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Structural, mechanical and in vitro characterization of individually structured Ti–6Al–4V produced by direct laser forming

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Abstract

Direct laser forming (DLF) is a rapid prototyping technique which enables prompt modelling of metal parts with high bulk density on the base of individual three-dimensional data, including computer tomography models of anatomical structures. In our project, we tested DLF-produced material on the basis of the titanium alloy Ti–6Al–4V for its applicability as hard tissue biomaterial. To this end, we investigated mechanical and structural properties of DLF-Ti–6Al–4V. While the tensile and yield strengths of untreated DLF alloy ranged beyond 1000 MPa, a breaking elongation of $6.5 \pm 0.6\%$ was determined for this material. After an additional post-DLF annealing treatment, this parameter was increased two-fold to $13.0 \pm 0.6\%$, while tensile and yield strengths were reduced by approx. 8%. A Young's modulus of 118.000 ± 2.300 MPa was determined for post-DLF annealed Ti–6Al–4V. All data gained from tensile testing of post-DLF annealed Ti–6Al–4 V matched American Society of Testing and Materials (ASTM) specifications for the usage of this alloy as medical material. Rotating bending tests revealed that the fatigue profile of post-DLF annealed Ti–6Al–4 V was comparable to casted/hot isostatic pressed alloy. We characterized the structure of non-finished DLF-Ti–6Al–4V by scanning electron microscopy and observed a surface-associated layer of particles, which was removable by sandblasting as a finishing step. We manufactured porous specimens with nominal pore diameters of 500, 700 and 1000 μm. The diameters were reduced by the used DLF processing by approx. 300 μm. In an in vitro investigation, we cultured human osteoblasts on non-porous and porous blasted DLF-Ti–6Al–4V specimens to study morphology, vitality, proliferation and differentiation of the cells. The cells spreaded and proliferated on DLF-Ti–6Al–4V over a culture time of 14 days. On porous specimens, osteoblasts grew along the rims of the pores and formed circle-shaped structures, as visualized by live/dead staining as well as scanning electron microscopy. Overall, the DLF-Ti–6Al–4V approach proved to be efficient and could be further advanced in the field of hard tissue biomaterials.

Keywords: Titanium alloy; Rapid prototyping; Structure; Mechanical properties; Osteoblast; Cell spreading

1. Introduction

Rapid prototyping (RP), also known as solid freeform fabrication, is a strategy to directly generate physical objects with defined structure and shape on the basis of virtual 3D model data. Among diverse established RP technologies, selective laser sintering (SLS) [1] or selective laser powder remelting/direct laser forming (DLF) [2] offers the advantage to make use of an extended range of basic materials including polymers,

Abbreviations: ASTM, American Society for Testing and Materials; DLF, direct laser forming; HIP, hot isostatic pressing; HOB, human osteoblasts; ILT, Fraunhofer Institute of Laser Technology; PBS, phosphate-buffered saline; RP, Rapid prototyping; SEM, scanning electron microscopy; SLS, Selective Laser Sintering; XTT, sodium 3′-[1-(phenylaminocarbonyl)-3, 4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate

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metals and ceramics. Comparing SLS and DLF techniques, SLS results in objects with sub-optimal bulk density due to partial melting and immediate sintering of the particles of the basic material. During DLF, on the other hand, the basic material particles are completely melted and fused in the laser focus resulting in objects with a density of almost 100%, resulting in a higher mechanical strength.

In principle, the DLF process can be subdivided into the following steps (Fig. 1). The structural information of the given 3D model is processed layer-wise. To this end, the model is split into layers with a defined thickness. In the production unit, the structure of the respective layer is selectively melted into a powder bed of the chosen material by a scanning laser beam. After the short exposure to the laser spot, the molten zones quickly solidify. In the next step, the production platform is lowered one layer thickness, a new powder layer is spread and scanned. The scanning orientation is altered by 90° after each layer. This process is repeated until the complete batch of layers has been transferred, resulting in a solid analog of the original 3D model, which usually only requires minimal surface finishing. Excessive basic material can be saved and reused, which additionally reduces manufacturing costs.

The prospect of instantaneously generating tailored parts via SLS/DLF has aroused much interest in the field of orthopedical and trauma surgery, where prostheses and implants have to be individually shaped in many cases. This is conventionally achieved by machining (e.g. milling, turning), which is time- and material-consuming and does not allow the realization of complex volumetric pore structures. With SLS/DLF technology, on the other side, parts with user-defined complexity can be fabricated almost realtime. The required 3D models can be directly derived from multiplanar 3D imaging of anatomical structures.

