

**INVESTIGATING THE EFFECTS OF SELECTIVE
LASER MELTING (SLM) PROCESS PARAMETERS
ON THE MICROSTRUCTURE, MECHANICAL
PROPERTIES, AND BIOCOMPATIBILITY OF 316L
STAINLESS STEEL IMPLANTS**

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**by
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ABSTRACT

INVESTIGATING THE EFFECTS OF SELECTIVE LASER MELTING (SLM) PROCESS PARAMETERS ON THE MICROSTRUCTURE, MECHANICAL PROPERTIES, AND BIOCOMPATIBILITY OF 316L STAINLESS STEEL IMPLANTS

Additive manufacturing allows the production of metallic biomaterials that mimic the anisotropic properties of bone. The effects of processing parameters on the material properties and biocompatibility of 316L stainless steel produced by the selective laser melting (SLM) method were investigated. In this thesis, SLM samples produced using three different parameter sets (1B, 2B, and 3B) were compared with 316L stainless steel manufactured by conventional methods. The microstructural properties of the materials were analyzed using optical microscopy, scanning electron microscopy (SEM), and electron backscatter diffraction (EBSD). Additionally, Vickers microhardness measurements, surface wettability, and cytotoxicity properties were thoroughly evaluated. Microstructural analysis revealed significant differences between the conventional and SLM samples. EBSD analysis indicated a high density of twin boundaries in conventional samples, attributed to recrystallization during production. In contrast, the high cooling rates in SLM samples resulted in fine sub-grain structures. Area fraction of grains having $\langle 110 \rangle$ BD orientation were 62.7 for sample 1B, indicating strong anisotropy. Mechanical tests showed that the hardness values of SLM samples were significantly higher than those of conventional 316L steel. The highest hardness value of 240.6 ± 19.3 HV was recorded for Sample 1B. Surface wettability results revealed that SLM produced samples exhibited more hydrophilic surfaces, enhancing their biocompatibility. Cell culture tests confirmed that SLM samples had higher cell viability compared to conventional sample. These findings highlight the potential of SLM to produce both anisotropic structures and biocompatible materials, making it a promising method for biomedical implant applications.

ÖZET

SEÇİCİ LAZER ERGİTME (SLE) ÜRETİM PARAMETRELERİNİN 316L PASLANMAZ ÇELİK İMPLANTLARIN İÇYAPI, MEKANİK ÖZELLİK VE BİYUYUMLULUĞU ÜZERİNDEKİ ETKİLERİNİN ARAŞTIRILMASI

Eklemeli imalat, kemiğin anizotropik özelliklerini taklit eden metalik biyomalzemelerin üretilmesine imkan tanır. Seçici lazer ergitme (SLE) yöntemiyle üretilen 316L paslanmaz çeliğin üretim parametrelerinin malzeme özellikleri ve biyoyumluluk üzerindeki etkileri incelenmiştir. Çalışma kapsamında, üç farklı parametre seti (1B, 2B ve 3B) kullanılarak üretilen SLM numuneleri, geleneksel yöntemle üretilen 316L paslanmaz çelik ile karşılaştırılmıştır. Malzemelerin mikroyapısal özelliklerini ortaya çıkarmak için optik mikroskop, taramalı elektron mikroskopu (SEM), elektron geri saçılım kırınımı (EBSD) yöntemleri kullanılmıştır. Vickers mikrosertlik ölçümleri, yüzey ıslanabilirliği ve sitotoksosite özellikleri detaylı olarak değerlendirilmiştir. Mikroyapı sonuçlarına göre, geleneksel ile SLM numuneleri arasında çok farklı yapılar olduğu sonucuna varılmıştır. SLM numunelerinin kendi içindeki mikroyapılarının değişmesinin sebebi de üretim parametrelerinin değişkenliğidir. EBSD analizlerine göre, geleneksel çelikte yüksek oranda twin boundariler görülmüştür, bunun sebebi üretim esnasında yeniden kristallenme oluşmasıdır. SLM numunelerinde yüksek soğuma oranlarından kaynaklı alt-tane denilen yapılar yüksek oranda bulunmuştur. 1B numunesinde, %62.7 oranında $\langle 110 \rangle // BD$ tekstürü gözlemlenmiş ve bu numunenin anizotropik özellikler sergilediği belirlenmiştir. Mekanik testler, SLM numunelerinin sertlik değerlerinin geleneksel çeliğe kıyasla önemli ölçüde yüksek olduğunu ortaya koymuştur. 1B numunesi, 240.6 ± 19.3 HV ile en yüksek sertlik değerine ulaşmıştır. Ayrıca, yüzey ıslanabilirliği sonuçları, SLM ile üretilen numunelerin daha hidrofilik yüzeylere sahip olduğunu ve bunun biyoyumluluğu artırdığını göstermiştir. Hücre kültürü testlerinde, geleneksele göre SLM numuneleri canlılık açısından daha iyi sonuç vermiştir. Bu da hem anizotropik yapı elde edilip hem de biocompatible bir malzemenin SLM ile üretilebileceğini göstermiştir.

