as scaffolds. Acropora coral has homogeneously dispatched and interconnected large pores (412 μm, standard deviation 212 μm) and high permeability (4.46x10\(^{-9}\) m\(^2\)), whereas *Porites* coral has homogeneously dispatched and interconnected smaller pores (154 μm, standard deviation 53 μm) and low permeability (0.12x10\(^{-9}\) m\(^2\)).\(^11\) For each genus, the cube specimens (n = 10) were imaged with high-resolution microCT (80 kV source voltage, 100 mA source current, 7.91 μm pixel size, 180° rotation, 0.3 second exposition), and reconstructed and analysed to determine their macroscopic architectures using dedicated software (Skyscan 1172, NRecon and CTAn; Skyscan, Aartselaar, Belgium).

All cubes were sterilised by autoclaving (at 121°C for 20 minutes), a method that is known to preserve coral composition and structure.\(^11,15\) Sterile coral cubes were washed with phosphate-buffered saline (PBS), immersed in culture medium for 24 hours, and subsequently loaded into a custom-made perfusion bioreactor containing culture medium, such as α-Minimum Essential Medium Eagle (Sigma-Aldrich, St Louis, Missouri) -10 %Fetal Bovine Serum (Sigma-Aldrich) at 10\(^5\) cells/cube, as previously described.\(^16\) The bioreactor was operated under sterile conditions at 10 ml/min flow at 37°C for seven consecutive days. The medium was changed every three days.

In order to control the distribution and presence of living MSCs onto TECs, three cubes from each group were randomly chosen on the day of surgery and the MSCs were labelled using carboxyfluorescein diacetate succininimidyl ester (CFSE) according to standard techniques. CFSE covalently labelled long-lived intracellular molecules with a fluorescent dye. Thus, the dye, examined...
under fluorescence microscopy, revealed living cells onto the cubes.16

**Surgical procedures.** Sheep were randomly assigned into three groups according to whether the CSD was filled with *Acropora*-TEC (n = 7), *Porites*-TEC (n = 6) or fragmented corticocancellous autograft harvested from the iliac crest (n = 2). In respect to the principles of the three Rs, as defined by Russell and Burch17 the number of animals in the positive control group was reduced because previous experiments, by our team, have shown this model to consistently result in bone union in the same model in sheep.6,7,9,14 The surgical procedures (supplementary material 2) were performed under general anaesthesia in aseptic conditions as previously described in previous experiments, by our team, have shown this model to consistently result in bone union in the same model in sheep.6,7,9,14 The surgical procedures (supplementary material 2) were performed under general anaesthesia in aseptic conditions as previously described and validated.14 In brief, a 25-mm long mid-diaphyseal ostectomy was performed in the metatarsal bone with full peristomial elevation. The so-created large defect was stabilised by plate (3.5 Dynamic Compression plate, Synthes, Etupes, France) and the resected bone was fully replaced by plate (3.5 Dynamic Compression plate, Synthes, Etupes, France) and the resected bone was fully replaced with TECs or autograft. Craniocaudal radiographs of the operated limb were obtained at the end of surgery, and at two and four months after surgery. The animals were killed after four months, using a barbiturate overdose.

**Specimen collection and analysis.** Immediately after killing, all the treated metatarsal bones were excised and fixed in 10% neutral buffered formalin for two weeks. The stabilisation plates were then removed and the implant sites, along with 2 cm of the surrounding host bone on each edge were removed. These pieces were embedded in methyl methacrylate resin according to established techniques6 and kept at room temperature until sectioning.

**MicroCT scan analysis.** All embedded specimens were imaged and analysed with a high-resolution microCT (Skyscan1172; Skyscan) with 80 kV source voltage, 100 mA source current, 26.6 μm pixel size, 180° rotation, 0.2 seconds exposition time, frame averaging 20, and aluminium-copper filters. On average, 1260 slides per sample were reconstructed using NRecon software (Skyscan). Data were treated using a global fixed threshold (60 to 220 grey levels) with the same volume of interest, corresponding to a cylinder centred in the middle of the defect with a length equal to the longest defect.

