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A new bone adhesive candidate- does it work in human bone? An ex-vivo preclinical evaluation in fresh human osteoporotic femoral head bone

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ABSTRACT

Introduction: The fixation of small intraarticular bone fragments is clinically challenging and an obvious first orthopaedic indication for an effective bone adhesive. In the present study the feasibility of bonding freshly harvested human trabecular bone with OsStic[R], a novel phosphoserine modified cement, was evaluated using a bone cylinder model pull-out test and compared with a commercial fibrin tissue adhesive.
Methods: Femoral heads (n=13) were collected from hip fracture patients undergoing arthroplasty and stored refrigerated overnight in saline medium prior to testing. Cylindrical bone cores with a pre-inserted bone screw, were prepared using a coring tool. Each core was removed and glued back in place with either the bone adhesive (α-tricalcium phosphate, phosphoserine and 20% trisodium citrate solution) or the fibrin glue. All glued bones were stored in *bone medium* at 37°C. Tensile loading, using a universal testing machine (5 kN load cell), was applied to each core/head. For the bone adhesive, bone cores were tested at 2 (n=13) and 24 (n=11) hours. For the fibrin tissue adhesive control group (n=9), bone cores were tested exclusively at 2 hours. The femoral bone quality was evaluated with micro-CT.
Results: The ultimate pull-out load for the bone adhesive at 2 hours ranged from 36 to 171 N (mean 94 N, SD 42 N). At 24 hours the pull-out strength was similar, 47 to 198 N (mean 123 N, SD 43 N). The adhesive failure usually occurred through the adhesive layer, however in two samples, at 167 N and 198 N the screw pulled out of the bone core. The fibrin tissue adhesive group reached a peak force of 8 N maximally at 2 hours (range 2.8-8 N, mean 5.4 N, SD 1.6 N). The mean BV/TV for femoral heads was 0.15 and indicates poor bone quality.
Conclusion: The bone adhesive successfully glued wet and fatty tissue of osteoporotic human bone cores. The mean ultimate pull-out force of 123 N at 24 hours corresponds to ~ 300 kPa shear stress acting on the bone core. These first ex-vivo results in human bone are a promising step toward potential clinical application in osteochondral fragment fixation.

Introduction

An advanced adhesive “superglue”, capable of both immediate and sustained bonding of broken bone, would enable orthopaedic trauma surgeons to treat clinical needs that standard implant hardware cannot presently adequately meet. The use of adhesives for fixation of smaller more delicate osteochondral fragments is a very compelling and clinically relevant indication, as the number and/or size of fragments are often challenging to stabilize with standard orthopaedic implants without violating the cartilage surface [1]. The concept of an adhesive solution has been proposed in orthopaedic research over many decades, yet such an adhesive does not currently exist [2]. The first clinical report of bone adhesive, described by Hédri in 1931 [3], was a mixture of collagen and fibrous protein, Ossocol. Good initial bonding strength and frac-

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ture healing was reported, but severe allergic reaction prevented its further clinical use. A further adhesive candidate, Ostamer [4], a polyurethane foam believed to be biodegradable was widely tested in clinical studies. This, however, resulted in non-unions, infections, tissue necrosis and healing difficulties and was subsequently denied approval for use by the US Food and Drug Administration in 1963.

Today, researchers and clinicians have comprehensively identified the challenges and safety and efficacy requirements for such tissue adhesive biomaterials. An ideal bone adhesive would be non-toxic, easy to apply, bind wet and fat-covered surfaces sufficiently together at surgery to stabilise and allow healing without impediment and, ideally, be resorbable. It would also have to show relevant clinical value compared with existing gold standard implant hardware treatments [2].

Modern tissue adhesives are either synthetic or biologically-inspired materials [5]. The former includes cyanoacrylates, polyurethane derivatives, thiol-ene etc., whilst the latter encompasses fibrin adhesives, mussel adhesive proteins, "sandcastle worm glue" and castor oil derivatives (Kryptonite^{TM}). Currently, none of these novel materials are approved for orthopaedic clinical practice. A fibrin tissue adhesive (Tisseel^{TM}), approved only as a tissue sealant, has often been used for its adhesive properties in preclinical studies in orthopaedic applications. The bond strength of fibrin sealants is very low and unlikely to maintain initial bone fragment reduction, therefore in combination with its early degradation, it would not meet the minimal requirements for orthopaedic application [6].

Recently, a novel bone adhesive (hereafter abbreviated to bone adhesive) composed of a phosphoserine modified cement (PMC) has shown strong tissue adhesion to animal bone under both ex-vivo and in-vivo laboratory conditions [7,8]. The adhesive formulation used in the current study (OsStic^{R}, Biomimetic Innovations Ltd, Ireland) consists of an amino acid – phosphoserine, alpha-TCP (tricalcium phosphate), calcium silicate and water. The amino acid is of particular interest as it is a component of many human proteins, has a role in cellular signal transduction and mineralization, contributes to tissue and bone healing [9,10], and furthermore, is suggested to be part of a molecular self-healing mechanism of bone [11]. The safety and non-toxicity of the adhesive has been demonstrated in a subcutaneous murine model [12], along with good bonding strength demonstrated in a novel ex-vivo murine femoral condyle bone core model [13]. Wu et al. presented superior screw augmentation effect of the adhesive in osteoporotic human femoral head bones compared to a calcium phosphate cement [14]. In a recent murine in-vivo study with the bone adhesive, good bonding strength (estimated to be up to 14.4 MPa in cancellous bone) and uneventful fracture healing was shown over a time period of 6 weeks [8]. In addition, long term biocompatibility was shown in a lapine model up to 52 weeks for a similar class of PMC adhesive where tetra-calcium phosphate was used [15]. At 2 years PMC adhesive, in a large animal (ovine) model [16], showed no adverse local effects, signs of infection or cytotoxicity in tissues either at or adjacent to implantation sites. It should be noted that at the time of writing there is no human tissue data that confirm these results to the appropriate ISO standards for genotoxicity, carcinogenicity and reproductive toxicity of the adhesive biomaterial and its degradation products.

