Mechanical property and biological behaviour of additive manufactured TiNi functionally graded lattice structure

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Abstract
Bio-inspired porous metallic scaffolds have tremendous potential to be used as artificial bone substitutes. In this work, a radially graded lattice structure (RGLS), which mimics the structures of natural human bones, was designed and processed by laser powder bed fusion of martensitic Ti-rich TiNi powder. The asymmetric tension-compression behaviour, where the compressive strength is significantly higher than the tensile strength, is observed in this Ti-rich TiNi material, which echoes the mechanical behaviour of bones. The morphologies, mechanical properties, deformation behaviour, and biological compatibility of RGLS samples were characterised and compared with those in the uniform lattice structure. Both the uniform and RGLS samples achieve a relative density higher than 99%. The graded porosities and pore sizes in the RGLS range from 40\%–80\% and 330–805 \( \mu m \), respectively, from the centre to the edge. The chemical etching has significantly removed the harmful partially-melted residual powder particles on the lattice struts. The compressive yield strength of RGLS is 71.5 MPa, much higher than that of the uniform sample (46.5 MPa), despite having a similar relative density of about 46\%. The calculated Gibson–Ashby equation and the deformation behaviour simulation by finite element suggest that the dense outer regions with high load-bearing capability could sustain high applied stress, improving the overall strength of RGLS significantly. The cell proliferation study suggests better biological compatibility of the RGLS than the uniform structures. The findings highlight a novel strategy to improve the performance of additively manufactured artificial implants by bio-inspiration.

Keywords: additive manufacturing, bio-inspired, graded lattice, mechanical properties, biological compatibility

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1. Introduction

Bone defect repair is a challenge in orthopaedics, but metallic porous scaffolds offer great potential for the treatment of osseous defects. Numerous studies have been conducted to investigate the performance of porous biomaterials with uniform lattice structures. However, the monotonous structural parameters, including porosity and pore size, could not achieve a balanced combination of sufficient porosity and capability to withstand various mechanical stresses in different regions [1]. To improve the overall performance of artificial scaffolds, the porous biomaterials that mimic the natural bone both in morphology and mechanical properties are critical. The complex architectures and porosity variations in bones (a lower porosity cortical bone in the peripheral and a higher porosity trabecular bone in the centre) indicate the requirement for a bone scaffold with radially graded lattice structures (RGLSs) [2, 3]. Laser powder bed fusion (LPBF) additive manufacturing processes 3D objects by using laser to melt powder particles on powder bed in a layer-wise manner [4, 5], which has significant advantages in the freeform design and manufacture of novel materials [6], composite materials [7], multi-materials [8], and complex structures with high structural resolution and controllable architectures [9, 10]. Hence, applying LPBF for bone scaffold manufacture brings more possibilities for mimicking this complex structure in the bone [11]. The bio-inspired design for porous metallic scaffolds shows tremendous potential in this field, especially when combined with novel biomaterials.

Although lattice structure can reduce the modulus of biomaterials to avoid the stress shielding effect between the artificial implants and human bones, the strength, particularly fatigue strength, in porous metals significantly decreases with the increase in porosity [12]. To improve fatigue resistance and mechanical biocompatibility, the low-modulus metallic material is essential for the artificial implant. As a typical biomaterial, titanium (Ti) and its alloys have been widely applied for tissue engineering, including skeletal repairing owing to its outstanding biocompatibility. However, the pure-Ti (α type) and Ti-6Al-4V (α + β type) have a high Young’s modulus of 105–110 GPa [13, 14], which leads to stress shielding effects in implants due to the much higher modulus than human bones. To reduce the modulus in Ti alloys, the development of β-Ti alloys attracted significant attention among researchers, in which the Ta, Mo, Nb, etc, were used to stabilise the β phase. The metastable β-Ti alloys have a lower elastic modulus of about 55–85 GPa, demonstrating good biomechanical compatibility [13–15].