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CHAPTER 1

INTRODUCTION

By 2050, the number of centenarians is expected to rise to 3.7 million, up from 722,000 in 2024, driven by longer life expectancy. Hip fractures, often caused by osteoporosis, affect not only centenarians but also individuals over 50.¹ Therefore, the demand for medical implants, especially orthopedic implants, is estimated to increase significantly in the future due to the aging population, and the rising prevalence of musculoskeletal disorders. Studies show that 20–24% of patients die within the first year after a hip fracture. The causes can be attributed to the inconvenient usage of implants for different patients, as well as implant loosening and wear.²

Biomaterials are categorized into four main groups: metallic, ceramic, polymeric, and composites.³ In 2022, metallic biomaterials dominated the market with a 59% share, owing to their significant advantages over other orthopedic implants.⁴ Therefore, selecting the right implants remains a crucial factor in effective treatment.

Mechanical properties are one of the essential considerations when designing metallic biomaterials. Table 1.1 presents the mechanical properties of commonly used metallic biomaterials and cortical bone. CoCrMo alloys are widely used as a stem material in hip replacement.

The high chromium content in this alloy provides superior corrosion resistance through the formation of a chromium oxide passive layer. However, the significant mismatch between the elastic modulus of CoCrMo alloys (240 GPa) and cortical bone (10–30 GPa) leads to bone weakening caused by the stress shielding effect.⁵ The most widely used titanium alloy is forged Ti-6Al-4V (Ti64) in hip replacement applications due to its improved mechanical properties.⁶ Compared to CoCrMo alloys, Ti64 has a relatively lower elastic modulus of 110 GPa, which is more favorable for reducing the stress shielding effect. Despite this, there are still concerns about the alloying elements in Ti64, particularly the potential toxicity of aluminum and vanadium.^{5,6}

Table 1.1. The mechanical properties of metallic implant materials and cortical bone
(Source: Q. Chen and Thouas 2015)

Material Type	Elastic Modulus (GPa)	Ultimate Tensile Strength (MPa)
CoCrMo Alloys	240	900-1540
Stainless Steel 316L	200	540-1000
Ti Alloys	105-125	900
Cortical Bone	10-30	130-150

Another metallic biomaterial is austenitic 316L stainless steel, which has an elastic modulus in the medium range compared to CoCrMo and Ti alloys. This type of stainless steel can be used in bone fixation applications such as bone plates, screws, and wires as shown in Figure 1.1. ⁷⁻⁹ Stainless steel 316L, nonetheless, is highly susceptible to localized corrosion, such as pitting and crevice corrosion. However, 316L is the most widely used metallic biomaterial due to its low cost, machinability, and accepted biocompatibility.

