Primary stability of a press-fit cup in combination with impaction grafting in an acetabular defect model

Ronja A. Schierjott1,2 | Georg Hettich1 | Marc Baxmann1 | Federico Morosato3 | Luca Cristofolini3 | Thomas M. Grupp1,2

Abstract
The objectives of this study were to (a) assess primary stability of a press-fit cup in a simplified acetabular defect model, filled with compacted cancellous bone chips, and (b) to compare the results with primary stability of a press-fit cup combined with two different types of bone graft substitute in the same defect model. A previously developed acetabular test model made of polyurethane foam was used, in which a mainly medial contained defect was implemented. Three test groups (N = 6 each) were prepared: Cancellous bone chips (bone chips), tricalciumphosphate tetrapods + collagen matrix (tetrapods + coll), bioactive glass S53P4 + polyethylene glycol-glycerol matrix (b.a.glass + PEG). Each material was compacted into the acetabulum and a press-fit cup was implanted. The specimens were loaded dynamically in the direction of the maximum resultant force during level walking. Relative motion between cup and test model was assessed with an optical measurement system. At the last load step (3000 N), inducible displacement was highest for bone chips with median [25th percentile; 75th percentile] value of 113 [110; 114] µm and lowest for b.a.glass + PEG with 91 [89; 93] µm. Migration at this load step was highest for b.a.glass + PEG with 868 [845; 936] µm and lowest for tetrapods + coll with 491 [487; 497] µm. The results show a comparable behavior under load of tetrapods + coll and bone chips and suggest that tetrapods + coll could be an attractive alternative to bone chips. However, so far, this was found for one specific defect type and primary stability should be further investigated in additional/more severe defects.

KEYWORDS
acetabular bone defect model, impaction grafting, optical measurement, press-fit cup, primary stability, synthetic bone graft substitute

INTRODUCTION
Revision hip surgery is still challenging and often associated with acetabular bone defects which make subsequent implant fixation even more difficult. The large variation in bone defects requires a broad range of specific treatment options, such as revision cups, reconstruction shells or impaction bone grafting (IBG). Using IBG, cancellous bone chips are compacted into a defect and combined with cemented polyethylene (PE) cups, press-fit cups or reconstruction shells. This technique enables bone stock reconstruction and...
has shown satisfying clinical results in acetabular and femoral hip revision surgery.\textsuperscript{5,7}

However, supply of donor bone is limited and very expensive. Furthermore, the preparation of bone chips in the operating room is time consuming and the quality of the produced bone chips differs widely due to biological variation and preparation technique.\textsuperscript{8} Finally, an infection risk remains.\textsuperscript{8}

Synthetic bone graft substitutes (BGS) may represent an attractive alternative or supplement to bone chips to increase reproducibility of mechanical properties and to decrease infection risk. Numerous BGS have been developed and tested. Most previous in vitro studies focused on mechanical properties in a simplified setup or primary stability of bone chips and BGS in combination with a cemented cup.\textsuperscript{9,12} Primary stability is an important prerequisite for clinical long-term success and to the authors’ knowledge, primary stability of these defect filling materials has yet hardly been assessed in combination with a press-fit cup.\textsuperscript{13,14}

The objectives of this study were to (a) assess primary stability of compacted bone chips with a press-fit cup using a previously presented reproducible acetabular test model, and to (b) compare the results with two BGS materials.

2 | MATERIALS AND METHODS

2.1 | Acetabular test model and test setup

An artificial acetabular test model made of polyurethane (PU) foam was used, which replicated the main support structures of the pelvis os ilium, os pubis and os ischii.

This model was oriented towards a previously developed surrogate model\textsuperscript{15,16} with the basic idea of mimicking the main support structures, while reducing the support in the remaining areas, especially in the incisura acetabuli (Figure 1A-D).\textsuperscript{14-16} In the herein applied acetabular test model, a diameter of 10 mm was chosen for the incisura acetabuli, as described by Jamieson et al., 2011.\textsuperscript{15} In order to represent a real pelvis as well as possible, the dimensions of the os ilium, os pubis and os ischii were derived from a clinical computed tomography (CT)-data set of a pelvis with acetabular bone defect. The latter was obtained within a previously conducted study in which 50 acetabular bone defects were quantitatively analyzed based on CT-data,\textsuperscript{2} whereby use of the CT-data was approved by the Ludwig-Maximilians-University Munich ethics committee (project no. 18-108 UE).