Whilst the foregoing published studies are encouraging, the pre-clinical evidence that such a material will meet all the requirements of a bone adhesive in a human clinical situation is still incomplete. This led the authors to propose the research goal in the present study to undertake the first human bone evaluation of this particular adhesive formulation.

The research goal of the present study is to evaluate the application of the novel PMC bone adhesive in a freshly harvested human bone cylinder pull-out test and to determine its bonding strength in the first 24 hours. It is benchmarked against a Fibrin glue control as this is the most frequently reported adhesive despite its low strength and that its main application is as a hemostatic agent.

Methods

Overall design

There are no standard testing methods for bone adhesives, therefore we developed the osteochondral bone core model, inspired by other studies [8,13,17,18], as a first step toward modelling the fixation of small intraarticular bone fragments. In this model bone cylinders were cored out and glued back in place and the axial pull-out strength was tested in a mechanical testing machine after 2 and 24 hours. The control group with fibrin tissue adhesive (Tisseel ^{TM}, Baxter) was compared only at 2 hours as in an earlier proof of concept pre-test there was no detectable difference between the 2 and 24 hour result.

Specimens

Thirteen femoral heads were harvested from patients with cervical hip fractures undergoing hemiarthroplasty at the Orthopaedic Department, Sahlgrenska University Hospital. The quality of such bone is assessed to be too poor for donation to the bone bank and as such is considered as biological waste. Nevertheless, an ethical committee approval was obtained (T600-18) and each patient was informed and signed a consent before the procedure. The median age of the 13 patients (8 male, 5 female) was 84 years.

The femoral heads were collected and stored in sterile plastic containers in phosphate buffered saline (PBS) overnight in the fridge at 4°C. The following morning, bone quality of the intact femoral heads in the bone adhesive group (n=10) was analysed with micro computed tomography (microCT) (XtremeCT, SCANCO Medical, Bruettisellen, Switzerland) with the following parameters: voxel size–82 µm; source voltage–60 kV; current–901 µA; filter—0.1 mm Cu; exposure time – 100 ms; frame averaging–1; rotation–180°; 750 projections. Trabecular bone volume fraction (bone volume (BV) over total volume (TV), BV/TV) was evaluated in 300 slices in the center of each femoral head, using the standard HRpQCT analysis software which uses a Laplace-Hamming filter to smooth the image and enhance edges, with application of a 40% fixed global threshold to segment the bone from marrow phase [19,20].

Subsequently, one to three bone cylinders per femoral head were drilled (from the cartilage surface towards the centre of the head) using a dental trepan burr (outside diameter 10 mm and inside diameter 8.9 mm, max drilling depth 12 mm, Komet Dental, Gebr. Brasseler GmbH) in the hospitalś wet lab. Firstly, the core was drilled, the original position of the cylinder was marked on the cartilage surface with an oscillating saw and then a canal along the centre axis of the core was drilled with 2.8 mm drill (Fig. 1a). A 4.0 mm cannulated cancellous screw, 20 mm in length was inserted with half of the thread length engaging in the cylinder (7 mm). The bone core was then eased out from the femoral head with the help of a cement chisel (Whelan curved chisel blade, Innomed, Inc., USA) thus creating a fracture surface at the bottom of the core cavity (Fig. 1b). By the introduction of the chisel tip into the osteotomy gap, this places the cancellous bone core into bending and it fractures due to tensile loading on the cancellous bone at the base of the bone core. In case of multiple cores per femoral head, the core locations were individually marked for identification and the geometric orientation of the corresponding

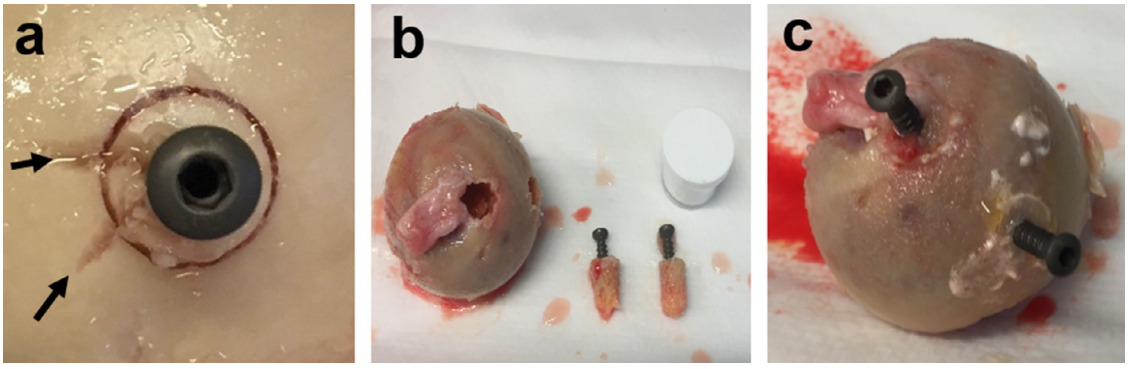

Fig. 1. Preparation of femoral head cylinders for testing. (a) The orientation of the core in relation to the cavity marked on the cartilage surface (arrows) with 4.0 mm cancellous screw inserted axially in the core centre; (b) Femoral head with two cylindrical cores removed; (c) Bone cylinders glued back in place.

