Influence of antibiotic pellets on pore size and shear stress resistance of impacted native and thermoisinfected cancellous bone: An in vitro femoral impaction bone grafting model

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ABSTRACT

Introduction: Morphology and mechanic properties of impacted cancellous bone are affected by carrier substances which provide high local concentrations of antibiotics.

Methods: Bone chips were taken from the femoral head of 6–7 months old piglets. One half was thermoisinfected and the other remained native. Ten specimens each were mixed with Herafill® antibiotic pellets and a control group of each 10 specimens respectively was examined. The cancellous bone was impacted according to Exeter technique and the implants were cemented. The distribution of the particles and the pores were defined with three dimensional computertomographic scan and shear force resistance was measured until failure.

Results: Shear force resistance was not measured significantly less for thermoisinfected (2.7 Nm) compared with native bone (3.5 Nm) and addition of antibiotic pellets reduced shear force resistance in both groups since this was significant for the native group. The average pore volume of the native bone specimens appeared significant smaller compared to the thermoisinfected group ($p = 0.011$) and the pore volume showed a negative correlation with shear force resistance ($p = 0.044$). Pore volume around the pellets was found significantly increased and it appeared smaller for native bone. The number of pellets located next to the implant showed a negative correlation with shear force resistance ($p = 0.034$) and the negative correlation increased for pellets below the tip of the shaft model ($p = 0.024$).

Conclusion: Adding antibiotic pellets to native and thermoisinfected impacted cancellous bone increased pore volume since the area around the pellets showed increased porosity which correlated with reduced shear force resistance. Computertomographic three dimensional measurement of porosity might predict shear force resistance of impacted cancellous bone and improve impaction of bone grafting intraoperatively.

1. Introduction

The necessity of filling bone defects during revision surgery of joint replacements is increasing and reconstruction of bone stock is important for longevity of implants.^{1–3} Addition of antibiotic carrier substances which can release high local antibiotic concentrations into impacted cancellous bone graft seems beneficial in case of hip revision surgery in infectious disease.^{4–6} Good long term results have been reported for

femoral impaction bone grafting.^{1,7–9} Primary stability of impaction bone grafting depends on the heterogeneity of bone grafts and the operative technique since relevant differences of mechanic properties of impacted bone grafts were found.^{1,10–18} Different degrees of impaction for processed bone including higher impaction than for native bone were described and no significant mechanic difference between native and processed bone was shown.^{1,3,13–15,17,18} The technique of femoral impaction bone grafting included rinsing of bone chips within warm

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water.^{1,12,19} Processing of bone and reducing fat and fluid of bone transplants increased the degree of impaction since bone density and the reduction of height of cancellous bone correlated with mechanic properties including an increase of rotational stiffness.^{1,3,13–22} Accordingly processed thermoisinfected bone might offer advantages compared with native cancellous bone since thermoisinfection caused a marginal reduction of mechanic properties of cancellous bone.^{12,23,24}

Porcine cancellous bone was chosen to examine the influence of addition of antibiotic pellets (Herafill®) on morphology and mechanic behaviour of impacted bone since it resembles geometry of human bone and showed results of cement distribution and impaction comparable with the human *in vitro* model.^{25,26} The set up of the impaction bone grafting model was related to a previous study which showed a comparable distribution of native and thermoisinfected cancellous bone since a variable size of particles improved density of impacted cancellous bone.^{1,12,18,27,28} A suitable mixture with different sizes of native and thermoisinfected bone chips was chosen for the present study accordingly.^{1,12} Local application of antibiotics within the impacted bone graft might be useful in case of revision surgery of infected joint replacements.^{4–6} The carrier substance should improve mechanic stability of the bone graft and promote osseous integration since adding hydroxyapatite granules to allogeneic bone improved bone mineral density and bone volume fraction.^{29–31} The influence of addition of gentamicin containing pellets (Herafill®) consisting of calciumcarbonate and calciumsulfate on mechanical properties and morphology of impacted native and thermoisinfected cancellous bone should be evaluated. Three dimensional computertomography was performed to analyze the distribution of the bone particles and to define porosity within the impacted bone related to addition of pellets within native and thermoisinfected cancellous bone. Pore size and its correlation to addition of pellets were compared with shear force resistance as well as the influence of the location of the pellets on the mechanical parameter were examined. The study should gain information to design suitable carrier substances to improve mechanic properties of its mixture with impacted native and thermoisinfected cancellous bone. Three dimensional computertomography examined the possibility to predict shear force resistance related to porosity since it would be of clinical importance to verify independent parameter correlated with mechanic stability of impacted bone graft.

