The design and in vivo testing of a locally stiffness-matched porous scaffold

Shaaz Ghouse a, Natalie Reznikov b, Oliver R. Boughton c, Sarat Babu d, K.C. Geoffrey Ng a, Gordon Blunn e, Justin P. Cobb c, Molly M. Stevens b, Jonathan R.T. Jeffers a,∗

a Department of Mechanical Engineering, Imperial College London, UK
b Department of Materials, Department of Bioengineering and Institute for Biomedical Engineering, Imperial College London, UK
c Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, UK
d Betatype Ltd, Unit 4 Bow Enterprise Park, London, UK
e Department of Biomedical Engineering, University College London, UK

A R T I C L E   I N F O

Article history:
Received 30 November 2018
Received in revised form 18 February 2019
Accepted 22 February 2019

Keywords:
Porous titanium Scaffold Mechanobiology Bone ingrowth Additive manufacturing

A B S T R A C T

An increasing volume of work supports utilising the mechanobiology of bone for bone ingrowth into a porous scaffold. However, typically during in vivo testing of implants, the mechanical properties of the bone being replaced are not quantified. Consequently there remains inconsistencies in the literature regarding ‘optimum’ pore size and porosity for bone ingrowth. It is also difficult to compare ingrowth results between studies and to translate in vivo animal testing to human subjects without understanding the mechanical environment. This study presents a clinically applicable approach to determining local bone mechanical properties and design of a scaffold with similar properties. The performance of the scaffold was investigated in vivo in an ovine model. The density, modulus and strength of trabecular bone from the medial femoral condyle from ovine bones was characterised and power-law relationships were established. A porous titanium scaffold, intended to maintain bone mechanical homeostasis, was additively manufactured and implanted into the medial femoral condyle of 6 ewes. The stiffness of the scaffold varied throughout the heterogeneous structure and matched the stiffness variation of bone at the surgical site. Bone ingrowth into the scaffold was 10.73 ± 2.97% after 6 weeks. Fine woven bone, in the interior of the scaffold, and intense formations of more developed woven bone overlaid with lamellar bone at the implant periphery were observed. The workflow presented will allow future in vivo testing to test specific bone strains on bone ingrowth in response to a scaffold and allow for better translation from in vivo testing to commercial implants. © 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Historically it has been reported that one of the requirements for bone ingrowth into a porous scaffold or into a porous coating of an orthopaedic implant is that it should match the mechanical properties of the surrounding bone tissue [1–3]. However, an increasing volume of work supports taking advantage of the mechanobiology of bone, that the lower the stiffness of a porous scaffold is, relative to the bone it is replacing, the more bone ingrowth occurs [4,5]. The challenges are then to ensure porous materials retain sufficient mechanical competence at low stiffness and to know the properties of the bone being replaced. However, not only are the mechanica-}

Properties of bone difficult to measure, resulting in variations in reported properties in literature [1,6], but bone properties vary between species and anatomical site. Bone properties also vary due to age, sex, nutrition, activity level, bone health and due to diseases [1,7,8]. Typically, when porous materials are tested in vivo or implanted during surgery the mechanical properties of the animal’s or patient’s bone being replaced are unknown and not quantified. It is therefore difficult to compare ingrowth results between studies and to translate in vivo animal testing to human subjects.

Bone is a complex tissue that continually undergoes dynamic biological remodelling, the coupled process whereby osteoclasts resorb mature or damaged bone tissue followed by osteoblasts that generate new bone to maintain homeostasis [9,10]. Generally, bone adapts to mechanical stimulus by apposition under high loading and by resorption under disuse (or less than habitual loading [11]). It is likely that osteocytes respond to bone tissue strain by recruiting osteoclasts to sites where bone remodelling is required [12,13], indeed, there are both theories (Wolff’s Law, Perren’s strain theory, Frost’s “mechanostat”) [14] and experiments that corroborate the effect of mechanical loads and strain on bone adaptation [15,16].

∗ Corresponding author.
E-mail address: j.jeffers@imperial.ac.uk (J.R.T. Jeffers).

https://doi.org/10.1016/j.apmt.2019.02.017
2352-9407/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
Adaptation and remodelling is hypothesised to optimise the stiffness and strength of bones, while minimising the metabolic cost of maintenance and ensures that animal skeletons are continuously adjusted to control strain [15]. It is also why the mechanical properties and structure of bone vary both locally at joints and globally between joints. For humans, the modulus of cortical bone varies between ~7 and 30 GPa whilst trabecular bone has a modulus between ~0.05 and 0.5 GPa [17]. The order-of-magnitude(s) differences between the modulus of solid metal implants (~110 GPa for Titanium and 210 GPa for Cobalt-Chrome) and bone can result in localised reduction in bone density. This is due to a diversion of loads and thus strains from the bone to the implant, known as stress-shielding, which can lead to bone resorption [1,18]. Ultimately, this can result in aseptic loosening of the implant or fracture of the bone around the implant [19].

Porous metals allow the apparent modulus (i.e. modulus of the porous structure treated as a homogenous material, and not the modulus of the base material) of the implant to closer match that of the bone it will be replacing whilst still maintaining mechanical competence. They provide an interconnected structure, allowing bone tissue to grow deep into the implant forming a strong bone-implant interface. Thus, in addition to reducing stress-shielding, they have applications in repair of massive bone defects and fixation of cementless joint replacements [20]. Additive manufacturing (AM) of these materials provides an opportunity for precise control over the porous structure. The size, shape, distribution and interconnectivity of pores and struts can be finely tuned, as well as the overall porosity and apparent modulus. It allows for gradients or local variations in the structure and can provide a strong interface between porous features and solid monolithic geometries. The requirements for bone ingrowth into porous materials are that they should be biocompatible, have a suitable surface for cell attachment, proliferation and differentiation, have an open interconnected porous network for cell ingrowth and transport of nutrients and metabolic waste, have sufficient mechanical strength and provide a strain gradient to encourage bone remodelling throughout the porous material [1,8,17,18].