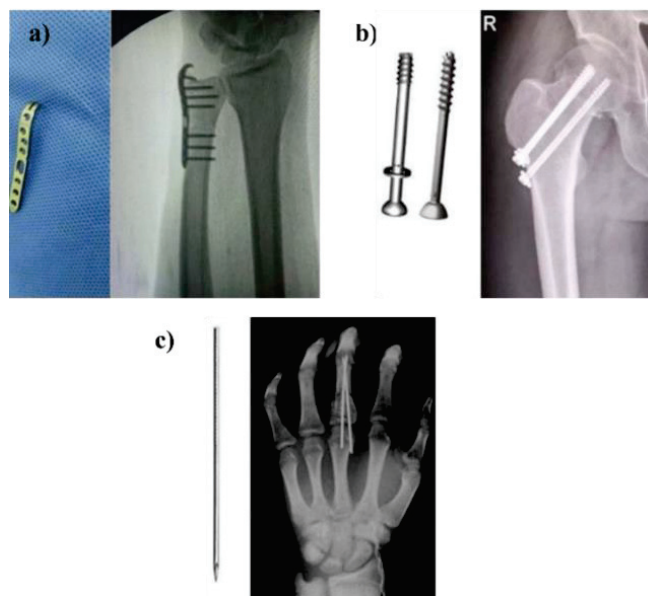


Figure 1.1. a) Bone plate, b) Screw, and c) Wire

(Source: J. Li et al. 2020; Navarro et al. 2008; Niinomi, Nakai, and Hieda 2012)

Another important criterion when selecting metallic biomaterials is osseointegration, which can be defined as the formation of new bone in orthopedic applications.⁵ Considering all of these, stainless steel 316L has been chosen to improve biocompatibility. Normally, it has isotropic mechanical, and microstructural properties when manufactured by traditional methods such as casting, and forging. Isotropic properties are independent of direction. In addition to these conventional methods, advanced manufacturing techniques like additive manufacturing have emerged.

Additive manufacturing (AM) methods enable the production of materials with anisotropic properties.¹⁰ Anisotropic properties depend on the crystallographic direction, they have different values for each direction.¹¹ As illustrated in Figure 1.3, anisotropic structures exhibit alignment, while isotropic crystal structures are uniformly distributed. Consequently, anisotropic structures demonstrate higher mechanical properties along the alignment direction.¹² Anisotropic properties are a crucial factor due to their relevance to bone characteristics. As shown in Figure 1.2, human bone exhibits anisotropic properties due to the alignment of collagen and apatite molecules along the longitudinal axis of the bone.¹³

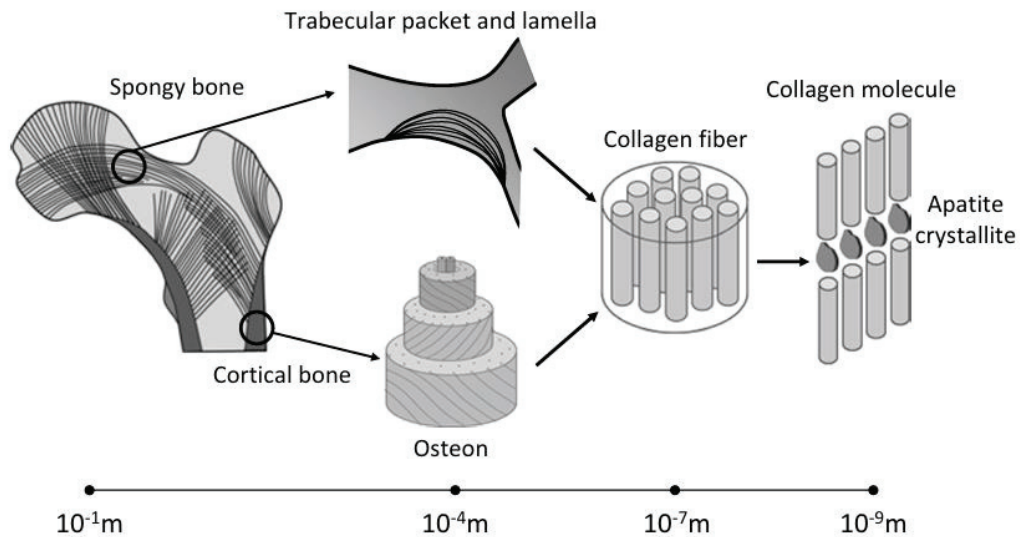


Figure 1.2. Schematic representation of bone structure from macro-to-nano scale
(Source: Nakano, 2015)