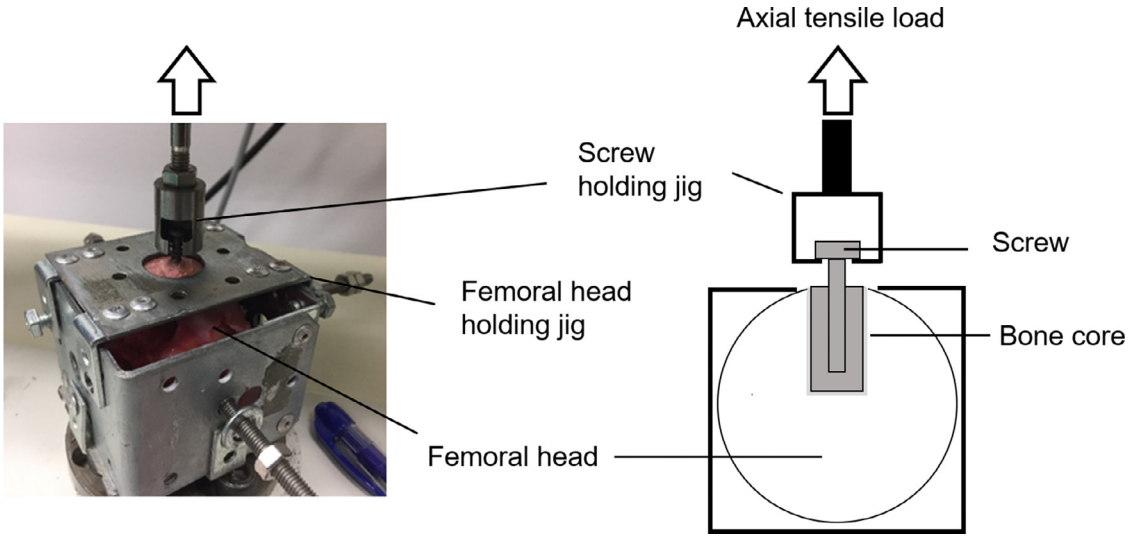

Fig. 2. Mechanical testing setup: A custom cage for retaining the femoral head during pullout. The screw at the center of the glued cone cylinder attaches to the load cell of the test machine.

bone cores in the surface of the femoral head was photographically documented.

Adhesive procedure

The femoral heads were placed in plastic containers and heated in a bath at 37 °C for 30 minutes. Under clean conditions, the bone cores were glued back into the femoral heads using the OsStic adhesive. The powder components, alpha tricalcium phosphate and O-phospho-L-serine (Flamma AB), were mixed at a 30% molar ratio. The powders were then combined with the liquid, a 20% (w/v %) solution of trisodium citrate (Fluka), at a liquid to powder ratio of 0.25 mL/g. The adhesive was mixed for 20 seconds at room temperature and the whole volume (ca. 1 ml) was applied by spatula into the core cavity in the femoral head. Subsequently, the cylinder was pushed into the cavity respecting its original orientation under slight constant pressure, which was maintained during 60 seconds (Fig. 1c).

At this time the bond strength was sufficiently strong that the glued titanium screws could support the entire weight of the femoral head when lifted by the screw.

For the bone adhesive**,** the femoral heads were submerged in *bone medium* and left for 2 hours (n=13) and for 24 hours (n=11) in the incubator of 37 °C, respectively. The bone medium consisted of MEM alpha, 50 µg/ml gentamicin, PEST (100 U/ml penicillin, 100 µg/ml streptomycin) and 2 mM GlutaMAX (Gibco), 10% heat-inactivated FCS (at 56 degrees for 30 minutes, Sigma Aldrich).

For the control group, the fibrin tissue adhesive (Tisseel) was prepared according to the manufacturer's instructions for use and the bone cylinders glued in place and stored for two hours before testing as described above. The fibrin control group was only tested at 2 hours, which was based from a pilot test showing that the mean peak bond strength at 2 hours did not change after 24 hours of curing.

Mechanical testing

The adhesive samples were tested for pull-out strength after 2 and 24 hours from gluing respectively, using a universal testing machine (AGS-H, Shimadzu, Tokyo, Japan), equipped with a 5 kN load cell. The test method was based upon ASTM F 543 – 07 (Standard Specification and Test Methods for Metallic Medical Bone Screws). A custom-made jig was constructed that only constrained motion of the femoral head in the direction of axial loading during testing. To ensure that alignment of the pull-out direction along the cylindrical axis of the bone cores the following procedure was followed: the femoral head was placed in the holding jig. Then the bone core screw was placed in its holding jig. The bone core and