Unlike the mainstream Ti alloy, this work focuses on the martensitic Ti-rich TiNi alloys, which have a lower modulus (about 50 GPa [16]), showing more promising biomechanical compatibility than the traditional Ti-alloys and reducing the likelihood of stress shielding. Moreover, similar to human bone, the martensitic Ti-rich TiNi alloys have unique asymmetric tensile-compressive behaviour (compressive strength higher than tensile) [17]. In this work, the bio-inspired RGLS, by tuning the localised porosity of lattice structures to mimic bone architecture, was designed and manufactured by LPBF of martensitic TiNi. The morphology, mechanical property, compressive deformation behaviour, and biocompatibility were assessed and characterised to evaluate the potential medical application as an artificial bone substitute.

2. Experimental

2.1. Materials and processing

The pre-alloyed Ti54.8Ni45.2 (at %) powder with a size range of 25–50 µm was used for lattice structure manufacture using a Concept Laser M2 Cusing LPBF system. All the computer-aided design (CAD) models of samples were designed by Unigraphics NX 8.5 software. The laser parameters are laser power 60–75 W, scan speed 400 mm s⁻¹, hatch spacing 75 µm, and layer thickness 30 µm, based on the previous studies [9]. A post-process chemical etching was applied to the as-fabricated (AF) lattices to remove the partially melted particles from the strut surfaces by immersing the lattices in a reagent (HF:HNO₃:H₂O = 1:2:3, vol %).

2.2. Density, morphology and mechanical properties

Archimedes densities of samples were averaged from three repeated tests measured using an OHAUS Adventurer® analytical balance using pure ethanol, as per ASTM B962-08. The morphologies of AF and post-etched samples were observed using a Hitachi TM3000 scanning electron microscope from the top surface. The tensile test on bulk TiNi sample (produced by the hot isostatic press (HIP) as illustrated in [16]) and compression tests on both bulk and lattice samples were conducted at room temperature on an Instron 5969 universal testing machine with 1 mm min⁻¹ load speed. The compression samples are cylinders with a diameter of about 5 mm and a height of about 15 mm. The round tensile specimens with a diameter of about 4 mm and a gauge length of 20 mm were used. The extensometer was used during the tensile and compression testing on bulk TiNi samples. The results for compression and tension tests of bulk TiNi were averaged from two repeated tests, and the results of compressive tests of lattice TiNi were averaged from three repeated tests.

2.3. Finite element (FE) simulations

FE simulations were carried out using ABAQUS to understand the stress distribution and deformation behaviour during compression of the RGLS. A 1/8 symmetric model with a mesh size of 0.2 mm was used, with the input material properties being from the testing properties of the hot isostatic pressed TiNi sample. During the simulation, the bottom surface was clamped, and a connecting plate was modelled on the top to incorporate boundary conditions analogous to the experiment test. A vertical displacement is applied to the plate, which then compresses the lattice structure.

2.4. Cell proliferation study

The lattice samples were sterilized by autoclaving at 120 °C for 20 min, then irrigated three times with phosphate buffered solution (PBS) prior to co-culture with cells. To assess the
effects of martensitic TiNi alloy on cell proliferation and viability, cell counting kit-8 (CCK8) was employed. To obtain the extract of TiNi alloy, the samples were added to a six-well plate with 2 ml fresh cell culture medium for 48 h in 5% CO\text{2} at 37 °C. The extract was gently collected for further use. The human bone marrow mesenchymal stem cells (hBM-MSCs) were seeded in a 96-well plate for 24 h in a standard cell culture atmosphere. Subsequently, each extract of samples was added to 96-well plates plated with hBM-MSCs for another one, three, or five days. At the predetermined time points, 10% CCK8 solution (Beyotime, Shanghai, China) was added to each well and incubated for an additional 2 h. The absorbance at 450 nm was detected using a microplate reader (ELX808; BioTek, Winooski, VT, USA). Each measurement was repeated in triplicate. To validate the effect of structural porosity on the proliferation of different kinds of cells. The hBM-MSCs and Human Embryonic Kidney Cell-293 (HEK-293) cells were seeded on the surfaces of RGLS and uniform lattice structures and then cultivated in fresh dulbecco’s modified eagle’s medium (DMEM), i.e. 10% fetal bovine serum + 100 U ml\text{−1} penicillin + 100 μg ml\text{−1} streptomycin. Fluorescent images of cells on the samples with different porosities and pore sizes were captured by a fluorescence microscope (Leica DMI8-M) after being cultured for three and seven days.