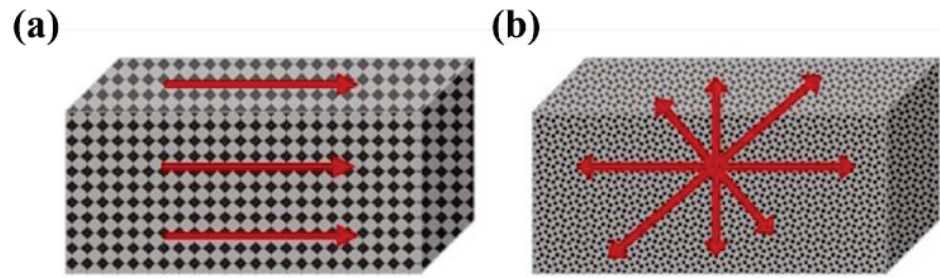


Figure 1.3. (a) anisotropic layered structure (b) isotropic uniform structure

(Source: Iyer et al. 2020)

There are different types of additive manufacturing methods, but, selective laser melting (SLM) is the most frequently used type AM type.¹⁴ Therefore, the motivation of this thesis is to manufacture metallic biomaterials with a texture orientation (anisotropic properties) and to examine how this texture influences the material properties and biocompatibility. Three different process parameter sets were selected from the literature to achieve anisotropic properties in 316L stainless steel. Microstructural features were analyzed using optical microscopy, scanning electron microscopy (SEM), and electron backscatter diffraction (EBSD). Hardness tests were conducted to assess localized plastic deformation behavior. Contact angle measurements were performed to determine whether the material was hydrophilic or hydrophobic. In vitro cell viability tests were carried out to evaluate the biocompatibility of the metals.

CHAPTER 2

LITERATURE REVIEW

2.1. Crystal Structure of Stainless Steel 316L

Metals have three types of crystal structure: body-centered cubic (BCC), face-centered cubic (FCC), and hexagonal close-packed. There are five types of stainless steel. These are ferritic, austenitic, martensitic, duplex, and precipitation-hardening (PH) steel. According to the American Iron and Steel Institute (AISI) numbering system 300-series chromium-nickel iron-based alloys are austenitic type stainless steel.¹⁵ Therefore, AISI 316L is an austenitic phase stainless steel. L refers to low carbon content (max. 0.03 wt %). Austenitic stainless steel 316L has an FCC type crystal structure. There are six atoms in each face, and eight atoms for each corner, however, atoms are in one-half for each face, and one-eighth for corners as can be seen in the Figure 2.1. Red and blue circles represent the face and corner atoms, respectively.¹¹

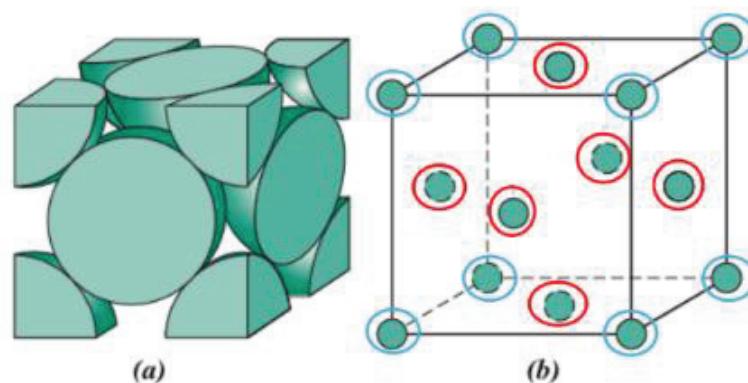


Figure 2.1. FCC crystal structure (a) original sphere illustration of unit cell (b) reduced sphere unit cell

(Source: Callister and Rethwisch 2018)

2.2. Microstructure of Conventional Austenitic Stainless Steel 316L

Austenitic stainless steels are the most widely used steel alloys, known for their good corrosion resistance and versatility in various environments. As aforementioned, austenitic 316L has an FCC crystal structure, achieved by adding nickel (austenite stabilizer) and manganese, which makes them tough, formable, and weldable. Nickel improves resistance to mild corrosives, while molybdenum enhances protection in oxidizing environments by strengthening the passive surface film. Stainless steel 316L type is a low carbon version of 316. The AISI standard stainless steel 316L compositional information can be found in Table 2.1.¹⁶

Table 2.1. Chemical Composition of the conventional stainless steel 316L

(Source: Schweitzer 2003)

Element	Cr	Ni	Mo	Mn	Si	C	P	S	Fe
Wt %	16-18	10-14	2-3	2	1	0.03	0.045	0.03	Bal.