2. Materials and methods

2.1. Construction of *in vitro* model

Porcine cancellous bone was obtained from femoral heads of 6–7 months old piglets with a weight of 90–100 kg. The femur was stored at –20 °C during transport and the femoral head was removed with an oscillating saw (Multitalent FMT 250 SL Start – Oszillierer – 250 W, Fein, Schwäbisch Gmünd-Bargau, Germany) and soft tissue was removed in the laboratory within 12 h after slaughter. One half of the specimens were thermoisinfected at 82.5 °C for 94 min (Lobator sd-2, Telos, Marburg, Germany) and the other half remained native and both were stored at –20 °C. Based on previous studies the minimum number of specimens within each group was calculated $n = 4$ according to statistical analysis (Power-Analysis G*Power Vers. 3.1.9.2., A. Buchner, E. Erdfelder, F. Faul, A. Lang, HHU Düsseldorf, Germany) and ten samples were chosen to improve significance.^{12,24}

2.2. Preparation and impaction of cancellous bone

Bone chips with defined size of 3–5 mm, 5–8 mm and 8–10 mm were manufactured (Noviomagus Bone Mill, Spierings Orthopaedics Nijmegen, Netherlands). According to a previous study the composition of native and thermoisinfected cancellous bone with the resulting highest density following the impaction was used for the examination.¹² The native cancellous bone graft consisted of 70% bone chips of size 3–5 mm

and 30% 8–10 mm since the thermoisinfected bone graft contained each a third of bone chip size 3–5 mm, 5–8 mm and 8–10 mm.¹² The bone chips were immediately stored at –20 °C. Prior examination the bone chips were thawed in normal saline solution (Braun Melsungen, Germany) at 21 °C (± 1 °C) for 3 h and then put into a towel (Telasorb®, Hartmann AG, Heidenheim, Germany) for rinsing procedure to reduce fat and water. Bone particles which were smaller than 2 mm were removed with a sieve and then mixed homogeneously and randomized into four groups of 10 specimens each of which two groups received additional 80 pellets loaded with gentamicin for each specimen according to recommendations for clinical use (Fig. 1)(Herafill® beads G, Heraeus Medical GmbH, Wehrheim, Deutschland). Impaction in an oval shaped polypropylene tube (Fig. 2) with a thermoplastic range between 155 °C and 200 °C (DIN EN 1451–1; Marley GmbH, Wunstorf, Germany) was done in three portions for the native and one for thermoisinfected bone. After the tube had been fixed to a socket a weight of 1450 g falling from a height of 180 mm was used until the impactor was exactly 1 cm above the tube for impaction (Fig. 3). Cement (Palacos® R + G 40, Heraeus Medical GmbH, Wehrheim, Germany) was mixed with vacuum technique (Palamix® Vakuummischsystem, Heraeus Medical GmbH, Germany) and filled retrograde into the prepared canal followed by rectangular implantation of specially designed implants (Fig. 4).