Most in vivo testing of porous materials for bone ingrowth has focused on the structural features of the material, typically pore size and porosity. Implants have included solid titanium plates with holes of increasing diameter [21], porous sintered titanium coatings, hydroxyapatite scaffolds and conventionally [20] and additively manufactured [2,4,22] porous metal scaffolds. There has also been a wide array of animal models and implant locations (e.g. skeletal location, cortical versus trabecular bone) used [1,18]. Consequently, the differences in mechanical environments and loading likely contribute to why inconsistencies in the literature still exist regarding the ‘optimal’ scaffold pore size and porosity for bone ingrowth [8]. Some work has claimed 100–400 μm size pores to be ideal, whereas other studies have found larger pore sizes of 600–1200 μm to be preferential for bone ingrowth [1], with some researchers even finding bone ingrowth to occur in pores of less than 100 μm [18,21]. However, as bone fills in small sized pores, it may hinder the transportation of oxygen and nutrients to the centre of the scaffold, inhibiting cell proliferation and maturation, and resulting in poor bone-implant bonding [1]. There are also practical concerns about removing loose un-sintered powder from porous materials, particularly if the pore size is too small. Conversely, larger pore sizes are associated with a low short term bone ingrowth ratio, as in the initial stages of bone formation, only a small quantity of bone occupies the implant pores [3]. Similarly with regards to the role of scaffold porosity on bone ingrowth, typically higher porosity scaffolds (>70% porosity) have been shown to have better ingrowth than low porosity scaffolds (<70% porosity) [3,18]; however in the range of 70–90% researchers have found opposing results [18]. Without understanding the mechanical properties of the tissue being replaced and the loading environment, it is difficult to make determinations regarding “optimal” pore size and porosity for scaffolds.

Most commercial or research-grade porous scaffolds have a homogenous structure and are consistent regardless of skeletal location or animal used despite bone varying locally at joints, globally through-out the skeleton and between species. As an increasing volume of work indicates mechanobiology is an important factor in how bone responds to an implant [4], there is a need for porous implants with stiffness gradients tailored to invoke the desired mechanobiological response from bone, specific to the site being investigated. More so, there is a need for a method to create such structures in a pragmatic way that can be deployed clinically in humans. This study will present the design and development of a locally stiffness-matched scaffold. The mechanical properties of trabecular bone in the medial condyle of the femur of skeletally mature ewes will be characterised; consequently a scaffold will be produced with local variations in stiffness equivalent to the bone it will be replacing and its performance will be discussed in vivo in an ovine model.

2. Materials and methods

2.1. Specimen design

2.1.1. Coarse resolution bone property quantification

In order to produce a locally stiffness-matched bone scaffold, the properties of the bone that is to be replaced need to be quantified. Eight ovine femora were CT scanned (SOMATOM Definition AS; SIEMENS AG) using a clinical protocol (512 × 512 resolution, 140 kVp, 0.6 mm slice thickness, and ∼0.5 mm pixel spacing). A five-material calibration phantom (Model 3; Mindways Software Inc) was placed under the specimens for bone mineral densitometry and was visible on each CT slice (Fig. 1.1). A 10 mm thick slice approximately perpendicular from the mechanical axis of the femur and 10 mm from the femoral condyle joint line was cut from each specimen. Four bone cores of Φ5 mm were extracted from the medial condyle of each of these slices (Fig. S1). Each bone core was mechanically tested as per Section 2.2 to determine elastic modulus (Fig. 1.2a).

As the CT scans of the ovine femur were performed with a calibrated phantom, the material properties of the tested trabecular bone cores could be determined. However, in order to do this the location of each bone core needed to be known. Therefore, after removal of the bone cores from each of the 10 mm thick slices, the slices were CT scanned again as per the protocol described earlier. The CT scans of the bone slices were registered to their parent bone in order to accurately determine the location of the bone cores being tested (Fig. S1). Solid Φ5 × 10 mm cylinders were then computationally modelled and meshed in a finite element simulations programme (ANSYS 13). These cylinders were then imported into bone density mapping software (Bonemat v3.2) at the locations of the removed bone cores to determine apparent density (Fig. 1.2b). Consequently a relationship between apparent density and modulus could be produced for trabecular bone. This relationship was combined with one for ovine cortical bone [23] with a linear interpolation between the trabecular and cortical bone relationships in a similar manner as described in [6] in order to have a continuous apparent density–modulus relationship covering the entire density range of ovine femoral bone (Fig. 1.3).

2.1.2. Fine resolution bone property quantification

The porous scaffold used in this study would have global dimensions of Φ16 × 15 mm and it would be implanted in the medial femoral condyle 20 mm from the joint line along the medial-lateral
axis (Fig. 1.4). As a continuous relationship has been established between apparent density and modulus for ovine bone, the local mechanical properties of the bone to be replaced by the scaffold can be determined at the desired resolution. The scaffold was first modelled as a solid object, discretised into 3 equally sized sections in all 3 axes (medial-lateral, anterior-posterior, distal-proximal), i.e. a $3 \times 3 \times 3$ array (27 sections) and placed at the surgical site in the bone density mapping software (Fig. 1.4). Each section extracted the average bone density ($\rho_{CT}$) based on the densitometry calibration and consequently the modulus could be determined using the apparent density–modulus relationship derived (Fig. 1.5).

