Mechanical and morphological characterization of PMMA/bone composites in human femoral heads

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Abstract

PMMA has gained an important place in a variety of orthopaedic applications in the femur. However, appropriate data on the mechanical properties of bone-cement composites from the human femur are lacking. Therefore, the goal of this study was to determine the morphological and quasi-static compressive properties of proximal femoral bone-cement composites. Thirty trabecular bone specimens were extracted from fifteen pairs of human femoral heads using specimen-specific cutting guides to ensure an accurate alignment with the main trabecular direction (MTD). One specimen from each pair was augmented with PMMA bone cement, while the other one was left untreated. Specimens were scanned with μ CT to determine morphological parameters and tested in quasi-static compression until failure. We found that the long axis of the specimens was highly aligned with the MTD (mean error $< 5^\circ$). A higher compressive modulus and ultimate strength were observed for the bone-cement composite specimens ($E = 5.7 \pm 0.4$ GPa; $\sigma_u = 77.9 \pm 5.1$ MPa) compared to the bone only specimens ($E = 2.9 \pm 0.7$ GPa; $\sigma_u = 19.0 \pm 5.8$ MPa). Furthermore, the composites had a higher modulus, but lower strength than cement itself ($E = 5.0 \pm 0.3$ GPa; $\sigma_u = 85.9 \pm 2.7$ MPa) and the composite modulus was significantly correlated with the bone volume fraction (BV/TV). These results are in contrast to previous findings on human vertebral bone, where the composite was more compliant than cement and no correlation was found between BV/TV and the composite modulus. Thus, properties of bone-cement composites cannot simply be applied across different anatomical sites; the site-specific differences in bone density and trabecular alignment should be taken into account. Collectively, the present results suggest that at low BV/TV, cement dominates the composite properties, while at high BV/TV, the contribution of bone becomes apparent, revealing a positive relationship between BV/TV and the on-axis modulus.

Keywords: Femur, Bone, Cement, PMMA, Material properties

1. Introduction

Poly(methyl methacrylate) (PMMA) bone cement was introduced in 1960 for the fixation of a femoral hip prosthesis [1]. Ever since, PMMA has gained an important place in a variety of applications in orthopaedic and trauma surgery [2]. PMMA is an inert material that consists of two components, a liquid and a powder, that are mixed in the operating room to form a dough-like material which is then applied to the bone and subsequently hardens to provide mechanical stabilization of the bone and/or prosthesis.

In the femur specifically, PMMA is currently used in applications such as total hip arthroplasty, fracture fixation and femoroplasty of metastatic bone lesions. These treatments are often modelled with finite element (FE) analysis for the purpose of evaluating the mechanical effect and/or optimizing the treatment method [3,4]. A variety of composite bone-cement material properties have been assumed in these FE studies, ranging from those of bone cement [4–6], to those of bone-cement composite based on results from vertebral bone studies [7] and to the summed stiffness of bone and cement [8]. It remains to be determined whether these assumptions are appropriate for modeling the behavior of augmented bone in the proximal femur, because accurate knowledge on the material properties of PMMA/bone composite in human femoral bone is lacking.

Thus far, the compressive behavior of PMMA/bone composites has been investigated mainly in the context of vertebroplasty in spinal surgery [9–12]; all these studies have made use of specimens from human vertebral or bovine tibial bone, which differ from human femoral bone in both density and structure. These site-specific differences in bone structure are well known and have already been shown to influence the material properties of human trabecular bone [13,14]. It is very likely that these differences also influence the cement distribution and mechanical behavior of bone-cement composites. Indeed, conflicting findings in literature regarding composite stiffness suggest that this is the case. Specifically, several studies on human vertebral PMMA composites found no influence of the bone volume fraction (BV/TV) on the material properties and observed a significantly lower stiffness for bone-cement

47 composite compared to cement itself [9–11,15]. In contrast, for bovine tibial bone, Williams and Johnson
48 found a strong correlation between BV/TV and the modulus in the direction of major trabecular
49 alignment, and their composites showed a higher compressive stiffness compared to cement [16]. These
50 data imply that the results based on human vertebral bone might not apply across different anatomical
51 sites with a higher BV/TV, such as the proximal femur [9].

52 Another important aspect that can influence the material properties of bone-cement composites is the
53 alignment of the specimens with the main trabecular direction (MTD). Off-axis alignment of approximately
54 20° can already result in 40% difference in the mechanical properties of native trabecular bone in the
55 femoral head [17]. Furthermore, specimen alignment has been shown to influence the mechanical
56 properties of PMMA/bone composites [12,16]. The observations of Williams and Johnson, pertaining to
57 the composite modulus being correlated with BV/TV and being higher than the cement modulus, only
58 held true when testing along the MTD and not when testing in the transverse directions [16]. Additionally,
59 Helgason et al. [12] suggested that the reason they did not observe a strong correlation between BV/TV
60 and the composite modulus in bovine tibial specimens was due to the large variation in alignment of their
61 specimens. Thus, there is a scope for developing methods that may be used to prepare bone and bone-
62 based composite specimens with an accurate alignment with the MTD. In turn, this should contribute to
63 the accurate determination of on-axis material properties and will likely result in stronger correlations
64 between BV/TV and the mechanical response of bone-cement composites [12,17].

65 The purpose of the present study was to determine morphological and quasi-static compressive
66 properties of PMMA/bone composites in human femoral heads. More specifically, the purposes were (1)
67 to develop a method for extracting proximal femoral bone specimens with an accurate on-axis alignment
68 with the MTD, (2) to compare the on-axis compressive stiffness, strength and energy to failure of proximal
69 femoral bone-cement composites to those of native femoral bone and cement, and (3) to determine the
70 influence of bone and cement morphological parameters on these compressive properties.