3. Results and discussion

3.1. Bio-inspired design and sample morphology

The skeletal structure unit with a central cube, four struts, and four nodes was created. As shown in figure 1(a), five types of cuboid units (13 mm3) with structural porosities (Ps) (i.e. void volume versus containing volume) of 40%, 50%, 60%, 70%, and 80% were designed via changing the central cubic dimension and strut sizes. Inspired by the porosity distributions in bone (figure 1(b)), an RGLS with graded structural porosity (Ps) and pore sizes were designed by assembling five units following the pattern in figure 1(c). The pore sizes of the RGLS decreased from 805 μm in the central region to 330 μm in the periphery, which followed the guidance that the typical pore size for bone tissues varies between 200 and 900 μm [18]. To investigate the difference in biomechanical properties and biological compatibility, the uniform lattice structures (figure 1(a)) and RGLS (figure 1(c)) were both designed and fabricated by LPBF. The AF samples are shown in figure 1(d).

3.2. Laser parameter optimisation and chemical post-processing

The benchmark of mechanical density for this TiNi material was estimated at 6.223 g cm\text{−3} from the powder HIPped sample, which was measured as 6.219 ± 0.005 g cm\text{−3} with a porosity of 0.07% measured using image analysis. The Archimedes density of the uniform lattices and RGLS produced by 60 W and 75 W is shown in figure 2(a). Despite using the same laser parameters (e.g. 60 W), the 80% Ps sample achieved a density of 6.201 g cm\text{−3}, while the 40% Ps sample reached only 6.092 g cm\text{−3} since thicker struts (470 μm) need higher energy to density. The density of the RGLS sample achieved a density of 6.168 g cm\text{−3}, which was slightly higher than that of the 50% Ps uniform lattice. Slightly increasing the laser power to 75 W improved the density in all types of lattices, and all the samples achieved a relative density higher than 99.1%. Besides, the RGLS achieved a density of 6.186 g cm\text{−3}, almost the same as the 50% Ps sample (6.184 g cm\text{−3}). Figure 2(b) shows the solid volume fraction (i.e. 1−Ps) of the uniform lattices and RGLS produced by 60 and 75 W. The solid volume fraction gradually decreases with an increase in structural porosity. Slightly increasing the laser power to 75 W improved the solid volume fraction in all types of lattices. The solid volume fraction of RGLS almost reaches the same level as the 50% Ps uniform lattices, which are about 46% (i.e. Ps 54%). Considering density and geometry integrity, the 75 W laser power is a better parameter for fabricating RGLS. Notably, the LPBF-processed 50% Ps uniform lattice and RGLS have almost the same Archimedes density level and Ps, which makes the comparison between these two lattice structures in the following sections more reasonable and reliable.

Figure 2(c) shows the surface morphology of the RGLS after chemical etching, which exhibits variable pore size, strut diameter, and Ps from the centre to the edge of the sample, following the designed pattern (figure 1(c)). As spotted in figure 2(d), random partially melted powder particles attached to the lattice struts in AF conditions may disintegrate into the body and become detrimental to surrounding tissues following implantation. Chemical etching could be developed as a simple, low-cost, and scalable method for surface treatment of artificial implants, which can remove the residual powder particles without changing the dimensions of the lattices. Hence, chemical etching was carried out on the graded sample. As compared in figure 2(d), the residual powder particles on the struts of the etched sample were significantly reduced, achieving a much smoother surface. Besides, post-process chemical etching will not cause biotoxicity since the sample will undergo washing, eluting, and terminal disinfection before implementation. Instead, the much smoother surface achieved chemical etching which benefits cell ingrowth since cell proliferation and attachment are more active around smoother surfaces and curved surfaces [20].