2.3. Measurement procedures

Computertomographic measurement (Somatom® Force, Siemens Healthcare AG, Zürich, Switzerland) was done with slice diameter 292.969 μm to measure morphometric parameter. No relevant artificial changes of the imaging were correlated to the implant. The three dimensional distribution of the pellets and air was analyzed from DICOM data (Analyze V. 11.0, Biomedical Imaging Resource, Mayo Clinic, Rochester MN, USA) which were converted into Bitmap data to create isotropic Voxel cube length followed by three dimensional orientation of the implant (Data Viewer V. 1.5.6.2, Bruker microCT, Billerica MA, USA). The tip of the implant was used for reference and accordingly the region of interest (VOI) was defined within 120 mm (CTAnalyser V. 1.18.8.0, Bruker microCT, Billerica MA, USA)(Fig. 5). The data of the tube and the implant were subtracted from the data creating a corresponding region of interest. The three dimensional orientation of the pellets should be analyzed corresponding to rotational stability. Five regions referenced from the tip of the endoprosthesis were defined as proximal, middle, distal, periprosthetic and control (Fig. 5) each with a length of 30 mm corresponding to 103 slices of the CT-scan since additional transversal slices were measured in each area. The central and external area of the bone graft contained each 50% of the volume (Fig. 5). Each pellet was assigned coordinates related to the tip of the prostheses to compare their location within the groups (Excel 2016, Microsoft Corporation, Redmond, USA) and pores corresponding to areas filled with air within the bone mixtures were analyzed to determine the volume of air related to the complete volume (V.1.18.8.0, Bruker microCT, Billerica, MA, USA). Data of implant and cement were removed by calculation to allow measurement of the distribution and volume of air and hard material using binarization. At a distance of 3 and 5 Voxel corresponding to 0.9 and 1.5 mm around the pellets the proportion of air was defined (Fig. 6). The rotation stress was measured with a universal testing machine range 0–20 kN and sensitivity 0.04% at 2000 N (Inspect table Blue 20 EDC 222, Hegewald & Peschke Meß- und Prüftechnik GmbH, Nossen, Germany) after mounting the measurement device to the implant. Measurement was done until failure of implant.

2.4. Statistical analysis

SPSS Statistics (V.26.0.0.0, IBM, Armonk, USA) and univariate variance analysis (ANOVA) were performed. Standard deviations were calculated. The LSD-post-hoc-test was used to examine differences in pairs and the Levene test was performed to judge homogeneity of

Fig. 1. Mixture of cancellous bone chips and Herafill®.

Fig. 2. Impacted cancellous bone within the model.

variance. The descriptive analysis of the computertomographic measurements and the analysis of the pores were correlated with the measurements of the rotation forces and calculated according to Pearson. The level of significance was defined with $\alpha = 0.05$.

3. Results

3.1. Shear force

The median value of shear force resistance was reduced for thermoisinfected ($2.7 \text{ Nm} \pm 1.4 \text{ Nm}$) compared with native bone ($3.5 \text{ Nm} \pm 1.4 \text{ Nm}$) since the measurement of the mixtures with pellets revealed comparable results for native ($1.7 \text{ Nm} \pm 0.8 \text{ Nm}$) and thermoisinfected bone ($1.7 \text{ Nm} \pm 0.9 \text{ Nm}$) (Fig. 7). The Kolmogorov-Smirnov test showed a normal distribution. The post-hoc-test revealed no significant difference ($p > 0.05$) of shear force resistance between native and thermoisinfected bone and between the native and thermoisinfected bone each supplemented with pellets. Adding pellets to native bone reduced shear force significantly ($p < 0.001$) since no significant difference was found between thermoisinfected bone and its mixture with pellets ($p = 0.053$). The number of impactations showed a wide range similar to a previous examination being decreased for thermoisinfected compared with native cancellous bone.¹² The 95% confidence interval for native bone ranges from 2.6 Nm to 4.2 Nm and from 1.9 Nm to 3.4 Nm for

Fig. 3. Model of the technical device for the impaction procedure including dimensions of the tube (mm) and an impacted thermoisinfected specimen.

thermoisinfected bone and the range is 0.9 Nm to 2.5 Nm for native bone mixed with pellets and 1.0 Nm to 2.7 Nm for the mixture of thermoisinfected bone and pellets.

Fig. 4. Cement being filled into the impacted cancellous bone graft.

Fig. 5. Areas of measurement (Table 1) within the impacted cancellous bone.

Fig. 6. Measurement of pore size within two defined areas next to antibiotic pellets (Herafill®).

Fig. 7. Torque resistance (Nm) of native (nwoA) and thermoisinfected (thwoA) impacted cancellous bone without antibiotic pellets as well as with addition of antibiotic pellets (nwa/thwa).