2.1.3. Quasi-static testing

Quasi-Static compression testing was performed to ISO 13314:2011 using a materials testing machine (Instron 8872) with a 1 kN load cell. Specimens were centred between hardened (>62 HRC) lubricated platens, equipped with a spherical seating, and crushed at a constant strain rate of 1 mm/min ($\sim$0.1 strain/min). For each specimen, every 100 N a hysteresis loop was present reversing at 20% of the load to account for the localised plasticity in porous materials and broken cell effects both of which reduces the slope of the initial loading curve. Displacement between the platens was measured at 30 Hz by two LVDTs (RDP D605000A) to reduce test-machine compliance errors (Fig. 1.2a). Strain was the average LVDT displacement divided by the specimen’s initial height and stress ($\sigma$) was the measured load divided by the specimen’s initial cross-sectional area (calculated from the initial diameter of the specimen). Stress–strain curves were produced for each test; elastic modulus ($E$) was the linear regression of the last hysteresis loop whilst the yield strength ($\sigma_y$) is determined as compressive stress at a plastic compressive strain of 1.0% relative to the elastic modulus.

2.1.4. Design of a locally stiffness-matched porous bone scaffold

A stochastic porous scaffold was designed by filling a $16 \times 15 \times 15$ mm 3D volume with a random distribution of $\sim$5000 points using a Poisson disc algorithm and consequently connecting these points to each other as zero-thickness lines (Fig. 2.1) at an average connectivity of 4.5. In previous works, the authors additively manufactured stochastic porous materials via laser powder bed fusion in a range of relative densities and moduli by altering the laser parameters [24,25]. This was done via Engine (Betatype Ltd.), which is a software platform that creates slice data (build files) directly from line geometry for AM and allows laser parameters and scan strategies to be controlled on a line-by-line basis and
thus avoiding the high computational cost of solid CAD modelling of complex porous materials. Previously, in these works a single laser parameter was used per porous material thus giving a structure with a uniform apparent relative density and apparent modulus (Fig. 2.2). In order for the scaffold in this study to locally match the modulus of the bone it would be replacing, it was discretised into $3 \times 3 \times 3$ sections, as described earlier (Fig. 2.3). In each of these 27 sections modulus was controlled by changing the laser parameters based on the relationships established in [24, 25]. Right and left leg versions of the scaffold were created which were mirrored versions of each other. The scaffold also contained a geometric feature to ensure correct orientation upon implantation and to distinguish between left and right versions.

2.2. Scaffold manufacture

The scaffolds were manufactured on a Renishaw AM250, a metal powder bed laser fusion system, onto a titanium substrate. The workings of the system have been described previously in [24, 25]. Commercially Pure Titanium grade 2 (CP-Ti) powder was used having a particle size range of 10–45 μm ($D_{50} \approx 27$ μm). After production, items were removed from the substrate by electro discharge machining and shot-blast. Specimens were rinsed and cleaned ultrasonically in a cleaning solution (0.2 μm filtered water and Decon Neutracon) followed by sterile isopropanol to remove all contaminants (Hunt Developments UK Ltd). Following filtered drying, specimens were vacuum packed/sealed in pouches and sterilised via Gamma Irradiation (25–35 kGy).

2.3. Surgical procedure

Six skeletally mature non-pregnant female sheep (older than 4 years) were enrolled in the study. Ethical approval for this study was granted by the United Kingdom Home Office (Project License Number 70/8247). A single hind leg of each sheep was used for this study, whilst the other hind leg was used for another study investigating different scaffold material, architecture and stiffness as described in [5]. One scaffold was inserted into the designated hind leg, in the distal medial femoral condyle (Fig. 1.4, S2). Of the six sheep, 3 right legs and 3 left legs were used for implantation with the respective (left or right version) stiffness-matched scaffold.

Antibiotics (Cefalexin 1 ml/25 kg animal once a day) were administered to each sheep pre-operatively and continued for 3 days post-surgery. The distal medial femoral condyle was exposed and the periosteum was removed over the surgical site under general fentanyl anaesthesia. A flat bottom $15 \times 15$ mm cylindrical defect (1 mm under-ream) through the medial-lateral femoral axis was created by use of drills and reamers of increasing diameter equipped with a depth gauge (Fig. S2, S4). Simultaneously, a scaffold was centrifuged in 30 ml of blood from the animal’s jugular vein in a sterile test tube for 3 min at 3000 RPM in order to displace micro air bubbles from the surface of the scaffold. Consequently, the scaffold was press fit by hand into the defect, followed by gentle impaction from a surgical mallet. The wound was closed in layers; first the fascia, then the subcutaneous soft tissue and finally the skin, using resorbable sutures. Consequently the wound was covered with an aseptic spray. Once a swallowing reflex had been regained post-operatively, the animal was returned to a single pen with straw bedding. The animals recovered in sternal recumbency with their normal feeding regime (hay, food concentrate...
and water). For 60 h postoperatively, the sheep received analgesia (Fentanyl 75 mcg patches). Two weeks prior to euthanasia, oxytetracycline was injected intravenously at 30 mg/kg of body weight to help identify the site of active osteogenesis with a fluorophore.

2.4. Explant analysis

At 6 weeks postoperatively all animals were euthanized. The relevant hind-leg femur was disarticulated and its distal metaphysis was removed and trimmed to a block of ~5 cm in all dimensions and stored in formalin at room temperature (Fig. S5).