While SLS-fabricated implants on the basis of polymers, polymer/ceramic composites and ceramics are investigated since the 1990s [3–6], the realization of metal-based implants by DLF is a more recent approach. At the Fraunhofer Institute of Laser Technology (ILT), Aachen, a DLF strategy has been developed to manufacture hard tissue implants on the basis of titanium and its alloys [7]. In our present investigation, DLF titanium with various structures, porosities and post-DLF treatments is produced and investigated with regard to hard tissue substitution, which comprises structural characterization, mechanical testing as well as in vitro investigation. The aim of the project is to help establish the DLF technique as an alternative to conventional manufacturing in the field of metallic hard tissue biomaterials. In this publication, we present structural and mechanical properties of DLF-fabricated titanium including tensile strength, rotating bending fatigue and Young's modulus. As in vitro model, we used human osteoblast culture to investigate spreading, vitality, proliferation and differentiation of the cells on DLF titanium.

2. Materials and methods

2.1. DLF production

Ti–6Al–4V-powder with a particle size of 25–45 μm was used as basic material. Processing was carried out in an argon atmosphere using a Nd:G laser system. For mechanical testing, round tensile specimens according to DIN 10002-1 with diameters of 4 mm and lenghts of 20 mm (overall length: 54 mm) were fabricated. Additionally, for fatigue property testings, round specimens according to DIN 50113-A with diameters of 4 mm and lenghts of 60 mm were manufactured. For the fabrication of specimens for mechanical testing the DLF process was set to exclusively produce dense parts (>99.5%). For tensile testing, some of the specimens underwent an annealing heat treatment which was carried out at 950 °C for 30 min in order to homogenize the metallic microstructure. The specimens were built with an oversize of 1 mm in diameter. Adjacently finish turning was conducted in order to match standard surface and tolerance requirements. Both types of round specimen were built up in a lying position which means that their longitudinal axis was parallel to the substrate's surface. For the in vitro experiments, discs with a thickness of 2 mm and a diameter of 20.5 mm were manufactured. Porosity was modelled according to a regular offset array of parallel cylindrical pores.

2.2. Mechanical testing

For all mechanical tests, specimens were DLF-processed in oversize and turned to match the appropriate dimensions. Tensile testing was conducted on the basis of DIN 10002-1. The main focus of mechanical testing was to determine basic material properties to match with American Society for Testing and Materials (ASTM) standards required for Ti–6Al–4V implants [8]. Untreated and post-DLF annealed DLF-Ti–6Al–4V was tested and compared to the literature results for wrought annealed Ti–6Al–4V. For each condition, three specimens were tested.

In order to determine fatigue properties of DLF-Ti–6Al–4V under standard geometric and environmental conditions 28 specimens were fabricated in charges of four pieces. All 28 specimens were annealed and adjacently turned into shape. Subsequent fatigue testing was carried out according to DIN/EN 50113 rotating bending fatigue testing standards at 50 Hz testing frequency. In each charge specimens were tested until failure starting with constant stresses from 500 to 700 Mpa. Moving from one specimen to the next, stresses were reduced successively to 350–450 Mpa. Using this procedure, a complete array of stresses and corresponding cycles to failure was covered with up to 1×10^7 cycles.