3.2. Distribution of pellets

The number of pellets within the measured areas appeared comparable between the native (61.5 ± 9.2) and thermoisinfected bone group (61.2 ± 4.9) with an average content of overall 61.4 ± 7.2 pellets. In both groups 67.2% (mean 41.2) of the pellets were localized in the periphery since 32.8% (mean 20.2) were located central. Most of the pellets (15.3 ± 5.2) were observed in the region below the tip of the implant showing no significant difference ($p > 0.05$) between native and thermoisinfected bone specimens (Table 1). The number of pellets located in the inner region showed a negative correlation with shear force resistance ($p = 0.034$) since the correlation increased for the region below the tip of the implant ($p = 0.024$) and the pellets located in the periphery did not show a correlation with shear force resistance. No significant difference of distribution of the pellets was measured between the different regions (Fig. 5).

3.3. Measurement of pore volume

The volume of the pores was measured within an average volume of 106.4 ± 1.7 ml in each model and the dimension of pores was distributed normally according to the Kolmogorov-Smirnov test (Table 2). The pore volume was not significantly different after pellets had been added ($p > 0.05$) for both groups. The native specimens showed a pore volume of 11.4 ± 2.5 ml and 11.4 ± 2.3 ml were measured with pellets being added. The thermoisinfected specimens had a median pore volume of 14.7 ± 2.0 ml and the mixture with pellets showed 13.6 ± 2.0 ml of pores. The main pore size diameter without a significant difference between native and thermoisinfected cancellous bone ranged between 0.9 mm and 1.5 mm. In the native group the proportion of that diameter was $46.4 \pm 1.8\%$ and $45.4 \pm 1.5\%$ in the group of thermoisinfected bone. The average pore volume of the native bone specimens appeared significant smaller compared with the thermoisinfected group ($p =$

Table 1

Number of antibiotic pellets (Herafill®) within 5 defined regions (proximal (1), middle (2), distal (3), periprosthetic (4), control (5))(Fig. 5) of native and processed (thermodisinfected) cancellous bone divided up for the external (out) region and the center (in) of the impacted bone.

Region	out	in	1	out	in	2	out	in	3	out	in	4	out	in	5
Native	8,3	4,0	12,3	8,5	3,3	11,8	8,8	3,3	12,1	8,9	6,1	15,0	7,0	3,3	10,3
Processed	7,2	3,8	11,0	8,5	3,0	11,5	7,9	3,4	11,3	9,6	5,9	15,5	7,7	4,2	11,9

Table 2

Distribution of different dimensions of pore diameter within impacted cancellous bone: The pore volume (ml) shows the complete volume and standard deviation (SD) of mean within the measured region (ml) of interest (VOI). The partial volumes (ml) are related to diameter 1 (pore size $0.293 \leq 0.879$ mm), diameter 2 (pore size $>0.879 \leq 1.465$ mm), diameter 3 (pore size $>1.465 \leq 2.051$ mm) and diameter 4 ($>2.051 \leq 4.395$ mm). Measurement of pores smaller than 0.293 mm was not technically feasible.

Bone specimen	VOI measured	Pore volume	Diameter 1	Diameter 2	Diameter 3	Diameter 4
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Native	106,1 $\pm$ 1,90	11,43 $\pm$ 2,49	3,66 $\pm$ 0,55	5,34 $\pm$ 1,20	1,78 $\pm$ 0,53	0,16 $\pm$ 0,25
Processed	106,3 $\pm$ 2,39	14,73 $\pm$ 1,99	3,57 $\pm$ 0,59	6,66 $\pm$ 0,82	3,00 $\pm$ 0,54	0,37 $\pm$ 0,54
Native & pellets	107,0 $\pm$ 1,16	11,39 $\pm$ 2,25	3,51 $\pm$ 0,64	5,29 $\pm$ 1,16	1,90 $\pm$ 0,40	0,17 $\pm$ 0,25
Processed & pellets	106,0 $\pm$ 1,17	13,62 $\pm$ 1,97	4,07 $\pm$ 0,49	6,21 $\pm$ 0,89	2,31 $\pm$ 0,46	0,26 $\pm$ 0,36

0.011)(Table 2) and the pore volume showed a negative correlation with shear force resistance ($p = 0.044$).