20 pounds per cubic foot (PCF) (0.32 g/cm\textsuperscript{3}) solid rigid PU foam (Sawbones Malmö, Sweden) with Young’s modulus of 210 MPa and compressive strength of 8.4 MPa was chosen\textsuperscript{14} to simulate slightly weakened bone as expected in revision surgery. This was oriented towards a study by Crosnier et al.\textsuperscript{17} who used 30 PCF and 15 PCF PU foam to simulate two different kinds of bone quality.

In the herein used acetabular test model, medial wall thickness of 5.6 mm was chosen to enable compaction of the defect filling materials with impacts similar to those applied during surgery.

A standardized, mainly medial contained defect with rim damage in the posterior-inferior aspect of approximately 1/3 of the circumference was created.\textsuperscript{14} This defect could be categorized as a variant of Paprosky 1 with some aspects of Paprosky 3 A, that is, rim damage of 1/3 of the circumference.\textsuperscript{1} Paprosky 1 is among the most commonly observed defect types based on studies analyzing radiographs in association with acetabular revision surgery.\textsuperscript{18,19}

The acetabular test models were prepared with a random spray pattern for strain analysis and were fixed in an acrylic resin block (Figure 1D). The latter was placed in an orientation block aligning the specimen with the load axis for dynamic testing (Figure 1E).

In order to ensure that the herein applied artificial test model showed a behavior under load comparable to a real pelvis, total relative motion between a press-fit cup and the artificial test model without defect, measured within a previous study of our research group,\textsuperscript{14} was compared with relative motion between a press-fit cup and the surrounding bone in human donor specimens, relating to a study of Beckmann et al. 2018.\textsuperscript{20}

Beckmann et al. compared relative motion between two different types of press-fit cups and the surrounding bone in 10 fresh-frozen human donor specimens.\textsuperscript{20} Using a multi-axial testing machine, they simulated 1000 repetitions of a normal gait cycle, whereby minimum and maximum load were restricted to 8.71% body weight (BW) and 69.93% BW, respectively. Based on the average donor weight, this corresponded to approximately 54 and 437 N, respectively. Relative motion was assessed using the optical measurement system GOM Pontos (GOM GmbH Braunschweig, Germany) and was found to be 36.03 ± 16.83 µm (Gription cup) and 29.27 ± 14.97 µm (Porocoat cup) after 1000 load cycles.\textsuperscript{20}

In a previous study of our research group, relative motion between a press-fit cup and the artificial test setup without defect was assessed under dynamic loading in direction of the maximum resultant force during level walking. At the lowest defined load increment (600 N), mean (maximum) relative motion were found to be 24.5 ± 3.8 µm (45.7 ± 5.6 µm) and were hence in a range comparable to the relative motion measured in human donor specimens.\textsuperscript{14,20}

2.2 | Test groups and specimen preparation

Three test groups were defined for this study: Morzellized allografts (bone chips), bioactive glass in a polyethylene glycol and glycerol matrix (b.a.glass + PEG), and ceramic tetrapods in a collagen matrix (tetrapods + coll) (Figure 1A-C). Cancellous bone chips were prepared from eight fresh frozen human donor femoral heads, retrieved within the ethics vote 5-170/2016 (University of Heidelberg, Germany). They were first sawed into slices. Using a bone nibbler, the cortical bone was removed and bone chips were nipped from the remaining cancellous bone.\textsuperscript{4} A bone nibbler was used in order to produce relatively large bone chips which are most suitable for acetabular impaction grafting.\textsuperscript{8,11,21} Bone chips size and variation were assessed by measuring the maximum edge length of 100 exemplary chips with a ruler.\textsuperscript{21} Mean bone chips size was 7 ± 2 mm (range: 3 to 12 mm). Bone chips of all eight femoral heads were mixed to reduce inter-specimen variability (Figure 1A).
B.\textit{alglass} + PEG consisted of S53P4 bioactive glass granules based on silicon-oxide, sodium-oxide, calcium-oxide, and phosphorus pentoxide with granule size 1.0 to 2.0 mm (80 wt.\%) and a PEG matrix (20 wt.\%) (Bonalive Biomaterials Ltd, Turku, Finland). The glass granules can inhibit bacterial growth by an elevation of pH and osmotic pressure and have already been used as bone void filler in infection treatments.\textsuperscript{22,23} Tetrapods + coll consisted of powder-injection-molded and sintered tricalciumphosphate (\(\beta\)-TCP) tetrapods with an edge-length of 3.3 mm (93 vol\%) (IFAM, Bremen, Germany)\textsuperscript{9} and a collagen (primarily type I derived from bovine tendon) matrix material (7 vol\%) (Collagen Solutions Plc, Glasgow, UK).