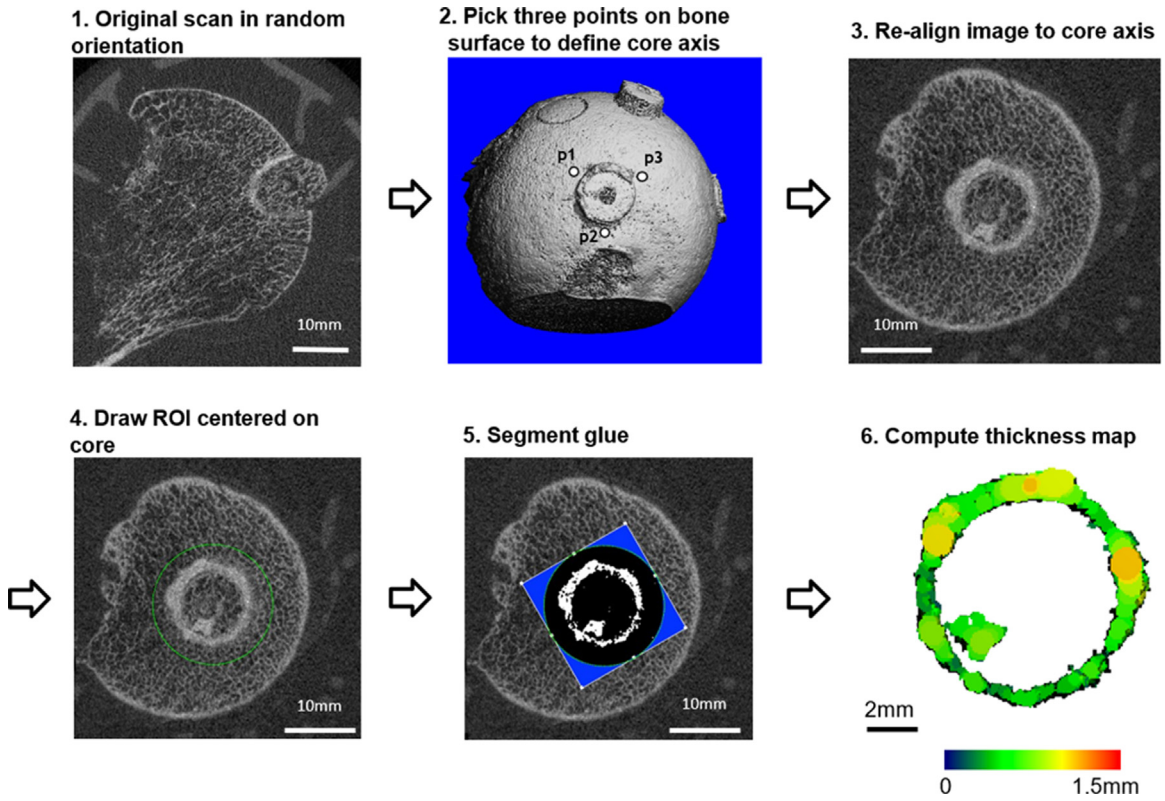

Fig. 3. Workflow for glue volume and thickness analysis. (1) The scans are acquired with random orientation. (2) Each core was re-aligned using the coordinates of three points at its surface and computing an axis of symmetry from them. (3) A rotation matrix was applied to align the axis to the Z axis. (4) ROIs were drawn in the aligned images. (5) Glue was segmented within these ROI with a 790 mgHA/cm^3 threshold. (6) A thickness map was computed. (ROI; region of interest)

the circular hole in the bone holding jig were then concentrically aligned (Fig. 2). The bone cylinders were pulled out of the trabecular bone by the cancellous screw placed in the centre of the cylinder at a constant cross-head speed of 1 mm/minute. The peak load obtained is defined as the pull-out force [N].

Post-testing microCT scanning

Following pull-out, the screws were manually unscrewed from the bone cores and the cores were placed back in the femoral head at their original position/orientation. The heads were scanned using microCT with the same parameters (*Sec 2.2*). These images were used to evaluate the volume and depth penetration of the glue.

For image analysis, each bone core was assessed individually. The image was aligned to the core axis of rotation determined from three points on the bone surface. Axial and longitudinal views of the core were acquired for visualizing the glue distribution. A cylindrical region of interest (ROI) (ø15mm centred on the screw hole, length adjusted to each core) was manually drawn. The glue was segmented within the ROI with a threshold of 790 mgHA/cm^3 (for comparison trabecular bone tissue density in these samples was ~480 mgHA/cm^3) followed by a component labelling filter to remove speckles of dense bone mistaken for glue. The glue volume was evaluated by voxel counting and the glue thickness distribution by a distance transformation method [21] (Fig. 3). Image processing algorithms were developed with EasyIPL v1.0.2 (available at easyipl.com), a high-level library of macros using the scanner software (Image Processing Language, IPL V5.42, SCANCO Medical) and OpenVMS DIGITAL Command Language, DCL V8.4-1H1, Hewlett Packard).

Statistical analysis

IBM SPSS Statistics (v.22 IBM Corp, Armonk, NY, USA) and R v3.6 were used for statistics. The data sets were assessed for normality by performing a Shapiro-Wilk test. Pearson's correlation for bone quality, glue volume and thickness versus pull-out strength was performed along with Tukey's Test for post-hoc analysis to compare mean variance between groups. Significance was set at $p < 0.05$.

Results

Bone adhesive characteristics during mixing, application and gluing

The bone adhesive behaved similarly to other calcium phosphate bone substitutes during mixing and application. A cohesive toothpaste-like consistency was easily achieved after 20 seconds of mixing using a metal spatula. Subsequently, application of the adhesive in the bone cavity and placing the bone cylinder in its original position took another 10-15 seconds. After a further 60 seconds the adhesive hardened sufficiently to bond the bone fragments and enable returning of the glued tissue to the warm bone medium bath without risk of any displacement. Mixing of the adhesive by hand resulted in variable consistency i.e., too liquid or too viscous. One sample was discarded due to addition of excess liquid in the glue mixing procedure.