3.4. Pore volume around pellets

Within a radius of 0.9 mm corresponding to 3 Voxel around the pellets the average pore volume was $28.8 \pm 3.1 \mu\text{l}$ in the thermoisinfected (612 pellets) and $25.6 \pm 4.4 \mu\text{l}$ in the native group (615 pellets) which was not significantly different ($p = 0.068$). Within a radius of 1.5 mm corresponding to 5 Voxel the average pore volume was $24.9 \pm 2.8 \mu\text{l}$ in the thermoisinfected group and $21.7 \pm 4.0 \mu\text{l}$ in the native group which was significantly different ($p = 0.042$) and a reduction of the pore volume with a further distance from the pellets was shown for both groups since the average pore volume appeared smaller for native bone.

4. Discussion

The proportional difference of shear force resistance between native and thermoisinfected impacted cancellous bone seems comparable with the marginal reduction of mechanic properties following thermoisinfection (Fig. 7). The present study did not show a significant difference between native and thermoisinfected bone concerning shear stress resistance since two statistical outliers with high values in both thermoisinfected groups (Fig. 7) and the wide range of the 95% confidence interval revealed a relevant variability. The heterogeneity of the cancellous bone graft and differences of the impaction technique have to be considered since the number of impaction procedures seemed to be related to the quality of bone.^{1,10,12,14,25,32} The size of the particles was shown to be relevant for mechanical parameters of the impacted cancellous bone since a comparable distribution of thermoisinfected and native cancellous bone was achieved along the implant using different size particles.^{12,13–15,18,28,32} The comparable distribution of pellets in native and thermoisinfected bone indicates a uniform impaction behaviour. Improvement of the impaction was found to depend on the technique and the number of impactions since the necessary number of impactions appeared limited.^{1,10,14,25–27,32–35} Similar to a previous study the number of impactions for thermoisinfected bone tends to be less indicating a faster impaction process comparable with lyophilized bone graft.^{12,16} The degree of impaction correlated with mechanic stability and inversely with porosity of bone since multiple impactions were found necessary.^{1,10,12,14,15,25,32} This might influence mechanical behaviour in femoral bone impaction grafting since a higher degree of impaction of processed bone has been described and the porosity of the impacted bone decreased towards the tip of the stem as well as the performance of impaction was not found to

be correlated with the degree of the impaction.^{12–15,17,18,36}

Reduction of soft tissue and water due to thermoisinfection might improve mechanic properties since shear stiffness was found increased after cleaning allograft bone and in presence of bone replacement material different results were shown.^{1,3,10,12–15,17,20–24} Different distribution of air needs to be considered since an increased proportion of air within the proximal area of impacted thermoisinfected bone was shown and the density of bone increased towards the tip of the implant in native and thermoisinfected bone.¹² The proportion of air and the degree of impaction might be one reason for the reduced shear force resistance of thermoisinfected bone in the present study since an improved resistance against shear force was described following cleaning of bone grafts which increased density.^{10,17,20–22} The transmission of impaction energy appeared different at the distal and proximal region since the density of bone was found increased distal in native and thermoisinfected cancellous bone after femoral impaction bone grafting and the distribution of pellets appeared different accordingly (Table 1).^{11–13} Differences of impaction technique might account for variability of mechanic properties.

Quality and geometry of bone graft were shown to influence deformation behaviour during impaction bone grafting and the impaction of processed bone grafts could reach more stiffness than native bone since more deformation energy was absorbed by native bone.^{13–16,35} Freeze dried bone provided more stability for cemented stems than fresh frozen bone graft since the degree of impaction seemed to correlate inversely with porosity.^{25,35} The measurement of pore volume within the impacted bone graft seems relevant since the cement penetration was found decreased in areas of reduced porosity of impacted bone grafts.¹² The cement mantle was found to be thicker in the proximal region of femoral impaction bone graft compared with the distal part of the stem since the amount of air within the impacted bone was found significantly increased in the proximal region of thermoisinfected cancellous bone and the impaction of thermoisinfected bone appeared increased distally compared with native bone.¹² This indicates different behaviour of native and thermoisinfected allograft related to the degree of impaction and the resulting porosity showing the relevance of the parameter pore volume to predict mechanic stability (Table 2).