For qualitative analysis of bone formation, lateral, medial, cranial and caudal cortices were examined, and the number of united cortices was recorded for each specimen. We considered two parameters for the qualitative evaluation of the bone formation: (i) if at least one of the four cortices was united by a bony bridge, we called it “bone union”, and (ii) if all the four cortices presented union, we considered that “bone regeneration” was achieved.

The diameters of both the proximal and distal parts of the metatarsal bone and the volume of the resected bone were measured to access group comparability.

For quantitative volume analysis of the new bone and for the residual coral within the region of interest (ROI), dedicated software (CTAn; Skyscan) was used to obtain the bone volume (BV) with bone-specific threshold (60 to 140 grey levels) and the coral volume (CoV) with coral-specific threshold (140 to 220 grey levels). The scaffold biodegradation rate was measured and expressed as a percentage of the initial coral volume iCoV (125 coral cubes imaged by microCT without being implanted), as followed: (iCoV-CoV) / iCoV. Subsequently, the ROI region was divided into three equal parts corresponding to the proximal, central and distal areas. The same microCT analyses used for BV and CoV were performed.

Concerning the autograft group, the volume of newly formed bone could not be distinguished from the implanted bone, thus the overall bone volume was assessed, but statistical comparison could not be performed.

**Undecalcified histology.** All embedded metatarsal bone specimens were cut lengthwise using a circular saw (200 to 300μm, Leitz 1600; Leica Biosystems, Nussloch, Germany). The section closest to the longitudinal mid-sagittal plane was selected for histological analysis, ground down to 100 μm thick, polished and stained. The staining protocol included successive baths: Stevenel blue bath at 60°C during 15 seconds, water, van Gieson picrofuchsin during 60 seconds and 100% alcohol. It permitted to discriminate by staining bone matrix, cell nuclei, and coral scaffold (in red, blue and brown, respectively).

**Statistics.** Statistical analyses were performed using a commercially available software package (GraphPad Prism V6.0c; GraphPad Software Inc., La Jolla, California). Quantitative data were expressed as mean and SD and analysed using the t-test or the Mann-Whitney test depending on the results of the normality test. The Kruskal–Wallis test was performed for comparison between more than two groups. Linear regression was

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**Table I.** MicroCT analysis of ten cube specimens of *Acropora* and *Porites* coral. Using dedicated software, total porosity, volume of open (or interconnected) pores, volume of closed pores, pore size (trabecular separation) and trabecular thickness were assessed and compared. Statistical analyses were performed using t-tests.

| Parameters                | *Acropora*     | *Porites*     | p-value    |
|---------------------------|----------------|---------------|------------|
| Total porosity (%)        | 36.3 (± 8.7)   | 55 (± 5.8)    | < 0.0001   |
| Volume of open pores (mm³) | 7.6 (± 1.2)    | 11.6 (± 1.5)  | < 0.0001   |
| Volume of closed pores (µm³) | 2.3 (± 4.8)   | 1.0 (± 1.6)   | 0.42       |
| Trabecular separation (µm)| 203 (± 27)     | 113 (± 12)    | < 0.0001   |
| Trabecular thickness (µm) | 326 (± 58)     | 89 (± 10)     | < 0.0001   |
Porites exhibited larger and more irregular pore size; Porites had a more homogeneous structure with smaller pores. The porosity of Porites was lower than that of Acropora.

**Results**

**In vitro evaluation.** The two scaffolds exhibited different microCT architecture (Fig. 1 and Table I): Acropora displayed large and irregular pores with heterogeneous distribution, whereas Porites had more homogeneously distributed smaller pores; Acropora porosity was also lower than that of Porites. For the two types of coral genera tested, fluorescent microscopy observations of scaffolds loaded with CFSE-labelled MSCs revealed the presence of adherent living cells with uneven MSC distribution, i.e. cells remaining mostly in the periphery of the scaffolds (supplementary material 3).