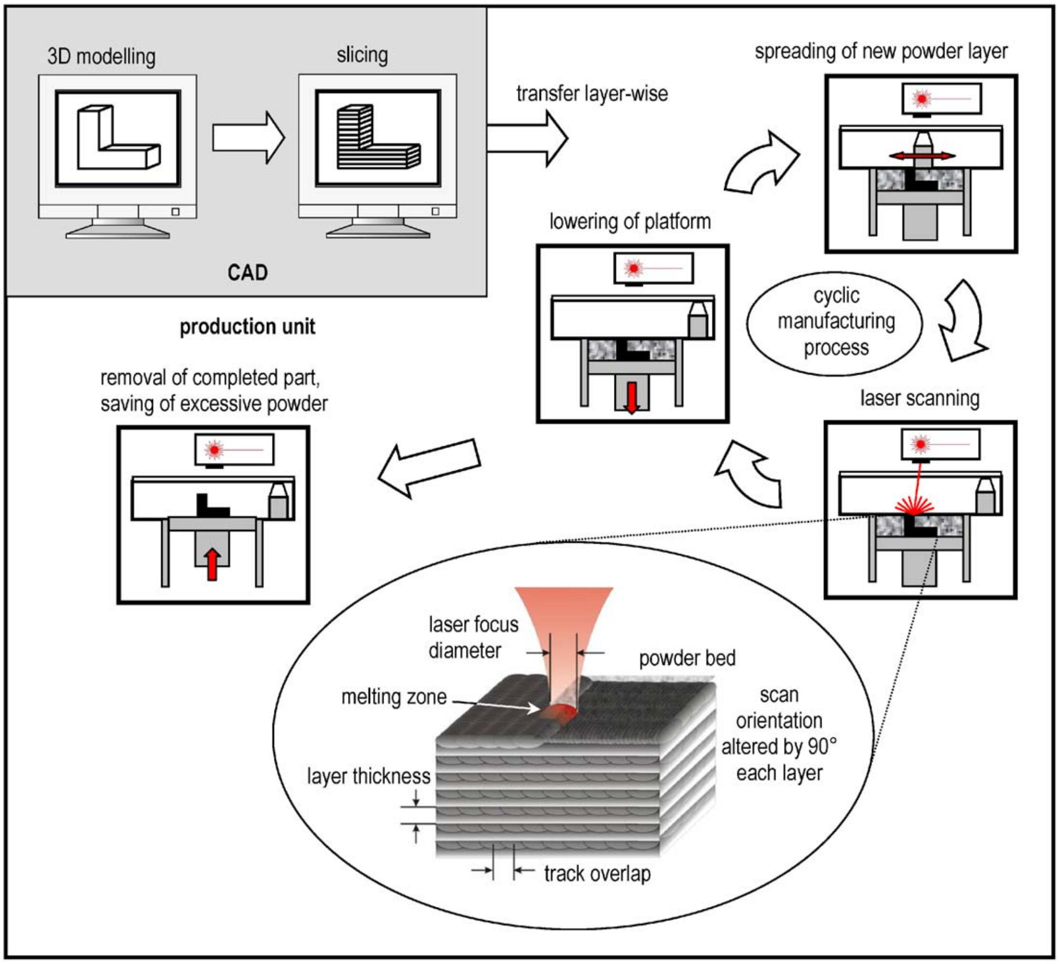

Fig. 1. Rapid prototyping of metal objects by direct laser forming (DLF), scheme.

2.3. *In vitro experiments*

Human primary osteoblasts (HOB) were isolated from cancellous bone which was obtained from femur heads after hip arthroplasty. Informed consent of the patients had been obtained before. Patients with generalized infections or known bone pathologies were excluded. The osteoblasts were cultured according to an established method [9]. All cultures were maintained at 37 °C in a humidified atmosphere supplied with 5% CO_2. Medium change was carried out twice a week. Cells were counted using a Casy counter (Schärfe System GmbH, Reutlingen, Germany). One cell donor was used for each experiment.

All Ti–6Al–4V discs were sonicated in acetone for 10 min to remove fatty residues. The discs were autoclaved, rinsed with sterile PBS/amphotericine B and transferred into 12-well culture dishes. Prior to cell contact, the specimens were equilibrated in culture medium for 10 min. Onto each specimen, 5×10^4 cells were seeded. Standard cell culture dishes were used as control. The cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The medium was replaced twice a week. At 3, 7 and 14 days after seeding, the cultures were assayed as follows. The discs were transferred into new 12-well dishes to separate them from dish-adhered cells. To assess metabolic activity and vitality of the HOB cultures, XTT assays (colorimetric assay based on the oxidation of the tetrazolium derivate XTT by vital cells, Roche, Switzerland) and subsequent vital staining (live–dead staining procedure using fluoresceine/propidium iodide double labelling, both ICN Biomedicals GmbH) combined with fluorescence microscopy were performed. Finally, alkaline phosphatase was determined using an enzymatic photometric assay including the substrate 4-nitrophenyl phosphate (Sigma) according to a described method [10].

In all quantitative determinations, triple estimates were used. All numeric data are given as means and standard deviations.

3. Results

3.1. *DLF manufacturing*

With the established DLF strategy, parts ranging from simple discs to more complex objects like cylinders with regular porosity were produced (Fig. 2a), which took 3 h per single part. For a batch of 40 discs with a

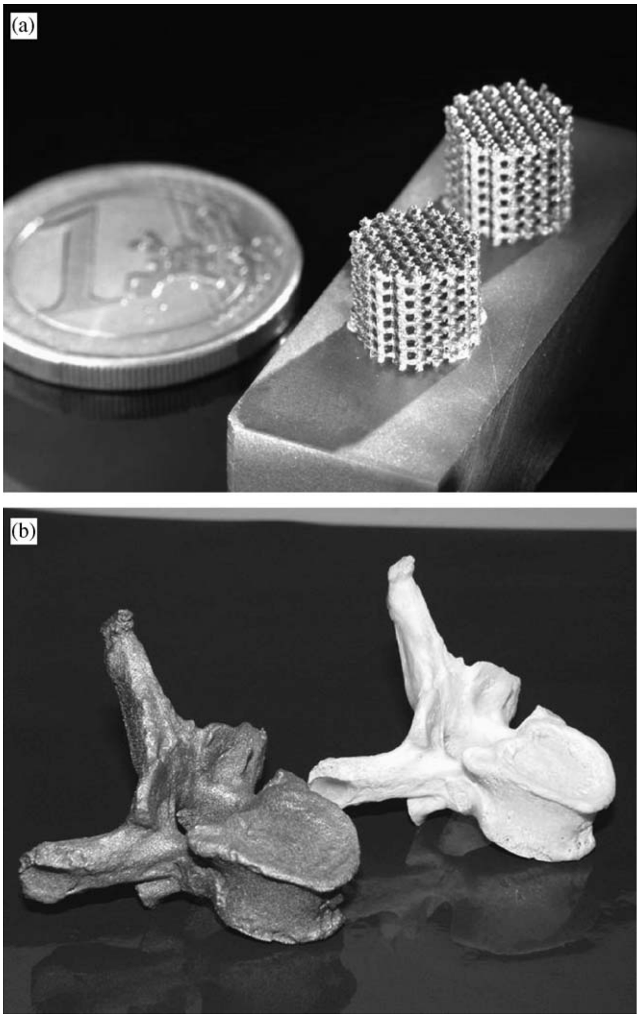

Fig. 2. Examples of DLF-fabricated Ti–6Al–4V parts, cylinders with cubic pore pattern (a), production time approx. 3 h, human vertebra (b) with original (right) and Ti–6Al–4V analog (left), production time approx. 12 h.