2. Materials and Methods

Fifteen pairs of fresh-frozen (-20°C) human cadaveric femoral heads were obtained from the Department of Anatomy KU Leuven (age 66-98 years, 11 males and 4 females) through their Body Donation Program. Only adult specimens without any abnormalities were included. Ethical approval was granted by the Ethics Committee of the University Hospitals Leuven (reference number NH019 2019-06-01).

2.1 Design of cutting guides

From each femoral head, a cylindrical trabecular bone specimen was extracted with its axis aligned with the main trabecular direction (MTD). To assure a precise on-axis location of the cylindrical specimen, cutting guides were prepared using additive manufacturing [18] (Fig. 1). Specifically, computed tomography (CT) scans of the femoral heads were acquired (Siemens Somatom Force, Siemens AG, Germany) to determine the desired location of the specimen. The following scanner settings were used: 120 ref kV, 250 ref mAs, slice thickness 0.4 mm, slice increment 0.2 mm, pitch 0.85, bone kernel, in-plane pixel size 0.4 mm. The CT scans were imported into Mimics 22.0 (Materialise NV, Leuven, Belgium) and the MTD was identified by the operator through visual inspection of two orthogonal planes (Fig. 1A). A virtual cylinder representing the specimen was positioned on the scans such that its axis was aligned with the MTD. Perpendicular to the MTD, a plane was drawn to indicate the position for cutting the specimen such that inclusion of cortical bone was prevented. The surface contour of the femoral head was segmented from the CT scan and converted into a 3D solid part. Using the femoral head geometry, together with the position of the cylinder and the cutting plane, a specimen-specific cutting guide was designed in 3-matic 14.0 (Materialise NV, Leuven, Belgium) (Fig. 1B). The cutting guide consisted of two parts, each with a concave side that fitted onto the femoral head. The circular opening at the top and the cutting slot on the sides indicated the coring direction and the cutting position respectively. The parts

were converted to stereolithography (STL) files and additively manufactured in polylactic acid (Ultimaker 3, Ultimaker, Utrecht, The Netherlands) (Fig. 1C).

Fig. 1: Procedure for manufacturing a specimen-specific cutting guide to assure specimen extraction along the main trabecular direction (MTD). (A) A cylinder representing the specimen (red) was positioned on the CT scan such that it was aligned with the MTD and a cutting plane (blue) was defined to eliminate cortical bone within the specimen. (B) Based on these references, a specimen-specific cutting guide was designed that indicated the coring direction and cutting position. The guide consisted of two parts that fitted to the shape of the femoral head. (C) The cutting guides were additively manufactured and assembled on the femoral heads.

2.2 Preparation of bone specimens

After assembling the specimen-specific cutting guides on the femoral heads (Fig. 1C), cylindrical bone specimens were cored along the indicated direction using a diamond-coated coring tool (BREU Diamantwerkzeug GmbH, Arbon, Switzerland) with an inner diameter of 8 mm. During coring, the head and cutting guide were fixed on a clamping device. Coring was performed under continuous irrigation with distilled water until a depth just below the cutting plane. Subsequently, the specimens were cut along this plane with a pneumatic oscillating saw (Bosch PMF 180E Multi, Gerlingen, Germany) to detach the cylindrical bone specimens. The specimens were placed in a custom-made sawing guide and trimmed to a length of 26 mm using a diamond-coated bandsaw (Proxxon MBS 240/E, Proxxon GmbH, Niersbach, Germany). Continuous cooling was provided with distilled water.

Bone marrow was thoroughly removed to facilitate a homogeneous cement augmentation. First, a toothbrush was used to brush away superficial marrow from the specimens while they were submersed

in a smooth soap solution [10]. Subsequently, the specimens were placed with the soap solution in closed vials inside an ultrasonic water bath for 20 min (VWR International Ltd., Lutterworth, UK), and afterwards rinsed with a high-pressure pulse lavage system (Palavage Heraeus Holding GmbH, Hanau, Germany). This process of ultrasonic and water jet cleaning was repeated at least two times until complete marrow removal was visually confirmed.

After cleaning, each specimen was submerged in phosphate buffered saline (PBS) solution and scanned inside a μ CT scanner (μ CT 100, Scanco Medical AG, Bassersdorf, Switzerland) to obtain bone morphological parameters. The following scanner setting were used: 70 kVp, 114 μ A, 14.8 μ m voxel size, 200 ms integration time and 0.5 mm aluminium filter.

After scanning, the length and the diameter of the specimens were measured with a digital caliper (Mettler Toledo, Greifensee, Switzerland, accuracy ± 0.01 mm). Finally, the specimens were dried on a napkin for a short time and wrapped in PBS soaked bandage for storage in separate plastic bags in a freezer at -20°C until the day of augmentation. In total, 30 bone specimens were successfully prepared.

2.3 Cement augmentation

The paired specimens were divided in two groups such that one specimen of each pair was randomly assigned for cement augmentation (composite group), while the other was left untreated (bone group). Additionally, 15 cement only specimens were prepared (cement group). All bone specimens were thawed overnight in a refrigerator at 4°C before the day of cement augmentation.