3.3. Mechanical property and deformation behaviour

Engineering tensile and comparison stress–strain curves of the powder HIPped TiNi solid samples are shown in figure 3(a). The ultimate compressive strength (UCS) and elongation (El) obtained by compression are about 1.6 GPa and 10.2%, respectively, much higher than the ultimate tensile strength (452 MPa) and El (2%) measured by tensile tests, disclosing a typical asymmetric behaviour in this materials. Notably, the mechanical properties in one material between lattice structure and bulk solid form follow the Gibson–Ashby equations, which suggests that the lattice
Figure 1. (a) Bio-inspired unit cells and 3D models of uniform lattice samples with different structural porosity levels, (b) structure of natural human bone (image adapted from [19]), (c) 3D model radially graded lattice structures (RGLS) with grading porosity pattern, and (d) LPBF-processed uniform and RGLS samples.

Figure 2. (a) Archimedes density of uniform and RGLS samples fabricated by 60 W and 75 W laser power, (b) solid volume fraction of different uniform and RGLS samples produced by different laser power, (c) the surface morphologies of the RGLS after chemical etching, and (d) close-up view of a selected unit pre- and post-etching.

form will not change the tension-compression asymmetry behaviour [21].

Figure 3(b) summarised the compressive properties of different lattice samples. The strength of lattices decreases significantly with the increase of Ps. Besides, the UCS and yield strength (YS) of the RGLS were higher than the 50% Ps uniform lattices despite sharing the equivalent Ps. The mechanical properties of porous materials are governed by the relative density, and the relationship between YS and relative density follows the Gibson–Ashby equation [21]. The Gibson–Ashby equation in this work was calculated as:

$$\sigma/\sigma_0 = 0.67(\rho/\rho_0)^{2.80}$$
where \( \sigma \) and \( \rho \) represent the YS and apparent density of lattice samples; \( \sigma_0 \) and \( \rho_0 \) are the YS and density of fully dense solid material. The \( \rho_0 = 6.223 \text{ g cm}^{-3} \), and \( \sigma_0 = 1770 \text{ MPa} \) is measured by compression of micropillar extracted from the lattice struts, which was elucidated in previous work [16]. This formula revealed the logarithmic relationship between YS and relative density, i.e. the YS increased exponentially by reducing Ps of lattices. Hence, the YS of RGLS is enhanced compared with the 50% Ps uniform lattice since the dense outer regions (Ps 40%) sustained the load and enhanced the overall strength.

FE simulation was conducted by static analysis to understand the deformation behaviour of the uniform and graded lattice samples. To reduce the computation time of the simulation, as shown in figure 3(c), 1/8 of the lattice structure was modelled. The boundary conditions are also indicated in figure 3(c), and the bottom surface is clamped, therefore constraining all active structural degrees of freedom. Symmetry boundary conditions are applied on the lateral surfaces that are internal to the lattice structure. A vertical displacement is applied to the plate, which compresses the lattice structure. As shown in figure 3(d), the lattice structures were meshed using linear tetrahedral elements of type C3D4 in Abaqus CAE. Figures 3(e) and (f) display FE results for the uniform lattices (50% Ps) and RGLS. Under the same deformation, the uniform lattice sample shows even stress distribution; in contrast, the high stress was concentrated in the outer region, while lower stress was mainly distributed in the central region in the RGLS sample. Besides, the maximum principal strain was distributed at the strut conjunctions, which are the weakest part of the structure. This region could exhibit a similar strain level at the beginning of compression. However, with deformation going on, the principal strain concentrated at strut conjunctions in the outer region. The FE simulated deformation behaviour suggests that the dense outer regions (Ps 40%) act as a support component during compression. This is because the YS achieves exponential growth by increasing the relative density as explained by the above Gibson–Ashby equation, and the dense outer regions with high load-bearing capability could sustain high applied stress, therefore, improving the overall strength of RGLS significantly.