All materials were applied using a template to provide reproducible filling of the defects (Figure 2). The materials were compacted manually step-by-step with a hemispherical impactor and an orthopedic hammer. Final compaction was performed with a weight of 456 g which was dropped 10-times\textsuperscript{24} from a height of 26 cm on an acetabular cup shaped impactor. 456 g corresponded to the weight of a standard orthopedic hammer and height of 26 cm was chosen for a standardized impulse of 1 Ns. The titanium press-fit cup Plasmafit Ø48 mm (Aesculap AG Tuttingen, Germany) with a nominal external diameter of 49.2 mm (NV148T) and a resultant press-fit of 1.2 mm related to the diameter was pressed on the filling material using the Zwick/Roell material testing machine Z005.
ZwickRoell GmbH & Co. KG, Ulm, Germany), with 2 kN and displacement of 1 mm/min. This aimed at performing the implantation as controlled and reproducible as possible and was oriented towards the implantation procedure described in previous studies assessing primary stability of acetabular cups, in which a static load of 2 to 3 kN was used to insert the cups.\textsuperscript{16,25}

Specimens of the test groups \textit{b.a.glass + PEG} and \textit{tetrapods + coll} were then conditioned in purified water at 37°C for 75 minutes and air-cured for 24 hours at room temperature. Specimens of all test groups were prepared with tracking points and the polyethylene inlay was inserted prior to testing [Color figure can be viewed at wileyonlinelibrary.com]

\textbf{FIGURE 2} Preparation matrix for the three test groups. In all specimens, the filling material was implanted using a template, which was followed by manual pre-compaction with a hammer and a standardized compaction by dropping a 456 g weight 10-times (Arts et al\textsuperscript{24}) from a height of 26 cm. 456 g corresponded to the weight of a standard orthopedic hammer and a height of 26 cm was used to achieve a standardized impulse of 1 N·s. Using a material testing machine, the cup was pressed on the filling material in the acetabular test model with 2 kN. Test groups \textit{b.a.glass + PEG} and \textit{tetrapods + coll} were then conditioned in purified water at 37°C for 75 minutes and air-cured for 24 hours at room temperature. Specimens of all test groups were prepared with tracking points and the polyethylene inlay was inserted prior to testing [Color figure can be viewed at wileyonlinelibrary.com]

Specimens of the test groups \textit{b.a.glass + PEG} and \textit{tetrapods + coll} were then conditioned in purified water at 37°C for 75 min including four (\textit{tetrapods + coll}) and eighteen (\textit{b.a.glass + PEG}) rinsing procedures to simulate matrix dissolution in the body after surgery. Eighteen rinsing procedures for \textit{b.a.glass + PEG} were defined based on a pretest to remove as much of the water-soluble PEG-matrix as possible. For \textit{tetrapods + coll}, the number of rinsing procedures was reduced to four, as the collagen matrix was not expected to be removed by water anyway.

A central hole at the bottom, that is, at the pole of the cup and several holes drilled in the ground of the acetabular test model allowed the fluid to get around the back of the cup. However, this was partially restricted by the tight press-fit between the cup and the BGS. Hence, the fluid had full access to the BGS in the central area, that is, near the pole of the acetabular cup, but reduced access to the BGS in the remaining areas behind the cup. The specimens were air-cured at room temperature for 24 hours before testing.
In all three test groups, a PE inlay was inserted and tracking points for optical motion measurement were placed on the acetabular test model, cup and inlay (Figures 1D and 2).

2.3 | Load protocol and relative motion measurement

The specimens were loaded dynamically with the servo-hydraulic testing machine MTS 858 Mini Bionix II (MTS, Minneapolis) in the direction of maximum resultant force during level walking, that is, during contralateral toe-off,\textsuperscript{14,26} with a normalized force vector of
\[
\begin{pmatrix}
-0.86 \\
+0.21 \\
+0.46
\end{pmatrix}
\]
in relation to the loading coordinate system (Figure 1 and 3).