2.4.1. Micro-computed tomography (micro-CT)

Tomographic imaging was conducted in an Xradia Versa 510 (Zeiss) at 140 kV, 70 μA at 3201 projections and a pixel size of ~25 μm. The 3D reconstructed tomographic images were segmented and analysed using CT-analyser v.1.18.4.1 (Bruker N.V.). Segmentation of the sample contained 3 steps. The defect (region of interest) was delineated as a cylindrical volume around the diameter and length of the scaffold and a 3 level multi-level Otsu segmentation was used to distinguish between the scaffold, bone and other. As the CT scans revealed beam hardening, visible in a tomogram as a halo of higher pixel values around the titanium scaffold elements, a 2 pixel dilation was applied to the scaffold segmentation and subtracted from the images prior to quantifying the new bone ingrowth. This labelling of bone based on the local grey values was necessary to avoid the inclusion of bright pixels around the metal elements that were not bone, but the effect of beam hardening.

2.4.2. Microscopy

To prepare samples for embedding, after μ-CT scanning, the samples were dehydrated by immersion in methylated spirits of increasing concentration (50%, 75%, 85%, 95% and 2 × 100%) followed by 24 h of chloroform immersion, and then methyl-metacrylate resin (LR White) for 72 h. Following saturation of the resin, the accelerator was added and the blocks were polymerised at ~20 °C. The blocks were trimmed and then sectioned by cutting perpendicular to the defect axis (medial-lateral axis) and therefore, parallel to the scaffold face (Fig. S5). For all the samples two central slices were prepared for microscopy. Slices were mounted, ground and polished with one of the slices being thinned down to a thickness of approximately 50 μm. The thinned sections were initially imaged via fluorescent microscopy. Fluorescent microscopy was performed using a Zeiss Wide-Field light microscope (WF3 Zeiss Axios Observer, Zeiss Germany). After fluorescent microscopy, toluidine blue and paragon were used to stain the samples to identify histological features. Histological microscopy was performed identical to the fluorescent microscopy. The non-thinned section was gold sputter coated and was imaged by scanning electron microscopy using a back scatter detector (Hitachi S-3400N).

3. Results

3.1. Bone core testing

Stress-strain curves of the trabecular bone cores and commercially pure titanium specimens of uniform modulus (manufactured and tested in [25]) indicate similar behaviour under compression as seen in Fig. 3. Two distinct regions are seen, an initial linear portion and a plateau of almost constant stress. If tested to higher levels of strain, a third region of densification and rapidly increasing stress would be seen [26]. The hysteresis loop for both bone and the CP-Ti specimens indicates there is localised plasticity at stresses well below the compressive strength of the specimen as well as settling due to broken cell edges. This is evident as the hysteresis loop has a steeper gradient than the initial loading portion. The plateau region of the trabecular bone indicates slightly more brittle failure of the trabecular structure compared to the more ductile collapse of the porous CP-Ti specimens.

Apparent density of the bone core specimens, obtained from CT-scans, ranged between 0.2 and 0.8 g/cm³ whilst modulus, obtained from the mechanical testing, ranged between 56.5 and 2737.7 MPa. A power–law relationship was modelled between the apparent density and modulus of the specimens as described by Gibson and Ashby [26,27] and seen in Fig. 4a.

If the apparent density-modulus trend, as seen in Fig. 4a, is extrapolated further it would underestimate modulus values for cortical bone. Therefore in a similar manner as was presented in [6], the cancellous bone data for the ovine medial femoral condyle was combined with ovine femoral cortical bone data also obtained by mechanical testing, reported in [23] with a linear interpolation between the two datasets (Fig. 4b). This produces a continuous relationship covering the entire apparent density range of ovine bone. A power–law relationship, as per Gibson and Ashby [26,27], or a linear relationship, both accurately model the relationship between yield strength and modulus for the trabecular bone cores, Fig. 5. Compared to the uniform moduli porous CP-Ti scaffolds produced in [25], over the modulus range tested, ovine trabecular bone displays greater strength for a given modulus, on average 24%±2% S.D. difference greater.

3.2. Locally stiffness-matched scaffold

Apparent density for the surgical site ranged on average from 0.27 to 0.76 g/cm³ with a modulus varying between 262.2 and 3644.22 MPa respectively. The local apparent density and
modulus variations for the surgical site, discretised into 27 sections, are presented in Table 1, as are the designed moduli of each of the 27 sections of the scaffold, where the laser parameters and consequent modulus values chosen were based on testing and relationships established in [24,25]. The produced scaffold (Fig. 2.4) had a modulus ranging from 566.0 to 3584.4 MPa with an average modulus over the 27 sections of 1435.2 MPa. As determined from the µ-CT scans, the scaffold had an average relative density of 12.7% (87.3% porous) with strut size ranging from ~80 to 400 µm (D90 = 210 µm) and pore size ranging from ~80 to 1300 µm (D50 = 830 µm). The strut size and pore size distribution can be seen in Fig. 6. The µ-CT scans also revealed that no loose powder was present in the scaffold or visible in the surrounding bone and therefore the scaffolds had been properly cleaned. SEM images of the scaffold can be seen in Fig. S6.

3.3. In vivo results

All sheep were euthanised after 6 weeks following implantation of the stiffness-matched scaffold. The volume of bone ingrowth into the scaffolds (BV) was quantified through µ-CT scans, and was divided by the total defect volume (TV) minus the scaffold volume (SV), both also quantified through the µ-CT scans per animal and implant, to determine the percentage of new bone ingrowth. Bone ingrowth ranged from 7.69% to 16.38% with an average of 10.73% ± 2.97% S.D. (Table 2).