The cement mantle depends on impaction technique and seems important for stability.^{12,37–39} Bone incorporation and cement mantle are not expected to be uniform and torsion seems to be a relevant parameter.^{40,41} The variations of cement application in different Gruen zones have to be considered since the lack of cement in some Gruen zones is an important reason for failure and accordingly the relevant parameter shear force resistance varies significantly along the femur.^{10–12,20,22,25,40–43} Therefore the diameter of pores and the total

pore volume seems to be a relevant parameter influencing mechanical properties of the bone graft and the interface between cement and bone since a different penetration of cement into native and thermoisinfected bone was shown.¹² The cement penetration into the bone graft might be modulated adding synthetic material since the cement penetration was found increased in presence of tricalciumphosphate and hydroxyapatite particles.^{10,21,44} Pellets (Herafill®) consisting of calciumsulfate and calciumcarbonate used for local delivery of antibiotics in bone might have an influence on mechanical behaviour of impacted bone graft and can be expected to be reabsorbed completely. Ceramic bone material was shown to reduce cohesion and the alteration of surface properties of the bone graft might influence cohesion which appears relevant for impaction behaviour.^{12–15,17,21,29–31}

Shear force resistance was not significantly found higher for native compared with thermoisinfected cancellous bone and the number of pellets in the inner area beneath the implant correlated significantly negative with shear force resistance (Fig. 7) since the contact volume of cement and bone was found significantly higher in native compared with thermoisinfected bone in a previous study.¹² The thickness of the cement mantle was found variable and reduced distally correlated with reduced penetration of cement according to lower porosity.^{12,36,42,43} Processing of bone graft might be related to reduced mechanical strength of the thermoisinfected bone since the distribution of the bone particles and Herafill® pellets did not reveal a significant difference between native and thermoisinfected bone.¹² An increase of bone density related to the impaction might improve mechanical properties since increased impaction of thermoisinfected bone reduces porosity of the bone graft although reduced cement penetration into bone has to be considered.^{12,35} Morphology and volume of pores within impacted bone graft might be an independent parameter to characterize mechanic behaviour. The average pore volume was significantly increased in thermoisinfected compared with native bone and the pore volume correlated significantly negative with shear force resistance since the addition of pellets did not change the distribution of pore volume in comparison of both groups. The average pore volume was around 20% larger in the impacted thermoisinfected cancellous bone which appears in a comparable dimension like the difference between shear force resistance of native versus thermoisinfected bone.²⁴ Around 50% of the pores had diameter ranging within a comparable range between 0.9 mm and 1.5 mm for native and thermoisinfected bone indicating a similar distribution of particles.

Addition of pellets (Herafill®) to thermoisinfected bone did not significantly reduce shear force resistance since a significant level might have been reached with a larger number of samples. Shear force resistance was reduced highly significant for native bone in composition with pellets indicating different interaction between the particles since the distribution of the pellets beneath the implant did not appear different between the native and thermoisinfected group as well as the proportion of pore size volume in different areas did not change relevant after addition of pellets. The increased size and volume of pores around the pellets seems to be relevant for the reduction of shear force resistance since a significant correlation between the pore volume and the shear resistance was shown as well as the average pore volume was significantly smaller for native compared with thermoisinfected bone (Table 2). The addition of pellets might reduce the ability of the particles to interlock during impaction indicating a primarily more stable connection between the native cancellous bone chips. A smaller size of the pellets should improve the mechanical properties of the impacted bone graft. The number of pellets near the implant correlated significantly negative with shear force resistance in both groups and the pore volume decreased with increasing distance from the stem (Table 1). This indicates the relevant influence of the pellets on the load transfer in the interface cement bone according to the location of failure since the absolute differences of the pore volume between both groups appeared small. The periprosthetic area (Fig. 5) near the tip of the stem seems important for mechanical behaviour since shear stress resistance in that

area appears susceptible to collection of pellets.