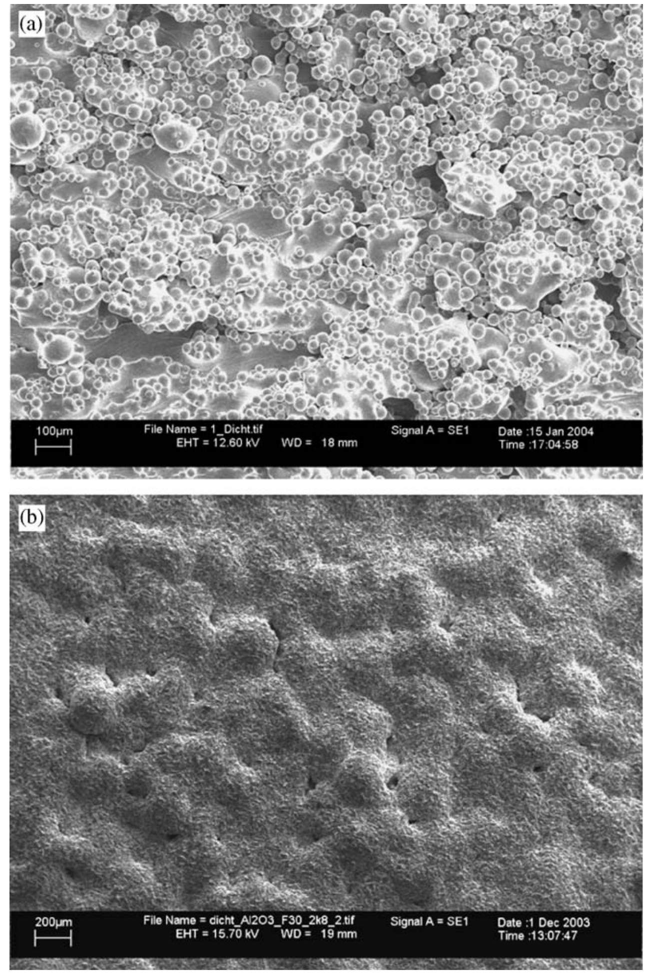

Fig. 3. DLF-fabricated Ti–6Al–4V, SEM imaging of surface before (a) and after sandblasting (b). Note the layer of globular particles on the untreated material, which was completely removed after the treatment.

height of 3 mm and a diameter of 20 mm, the production time was approx. 7 h. To demonstrate the potential of the DLF technology, we scanned the model of a human vertebra and produced a titanium analog via DLF (Fig. 2b), which took approx. 12 h.

Unfinished, the surface of DLF-fabricated objects typically had a granulated appearance, as visualized by scanning electron microscopy (SEM, Fig. 3a). Parts of the surface area were covered with spherical particles which had diameters from 25 to 45 µm. Macroscopically, we noticed a sedimentation of metal powder in incubation vessels in which DLF parts had been stored, which indicated that the observed particles were only *loosely* associated with the surface. As a consequence, further DLF batches underwent corundum and glas pearl blasting in order to remove the particle layer. The surface of blasted DLF material appeared to be almost particle-free (Fig. 3b) and showed a groove-like structuring. Additionally, small pinpoint defects were observed. After storage of such treated material, no visible sedimentation occurred in the respective vessels. We established sandblasting as a standard finishing step.

We produced discs with parallel cylindrical pores in a regular offset pattern and characterized them by SEM to estimate the resolution and accuracy of the DLF process. A SEM image of a disc with a nominal pore diameter of 1000 µm is shown in Fig. 4. The actual diameters of two of the pores were estimated. The circle was added to compare the nominal and actual dimension of one of the pores. In comparison with the nominal diameter, the actual pore diameter was reduced to approx. 700 µm. In other terms, the rims of the pores were thickened in the range of 150 µm. A comparable range of reduction was observed in DLF batches with nominal pore diameters of 500 and 700 µm, which resulted in actual diameters of approx. 200 and 400 µm, respectively (not shown). This result indicated that the DLF process caused a defined overhang during