A typical 3D reconstruction of new bone ingrowth into the scaffolds is seen in Fig. 7. For most specimens intimate contact was present between bone and the scaffold over the entire diameter along the entire scaffold length. However, for two specimens (Animal 1 and 5) this was not the case. Animal 1 was the pilot and used a conical drill (jobber) drill of 15.5 mm and consequently gaps are observed between the defect diameter and the scaffold. Following the pilot, the drill was changed to a flat bottom 15 mm drill. Defect creation and consequently implant placement on animal 5 was slightly too distal and not the entirety of the defect was surrounded by bone (Fig. S7). These issues likely contributed to lower bone ingrowth in these two animals. The µ-CT scans also revealed a gap between the lateral end of the scaffold and the end of the defect tunnel ranging between 3 and 5 mm between the animals. However, for animals 2, 3, 4 and 6 bone had almost bridged the 15.5 defect and there was evidence of growth into the lateral end of the implant and not just radially around the diameter (Fig S8).

Around the scaffold periphery bone ingrowth depth was consistently 1–2 mm and would encompass the outer strut elements (Figs. 8–10). In animals 3, 4 and 6 where a greater amount of bone ingrowth was found, thin branches or islets of bone were also observed deep within the scaffold interior. These are seen in the fluorescent microscopy image, which highlights mineralisation of nascent bone during the last 2 weeks of the study, as high intensity specks in the scaffold interior (Fig. 8) and in the histology images (Fig. 9) as thin branches between the struts.

The histology images provide additional information about the pathways of bone formation within the scaffold. The sections taken, through the middle of the scaffolds (7–8 mm deep from the peristeal surface), show the nascent bone ingrowth as woven bone tissue. The structure of which is a very fine, densely branching struts, forming a delicate mesh resembling embryonic bone. At the periphery of the defect (around the scaffold diameter) at the bone-implant interface is where the most intense formation of fine woven bone is observed overlaid with lamellar bone. Embryonic-looking woven bone (Fig. 9b) is seen in the interior of select scaffolds (animals 3, 4 and 6), however the networks of woven bone and titanium struts were intercalated without intimate contact.

Scanning electron microscopy images obtained with a back-scattered detector confirmed the histological findings. Fig. 10
Table 1
Localised density and modulus variations of bone at implant site and modulus variations of scaffold.

| Location | Apparent density of bone (g/cm³) | Modulus of bone (MPa) | Modulus of scaffold (MPa) | Difference (MPa) |
|----------|----------------------------------|-----------------------|--------------------------|------------------|
| 111      | 0.56 ± 0.15                      | 1337.4 ± 678.4        | 1320.6                   | −16.8           |
| 112      | 0.65 ± 0.16                      | 2151.8 ± 1389.0       | 2263.9                   | +112.0          |
| 113      | 0.76 ± 0.16                      | 3550.2 ± 1822.9       | 3584.4                   | +34.2           |
| 121      | 0.51 ± 0.10                      | 998.4 ± 397.0         | 943.3                    | −55.1           |
| 122      | 0.61 ± 0.13                      | 1595.6 ± 700.2        | 1697.9                   | +102.3          |
| 123      | 0.72 ± 0.21                      | 3644.2 ± 4969.3       | 3584.4                   | −59.8           |
| 131      | 0.47 ± 0.12                      | 890.5 ± 510.5         | 943.3                    | +53.3           |
| 132      | 0.49 ± 0.09                      | 900.1 ± 406.7         | 943.3                    | +43.2           |
| 133      | 0.6 ± 0.15                       | 1873.2 ± 1800.6       | 1886.6                   | +13.3           |
| 211      | 0.51 ± 0.15                      | 1095.7 ± 641.9        | 1132.0                   | +36.3           |
| 212      | 0.62 ± 0.21                      | 2365.8 ± 2801.5       | 2452.5                   | +86.7           |
| 213      | 0.71 ± 0.15                      | 2757.6 ± 1045.7       | 2792.1                   | +34.5           |
| 221      | 0.42 ± 0.13                      | 691.6 ± 390.6         | 754.7                    | +63.0           |
| 222      | 0.52 ± 0.15                      | 1143.5 ± 620.3        | 1132.0                   | −11.5           |
| 223      | 0.6 ± 0.14                       | 1618.5 ± 1015.7       | 1697.9                   | +79.4           |
| 231      | 0.32 ± 0.09                      | 334.8 ± 195.9         | 566.0                    | +231.2          |
| 232      | 0.38 ± 0.10                      | 506.4 ± 294.3         | 566.0                    | +59.6           |
| 233      | 0.43 ± 0.15                      | 753.6 ± 753.3         | 754.7                    | +1.0            |
| 311      | 0.51 ± 0.10                      | 1011.3 ± 507.0        | 943.3                    | −68.0           |
| 312      | 0.56 ± 0.11                      | 1272.5 ± 616.6        | 1320.6                   | +48.1           |
| 313      | 0.7 ± 0.12                       | 2679.2 ± 1722.3       | 2678.9                   | −0.3            |
| 321      | 0.42 ± 0.11                      | 6567.2 ± 3044.4       | 641.5                    | −15.3           |
| 322      | 0.46 ± 0.08                      | 787.5 ± 274.4         | 754.7                    | −32.8           |
| 323      | 0.58 ± 0.14                      | 1648.5 ± 1374.8       | 1697.9                   | +49.4           |
| 331      | 0.27 ± 0.12                      | 262.2 ± 191.5         | 566.0                    | +303.8          |
| 332      | 0.32 ± 0.08                      | 344.2 ± 196.5         | 566.0                    | +221.8          |
| 333      | 0.36 ± 0.12                      | 480.1 ± 384.0         | 566.0                    | +85.9           |

Average ± S.D. 0.52 ± 0.13 1383.5 ± 923.7 1435.2 ± 897.2 ±51.8

| Location notation (Fig. S3): X: Posterior-Anterior; Y: Medial-Lateral; Z: Inferior-Superior; all going 1–3 respectively.