**In vivo evaluation**

**Scaffold material bioresorption.** X-ray longitudinal analysis revealed that while bioresorption of Porites-TEC could be visualised as early as two months post-implantation with the absence of visible granules and was nearly complete at four months post-implantation, only partial bioresorption of Acropora-TEC was observed at four months (Fig. 2). Residual coral quantities and bioresorption rates at four months differed significantly between Acropora- and Porites-TECs: 114 $\pm$ 92 mm$^3$ versus 5 $\pm$ 5 mm$^3$ ($p = 0.008$), 81 $\pm$ 5% versus 94 $\pm$ 6%, respectively ($p = 0.04$)(Fig. 3b). Due to the fast bioresorption of the Porites-TECs, distribution of the scaffold material bioresorption along the defect could only be assessed for Acropora-TEC and it was evenly distributed among the three defect areas. Remaining coral was surrounded by either bone or fibrous tissue (Fig. 4).

New bone tissue was present above and inside the remaining coral scaffolds (a, b). Both mature and immature bone tissue was observed, with, respectively, well orientated, small and dark cells (osteocytes in lacunae) forming a lamellar tissue (c, e), and disorganised, large-nucleated cells forming a non-lamellar tissue (b, f). Abundant osteoid (yellow arrow heads), encircled by bone-lining cells (black arrow heads), was present surrounding the bone tissue, revealing active bone formation (c, e, f). When bone tissue was absent, fibrous tissue filled the defect (d). The images were obtained from two sheep of the Acropora-TEC group. Stains: Stevenel blue and von Gieson picrofuschin. Bone, cells and coral stained red, blue and brown, respectively.

**Bone formation.** Early bone formation in Acropora-TECs was difficult to evaluate using radiographs because slow scaffold material bioresorption prevented distinction between the newly formed bone and the remaining substrate scaffold (Fig. 2). Similarly, with autograft, bone healing was not assessed. In contrast, bone formation could be observed as early as two months post-implantation on fast-resorbing Porites-TECs (Fig. 2).

At four months post-implantation, the results were highly variable in both TEC groups (Fig. 2). Bone deposition was either: scarce and confined to the bone edges (three of seven animals with Acropora-TECs, three of six with Porites-TECs); nonunion with defects mostly filled with fibrous tissue occurred in six of the tested animals (Fig. 4); or abundant and present at a distance from the bone edges (union of at least one cortical site in four of seven animals with Acropora-TECs and in three of six animals with Porites-TECs). Bone formation permitted full bone regeneration, defined as four cortices united, in two of four united defects with Acropora-TECs and one of three united with Porites-TECs. Moreover, remodeling with recorticalisation was observed in one of the animals with Acropora-TEC. Taken together, these findings suggest that bone union was more frequent with Acropora-TECs.

The occurrence of bone union affected the distribution of the newly formed bone, regardless of the TEC tested. When bone union was achieved, bone was uniformly distributed between the external (in the continuity of the cortices) and the inner (in the continuity of the medullary canal) parts of the defect. In contrast, when nonunion occurred, bone was limited to the inner part, with no bone tissue observed at the external part.

Histological analysis revealed in all examined sections that, irrespective of the TEC tested, mature and immature bone, osteoid, osteocytes, osteoblasts and bone-lining cells were present both in contact with the remaining coral scaffold (even in the core of the coral cubes) and in the bone defect edges (Fig. 4).

When compared with Porites-TECs, Acropora-TECs achieved a two-fold increase in the volume of newly formed bone (1437 $\pm$ 1089 mm$^3$ versus 782 $\pm$ 507 mm$^3$) (Fig. 3a). However, this trend did not show statistical significance ($p = 0.09$). In addition, two Acropora-TECs exhibited a larger amount of newly formed bone.
formed bone was similar in the central and distal areas of the defect, but in the case of Acropora-TECs, the rate of newly formed bone was significantly higher than that observed in Porites-TECs in the proximal area (p = 0.01).

**Bone formation/scaffold biore sorption coupling.** In our study, Acropora-TECs exhibited a slower rate of biore sorption than Porites-TECs and tended to result in more bone formation, suggesting an inverse coupling between the two processes. At four months, the biore sorption rates of the two best performing Acropora-TECs and Porites-TECs were 64% and 86%, and 85% and 87%, respectively (Fig. 3). The two Acropora-TECs and the four Porites-TECs which exhibited the highest rate of scaffold material biore sorption (more than 90%) presented the lowest rate of bone formation. These findings showed that there was no correlation between bone formation and scaffold biore sorption with either TEC, but premature scaffold material biore sorption impaired bone healing in many cases.