The composite and cement specimens were prepared using a polytetrafluoroethylene (PTFE) split-mold [19] (Fig. 2). For preparation of the composite specimens, a bone cylinder was positioned at the center of the mold before assembling. A copper mesh was placed between the mold and the bottom plate to allow air to escape during cement injection (Fig. 2A). The PMMA bone cement (Traumacem V+, Synthes GmbH, Oberdorf, Switzerland) was prepared by hand-mixing the powder and liquid components with a metal spatula for 30 s. The cement was then transferred into a 5 ml syringe with the tip cut off and immediately

131 injected into the bone cylinder via the top opening of the PTFE mold (Fig. 2B). The injection was stopped
132 when cement visibly penetrated the copper mesh at the bottom end of the molds. After 1 h of initial
133 setting at room temperature, the augmented bone specimens were removed from the molds. The ends
134 were trimmed to the desired specimen length (26 mm) with the diamond-coated bandsaw. Diameter and
135 length were measured with a digital caliper.

Fig. 2: PTFE split-molds used for cement augmentation. (A) Disassembled split-mold; for preparation of the composites, a bone specimen was placed at the center of the mold before assembly. (B) Assembled split-mold. (C) From left to right an example of a native bone specimen, a composite specimen and a cement only specimen.

136 The specimens in the cement group were prepared with a similar procedure. In this case, cement was
137 injected into an empty split-mold. The only difference in the procedure was a 30 s agitation on a vibration
138 plate (KV-26 plus, Wassermann Dental-Maschinen GmbH, Hamburg, Germany) immediately after cement
139 injection to remove large entrapped air holes.

140 All specimens were stored in individual plastic containers filled with PBS solution and left to set for
141 approximately 24h at 37°C. After setting of the cement, the specimens from the composite and cement
142 group were scanned using μ CT for quantifying cement porosity. Finally, aluminum endcaps (cap depth 5
143 mm) were glued onto the specimens using an epoxy-based adhesive (Araldit Rapid, Araldite, Basel,
144 Switzerland) to minimize structural end-effects [20]. Alignment of the end caps during gluing was
145 controlled by fixing the capped specimens in a custom-made alignment holder. The final dimensions of
146 the exposed bone between the end caps was 8 mm in diameter and 16 mm in height (aspect ratio = 2). In
147 between processes, the specimens were returned to a container with PBS at room temperature until
148 mechanical testing.

2.4 MicroCT analysis

The μ CT images of the native bone specimens were analyzed to quantify bone morphological parameters and specimen alignment with the MTD. The images were Gaussian filtered ($\sigma = 1.2$, support = 1) and segmented using a fixed global threshold of 22% of the maximum grey scale value (corresponding to 485 mg HA cm⁻³ according to a phantom calibration) [21] (Fig. 3A). Within a cylindrical volume of interest (VOI) of diameter 7 mm and height 16 mm, the following bone morphological parameters were quantified: bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), structure model index (SMI), connectivity density (Conn.D), and degree of anisotropy (DA). Trabecular orientation was assessed by calculating the mean intercept length (MIL) [22]. The MTD direction of the cylindrical bone specimen was defined as the direction of the eigenvector corresponding to the maximum eigenvalue of the MIL tensor. The misalignment of the specimen axis from this MTD (off-axis angle) was defined as the angle between the MTD and the long axis of the cylindrical specimen.

The μ CT images of the composite and cement specimens were analyzed to quantify the pore volume fraction (PV/TV). A Gaussian filter with a higher support level ($\sigma = 1.2$, support = 5) was applied since the heavy zirconium metal increased the amount of image noise. The porous domain was obtained by segmenting the bone and cement together with a fixed threshold value determined by visual inspection (28% of maximum grey value, 670 mg HA cm⁻³) (Fig. 3B-C). Within the cylindrical VOI (diameter 7 mm, length 16), the combined bone and cement volume fraction (BV+CV)/TV was then quantified and PV/TV was obtained by assuming that $PV/TV = 1 - (BV+CV)/TV$. In case of the cement only specimens (Fig. 3C), no bone was present and BV in the previous formula was equal to zero.

Fig. 3: Segmentation of the μ CT scans within the cylindrical VOI (blue contour). A) Bone was segmented from the native bone scans to extract bone morphological parameters. B) Bone and cement were segmented together from the composite scans to quantify the pore volume fracture (PV/TV). C) Cement was segmented from the cement only scans to quantify the PV/TV.

2.5 Quasi-static compression testing

Capped bone, composite and cement specimens were tested to failure in compression on an Instron E10000 materials testing machine (Instron, High Wycombe, UK). To avoid the influence of machine compliance and end-artifacts, strain was measured using an extensometer (Model 3442-006M-013-ST, Epsilon Technology Corp., Jackson) with a gauge length of 6 mm (Fig. 4). Five preconditioning cycles from 0.1 to 0.4% strain at a rate of 0.5% per second [23] were applied before the specimens were loaded to failure. The ramp to failure was applied in displacement control at a displacement rate of 0.13 mm/s (corresponding to approximately 0.5% strain per second). Failure was defined as a 100 N drop in the load as recorded by the load cell [24]. Load, displacement and strain data were acquired at 100 Hz.

Fig. 4: Mechanical testing setup. Aluminum endcaps were glued onto the specimens. The constructs were tested to failure under compressive loading. Strain was measured with a 6 mm extensometer attached to the center of the specimen.

Mechanical properties were extracted from the final ramp segment of the compression test results. Stress was defined as the load divided by the nominal cross-sectional area of the specimens. Strain was directly obtained from the extensometer output. The modulus of elasticity (E) was defined as the slope of the stress-strain curve between 0.2 and 0.4% strain [12]. Yield strength (σ_y) and yield strain (ϵ_y) were defined by the 0.2% offset method. Ultimate strength (σ_u) was defined as the maximal stress value and energy until failure (U) as the area under the stress-strain curve up to the ultimate strain (ϵ_u). U provides a measure of the resistance to fracture [25].