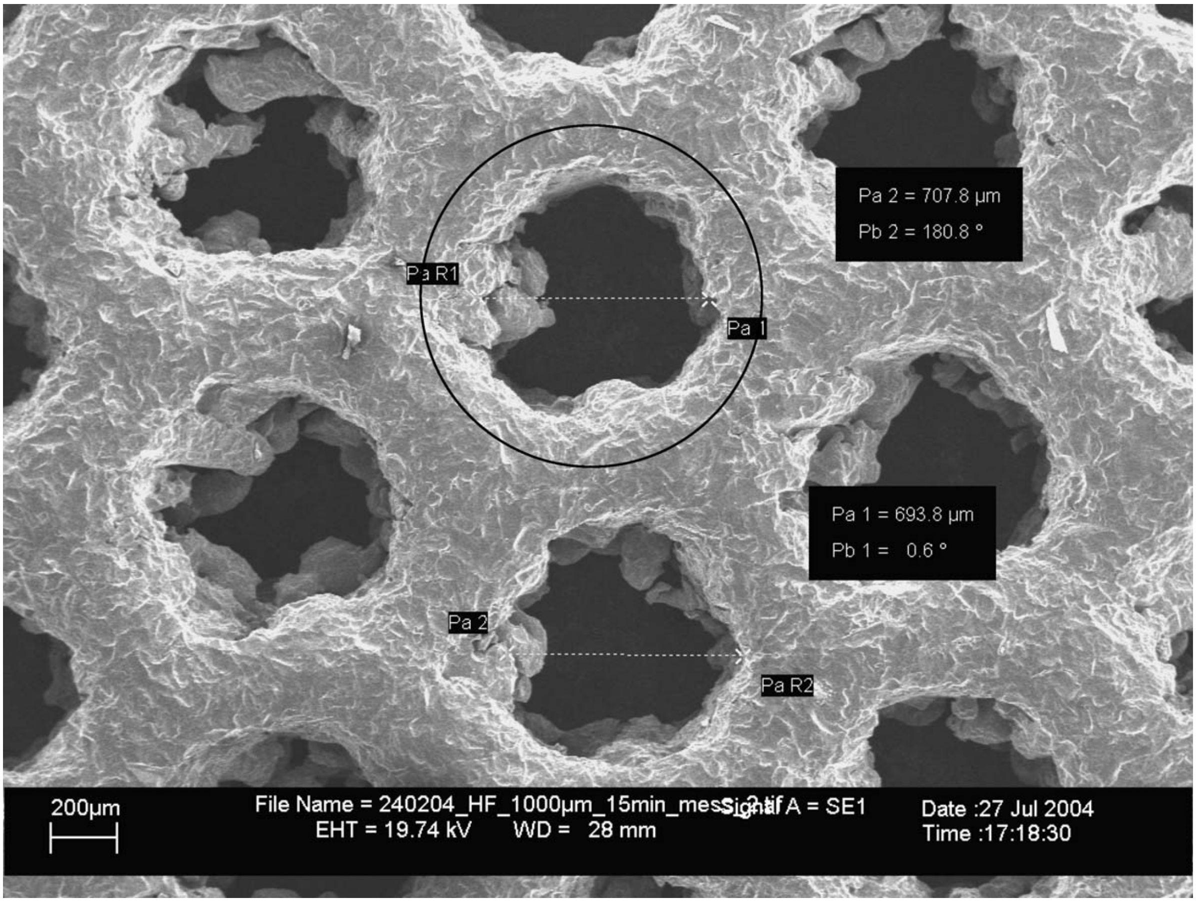

Fig. 4. DLF-fabricated porous Ti–6Al–4V disc with a nominal pore diameter of 1000 μm (indicated as black circle), measurement of actual pore diameters. The nominal pore dimensions were reduced to approx. 700 μm, indicating a defined processing overhang of approx. 150 μm.

sintering. While the pores were continuous and roughly cylindrical, the inner surface of the pores had an irregular appearance with some structures protruding into the pore lumens (dimensions max. 300 μm).

3.2. Mechanical testing

3.2.1. Tensile testing and Young's modulus

We tested tensile and yield strengths of DLF-Ti–6Al–4V with regard to ASTM specifications for the usage of this alloy as a medical material. The results are shown in Fig. 5. Tensile and yield strengths of untreated DLF-Ti–6Al–4V were 1211 ± 31 and 1100 ± 12 MPa, respectively, which clearly surpassed ASTM limits (860 and 795 MPa). With $6.5 \pm 0.6\%$, the breaking elongation of this material was below the respective ASTM limit (10%). Therefore, a set of DLF specimens underwent an additional annealing heat treatment. While the breaking elongation of this annealed DLF-Ti–6Al–4V was increased approximately two-fold to $13.0 \pm 0.6\%$, tensile and yield strengths were reduced by approximately 8% in comparison to untreated material (1042 ± 20 and 960 ± 19 MPa, respectively), but did not fall below the ASTM limits. All tensile testing data derived from annealed DLF-Ti–6Al–4V matched the mentioned ASTM specifications and were in the same range as respective values derived from conventionally wrought annealed titanium alloy, which is shown for comparison. We conclude that annealing of DLF-fabricated Ti–6Al–4V increased ductility, while tensile and yield strengths were moderately impaired by this treatment. Both untreated and annealed DLF material conditions showed a Young's modulus of 118.000 ± 2.300 MPa (standard: 110.000 MPa).