**Discussion**

Finding an ideal scaffold for TEC has emerged as a dominant issue in the field of bone regeneration. In this respect, recent reports from our group suggested that Porites and Acropora exoskeletons are acceptable scaffolds for the repair of large bone defects.6-8 However, their biore sorption and osteogenic capacities could not be compared based on these studies because of different experimental settings. Here, we have demonstrated that Porites and Acropora (processed similarly in a perfusion bioreactor to standardise their preparation16) are also promising TECs for the repair of large CSDs. Most importantly, compared with Porites-TECs, Acropora-TECs resorbed at a significantly slower rate and exhibited a trend towards increased bone formation.

Biore sorption is a crucial parameter for scaffolds, as TECs should degrade with time to create space for the new bone tissue to grow. In the present study, whereas important biore sorption of Porites-TEC occurred at two months post-implantation and was nearly complete at four months, almost no biore sorption of Acropora-TEC was observed at two months, and remaining coral granules were still present at four months. These remnants would most likely have disappeared completely at six months, as observed in a previous study using Acropora-TECs (99% resorption at six months),8 which is the recommended timeframe for scaffold biore sorption in clinical trials for TEC-based bone regeneration.11,18 Thus, Acropora-TECs and Porites-TECs exhibited significant differences in biore sorption rates at four months, and the data suggested that the kinetics of biore sorption of Acropora-TECs are slower than those of Porites-TECs. These results are in accordance with previous studies in sheep, in which cell-free or MSC-seeded scaffolds were implanted in small bone defects19 or subcutaneously.20 Thus, it seems that the resorption profile of coral-based TECs is not different depending on the site of implantation. Although further studies are mandatory to
investigate this issue, studies related to the coral resorption profile may be conducted in cheaper and easier, less invasive models than the orthotopic model. However, this is no true concerning bone formation, as it had been previously observed in mice.21

An important finding of the present study is that Acropora-TECs exhibited a trend towards an increase in the total amount of newly formed bone when compared with Porites-TECs. This trend even demonstrated significance in the proximal areas of the bone defects. Moreover, a higher number of animals exhibited full bone regeneration with Acropora-TECs (29% versus 17%) when compared with Porites-TECs. Put together, these findings support that the use of an Acropora template enhances the bone-forming capacity of TECs, at least in the proximal areas, which is consistent with findings from a previous study from our group.8 Obtaining conclusive evidence as to whether Acropora-TECs have an overall superior osteogenic capacity will require a larger number of animals. It is indeed very likely that the two-fold increase in bone quantification would have been statistically significant with groups including 30 animals, according to conventional standards of significance. However, this would rightly raise critical ethical issues, indeed until the results are not equivalent to autograft, it is not ethically warranted to kill so many animals. Therefore, the lack of significance in the present study could be the result of a type II error.
A rational delineation of the requirements for developing a successful MSC-delivery scaffold for bone repair, and its validation in clinically relevant animal models, is a critical challenge facing tissue engineers seeking to translate stem cell therapy to the clinic. In the present study, the two Acropora- and four Porites-TECs that exhibited the highest scaffold material biodegradation rate (> 90%) displayed the poorest bone formation and resulted in non-union. These observations provide an empirical validation of the claim that too high a rate of biodegradation can lead to poor bone formation in a CSD. Whether the poor bone-forming capacity of these highly resorbed constructs arises from destabilisation of early bone apposition through scaffold disintegration, and/or stimulation of an inflammatory response by elevated in situ levels of degradation products remains to be determined. In any event, in every case of nonunion associated with the premature biodegradation of the TECs, fibrous tissue was observed throughout the defect, especially at its external part, and a bony bridge closed the medullary cavity, thereby definitively preventing bone healing. This contrasted with the well-distributed bone tissue observed in regenerated defects, and may be the consequence of the less demanding fibroblastic—compared with osteoblastic—differentiation. It is therefore tempting to speculate that a suitable scaffold for delivering MSCs in large bone defects that would be able to compete with the bone-forming capacity of autologous bone grafts should exhibit a rate of scaffold material biodegradation less than 90%, at four months post-implantation. However, the two Acropora-TECs, which matched or superseded the osteogenic capacity of the autologous bone grafts, displayed a biodegradation rate between 64% and 86% at four months. This corresponds to a wide range of biodegradation rates in which non-united defects were also observed. Surprisingly, the implants associated with the greatest bone formation are not necessarily the ones with the lowest resorption rates. All of these results suggest that while persistence of the scaffold at four months is necessary, this is not sufficient for ensuring consistent bone regeneration when using coral-based TECs. Autograft performed better than TECs in bone formation. For all of these reasons, it is necessary to improve the coral-based TEC performance through other factors than biodegradation kinetics such as the improvement of its intrinsic osteo-inductive capacity through. Strategies pertaining to the cellular