Mechanical properties

For bone adhesive specimens, all but two cylinders failed through the adhesive interface (Fig. 4a). At 2 hours the force displacement characteristic of the bone adhesive was consistent with a strong, rigid, brittle material (e.g. ceramic cement with sharp and

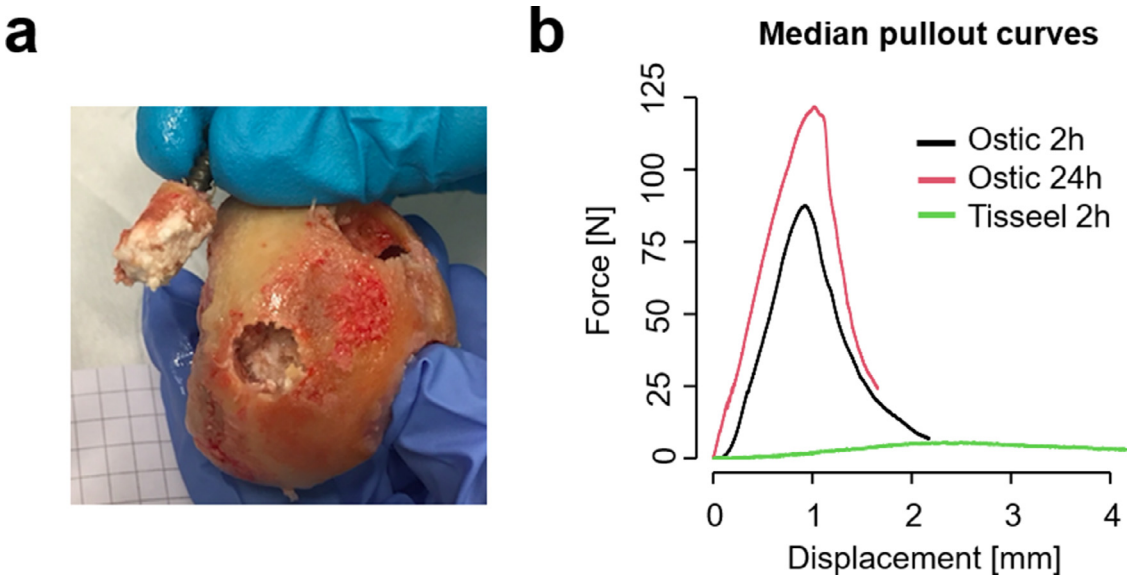

Fig. 4. Force displacement characteristics of OsStic: (a) Bone core after mechanical testing; bone cylinder and cylinder cavity covered with a layer of the white bone adhesive (b) Representative pullout curves for each group. The displayed specimens had the median ultimate pullout force of their respective group.

Table 1
Pullout forces (N). OsStic adhesive at 2h and 24 hours, Tisseel at 2 hours. (SD – standard deviation).

	n	Minimum (N)	Maximum (N)	Mean (N)	SD
OsStic 2h	13	36	171	94	42
OsStic 24 H	11	47	198	123	43
Tisseel 2 h	9	2.8	8.0	5.4	1.6

abrupt failure curve). Typically, an approximately linear load versus displacement curve was followed by a sharp peak and sudden decrease in force as the adhesive layer failed. The gradual tail off on some of the load displacement curves was attributed to the drag of the bone core cylinder (friction) as it emerged from the cored hole. The adhesive curves at 24 hours had a similar shape and the bone cores failed in the same manner (Fig. 4b). The peak pull-out force at 2 hours ranged from 36 to 171N (mean 94, SD 42). At 24 hours peak pull-out forces were similar (range 47 to 198N, mean 123, SD 43). In two specimens tested at 24 hours, the screw pulled out before the failure of the adhesive interface at 198N and 167N, respectively, implying that the adhesive strength was higher than the screw attachment in the two cores (plain dots in Fig. 5).

The load displacement characteristic of the fibrin tissue adhesive 2h group was substantially different, displaying a gradual increase to a maximum load, followed by a prolonged displacement and reduction in load compared to the bone adhesive (Fig. 4b, Table 1). The fibrin tissue adhesive control group reached a maximum 8 N at 2 hours (range 3-8, mean 5.0N, SD 2.0N) and the mean of the recorded peak values were significantly lower than those seen in the adhesive ($p < 0.0001$).

It should be noted that the bone adhesive peak pull-out force occurred typically at a displacement of 1mm whilst the peak pull-out force for the fibrin tissue adhesive was much higher at around 2.5mm.

Bone quality

The femoral heads were of poor bone quality with the mean bone volume fraction (BV/TV) of 0.15 as compared to BV/TV for the general population of 0.26 to 0.36 [22,23], suggestive of osteoporosis. There was no statistical correlation between the femoral head global bone volume fraction and the maximal pull-out load at either 2 or 24 hours (Pearson's correlation -0.4, p=0.2 and 0.3 respectively).

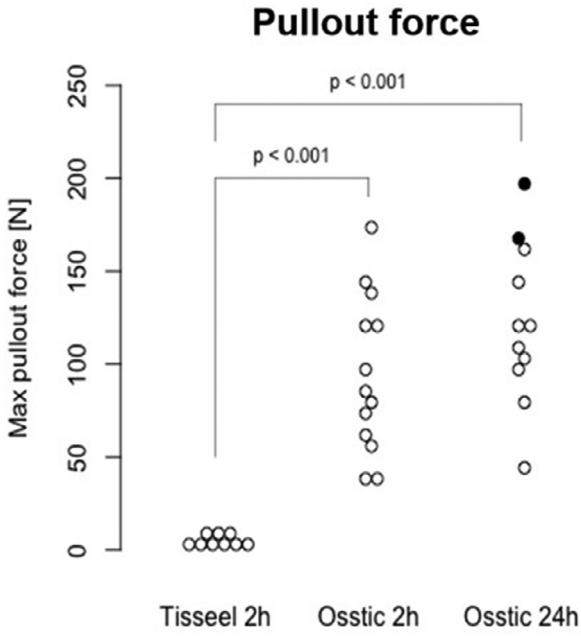

Fig. 5. Comparison of ultimate maximal pullout forces Maximum pullout force values where OsStic at 2 and 24h had significantly higher pullout force to Tisseel at 2h ($p<0.001$). There was no difference between OsStic 2h and 24h. Plain dots represent specimens where screws pulled out of bone (h; hours).