Relative motions were measured in 3D with the optical measurement system GOM Pontos (GOM GmbH Braunschweig) with two 5 MP cameras with 50 mm lenses (Figure 3). Twenty tracking points (size 0.4 mm, ID 35231) were placed on the press-fit cup and 38 on the acetabular test model, whereof 33 could be tracked throughout the tests (Figure 3B). Thereby, a series of images was acquired and load information from the testing machine was used to identify the images at maximum and minimum loads, which were then used to assess the relative motions between cup and test model in terms of inducible displacement and migration (Figure 3).

First, a reference image was taken at zero load (t0) and at static pre-load of 300 N (t1) (Figure 3A). Specimens were then loaded dynamically in a sinusoidal wave form, whereby minimum load was 300 N throughout the tests and maximum load was increased step-wise from 600 to 3000 N. Load was increased by 300 N every 1000 cycles, resulting in nine load steps with 1000 cycles each, whereof the first 900 were applied at 1 Hz and the following 100 at 0.5 Hz. Few cycles of the first load step (600 N) and the last load step (3000 N) are exemplarily shown (Figure 3A). In each load step, at 990 cycles, motion during one cycle of dynamic load was captured by a series of 40 images taken by the optical measurement system at 15 Hz. Images were taken at a dynamic load frequency of 0.5 Hz instead of 1 Hz to reduce the potential error in capturing the minimum and maximum load from 29.5 N at 1 Hz to 7.4 N at 0.5 Hz (Figure 5). In each load step, the image at maximum load and minimum load was identified and used to assess relative motion in the software Aramis Professional 2017 (GOM GmbH Braunschweig): Inducible displacement (I600 and I3000 in Figure 3A) as the motion between minimum and maximum load in each load step and migration (M600 and M3000 in Figure 3A) as the motion between pre-load and minimum load in each load step. The same acquisition and measurement principle applies to the other load steps, not shown in Figure 3A. Resultant relative motions, as well as motions in medial-lateral (x), anterior-posterior (y), and cranial-caudal (z) direction were assessed (Figure 3B). The defined coordinate system originates in the center of the acetabular cup plane. The x-axis represents the normal to this plane, pointing laterally (personal communication with Philipp Damm, Julius Wolff Institute Berlin). The y-axis points anteriorly and is based on the International Society of Biomechanics reference coordinate system.\textsuperscript{27} The z-axis results thereof, pointing cranially (Figure 3C).

After dynamic loading and a waiting time of at least 10 min to allow for viscoelastic recovery, an image at zero load (t11) was taken again for each specimen to measure the resulting cup position.

Relative motions presented within this study correspond to the average motions of the 33 tracking points on the acetabular test model relative to the cup. Values of the three test groups with N = 6 each are presented as median [25th percentile; 75th percentile].

2.4 | Cup tilt measurement

The angle between acetabular cup plane and fixation block surface was measured using Aramis Professional 2017 (GOM GmbH Braunschweig) prior to the test (t0) and after the test (t11) at zero load. The cup tilt represents the change in the measured angle between t0 and t11.

3 | RESULTS

3.1 | Cup translations

Relative motions were measured in terms of inducible displacement and migration, whereby the resultant displacement (Figure 4 and Figure 5), as well as its medial-lateral (x), anterior-posterior (y), and cranial-caudal (z) components were analyzed (Figure 6). Values are given as median [25th percentile; 75th percentile].

Resultant inducible displacement and migration increased with increasing load in all three test groups (Figure 4). Inducible displacement at the first load step (600 N) was lowest for \textit{bone chips} with 15 [14; 16] µm and highest for \textit{b.a.glass} + PEG with 19 [18; 19] µm (Figure 4A). At the last load step (3000 N), lowest inducible displacement was found for \textit{b.a.glass} + PEG with 91 [89; 93] µm and highest for \textit{bone chips} with 113 [110; 114] µm. Curves of all test groups showed a linear regression with inducible displacement being the dependent variable and the load level being the independent variable (\textit{bone chips}: $R^2 = 0.998$, \textit{b.a.glass} + PEG: $R^2 = 0.982$; \textit{tetrapods + coll}: $R^2 = 0.998$).

Migration at the first load step was lowest for \textit{bone chips} with 19 [19; 21] µm and highest for \textit{b.a.glass} + PEG with 48 [37; 50] µm (Figure 4B). At the last load step (3000 N), lowest migration was found for \textit{tetrapods + coll} with 491 [487; 497] µm and highest for \textit{b.a.glass} + PEG with 868 [845; 936] µm. Curves of \textit{tetrapods + coll} showed a linear regression ($R^2 = 0.996$), \textit{bone chips} trend to a power function ($R^2 = 0.983$), and \textit{b.a.glass} + PEG trend to a logarithmic function ($R^2 = 0.981$) with migration being the dependent variable and the load level being the independent variable (Figure 4B).