Representative histology of newly formed bone in the tested defects. New bone tissue was present above and inside the remaining coral scaffolds (a and b). Both mature and immature bone tissue was observed, with, respectively, well-oriented, small and dark cells (osteocytes in lacunae) forming a lamellar tissue (c and e) and disorganised, large-nucleated cells forming a non-lamellar tissue (b and f). Abundant osteoid (yellow arrow heads) encircled by bone-lining cells (black arrow heads) was present surrounding the bone tissue, revealing active bone formation (c, e and f). When bone tissue was absent, fibrous tissue was filling the defect (d). The images were obtained from two sheep of the Acropora-TEC group.

Stains: Stevanel Blue and von Gieson picrofuschin. Bone, cells, and coral stained red, blue, and brown, respectively.
fraction of the construct (i.e. co-cultures) or the adjunct
growth factors such as bone morphogenetic pro-
tins should be investigated.

Both bone formation and scaffold bioreosorption were
highly variable amongst animals, as was found to be the
case in previous studies with coral-based TECs.\textsuperscript{6-9} It is
tempting to postulate that sheep exhibit large diversity
affecting their intrinsic bone-forming capacity, however,
bone union was consistently achieved with autograft in
the present (n = 2) and past (n = 18) studies.\textsuperscript{7,9,14} Still,
diversity may affect the inflammatory response, which
should be further investigated.\textsuperscript{2,4,24} Differences into the
TECs may also account for these variations, for example,
the components of the TECs might suffer some heteroge-
neity and the preparation can lead to variation, especially
using autologous MSCs.\textsuperscript{25}

In conclusion, while searching for the ideal scaffold for
TEC-based bone regeneration, there are important fac-
tors that should be taken into account, especially the
kinetics of bioreosorption. We demonstrated that, when
associated with autologous MSCs in CSDs, premature
resorption of coral-based scaffolds consistently led to fail-
ure of bone union. The use of Acropora scaffolds, which
resorb more slowly than those of Porites, allowed us to
move closer to a clinical application. Moving forward,
osteo-inductive capacity of the scaffold material will be
the focus of future of research.

Supplementary material

A figure showing Acropora and Porites-TECs labelled
MSCs under fluorescent microscopy are available
alongside this article at www.bjr.boneandjoint.org.uk

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Author Contribution

A. Decambron: Study design, Implant preparation, Surgery, Data collection, Data
analysis and interpretation, Drafting the article.
B. Manassero: Study design, Implant preparation, Surgery, Data collection, Data
collection, Data analysis, Critical revision of the article.
C. Bensaid: Study design, Implant preparation, surgery, Data collection, Data
collection, Data analysis, Critical revision of the article.
D. Petite: Study design, Data analysis and interpretation.
E. Hing: Study design, Data analysis and interpretation.
F. Bensaid: Study design, Data analysis and interpretation.
G. Viateau: Study design, Surgery, Data collection, Data analysis and interpretation,
Critical revision of the article.
H. Mondello: Study design, Data analysis and interpretation.
I. Miellet: Study design, Data analysis and interpretation.
J. Manassero: Study design, Surgery, Data collection, Data analysis and interpretation.

ICMJE conflict of interest

None declared.

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