Adhesive volume and penetration

On average the measured volume of the bone adhesive was 382±73 mm^3 and thickness (i.e. a proxy for adhesive penetration in bone) was 0.83±0.29 mm. Of interest, the thickness distribution within a single specimen ranged from 0 to 3.5mm, illustrating the potential of the adhesive for deep local penetration and distribu-

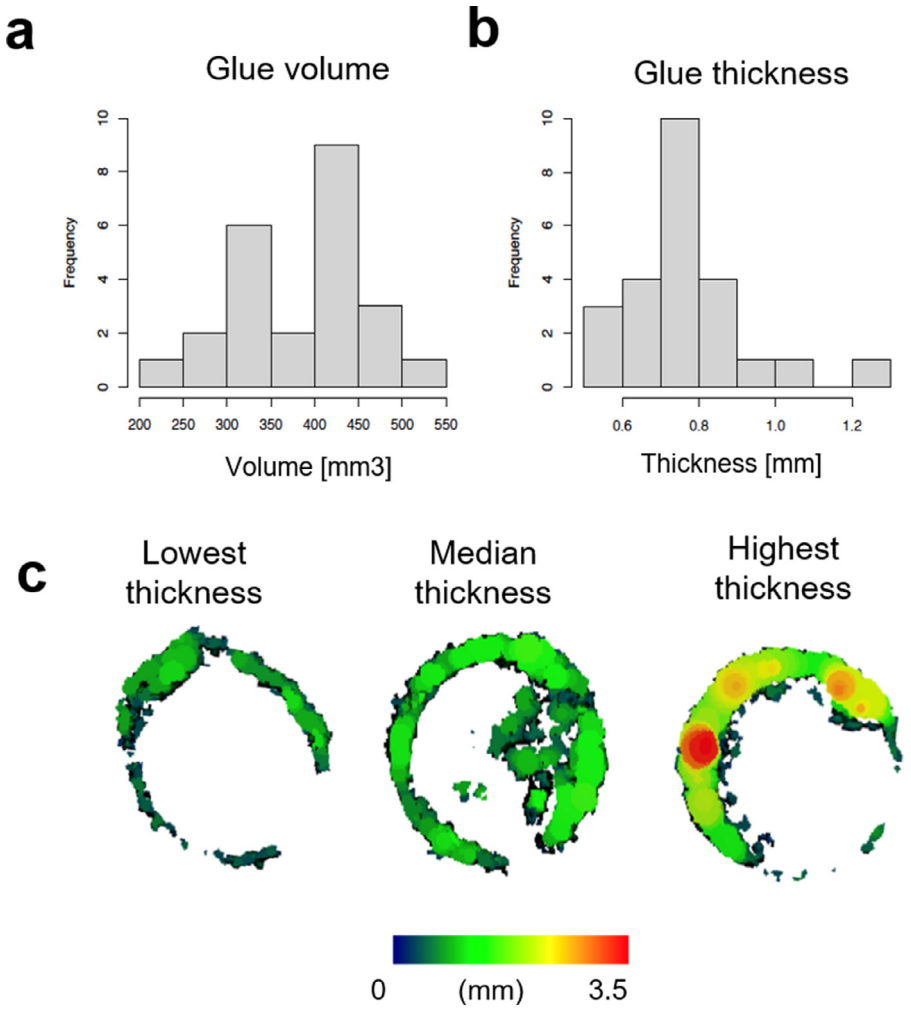

Fig. 6. microCT analysis of the glue volume and thickness. (a) Distribution of glue volume. The glue volume ranged from 248 to 510 mm3 (389±71, median 407). (b) Distribution of glue thickness (mean thickness of each specimen). The specimen average (ie. The distribution of mean thickness between different specimens) glue thickness ranged from 0.57 to 1.25 mm (0.78±0.15, median 0.77). (c) Representative thickness maps with lowest, median and highest mean thicknessses. Note large spacial variations in glue thickness within single specimens (up to 3.5mm).

tion (Fig. 6). We observed a weak correlation between adhesive volume and pull-out force (Pearson's correlation 0.36, $p=0.051$) (Fig. 7).

Discussion

In evaluating the application of the bone adhesive in a simulated clinical setting it was shown that the bone adhesive successfully bonded to freshly harvested human bone tissue surfaces at both 2h, and at 24 hours, ex-vivo. The peak pull-out force at 2 hours ranged from 36 to 171N (mean 94, SD 42). At 24 hours the mean peak pull-out force ranged between 47N and 198N with a mean of 123N, corresponding to average stress values of 118kPa, 498kPa and 309kPa respectively, based on the assumptions below[1]. Although the mean peak value appeared lower at 2h than at 24h, there was no statistical significance between the means ($p=0.13$). Whilst the maximum bond strength at both time points is substantial, it is the minimal bond strength that a clinician has to consider when assessing the suitability for clinical use in bonding bone fragments. To translate this to a more accessible measure, 100kPa can be visualized as a 1-kilogram weight attached to the end of a finger with a contact area of 1cm^2, whilst 500kPa would be 5-kilograms. Adhesive strength in this range might be sufficient to maintain the reduction of a small intraarticular bone fragment in the early phase of healing, especially where early postoperative loading is limited e.g., upper extremity metaphyseal fractures. According to Weber and Chapman [6] 200kPa was described as sufficient strength for clinical use, i.e. maintaining the intraoperative reduction whilst Farrar [6] suggests 500-1000kPa. However, there is little consensus in the literature on the strength of cancellous bone in osteochondral fragments. For isolated osteochondral bone fragments that reduce well, an adhesive would have the advantage of accurately joining adjacent surfaces together. This would also have the advantage of distributing the loading uniformly across the bone surfaces, which conventional plate and screw osteosynthesis fail to do. It should be emphasized that the strengths reported above were without any form of surface preparation or modification e.g. washing, pulsed lavage, suction etc. Recent studies on the bonding and material properties of the bone adhesive indicate that the bond strength may not depend on the volume or contact area [24] which would translate to identical pull-out force and higher force per area for smaller bone fragments. Indeed, there was no correlation found in the present study between overall bone vol-