3.2.2. Rotating bending fatigue testing

Post-DLF annealed Ti–6Al–4V underwent rotating bending fatigue testing. The results are shown in a standard Woehler diagram (Fig. 6). The array displayed contains all 28 tested experimental configurations of tensile strain and corresponding cycles to failure. The variation observed especially in the low cycles area ($< 10^7$cycles) ranged up to ± 110 MPa. The high cycle fatigue strength was 375 ± 25 MPa. In comparison to traditional fabrication technologies such as casting, casting/hot isostatic pressing (HIP) and

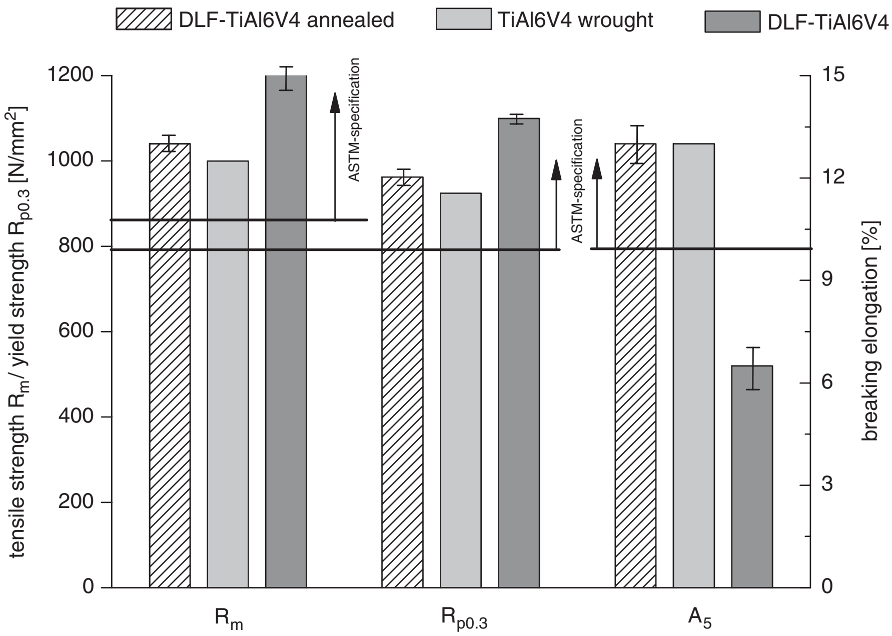

Fig. 5. Tensile and yield strengths and breaking elongation of DLF-Ti–6Al–4V before and after post-DLF annealing treatment in comparison to wrought annealed Ti–6Al–4V. Annealed DLF-Ti–6Al–4V surpassed the recommended ASTM limits of all investigated parameters.

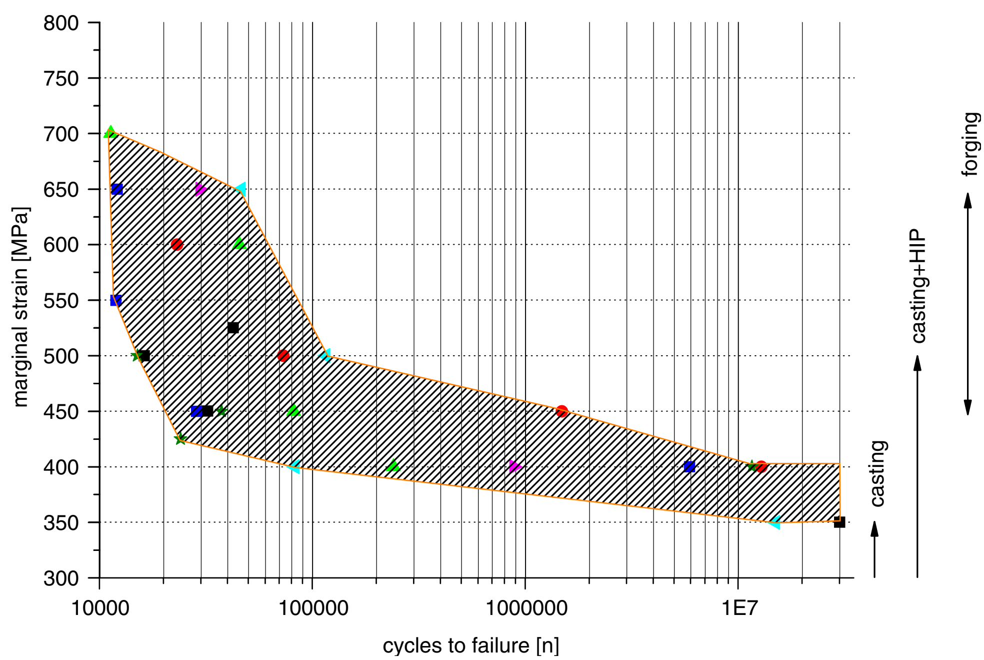

Fig. 6. Rotating bending fatigue testing of annealed DLF-Ti–6Al–4V. Ranges of casted, casted/hot isostatic pressed and forged titanium alloy are shown on the right hand. Fatigue characteristics of the DLF material ranged above casted and below forged Ti–6Al–4V.

forging, rotating bending fatigue of DLF-Ti–6Al–4V was in the same range as casted/HIP-treated alloy.