3.4. Cell proliferation behaviour

As biosafety assessment is necessary for potential clinical applications, cytotoxicity tests were carried out to evaluate the safety of the TiNi functionally graded lattice. As shown in figure 4(a), the results of the CCK8 assay suggest that the samples of both RGLS and uniform lattice have no impact on hBMMSCs proliferation and the cell viability of hBMMSCs remained above 90%. This is because the TiNi alloy has good biocompatibility (better than stainless steel) [22], and the Ti-rich system can form a TiO2-based oxide film on the surface of TiNi alloy [23], reducing the
Ni-ion release significantly and ensuring Ni-ion toxicity below cytotoxicity [24].

The variations in pore size and surface area have a significant influence on cell proliferation and attachment [25]. The optimal pore structure for bone ingrowth in AM-processed porous metallic implants remains unidentified. Previous studies have indicated that higher porosity and larger pore size are advantageous to achieve good bone ingrowth due to vascularisation and cell size-related factors [26]. Otsuki et al reported that in porous titanium, big pores (500–1500 µm) favoured bone formation more than small pores (250–500 µm) in the rabbit femur [27]. Meanwhile, Taniguchi et al indicated that the porous titanium implant with a pore size of 600 µm is more suitable for orthopaedic implants than with pore sizes of 300 µm and 900 µm [25]. Comparative experiments of cell proliferation between the RGLS and 50% Ps uniform lattice samples were provided in figure 4(b). The HEK293T cells, which are human epithelium kidney cells, showed similar proliferation capability on RGLS and uniform samples on day 3 and day 7. However, the HEK293T cells proliferate faster on the dense outside regions in the RGLS sample, which suggests that the low Ps with large surface areas favour the growth of the soft tissues. In contrast, the growth of hBMMSC (a typical bone integral cell) in the central region of the RGLS sample, which suggests that the low Ps with large surface areas favour the growth of the soft tissues. In contrast, the growth of hBMMSC (a typical bone integral cell) in the central region of the RGLS is better than the 50% Ps uniform sample, as the large space allows sufficient nutrient-waste exchange [25]. Our results show the complex relationship between cells and biomaterials and suggest that the optimum structural porosity for each cell may be different due to different cellular properties. Therefore, the RGLS has synergetic effects on different kinds of cells proliferation (HEK293T and hBMMSC) as the graded pores improved the biocompatibility of bone substitutes for the ingrowth of different kinds of cells.

4. Conclusions

In summary, the bio-inspired martensitic Ti-rich TiNi RGLS was designed and fabricated by LPBF. The main conclusions are:

(a) The processing parameter optimisations for lattice structures need to consider both the density and structure integrity. The residual powder particles on the lattice struts may disintegrate into the body and become detrimental to surrounding tissues, which could be significantly reduced by chemical etching.

(b) The tensile-compression asymmetry in bulk TiNi was observed. The strength of lattices decreases significantly with the increase of Ps, following the Gibson–Ashby equation \( \sigma/\sigma_0 = 0.67(\rho/\rho_0)^{2.8} \). The UCS and YS of the RGLS were higher than the 50% Ps uniform lattice despite sharing the structural porosity. The FE simulations demonstrated that the stress distribution in the RGLS was localised in the dense outer region, which enhanced the overall strength.

(c) Biological cytocompatibility showed that the RGLS has synergetic effects on different kinds of cells proliferation (HEK293T and hBMMSC) as the graded pores improved the biocompatibility of bone substitutes for the ingrowth of different kinds of cells.

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Credit author statement

C T—conceptualisation, fabricated the samples, wrote the manuscript, and performed majority of the experiments. C D—co-write the paper. J L—co-write the paper and conducted the cell proliferation study together with L W and G X. S L—lattice design, P J—chemical etching, and A A and K E—FE simulation. M M A led the programme at the University of Birmingham.

Conflict of interest

The authors declare that they have no competing interests.

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