[1] Using the typical bone core dimensions of diameter d 8.9mm and length l 12mm gives a contact area of 0.0004m^2 ($\pi d^2/4 + \pi dl$) and assuming that these forces were distributed equally over the engaged surfaces of the cylindrical bone core to give the values noted above in parentheses.

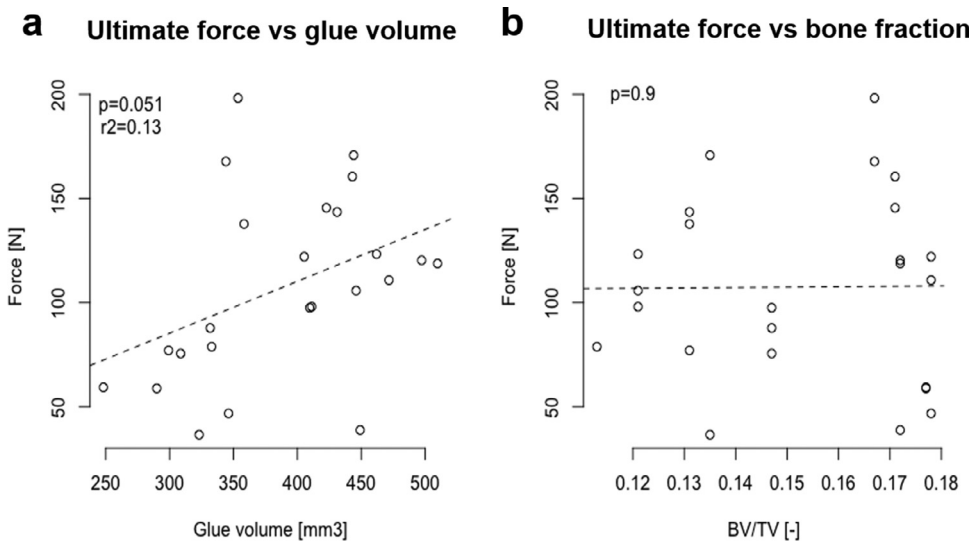

Fig. 7. Statistical correlation analyses. (a) Maximum pullout force against glue volume, where there was no statistical significance (p=0.051) and a slight correlation between glue volume and pullout force (Pearsons correlations 0.36. (b) Maximum pullout force against bone volume, where no statistical significant was detected (p=0.9).

ume fraction and peak force at the time points considered as noted above. Also, as it is reported that bond strength does not depend upon the surface properties, or the bond thickness [25] and so it can be expected to consistently bond to varied osseous surfaces, over a wide range of mineral densities, tissue architecture (e.g. porosity). It could be less sensitive to surgical handling error (e.g., very thick, thin, or uneven layering of the adhesive between tissues) although, a weak correlation was found between the maximal pull-out load and the adhesive thickness in the present study.

The fibrin tissue adhesive group mean peak force value at 2 hours was 5N approximating to 12.5 kPa average mean peak stress (see assumptions in footnote below[1]) which is in good agreement with an earlier ex-vivo murine bone core model [13] in which the average fibrin tissue adhesive mean peak stress at failure was estimated at 13kPa at 4 hours. It should be noted that the fibrin tissue adhesive samples typically required many millimetres of displacement before the peak force levels were reached. Expectedly, the peak force for fibrin tissue adhesive was statistically significantly lower than that of the bone adhesive. Tisseel^{TM}, known for its weak bond strength in bone [17], was chosen as a control group due to lack of a gold standard emphasising the novelty of this new group of phosphoserine-modified cement with bone adhesive properties. The low initial strength of fibrin would not meet the clinical need for adequate primary stabilisation.

There is a substantial unmet clinical need for the treatment of intraarticular fractures with adhesive application, as many osteochondral fragments are too small to stabilize with conventional screws or pins. Cartilage thinning and extensive subchondral remodelling can occur after internal fixation of an osteochondral fragment with bioabsorbable compression screws, potentially as a result of violating the biology of the vulnerable fragment [1]. Other authors also see this potential for an adhesive: for example, Böker et al. [2] considered smaller bone fragments, in lower loaded upper extremity applications such as hand and wrist, as a good application for a bone adhesive. They even suggest that stand-alone use of an adhesive could replace metallic implants entirely in some indications, however, this remains to be proven. In considering potential applications the in-vivo loading environment during the healing period will be a key factor in determining if the properties demonstrated here are sufficient. Whilst much higher strengths ~ 4000kPa are reported ex-vivo for this class of adhesive [7,15] such data is obtained under ideal laboratory conditions, in animal cortical bone which is less representative of typical human osteochondral fragments. It should be noted that far higher bond strengths (>100% increase, 4-6 000kPa or 4-600 N cm^{-2}) have been obtained through formulation enhancement, where the inorganic portion (calcium phosphate) is replaced with calcium silicate [24] or the organic portion (phosphoserine) is replaced by synthetic analogues [26]. Therefore, it is likely that future generations of the bone adhesive may provide even greater fixation strengths, in vivo.