3.3. In vitro experiments

Human osteoblasts (HOB) were cultured on sand-blasted DLF-fabricated Ti–6Al–4V discs for 3, 7 and 14 days. Standard cell culture plastic was used as control. Triple estimates were used. The HOB cultures underwent XTT assaying to investigate proliferation of the cells. Over 14 days of culturing, the relative XTT activity of HOB grown on DLF material was increased approx. 2.5-fold, which pointed out that the cultures were vital and proliferating. Vitality of the cells was proved by live/dead staining, which showed that vital cells had grown on the DLF material with only few isolated disrupted individuals (Fig. 7a). Spreading of single HOB on the surface was moderately less in comparison with cell culture plastic, which was used as control (Fig. 7b). Alkaline phosphatase was clearly detectable in the HOB cultures by the used enzymatic assay, which indicated osteoblast-specific differentiation of the cultures.

To investigate the influence of porosity, we cultured HOB on porous DLF-Ti–6Al–4V discs with nominal pore diameters of 500, 700 and 1000 μm, respectively. After 14 days, live/dead staining was performed to visualize the structure of HOB cultures. The specimens of the 500 μm group were apparently overgrown with cells (Fig. 7c). The cultures covered most of the pores and hid them from external view. In contrast, the HOB cultures on the specimens of the 700 and 1000 μm group did not completely overgrow the pores, but showed a circular-shaped growth pattern along the pore rims (1000 μm group, Fig. 7d). Some of the pores were completely filled with cells. SEM imaging corroborated the live/dead staining results (Fig. 7e,f). Again, an orientation of the cells along the pore rims and a vortex-like appearance was evident on the specimen with 1000 μm pores. The in vitro experiments demonstrated that DLF-fabricated Ti–6Al–4V allowed structure-oriented growth of human osteoblasts on its surface.

4. Discussion

While the RP method SLS has been described since the last decade [1], establishment of the related DLF is still under way. The performance of DLF processing depends on several parameters, which include dimension of the laser focus, power rating of the laser, scanning speed, average particle size of the used material powder, layer thickness, track overlap (Fig. 1) and process atmosphere conditions. At the ILT, DLF processing of Ti–6Al–4V is being optimized taking into account each of the mentioned parameters. Main focus is on proper material processing to reach homogeneous material deposition with a resulting density as close as possible to 100%, which is a premise for higher mechanical strength. The actually used DLF configuration proved to be effective for the rapid fabrication of individually structured Ti–6Al–4V parts with a resolution of 150 μm. The results gained from static mechanical testing of DLF-Ti–6Al–4V were satisfactory since tensile and yield strengths matched ASTM specifications, with the addition that post-DLF annealing was necessary to surpass the ASTM limit for breaking elongation. While the moderate reduction of tensile and yield strengths after annealing was expected, this did not lead to an exclusion according to ASTM criteria. On the other side, the fatigue test results did not fully comply with expectations. Taking into consideration that the fabrication process is carried out under inert gas atmosphere and thus no chemical contamination should occur, the question arises whether high cycle fatigue strength is comparable with casted parts rather than forged parts. Therefore, the main effort is put in investigations regarding chemical composition and microstructure composition to understand and improve the combination of process guidance and corresponding mechanical properties. Since the specimens used for mechanical testing were turned after DLF manufacturing, no statement can be made on the effect of surface structuring/defects of raw DLF material on the mechanical properties.

According to the microscopical characterization of the presented products, the resolution of the actually used DLF configuration was estimated to be approx. 150 μm, which equals the observed overhang at structure rims. Regarding the prospect of a porous biomaterial for hard tissue replacement, the described resolution allows pore structuring in a triple-digit micron range, which covers the dimensions considered to be effective for bone tissue ingrowth [11,12].

Unfinished, the surface of the DLF products was covered with loosely bound spherical metal particles. These particles were most likely remnants from the used basic material powder and may have sintered partially to the bulk material. With regard to the prospected use as biomaterial, the release of surface-associated particles in vivo would probably cause inflammation due to macrophage activation. Thus, finishing the DLF products by sandblasting was inevitable. While the grooves observed on the treated surface were caused by the sandblasting itself, the pinpoint defects were thought to be small isolated surface-accessible pores, which assumably have a negligible significance concerning the overall surface properties. Taking into consideration that the process time for rather complex parts like the human vertebra was assumably far below the prospected time for conventional fabrication, this additional step was thought to play a minor role concerning the total production time. On the other side, we assume that this finishing method may not be able to remove particles out of complex volumetric pore systems. Therefore, alternative methods have to be considered, which cover the entire accessible surface of the object. We currently compare sandblasting with chemical etching.