Fig. 6. Scaffold strut size (thickness) and pore size distribution. Bars indicate range of sizes in % of volume.

illustrates fine branching networks of woven bone within the titanium scaffolds. These bone formations contained irregularly shaped osteocytes, characteristic of woven bone (Fig. 10b). The variability between contact between the scaffold and bone is also clearly seen. Sometimes bone will engulf the scaffold elements without intimate contact (Fig. 10a), whilst there is also evidence of bone on-growth onto some scaffold elements (Fig. 10c) and furthermore in areas where newly formed bone is present, the bone has no to little association with the scaffold (Fig. 10b). The SEM back-scatter detector also highlights mineral density, thus the areas of newly formed bone have a less dense organisation and a lower degree of mineralisation leading to a darker appearance. On the scaffold periphery at the scaffold-bone interface, where more developed and mature woven bone is present, a denser woven–lamellar bone structure is seen. The mature woven bone is characterised by a higher degree of mineralisation and appears brighter (Fig. 10a, b).

4. Discussion

This study has presented a method to create implantable devices with a tailored stiffness gradient that controls the local strain environment to invoke a desired mechanobiological response from bone. The modulus and strength of trabecular bone from the medial condyle of skeletally mature ewes was characterised and by utilising a clinical CT protocol with a calibrated multi-material phantom a continuous relationship was established between apparent density and modulus. Consequently, a porous scaffold was produced to maintain the mechanical environment of homeostasis at the surgical site, i.e. a 0% strain difference between scaffold and bone it would be replacing. The short term in vivo performance of the scaffold in a load bearing environment was seen. The average bone ingrowth into the scaffolds pores was 10.73 ± 2.97% after 6 weeks. To be able to translate in vivo testing in animals to humans and to compare results between studies related to bone regeneration, the
mechanical environment should be considered and should replicate conditions close to clinical practice. The goal of this study was not to maximise bone ingrowth into a porous scaffold but rather to provide a methodology to better test the mechanobiology of bone. Yet, this study has demonstrated that an additively manufactured porous titanium scaffold that is stiffness-matched to the bone it is replacing is a suitable solution for repairing large bone defects; and by further tailoring the stiffness of the scaffold a desired strain in the bone can be produced to maximise the speed and quantity of bone repair [2,4,5].

4.1. Mechanical response of trabecular bone and AM porous materials

The behaviour of the trabecular bone tested and of the porous CP-Ti specimens were typical of open-cell stochastic materials [27]. The use of a hysteresis loop or preconditioning remains contentious in bone testing [28]. For porous metal specimens, a hysteresis loop is necessary as surface strain measurements have indicated that there is localised plasticity in the specimen at stresses well below the compressive strength of the foam, reducing the slope of the initial loading curve [29]. From the stress–strain plots in Fig. 3 it is clear that stiffness measured from the initial loading curve vs. the hysteresis loop will give different results, however to reduce structural edge effects and since bone is a material that is constantly under cyclic loading it seems appropriate to obtain stiffness from a hysteresis loop.

As bone is responsive to the loading environment, it has been found that the structure and density of trabecular bone will vary with anatomical site [7,26,30]. Regardless of the structure, the relationship between strength, modulus and relative density of bone or any porous structures has been described by models developed by Gibson and Ashby (Eqs. (1) and (2)). Stress/Strength (σ) and modulus (E) increase with density (ρ) by a power law. This relationship has also been found to be true for AM structures [24,25,31,32].

\[
\frac{\sigma}{\sigma_0} = C_1 \left( \frac{\rho}{\rho_0} \right)^n \]  \hspace{1cm} (1)

\[
\frac{E}{E_0} = C_2 \left( \frac{E}{C_2E_0} \right)^{n/m} = C_3 \left( \frac{E}{E_0} \right)^{n/m} \]  \hspace{1cm} (2)

\[
\frac{\sigma}{\sigma_0} = C_1 \left( \frac{E}{C_2E_0} \right)^{n/m} = C_3 \left( \frac{E}{E_0} \right)^{n/m} \]  \hspace{1cm} (3)

where \(\sigma_0, \rho_0 \) and \(E_0\) are the properties of the base material (i.e. solid titanium or the properties of the individual trabeculae) and \(C, m\) and \(n\) are constants found experimentally. If Eq. (2) is substituted into Eq. (1) for relative density, a relationship between strength and stiffness is obtained (Eq. (3)). This is the same relationship as the trend line seen in Fig. 5.

These relationships accurately describe the trends seen in Figs. 4 and 5. It is important to note that the bone relationships are not only animal specific but potentially site-specific and for on-axis loading only. It has been found that there is no single, universal modulus-density relationship across anatomic sites for on-axis loading, as predicted values of modulus at a given apparent density can differ between sites by 49% [30].

The mechanical testing results also highlight one of the key aspects of trabecular bone as a material; and that is its high strength to stiffness ratio. At low relative density and thus low stiffness the strength-to-stiffness ratio of bone was on average 24% ± 2% S.D. difference greater than the porous CP-Ti specimens. Previous work has shown that statically Ti64 has a 45% difference greater strength-to-stiffness than CP-Ti however in high cycle fatigue, CP-Ti was seen to have a 19% difference greater fatigue strength-to-stiffness [25]. Materials such as tantalum or titanium-tantalum alloys may be even better suited for low stiffness porous scaffolds that require high cyclic fatigue strength [25,33,34].