In the author's opinion, the aim of the bone adhesive would not be to replace all metal hardware used in fracture surgery, but to use it as a complement in difficult fractures where standard orthopaedic implants reach their limitations. Multiple small bone fragments could be glued together to make a smaller number of larger bone fragments that can then be reduced and repaired with conventional hardware. The clinical benefits might be immense, where younger patients with intraarticular fractures could mobilize the joints earlier and sustain less posttraumatic arthritis complications, avoiding or postponing the joint replacement. In older patients with osteoporotic bone, the simultaneous fixation and bone augmentation would improve the end result allowing earlier rehabilitation, thus improving the quality of life and prevent the typical complications and fracture collapse and implant cut-out.

The next steps toward human clinical use of the bone adhesive will be to demonstrate that fracture reduction is maintained through to bone healing in a cancellous fracture model and ideally, that the adhesive is transformed to normal healthy bone of normal quality. Although, this has been shown recently in the murine in-vivo model [8], larger standardized animal studies are needed to confirm these early promising results. Once this is achieved the adhesive can be considered for a first clinical pilot in an appropriate clinical indication.

Limitations of the study

The absence of standard testing protocols for bone adhesives resulted in the development of a new testing method appropriate for human bone and the given application in the present study. It should be noted that a similar model was developed for rodents, and when tested *ex vivo*, murine femora produced similar relative strength and performance profiles [13], which is strong evidence of the utility, predictive strength, and translational value (between species) of our test model. The aforementioned murine model has

recently been validated, *in vivo*, with similar mechanical testing results to the present study [8]. The present work is a new model in human bone tissue and there exists only one related study in human bone, to refer against our data [14]. In human femoral bone, harvested and prepared similarly to the present study, Wu et al. demonstrated significantly higher fixation strength when using Os-Stic, compared to conventional calcium phosphate cement, when augmenting cancellous bone screws.

Clinical and biological conditions were closely mimicked by harvesting fresh broken femoral heads from geriatric patients and processing them (all the steps in the current test model) within 48 hours from the patient's admission to the hospital. The bones were stored in the fridge at 4 degrees Celsius in PBS and brought to body temperature in the bone medium for the gluing procedure. The steps were performed under clean but not sterile conditions. Rollo et al. [27] in their clinical study on reimplantation of extruded bone fragments even after considerable time period show good healing outcomes. Correspondingly, the present model indicate that the local environment was favourable to maintain the bonding strength during the studied period. However, we still do not know how the adhesive would perform in living patients, taking into consideration an inflammatory environment and changing pH conditions. Further, as noted in the introduction, there is no human tissue data to confirm the absence of toxic and carcinogenic effects either short or long term. Nevertheless, the recent in-vivo murine model with the bone adhesive has shown promising results such as good adhesion, bioactivity, osseointegration, osteoconduction, and biodegradability, without inducing either local adverse effects or uncontrolled bone repair [8]. The adhesive was not sterilized for this study, although the aforementioned murine study suggests that the characteristics of the adhesive do not change.

Other technical issues in this study included the adhesive mixing procedure, which was done manually and could have been a source of important variability of the presented results (e.g., presence of air bubbles, incomplete mixing, etc.). In the future, a reliable application-guided mixing system should be developed.

It is known that trabecular microstructure of the femoral head varies considerably depending on the femoral head region [28,29]. It would thus be expected that a calcium phosphate-based adhesive that penetrates void spaces, might show a correlation between lower BV/TV (more void, more surface area to bond to) and bond strength. Additionally, whilst local bone density could be a factor influencing the bonding strength, due to data loss during the study, it was not possible to retrieve and analyse this factor.

The testing time at 2 and 24 post adhesion was chosen arbitrarily in order to demonstrate the durability of the bonding after the surgery, i.e., the osteosynthesis would not fail the moment the patient regains the muscle tonus after the anaesthesia and starts the loading of the fracture system. The adhesive works rapidly after the curing reaction begins i.e., after a two to five minutes delay depending on the formulation. This effect has been observed in both animal and human cadaver bone [13]. Nevertheless, it is critical for the surgeon to know the actual setting time of the bonding with adhesive, in order to proceed with surgery. It is recommended that future investigation should assess several factors: biodegradability and influence on bone healing, evolution of bonding strength over time and the possibility of reversing the adhesive effect at or after surgery.

Moreover, for the specific application of this bone adhesive such as osteochondral fragment fixation, its influence on the cartilage should also be investigated.

Conclusions

We were able to show that the bone adhesive develops bonding strength between untreated (wet and fatty) human bone surfaces when handled by a surgeon in a model closely mimicking a clinical setting. The bone adhesive demonstrated sufficient mechanical properties to be considered as a clinically useful tool and further showed that it was not detrimentally affected by poor bone quality, bone volume, or surgical technique (e.g., irregular shaped surfaces). The present results, the first ever in fresh human bone, are a very promising step way toward a bone adhesive becoming a clinically useful material in the operating theatre.

Conflict of Interest

Alicja Bojan (A.B.) is a consultant for PBC BioMed Limited, Ireland. The following authors declare partial ownership in a company that owns all related intellectual property (GPBio LTD): Michael Pujari-Palmer (M.P.), Gerard Insley (G.I.), Philip Procter (P.P.), Håkan Engqvist (H.E.).

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