2.6 Statistical analysis

Statistical analysis was carried out in Matlab v2020a (Mathworks, Inc., Natick, MA, USA). Statistical differences between groups were identified with Wilcoxon signed-rank tests for comparison of the paired specimens in the bone and composite group, and with Mann-Whitney U tests for comparison with the cement only group. P-values below 0.05 were considered statistically significant. Linear regression was applied to identify correlations between parameters. The coefficient of determination (R^2) was used to determine the goodness of fit.

3. Results

3.1 MicroCT analysis

The volume fractions, off-axis angle and bone morphological parameters as measured from the μ CT data are listed in Table 1. Statistically significant differences between the bone and composite group were not found for any of the parameters. The off-axis angle was $4.60^\circ (\pm 2.34^\circ)$ on average for the bone and composite specimens ($N = 30$) and never exceeded 10° . Between the composite and cement group, there was no statistical difference in the PV/TV.

Table 1: Summary of the microCT parameters and mechanical properties (mean $\pm$ SD; range in parentheses).

	Bone (N = 14/ ^Δ 10)	Composite (N = 15)	Cement (N = 15)
MicroCT parameters			
BV/TV (%)	27.3 $\pm$ 5.0 (18.8–36.8)	26.9 $\pm$ 4.6 (20.7–36.6)	–
CV/TV (%)	–	69.7 $\pm$ 4.8 (60.3–77.0) [§]	97.1 $\pm$ 0.1 (95.7–98.0)
PV/TV (%)	–	3.4 $\pm$ 1.0 (2.2–5.1)	2.9 $\pm$ 0.1 (2.0–4.3)
Off-axis angle ($^\circ$)	5.03 $\pm$ 2.34 (1.66–9.62)	4.16 $\pm$ 2.33 (0.68–8.42)	–
Conn.D	4.65 $\pm$ 1.40 (2.45–8.18)	4.33 $\pm$ 1.43 (2.54–8.43)	–
SMI	-0.01 $\pm$ 0.53 (-1.20–0.69)	0.03 $\pm$ 0.57 (-1.38–0.66)	–
DA	2.19 $\pm$ 0.16 (1.95–2.50)	2.23 $\pm$ 0.26 (1.86–2.69)	–
Tb.Th	0.21 $\pm$ 0.03 (0.16–0.29)	0.21 $\pm$ 0.03 (0.17–0.27)	–
Th.Sp	0.68 $\pm$ 0.07 (0.54–0.78)	0.70 $\pm$ 0.07 (0.59–0.86)	–
Th.N	1.35 $\pm$ 0.12 (1.21–1.66)	1.31 $\pm$ 0.11 (1.08–1.56)	–
Mechanical properties			
E (MPa)	2902 $\pm$ 742 (1633 – 4393)	5715 $\pm$ 417 (5106 – 6403) ^{*§}	4999 $\pm$ 256 (4574 – 5507) [*]
σ_y (MPa)	20.3 $\pm$ 5.0 (12.1 – 29.5)	50.8 $\pm$ 4.6 (41.5 – 59.6) ^{*§}	57.2 $\pm$ 2.7 (53.3 – 63.9) [*]
σ_u (MPa)	19.0 $\pm$ 5.8 (6.4 – 29.9)	77.9 $\pm$ 5.1 (65.0 – 86.8) ^{*§}	85.9 $\pm$ 2.7 (82.2 – 91.2) [*]
ε_y (%)	0.87 $\pm$ 0.04 (0.79 – 0.95)	1.09 $\pm$ 0.05 (0.99 – 1.19) ^{*§}	1.34 $\pm$ 0.04 (1.28 – 1.42) [*]
ε_u (%)	1.27 $\pm$ 0.26 (0.99 – 1.81)	4.52 $\pm$ 0.79 (3.62 – 6.33) [*]	4.08 $\pm$ 0.40 (3.44 – 4.78) [*]
U (J/mm ³)	0.18 $\pm$ 0.05 (0.11 – 0.24)	2.75 $\pm$ 0.60 (2.08 – 4.24) [*]	2.56 $\pm$ 0.30 (2.11 – 3.15) [*]

^Δ One specimen in the bone group was fully excluded due to preliminary failure during preconditioning. For 4 other specimens, the measurements of σ_y , ε_y , ε_u and U had to be excluded due to slippage of the extensometer.

^{*} Significantly different ($p < 0.05$) from bone

[§] Significantly different ($p < 0.05$) from cement

3.2 Mechanical testing

The mechanical test results for one specimen in the bone group were excluded since this specimen already failed during preconditioning. From the remaining 14 specimens in this group, σ_y , ε_y , ε_u and U could not be obtained for 4 specimens due to slippage of the extensometer prior to failure. In the pairwise comparisons between the bone and composite group, the specimen pairs corresponding to the unavailable measurements were left out.

A summary of the mechanical properties for the three groups (bone, cement and composite) is presented in Table 1. Representative stress-strain curves for each group are shown in Fig. 5. All mechanical properties in the bone group were significantly different from both the composite and cement group (Table 1). A higher compressive stiffness, strength, ductility and energy to failure were observed in the composite group compared to the bone group (Table 1 and Fig. 6). When comparing the composite and cement group, the composite specimens showed a significantly higher compressive modulus (E), but a lower strength (σ_y , σ_u) (Table 1 and Fig. 6). The cement specimens had a significantly higher ε_y than the composite specimens, yet no significant differences were observed for ε_u and U.

Fig. 5: Representative stress-strain curves for a paired bone and composite specimen, and a cement only specimen. The ultimate strength/strain point is marked with a dot.

Fig. 6: Comparison of the compressive modulus of elasticity E (A), ultimate strength σ_u (B) and ultimate strain ε_u (C) between the bone, composite and cement group. Refer to Table 1 for individual groups sizes in the statistical analyses.