4.2. In vivo performance of stiffness-matched scaffolds

There is difficulty in comparing in vivo studies as different animal models, study lengths and implantation sites and sizes are used; combined with varying mechanical properties of scaffolds there will be different biomechanical environments. However, there are two studies where appropriate comparisons can be made. Malhotra et al. investigated bone ingrowth in an empty defect model in a similar defect location and at a similar defect size, also in an ovine model [35]. The empty defect study had a far higher modulus difference between the original bone and the empty defect when compared to the modulus difference between the original bone and the stiffness-matched porous scaffold in this study. Consequently, they found greater bone ingrowth, BV/TV of 17.7% than this study. However, the bone was given a radiographic score of 1 (no visible bone formation within the defect, radiographic density considerably less than adjacent bone), all the new bone was centred around the periphery of the defect, whilst the centre was filled with fibrous tissue.

In a related study to this work, Reznikov et al. investigated scaffolds of different materials, porous architectures and moduli in an identical ovine model. Their study featured homogenous structures of varying moduli, namely, nylon scaffolds of ~200 MPa

![Fig. 7. CT-Renderings of bone ingrowth into a locally stiffness-matched porous scaffold (not shown).](image-url)
apparent modulus and a titanium scaffold with an apparent modulus of 7.1 GPa. The study reported two mechanisms of osteogenesis; that regardless of scaffold stiffness bone regeneration around the periphery of the bone-implant interface was consistent however bone ingrowth deep into the scaffold interior was inversely correlated to scaffold stiffness with the lower the stiffness of the scaffold the greater the bone ingrowth [5]. The low stiffness (∼200 MPa) scaffolds from the related study were significantly less stiff than the bone they were replacing (Table 1) and consequently lamellar bone was seen both on the periphery and deep within the scaffold.

In this work, the short 6 week study length allowed for a snapshot of bone regeneration to be observed. Embryonic-looking
woven bone in the interior of scaffolds to a more developed and mature structure of woven bone overlaid with lamellar bone at the periiphery of the bone-implant interface were present (Fig. 10). The structure and function of woven bone differs from lamellar bone. Woven bone is a trait of the initial, transient stage of osteogenesis and can be initiated by events such as an injury response or in foetal development. Thus, it normally precedes lamellar bone, in order to provide anchorage and be a suitable substrate for deposition of lamellar bone [36]. Whilst its mechanical properties are inferior to lamellar bone, woven bones provides a biological scaffold whose properties can be adapted by deposition of lamellar bone. “True osteoinduction” is the term used to describe the formation of woven bone within a fracture callus not associated with existing trabeculae. This also accurately describes the networks of woven bone within select scaffold interiors that were dissociated from the struts of the porous scaffold. Conversely, lamellar bone occurs as an adaptive response [37] and is typically deposited in response to bone strain exceeding about 0.1% [38]. This is consistent with the findings of the study, the stiffness-matched scaffold which was designed to maintain homeostasis saw little amounts of lamellar bone formation, whilst for scaffolds significantly less stiff than the bone it was replacing, such as in [5] vast amounts of lamellar bone were present due to the mechanical demands.

A similar pathway of bone regeneration was also found for porous titanium additively manufactured scaffolds implanted in the cortical bone of a critical segmental sized defect mid-tibia in an ovine model [4]. The study recapitulated the postulated “two waves theory” of cortical bone growth in an unimpaired defect healing situation [36,39]. Bone regeneration was found to begin with woven bone, which also showed a less organised and less mineralised structure, whereas the older bone at the interface between adjacent tibia and scaffold had already been augmented and overlaid with lamellar bone. Thus, the initially highly porous primary bone structure served as an endogenous scaffold to direct the formation of lamellar bone with improved mechanical properties. The results of the study also supported the consideration of mechanobiology and found the lower the stiffness of the implant the greater the bone ingrowth [4].

4.3. Considerations

There are a number of improvements that could be made to this study. Firstly, the stiffness-matched scaffold was designed based on an ex vivo cohort and not tailored on a sheep by sheep basis. Variability in bone ingrowth can be explained by a number of factors including the capacity for mechanosensation of the host, age and physical activity of the animal, however in this study the levels of mobility of the sheep were not monitored [13,40]. It has been reported that in sheep there is high variations in load at the knee, and that the inter-subject variation, which was also representative of human subjects, was attributed to individual gait patterns [41]. In this study, the apparent density, modulus and strength results (Fig. 4, 5) for the ovine bone mechanically tested showed a wide spread of data despite the trends for apparent
density vs. modulus and modulus vs. strength having high correlation coefficients. Therefore to better ensure a 0% strain difference between the scaffold and bone and that the local biomechanical demands for each individual subject were taken into account, each sheep could have been CT-scanned prior to surgery and based on those apparent density values a patient-specific scaffold could have been manufactured utilising the apparent density-modulus relationship derived earlier.

A limitation to the bone property quantification portion of this study is the large specimen size required for mechanical testing (Φ5 x 10 mm) relative to the fine CT-Scan resolution (0.5 mm voxels). The specimen size is necessary for accurate mechanical testing, as in order for a cellular structure to behave as a continuum and not be affected by individual trabeculae or edge effects of the structure it must be of sufficient size [28]. However, as trabecular bone varies locally and exists in different structures (rod-like or plate-like trabeculae), the results of the mechanical testing will reflect the least stiff and weakest portion of the bone core and not necessarily the average of the entire bone core. Future work should look at alternative methodologies to obtain bone mechanical properties at a finer resolution and consequently produce scaffolds discretised less coarsely than in this study.

Another limitation was that the bone and the porous scaffold were only stiffness-matched in one direction, for bone that was along the mechanical axis of the femur and for the porous scaffolds it was along the build direction. The isotropy/anisotropy of both the porous scaffolds and the bone in the medial femoral condyle needs to be characterised in order to truly ensure a 0% strain difference in all directions. Finally during surgery, patient-specific drill guides could have been used to better standardise defect locations and sheep weights and activity could be better monitored. Measuring the mechanical properties of the bone surrounding the implant as well as the new ingrown bone during regeneration would provide an even better understanding of the mechanobiology of bone in response to a porous scaffold. The last consideration regarding this study is how much the modulus of the base material of the porous scaffold and the stiffness mismatch between the individual titanium struts and bone trabeculae affects bone ingrowth. It is likely reducing this mismatch would improve osseointegration into the scaffold and on-growth onto the strut elements.