Inducible displacement vectors of the acetabular test model relative to the cup, exemplarily shown for one specimen of each test
A group (Figure 5, left) indicated a closing motion of the test model around the cup with main motion in the medial-lateral axis (Figure 6A). Migration vectors (Figure 5, right) indicated the movement of the cup into the acetabular cavity along the medial-lateral axis, which was predominant in the posterior and caudal aspect of the model and an additional movement of the cup out of the acetabulum in the cranial-anterior aspect, which was mainly present in baglass + PEG and bone chips (Figure 5, right). In all specimens, main motion was seen along the medial-lateral axis and lowest motion along the anterior-posterior axis (Figure 6A-C).
3.2 | Cup tilt

Cup tilt was lowest for tetrapods + coll with 0.97° [0.75°; 1.00°] and highest for b.a.glass + PEG with 2.51° [2.34°; 2.63°]. Cup tilt for bone chips was 1.40° [1.23°; 1.53°] (Figure 7).

4 | DISCUSSION

The objectives of this study were to assess the primary stability of a press-fit cup in a standardized acetabular defect test model, which was filled with compacted bone chips, and to compare it to the primary stability achieved by a filling with two different bone graft substitutes: B.a.glass + PEG and tetrapods + coll.

This study has some limitations. First, a simplified acetabular test model made of PU foam was used instead of human donor specimens, which represent the most realistic test models. However, the latter are limited in supply and associated with high inter-specimen variability and limited test time. PU foam models were used to increase reproducibility and comparability between the test groups and were designed to mimic the main support structures of the pelvis. Second, primary stability was assessed in one specific type of defect. The defect was chosen based on a previously conducted quantitative defect analysis and in consultation with four senior hip revision surgeons from four European clinical centers as a common and representative defect which is likely to be treated with bone chips or BGS. However, there is a wide variation of bone defects and results of the present primary stability tests might be different in a different kind of defect. Third, the three test groups could not be prepared in the exact same way, that is, test group bone chips could not be conditioned in water due to hygienic reasons and number of rinsing procedures of tetrapods + coll were less than for b.a.glass + PEG as the collagen matrix was not expected to dissolve significantly in deionized water with 5.5 to 7.0 pH, but by acidic or alkaline processing or enzymes. It is not expected that the described difference in soaking and rinsing has a significant influence on the test results, but that the interface characteristics at the cup-foam and cup-defect filling contact areas, that is, the (remaining) humidity is more relevant for the primary stability. Humidity was controlled by a drying period of 24 hours prior to testing for both soaked test groups (tetrapods + coll, b.a.glass + PEG) and by testing the test group bone chips, which was not soaked, directly after implantation. Due to the fact that cancellous bone chips from fresh-frozen femoral heads were used, humidity at cup-bone chips interface could be expected to be comparable to the other test groups. Fourth, relative motions were measured at the end of each load step and hence information...
FIGURE 5 Vectors of relative motion between cup and acetabular test model, that is, inducible displacement (left) and migration (right), shown in an exemplary way for one specimen of each test group at the last load step (3000 N). Relative motion of test model relative to the cup is shown based on the 33 tracking points. Arrow direction indicates direction of motion, arrow color and length indicate magnitude of motion at the corresponding tracking point [Color figure can be viewed at wileyonlinelibrary.com]
on their temporal progress cannot be provided. However, this does not jeopardize comparability among the test groups. Fifth, dynamic uniaxial loading was applied, although simulation of a complete motion cycle may induce higher relative motions. However, uniaxial loading was chosen in this study to allow optical relative motion tracking throughout the tests, which is in good accordance with previous primary stability studies and does not jeopardize comparability among the test groups.