3.3 Regression analysis

In the bone group, E and σ_u showed a strong linear relation to BV/TV ($R^2 = 0.81$ and 0.96 respectively) (Fig. 7A). A similar correlation was observed for σ_y ($R^2 = 0.91$). E , σ_y and σ_u were also correlated with SMI and Tb.Th ($0.58 < R^2 < 0.72$), but the latter two parameters were not independent from BV/TV. SMI and Tb.Th were strongly related to BV/TV ($R^2 = 0.76$ and 0.73).

In the composite group, again a linear relation was found between BV/TV and E ($R^2 = 0.51$) (Fig. 7B). Consequently, E was also correlated with SMI and Tb.Th ($R^2 = 0.61$ and 0.65). Yet, in contrast to the bone group, no correlation was found between BV/TV and σ_y and σ_u (Fig. 7B). The strength parameters σ_y and σ_u on the other hand did show a negative relation with the Conn.D ($R^2 = 0.53$ and 0.50) (Fig. 8). Furthermore, E also correlated with a cement related parameter CV/TV ($R^2 = 0.57$), but the latter was again not independent from BV/TV.

Other correlations between the μ CT parameters and mechanical properties were not significant or were weak ($R^2 < 0.42$).

Fig. 7: A) In the bone group, BV/TV showed a strong linear relation with the compressive modulus of elasticity (E) and ultimate strength (σ_u). B) In the composite group, BV/TV also showed a significant correlation with E , but not with σ_u .

Fig. 8: In the composite group, the yield strength (σ_y) and ultimate strength (σ_u) were linearly related with the connectivity density (Conn.D).

4. Discussion

The aim of this study was to characterize the mechanical properties of bone-cement composites produced with trabecular bone specimens retrieved from human femoral heads. These properties can be applied in finite element models to simulate surgical procedures in the human proximal femur that involve cement

augmentation with PMMA. We found proximal femoral bone-cement composites aligned with the MTD to have a higher compressive modulus, but lower strength than cement only specimens. Furthermore, the composite modulus showed a significant correlation with BV/TV.

An excellent alignment of the specimen axis with the MTD was obtained using specimen-specific 3D-printed cutting guides. Specimen malalignment was quantified with an off-axis angle of $4.6^\circ \pm 2.3^\circ$. This error is smaller than the one obtained in previous studies aiming for specimen alignment with the MTD [17,26–28]. Using steel markers as a reference, recognizable on CT images to compare the reference axis with the MTD, an off-axis angle of $6.1 \pm 3.3^\circ$ was found by Öhman et al. [17] and $7.7 \pm 3.7^\circ$ by Perilli et al. [28]. Another study made use of a specimen holder with an adjustable angle such that the position of the specimen before coring the bone cylinder could be accurately adjusted based on a μ CT scan. With this method off-axis angles of $5.35 \pm 3.17^\circ$ [26] and $6.9 \pm 3.3^\circ$ [27] were obtained.

The compressive modulus and strength of the native bone specimens showed a significant correlation with BV/TV, SMI and Tb.Th. The correlations were not independent; SMI and Tb.Th were strongly related to BV/TV. In comparison to the literature [20,29], high coefficients of determination were found for the density-modulus ($R^2 = 0.81$) and density-ultimate-strength relationships ($R^2 = 0.96$).

The compressive modulus of the bone-cement composites was significantly higher than the modulus of bone and cement only specimens. On average, the moduli of bone, composite and cement were 2.9 ± 0.7 , 5.7 ± 0.4 and 5.0 ± 0.3 GPa respectively. This result is in agreement with a study on bovine tibial bone [16]; yet, is in contrast to reported data on human vertebral bone, where the composite specimen was more compliant than cement (30-48% lower) [9–11]. Using a simple rule of mixtures, one would expect a higher modulus for bone-cement composites than for cement since the tissue modulus of trabecular bone (10 - 20 GPa [30,31]) is higher than the modulus of PMMA cement. In case of perfect bonding between bone and cement, the composite modulus could be calculated as $E_{\text{composite}} = BV/TV \cdot E_{\text{bone}} + CV/TV \cdot E_{\text{cement}}$

[16], which would correspond to 6.2 - 8.9 GPa based on the mean values of our study. However, the modulus we measured was lower, most likely because PMMA cement does not perfectly bond to bone [11]. In addition, some bone damage may have occurred during augmentation [32]. Yet, in the present study we still found the composite modulus to be higher than the modulus for cement alone, while studies on vertebral bone found the modulus of composite specimens to be even lower than for cement alone. An important factor contributing to this difference is the lower BV/TV of vertebral trabecular bone compared to trabecular bone in the femoral head, which reduces the contribution of bone to the composite modulus. In addition, vertebral bone has a different trabecular orientation, which, together with the BV/TV, have been shown to be the main factors influencing the mechanical properties of trabecular bone [29].

Similarly to the native bone modulus, the composite modulus was significantly correlated with BV/TV, SMI and Tb.Th. Again, this finding is in disagreement with studies on vertebral bone [9–11], that did not find a significant correlation between the composite stiffness and bone morphological parameters. As discussed in the previous section, this difference can be attributed to the site-specific differences in bone morphology between vertebral and proximal femoral bone. In low-density vertebral bone, cement dominates the mechanical properties, thereby suppressing the role of bone morphology. However, even for high-density bone from the bovine tibia, different findings have been reported [12,16,24]. Helgason et al. [12] found no significant correlation between the composite modulus and BV/TV, Lopez et al. [24] only found a significant correlation between the modulus and BV/TV for low-modulus cement, and Williams and Johnson only found a correlation along the MTD and not in the transverse directions [16]. Two other factors thus seem to influence the correlation between the composite modulus and BV/TV, namely the cement modulus and the alignment with the MTD. The effect of the cement modulus was attributed earlier to the increased relative contribution of bone when decreasing the cement modulus [24]. The effect of alignment can be explained similarly; the contribution of bone increases with an