Application of Herafill® of the size studied does seem to impair the stability of the interface between bone and cement since an influence on the pore diameter and pore volume of the bone graft could be observed. This appears relevant since a significant negative correlation between pore volume and resistance against shear force was found. A smaller size of the pellets should be expected to avoid this.^{13–15,18} The high initial release of gentamicin from Herafill® is related to the microstructure and hydroxyapatite seems less favourable for the purpose of fast release of antibiotics since mechanic stability of Herafill® appears minor. Hydroxyapatite has been shown to improve bone mineral density and stiffness and tricalciumphosphate and hydroxyapatite particles improved cement penetration.^{10,21,29–31,44} Processed cancellous bone showed an increased degree of impaction and this could have been expected for impaction of thermoisinfected bone since heterogeneity of bone graft following impaction has to be considered.^{10,13–15,17,18,32} Increased impaction correlated with improved mechanic behaviour and deformation of cancellous bone.^{10,15,16} The interaction between thermoisinfected cancellous bone and the pellets appeared different from native bone since the decrease of shear force resistance was increased significantly after adding Herafill® to native bone. This might be related to different cohesion after processing with thermoisinfection since morphology and distribution of the impacted bone showed no significant differences between native and thermoisinfected bone.¹² The reduction of shear force resistance of thermoisinfected bone due to addition of pellets is not significant since the addition of pellets to native bone reduced mechanic parameter significant indicating an effect related to interference of pellets with the surface of native bone. The overall distribution of particles and pore diameter shown by three dimensional computertomographic analysis seemed comparable between native and thermoisinfected bone indicating the relevance of surfaces and deformation behaviour on the mechanical parameter. Computertomographic procedures are firmly established as intra-operative diagnostic technique and in this respect analysis of porosity of impacted bone might allow immediate optimization of the bone graft and therefore contribute to improvement of mechanic properties and clinical outcome.⁴⁵

4.1. Limitations

The heterogeneity of bone specimens and variations of impaction related to technical reasons might be responsible for differences of shear force between native and thermoisinfected bone since density might be variable in different areas. The limitations to achieve a reproducible homogeneous impacted bone graft need to be considered regarding the results of the study and the clinical application. Further research should evaluate the influence of adding smaller particles of Herafill® to impacted native and thermoisinfected cancellous bone graft which might reduce porosity and influence distribution of particles. The influence of different suitable supplemented materials should also be considered.

5. Conclusion

The porosity of impacted cancellous bone grafts and the location of the pellets near the implant correlated significantly negative with the resistance to shear force. The average pore volume was significantly smaller for native compared with thermoisinfected impacted bone since the failure occurred in the bone graft near the interface of cement and bone. No significant difference of shear force resistance was found between native and thermoisinfected bone since the values appeared higher for native cancellous bone. The mixture with pellets (Herafill®) decreased shear force resistance in both groups and leveled the difference between them. A higher pore volume related to the pellets decreasing towards the periphery was shown for native and thermoisinfected bone since the distribution of pore diameter appeared

comparable. Suitable carrier material providing high local antibiotic release should reduce porosity of the impacted bone graft to improve mechanic stability. Three dimensional computertomographic measurement of porosity might be of clinical relevance to predict mechanic properties of impacted bone graft intraoperatively and this could be helpful to improve impaction and homogeneity of the transplanted bone.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Authors' contributions

CF, AJ and MR designed and wrote the study and it was performed in the laboratory by JB, AJ, GAA, CAFU, GAK, MK and CF. All authors read and approved the final manuscript.

Availability of data and material

The data measured and calculated in the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

The Authors declare that there is no conflict of interest.

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Key words

Femoral impaction bone grafting: Thermodisinfected bone

Porosity bone graft: Native cancellous bone

Antibiotic carrier: Shear force resistance