The in vitro experiments showed that DLF-fabricated sandblasted Ti–6Al–4V allowed spreading and growth of vital human osteoblasts on its surface, which was comparable to the well-known biological characteristics of conventionally fabricated Ti–6Al–4V [13–15]. We conclude that the DLF manufacturing process did not lead to any disadvantageous alteration of the chemical/biological properties of the material.

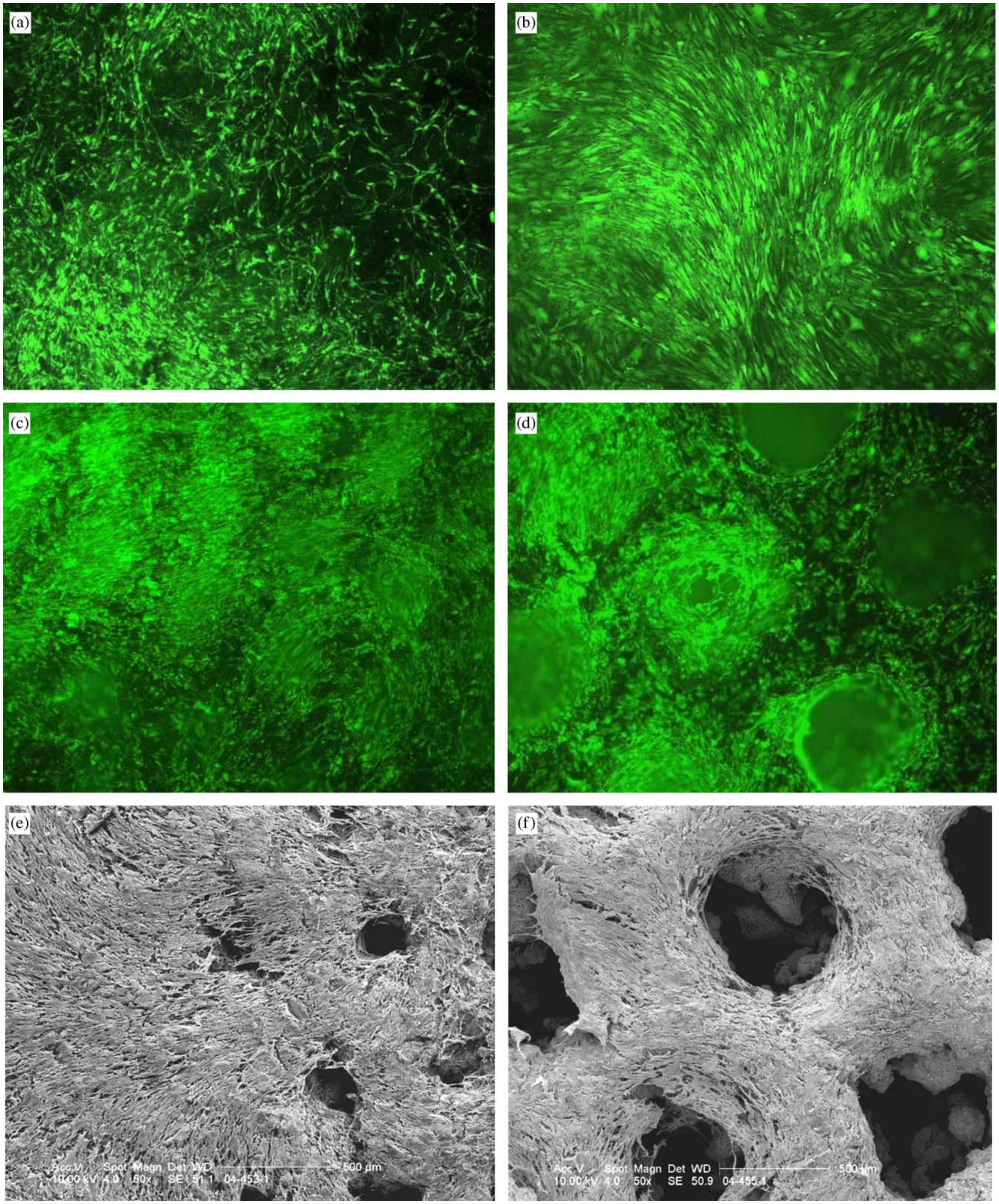

Fig. 7. DLF Ti–6Al–4V in vitro, human osteoblasts after 14 days of culturing, live/dead stain of cells on non-porous material (a) and cell culture plastic as control (b), live/dead stain of culture on porous substrate with a nominal pore diameter of 500 μm (c) and 1000 μm (d), SEM images from the same specimens (e, f). Some of the pores were filled with cells, which had grown along the pore rims in a circular-shaped manner.

As a conclusion, we state that the RP technique DLF proved to be reasonable and effective for the production of taylored Ti–6Al–4V parts, bearing in mind that additional treatment including annealing and surface blasting was necessary in order to meet essential mechanical and surface criteria recommended for biomaterials in the field of hard tissue substitution.

Acknowledgments

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