accurate alignment along the MTD since bone is known to be a lot stiffer along its MTD [17,29]. In case no accurate alignment was obtained, the variance caused by different alignments could mask the effect of BV/TV. In the study of Helgason et al. [12], a large variation was observed in the anisotropy ratio, indicating that some of their specimens were tested off-axis. This higher variation in alignment can potentially explain why they did not observe a significant correlation with BV/TV. That same study did find a significant correlation between the degree of anisotropy and the mechanical properties; a correlation we were not able to observe due to the very narrow range in degree of anisotropy. This implies that in order to define the material properties of bone-cement composites across varying directions, not only along the MTD, there will potentially be a need for a modulus-density-morphology relationship that takes into account both the effect of BV/TV and trabecular alignment [29]. In order to define such a relationship, additional testing will be required for specimens across a large spectrum of BV/TV and trabecular architecture.

In contrast to the modulus, the composite strength ($\sigma_y = 51 \pm 4.6$ MPa, $\sigma_u = 78 \pm 5.1$ MPa) was found to be lower than the cement strength ($\sigma_y = 57 \pm 2.7$ MPa, $\sigma_u = 86 \pm 2.7$ MPa), although the trabecular bone tissue strength ($\sigma_y = 83/134$ MPa in tension/compression according to [31]) is higher than the strength of cement. Still, the ratio of bone-to-cement strength is smaller (factor 1.5-2.4) than for the modulus (factor 2-4), which makes the relative contribution of bone smaller. In addition, no significant correlations with BV/TV, SMI and Tb.Th were observed. Analogously to the studies on vertebral bone-cement composites, the correlations with bone morphology seem to get masked if cement dominates the composite strength [9–11]. We did observe a negative correlation for σ_y and σ_u and the Conn.D, which is a measure of the connectivity of the trabeculae. A possible explanation is that highly connected trabecular bone structures cause more interruptions of the cement structure, which could decrease the strength of the latter.

No correlation with porosity was observed in both the composite and cement group. In contrast, Kinzl et al. did find a strong correlation ($R^2 = 0.91-0.98$) between the mechanical properties and porosity, defined as $PV/(TV - BV)$ to exclude potentially confounding influences of BV/TV [9]. Also for this porosity definition, no correlations were found in our study, although we believe this might be related to the very low porosity values as opposed to Kinzl et al. (range 3-8% versus 4-22%).

The focus of this study was on the mechanical evaluation of PMMA bone cement for augmenting proximal femoral trabecular bone, yet recent studies have also shown interest in the development of other functional properties of PMMA. For example, the inclusion of antibiotics as an additive has proven to be useful, although it has its share of shortcomings, including sub-optimal release of the antibiotic and very low effectiveness against bacteria commonly found in cases of periprosthetic joint infection of hip and knee arthroplasties [2,33]. More recently, several nanoparticles with antimicrobial activity have been emerging for reducing bacterial proliferation, i.e. silver and gold [33,34]. In addition to antibacterial activity, gold nanoparticles have also shown to influence the mechanical properties of PMMA, more specifically improving the punching strength of PMMA [34].

There are a number of limitations of our study. First, the augmentation was carried out in vitro after thorough removal of bone marrow. This protocol does not present the clinical situation where cement pushes marrow out of the cancellous bone pore space during the injection of the cement [12]. The study by Kinzl et al. [9] addressed this issue by injecting bone cement in a human vertebra without removing the bone marrow in advance. They observed a higher PVF than in our study and they reported that the compressive modulus and yield strength linearly decreased with increasing porosity. The injection into marrow-free cylindrical specimens in the present study will thus likely have underestimated the PVF and overestimated the mechanical properties of the composite.

Second, data in the bone group were lost due to slippage of the extensometer upon failure. Application of the extensometer at the center of the specimen is a good practice method, yet for rough surfaces as bone, it might be better to use a larger extensometer attached to the endcaps instead of the bone surface itself [20]. This would overcome the problem of slippage and prevent potential damage caused by the attachment of the extensometer to the surface. Another advantage of this technique is that it measures strain over the entire length of the specimen, rather than sampling at one location on the irregular specimen. In this study, only the central 6 mm of the specimen was captured for strain measurement. If the deformation took place outside this extensometer gage length, this might have caused less precise measurements of the yield and ultimate strain.

Third, the strain rate during the ramp to failure was not perfectly equal to 0.5% per second. Given the instability of the extensometer measurement towards failure, we chose for displacement control in the ramp to failure. We aimed for a displacement rate that would correspond to a strain rate of approximately 0.5% per second, as applied in previous studies [11,23]. Yet, the effective strain rate as measured from the extensometer signal was slightly lower ($0.4 \pm 0.07\%$ per second) and showed some variation between specimens. However, based on preliminary testing, this variation did not have a large effect on the mechanical properties.

Finally, the material properties found in this study depend on several variables of the PMMA bone cement, such as the composition, storage conditions prior to mixing and the method of mixing. The properties might deviate under different conditions.