In the present study, relative motion between press-fit cup and acetabular test model were successfully assessed in terms of inducible displacement and migration using an optical measurement system. Motion increased with increasing load (Figure 4), which is in good accordance with previous primary stability studies under dynamic load. At the first load step (600 N), inducible displacement and migration were lowest for bone chips and highest for b.a.glass + PEG. At the last load step (3000 N), inducible displacement was lowest for b.a.glass + PEG and highest for bone chips, whereas migration was lowest for tetrapods + coll and highest for b.a.glass + PEG. This is in contrast to a study by Morosato et al., who performed a left-right comparison of bone chips and tetrapods + coll with a press-fit cup.
Another study which applied the same direction of their behavior. This is in good accordance with the findings in the present study where \textit{tetroads}+\textit{coll} showed lower relative motion than bone chips towards the higher applied loads (Figure 4). In numerous studies which assessed relative motion of bone chips and different BGS (TCP/hydroxyapatite [HA] and titanium granules) in combination with a cemented PE cup, it was also found that relative motions were lower for BGS granules or bone chips-granules mixtures than for bone chips alone.\textsuperscript{10,12}

In the present study, median values of inducible displacement at 3000 N were 113 \( \mu \text{m} \) for bone chips and 103 \( \mu \text{m} \) for \textit{tetroads}+\textit{coll}, and therefore 25\% and 57\% lower than reported by Morosato et al.\textsuperscript{36} Median values of migration at 3000 N were 632 \( \mu \text{m} \) for bone chips and 491 \( \mu \text{m} \) for \textit{tetroads}+\textit{coll}, and therefore 25\% and 65\% lower than reported by Morosato et al., but in a range comparable to a study assessing migration of a PE cup cemented on bone chips and a combination of bone chips and titanium granules.\textsuperscript{10}

In the present study, it was found that relative motion appeared mainly along the medial-lateral axis (Figure 6), which is probably related to mainly medially directed load and the mainly medial defect. However, the observed medial migration in combination with cup movement out of the acetabulum in the cranial-anterior region, which is best visible in \textit{b.a.glass}+\textit{PEG}, can also be seen in clinical situations.\textsuperscript{37,38}

The results obtained within this study suggest that the bone graft substitute made of \( \beta \)-TCP-tetrapods in a collagen matrix shows a behavior under load comparable with bone chips. At loads larger than 2000 N, the behavior of \textit{tetroads}+\textit{coll} was even more favorable, that is, relative motions were smaller than for bone chips. This might be related to the fact that in the present study the cups were pressed into the acetabulum with 2 kN and although the bone chips were compacted prior to cup press-in, they may have been subjected to additional compaction within the dynamic testing at loads larger than 2 kN. However, mean inducible displacements of all three test groups were in a range in which osseointegration may potentially still be possible.\textsuperscript{39,40} Migration of \textit{b.a.glass}+\textit{PEG} was considerably higher than for \textit{tetroads}+\textit{coll} and bone chips, but still below the clinically defined radiographic thresholds critical for implant fixation\textsuperscript{41,43} and most likely related to the dissolution of the matrix (PEG), not to the bioactive glass granules.

BGS, such as \textit{tetroads}+\textit{coll} could represent an attractive alternative to bone chips, which are expensive and restricted in supply,
time consuming to produce, show an inconsistent quality due to biological variation and a remaining infection risk.\textsuperscript{8} The present study, alongside with previous studies, showed the favorable properties of bone graft substitutes, in terms of higher in vitro measured primary stability in comparison with bone chips.\textsuperscript{10,11,13} Satisfying clinical short- and mid-term results for BGS consisting of TCP and HA have also already been reported.\textsuperscript{44,45}

Nevertheless, it cannot be generally applied that bone graft substitutes are always superior to bone chips, but that the performance depends on the defect characteristics. Considering the results of Morosato et al.\textsuperscript{96} it could be concluded that a prerequisite for a good performance for BGS is a contained defect with enough remaining wall thickness for adequate compaction.

Future studies should assess the effect of collagen matrix dissolution on primary stability using enzymes or acidic/alkaline processing. In addition, primary stability of defect treatments with BGS and bone chips should be assessed in additional/more severe defects to further investigate the prerequisites and potential limitations of the different filling materials. Furthermore, osseointegration should be investigated in mechano-biological or large animal studies.

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CONFLICT OF INTERESTS
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AUTHOR CONTRIBUTIONS
All authors worked on research design, or acquisition, analysis or interpretation of data. RS worked on drafting the paper and GH, MB, FM, LC, and TG critically revised it. All authors have read and approved the final submitted manuscript.

ORCID
Ronja A. Schierjott \(\text{http://orcid.org/0000-0002-0624-1978}\)
Luca Cristofolini \(\text{http://orcid.org/0000-0002-7473-6868}\)

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