5. Conclusion

This study has demonstrated a workflow to more accurately test the mechanobiology of bone in response to an implanted porous scaffold. This work has also highlighted the importance of knowing the properties of the bone being replaced in order to drive the design of the mechanical properties of a porous scaffold. By characterising the local variation of trabecular bone properties in the medial femoral condyle of skeletally mature ewes a scaffold was created that matched the variations in moduli. The difficulty in material selection for porous scaffolds was also evident as the trabecular bone tested displayed higher strength at a given stiffness compared to commercially pure titanium porous scaffolds. The short term in vivo performance of the locally stiffness-matched scaffold (i.e. to maintain mechanical homeostasis) in a load bearing environment was seen. The average bone ingrowth into the scaffolds pores was 10.73 ± 2.97% after 6 weeks and displayed various stages of bone development from fine embryonic-looking woven bone in the interior of the scaffold to more developed and older bone structure of intense formations of fine woven bone overlaid with lamellar bone at the implant-bone interface. The clinically applicable and pragmatic workflow presented in this study will allow future in vivo testing to test specific local strain/modulus differences between a porous scaffold and bone as opposed to the global differences that have been tested historically and should allow for better translation from in vivo testing to commercial implants.

Data and materials availability

The raw data required to reproduce these findings are available to download from rdm-enquiries@imperial.ac.uk. The processed data required to reproduce these findings are available to download from rdm-enquiries@imperial.ac.uk.

Acknowledgements

The authors wish to gratefully acknowledge the following: The Engineering and Physical Sciences Research Council and Renishaw PLC for their financial and technical support of this study through grant number EP/K027549/1. The Wellcome Trust for their financial support through a Senior Investigator Award (098411/Z/12/Z) and a Value-In-People Award from the Institutional Strategic Support Fund (097816/2/11/B). The National Institute for Health Research (NIHR) UK Imperial BRC for Institutional Support. The Michael Uren Foundation, Royal College of Surgeons of England and Dunhill Medical Trust for financial support. The UK Regenerative Medicine Platform Ácellular/Smart Materials - 3D Architecture (MR/R015651/1). L. Collinson and A. Weston at the Francis Crick Institute for their help with μCT characterisation. The Francis Crick Institute receives its core funding from Cancer Research UK (FC001999), the UK Medical Research Council (FC01999), and the Wellcome Trust (FC01999).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.apmtd.2019.02.017.

References

[1] X. Wang, S. Xu, S. Zhou, W. Xu, M. Leary, P. Choong, M. Qian, M. Brandt, Y.M. Xie, Topological design and additive manufacturing of porous metals for bone scaffolds and orthopaedic implants: A review, Biomaterials 83 (2016) 127–141.
[2] J. Wieder, T. Lindner, P. Bergschmidt, R. Bader, Biomechanical stability of novel mechanically adapted open-porous titanium scaffolds in metatarsal bone defects of sheep, Biomaterials 46 (2015) 35–47.
[3] S. Wu, X. Liu, K.W.K. Yeung, C. Liu, X. Yang, Biomimetic porous scaffolds for bone tissue engineering, Mater. Sci. Eng.: R: Reports 80 (2014) 1–36.
[4] A.-M. Ploboh, S. Checa, H. Razi, A. Petersen, J.C. Weaver, K. Schmidt-Bleek, M. Windolf, A.A. Tatoa, C.P. Roth, K.-D. Schaser, G.N. Duda, P. Schwabe, Mechanobiologically optimized 3D titanium-mesh scaffolds enhance bone regeneration in critical segmental defects in sheep, Sci. Transl. Med. 10 (423) (2018).
[5] N. Reznikov, O.R. Boughton, S. Ghose, A.E. Weston, L. Collinson, G.W. Blunn, J. Jeffers, J.P. Cobb, M.M. Stevens, Individual response variations in scaffold-guided bone regeneration are determined by independent strain- and injury-induced mechanisms, Biomaterials 154 (2019) 183–194.
[6] M. Tancer, U.N. Hansen, A.A. Amir, Prediction of structural failure of tibial bone models under physiological loads: Effect of CT density–modulus relationships, Med. Eng. Phys. 36 (8) (2014) 991–997.
[7] S.A. Goldstein, The mechanical properties of trabecular bone: Dependence on anatomic location and function, J. Biomech. 20 (11–12) (1987) 1055–1061.
[8] K. Alvarez, H. Nakajima, Metallic scaffolds for bone regeneration, Materials 2 (3) (2009) 790.
[9] D.J. Hadjidakis, I.L. Androulakis, Bone remodeling, Ann. N. Y. Acad. Sci. 1092 (11) (2006) 385–396.
[10] N. Reznikov, R. Shaheer, S. Weiner, Bone hierarchical structure in three dimensions, Acta Biomater. 10 (9) (2014) 3815–3826.
[11] O.R. Boughton, S. Ma, S. Zhao, M. Arnold, A. Lewis, U. Hansen, J.P. Cobb, F. Giuliani, R.L. Abel, Measuring bone stiffness using spherical indentation, PLOS ONE 13 (7) (2018) e0200475.
[12] L.F. Bonevald, The amazing osteocyte, J. Bone Miner. Res. 26 (2) (2011) 229–238.
[13] L. Bonevald, Osteocytes, muscle and exercise: role in healthy aging, FASEB J. 31 (2017) 7.2-7.2, (1 supplement).