5. Conclusions

In conclusion, we found that (1) trabecular bone specimens that were highly aligned with the MTD (mean error $< 5^\circ$) could be obtained with a preparation procedure using specimen-specific 3D-printed cutting guides; (2) the compressive modulus, strength and energy to failure of trabecular bone were significantly

increased by cement augmentation; compared to bone cement itself, the proximal femoral bone-cement composites had a higher compressive modulus, but lower strength; (3) the composite modulus was significantly correlated with BV/TV. These results are in contrast with previous findings on human vertebral bone, where the composite was more compliant than bone cement and no correlation was found between BV/TV and the composite modulus. The mechanical properties of bone-cement composites thus cannot simply be applied across different anatomical sites; the site-specific differences in bone density and trabecular alignment should be taken into account. Collectively, the data suggest that at low BV/TV, cement dominates the composite properties, while at high BV/TV, the contribution of bone becomes apparent, revealing a positive relationship between BV/TV and the on-axis compressive modulus.

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7. References

- [1] J. Charnley, Anchorage of the femoral head prosthesis to the shaft of the femur., *J. Bone Joint Surg. Br.* 42 B (1960) 28–30. doi:10.1302/0301-620x.42b1.28.
- [2] R. Vaishya, M. Chauhan, A. Vaish, Bone cement, *J. Clin. Orthop. Trauma.* 4 (2013) 157–163. doi:10.1016/j.jcot.2013.11.005.
- [3] A. Sas, E. Tanck, A. Sermon, G.H. van Lenthe, Finite element models for fracture prevention in patients with metastatic bone disease. A literature review, *Bone Reports.* 12 (2020) 100286. doi:10.1016/j.bonr.2020.100286.
- [4] M. Taylor, P.J. Prendergast, Four decades of finite element analysis of orthopaedic devices:

368 Where are we now and what are the opportunities?, *J. Biomech.* 48 (2015) 767–778.
369 doi:10.1016/j.jbiomech.2014.12.019.

370 [5] T.S. Kaneko, H.B. Skinner, J.H. Keyak, Lytic lesions in the femoral neck: Importance of location and
371 evaluation of a novel minimally invasive repair technique, *J. Orthop. Res.* 26 (2008) 1127–1132.
372 doi:10.1002/jor.20555.

373 [6] E. Basafa, R.S. Armiger, M.D. Kutzer, S.M. Belkoff, S.C. Mears, M. Armand, Patient-specific finite
374 element modeling for femoral bone augmentation, *Med. Eng. Phys.* 35 (2013) 860–865.
375 doi:10.1016/j.medengphy.2013.01.003.

376 [7] P. Varga, J.A. Inzana, J. Schwiedrzik, P.K. Zysset, B. Gueorguiev, M. Blauth, M. Windolf, New
377 approaches for cement-based prophylactic augmentation of the osteoporotic proximal femur
378 provide enhanced reinforcement as predicted by non-linear finite element simulations, *Clin.*
379 *Biomech.* 44 (2017) 7–13. doi:10.1016/j.clinbiomech.2017.03.001.

380 [8] J. Kok, A. Širka, L. Grassi, D.B. Raina, S. Tarasevičius, M. Tägil, L. Lidgren, H. Isaksson, Fracture
381 strength of the proximal femur injected with a Calcium Sulfate/Hydroxyapatite bone substitute,
382 *Clin. Biomech.* 63 (2019) 172–178. doi:10.1016/j.clinbiomech.2019.03.008.

383 [9] M. Kinzl, A. Boger, P.K. Zysset, D.H. Pahr, The effects of bone and pore volume fraction on the
384 mechanical properties of PMMA/bone biopsies extracted from augmented vertebrae, *J. Biomech.*
385 44 (2011) 2732–2736. doi:10.1016/j.jbiomech.2011.07.028.

386 [10] A. Boger, M. Bohner, P. Heini, K. Schwieger, E. Schneider, Performance of vertebral cancellous
387 bone augmented with compliant PMMA under dynamic loads, *Acta Biomater.* 4 (2008) 1688–
388 1693. doi:10.1016/j.actbio.2008.06.019.

389 [11] A. Race, K.A. Mann, A.A. Edidin, Mechanics of bone/PMMA composite structures: An in vitro
390 study of human vertebrae, *J. Biomech.* 40 (2007) 1002–1010.
391 doi:10.1016/j.jbiomech.2006.04.003.

392 [12] B. Helgason, P. Stirnimann, R. Widmer, A. Boger, S.J. Ferguson, Influence of cement stiffness and
393 bone morphology on the compressive properties of bone-cement composites in simulated
394 vertebroplasty, *J. Biomed. Mater. Res. - Part B Appl. Biomater.* 101 B (2013) 364–374.
395 doi:10.1002/jbm.b.32847.

396 [13] E.F. Morgan, T.M. Keaveny, Dependence of yield strain of human trabecular bone on anatomic
397 site, *J. Biomech.* 34 (2001) 569–577. doi:10.1016/S0021-9290(01)00011-2.

398 [14] E.F. Morgan, H.H. Bayraktar, T.M. Keaveny, Trabecular bone modulus-density relationships
399 depend on anatomic site, *J. Biomech.* 36 (2003) 897–904. doi:10.1016/S0021-9290(03)00071-X.

400 [15] M.I.I. Jofe, T. Takuchi, W.C. Hayes, Compressive Behavior of human bone-cement composites,
401 (1987).

402 [16] J.L. Williams, W.J.H. Johnson, Elastic constants of composites formed from PMMA bone cement
403 and anisotropic bovine tibial cancellous bone, *J. Biomech.* 22 (1989) 673–682. doi:10.1016/0021-
404 9290(89)90017-1.

405 [17] C. Öhman, M. Baleani, E. Perilli, E. Dall'Ara, S. Tassani, F. Baruffaldi, M. Viceconti, Mechanical
406 testing of cancellous bone from the femoral head: Experimental errors due to off-axis
407 measurements, *J. Biomech.* 40 (2007) 2426–2433. doi:10.1016/j.jbiomech.2006.11.020.

- 408 [18] H. Lv, L. Zhang, F. Yang, Z. Zhao, Q. Yao, L. Zhang, P. Tang, Comparison of microstructural and
409 mechanical properties of trabeculae in femoral head from osteoporosis patients with and
410 without cartilage lesions: A case-control study *Pathophysiology of musculoskeletal disorders*,
411 *BMC Musculoskelet. Disord.* 16 (2015) 1–10. doi:10.1186/s12891-015-0530-5.
- 412 [19] I. Koh, Y. Gombert, C. Persson, H. Engqvist, B. Helgason, S.J. Ferguson, Ceramic cement as a
413 potential stand-alone treatment for bone fractures: An in vitro study of ceramic-bone
414 composites, *J. Mech. Behav. Biomed. Mater.* 6 (2016) 519–529.
415 doi:10.1016/j.jmbbm.2016.03.027.
- 416 [20] B. Helgason, E. Perilli, E. Schileo, F. Taddei, S. Brynjólfsson, M. Viceconti, Mathematical
417 relationships between bone density and mechanical properties: A literature review, *Clin.*
418 *Biomech.* 23 (2008) 135–146. doi:10.1016/j.clinbiomech.2007.08.024.
- 419 [21] F. Eckstein, M. Matsuura, V. Kuhn, M. Priemel, R. Müller, T.M. Link, E.M. Lochmüller, Sex
420 differences of human trabecular bone microstructure in aging are site-dependent, *J. Bone Miner.*
421 *Res.* 22 (2007) 817–824. doi:10.1359/jbmr.070301.
- 422 [22] A. Odgaard, Three dimensional methods for quantification of cancellous bone architecture, *Bone.*
423 20 (1997) 315–328.
- 424 [23] T.M. Keaveny, T.P. Pinilla, P.R. Crawford, D.L. Kopperdahl, A. Lou, Systematic and random errors
425 in compression testing of trabecular bone, *J. Orthop. Res.* 15 (1997) 101–110.
- 426 [24] A. López, G. Mestres, M. Karlsson Ott, H. Engqvist, S.J. Ferguson, C. Persson, B. Helgason,
427 Compressive mechanical properties and cytocompatibility of bone-compliant, linoleic acid-
428 modified bone cement in a bovine model, *J. Mech. Behav. Biomed. Mater.* 32 (2014) 245–256.
429 doi:10.1016/j.jmbbm.2014.01.002.
- 430 [25] N.H. Hart, S. Nimphius, T. Rantalainen, A. Ireland, A. Siafarikas, R.U. Newton, Mechanical basis of
431 bone strength: Influence of bone material, bone structure and muscle action, *J. Musculoskelet.*
432 *Neuronal Interact.* 17 (2017) 114–139.
- 433 [26] X. Wang, X. Liu, G.L. Niebur, Preparation of On-Axis Cylindrical Trabecular Bone Specimens Using
434 Micro-CT Imaging, in: *Trans. ASME*, 2004: pp. 122–125.
- 435 [27] Z. Wu, A.J. LaNeve, G.L. Niebur, In vivo microdamage is an indicator of susceptibility to initiation
436 and propagation of microdamage in human femoral trabecular bone, *Bone.* 55 (2013) 208–215.
437 doi:10.1016/j.bone.2013.02.019.
- 438 [28] E. Perilli, M. Baleani, C. Öhman, R. Fognani, F. Baruffaldi, M. Viceconti, Dependence of
439 mechanical compressive strength on local variations in microarchitecture in cancellous bone of
440 proximal human femur, *J. Biomech.* 41 (2008) 438–446. doi:10.1016/j.jbiomech.2007.08.003.
- 441 [29] I. Fleps, H. Bahaloo, P. Zysset, S.J. Ferguson, H. Pálsson, B. Helgason, Empirical relationships
442 between bone density and ultimate strength: A literature review, *J. Mech. Behav. Biomed. Mater.*
443 110 (2020) 103866. doi:10.1016/j.jmbbm.2020.103866.
- 444 [30] E.F. Morgan, G.U. Unnikrisnan, A.I. Hussein, Bone Mechanical Properties in Healthy and Diseased
445 States, *Annu Rev Biomed Eng.* 20 (2018) 119–143. doi:10.1146/annurev-bioeng-062117-121139.
- 446 [31] H.H. Bayraktar, E.F. Morgan, G.L. Niebur, G.E. Morris, E.K. Wong, T.M. Keaveny, Comparison of
447 the elastic and yield properties of human femoral trabecular and cortical bone tissue, *J. Biomech.*

448 37 (2004) 27–35. doi:10.1016/S0021-9290(03)00257-4.

449 [32] M. Kinzl, A. Boger, P.K. Zysset, D.H. Pahr, The mechanical behavior of PMMA / bone specimens
450 extracted from augmented vertebrae : A numerical study of interface properties , PMMA
451 shrinkage and trabecular bone damage, 45 (2012) 1478–1484.
452 doi:10.1016/j.jbiomech.2012.02.012.

453 [33] A. Bistolfi, R. Ferracini, C. Albanese, E. Vernè, M. Miola, PMMA-based bone cements and the
454 problem of joint arthroplasty infections: Status and new perspectives, Materials (Basel). 12
455 (2019) 1–16. doi:10.3390/ma12234002.

456 [34] T. Russo, A. Gloria, R. De Santis, U. D’Amora, G. Balato, A. Vollaro, O. Oliviero, G. Improta, M.
457 Triassi, L. Ambrosio, Preliminary focus on the mechanical and antibacterial activity of a PMMA-
458 based bone cement loaded with gold nanoparticles, Bioact. Mater. 2 (2017) 156–161.
459 doi:10.1016/j.bioactmat.2017.05.002.

460