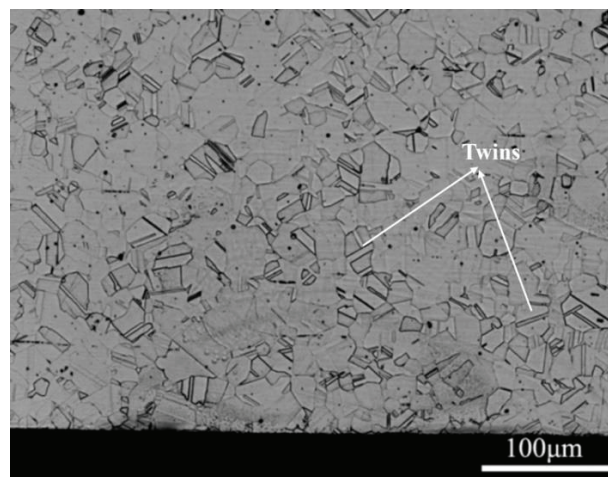


Figure 2.2. SEM image of conventional AISI 316L stainless steel

(Source: (Y. Wang et al. 2018))

Microstructures are changing based on manufacturing processes like casting, forging, and rolling. Heat treatment methods are generally applied to enhance mechanical properties. In Figure 2.2, a scanning electron microscopy (SEM) image of the annealed stainless steel 316L shows the microstructure. It is labeled with twin boundaries which are commonly observed in austenitic steel, especially annealing twins.¹⁷ Annealing twins are planar type defects that form during the recrystallization process.

Deformations, like twins and dislocations, are formed in specific planes and directions in crystal structure. The slip plane is the crystallographic plane that has the highest atomic density, the slip direction is the densest crystallographic direction within the slip plane. The slip direction is related to the atomic movement. A slip system is a combination of slip plane-slip direction.

For FCC structures, the primary slip plane is $\{111\}$ (close packed planes), slip direction is $\langle 1\bar{1}0 \rangle$ (closed pack directions). In the unit cell of the FCC structure, atoms are packed highest in 111 planes as illustrated in Figure 2.1. There are four slip planes: (111) and negatives of this, $(\bar{1}11)$, $(1\bar{1}1)$, and $(11\bar{1})$. In addition, there are six slip directions as illustrated in Figure 2.4. However, three of them lie along the same lines but in opposite directions. Therefore, only three distinct slip directions are considered.

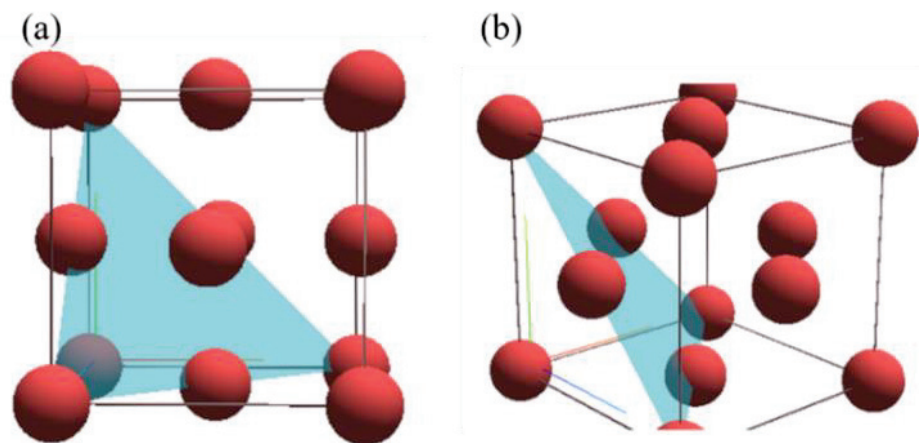


Figure 2.3. FCC structure (a) (111) slip plane (b) closer look of this plane
(Source: Callister and Rethwisch 2018)

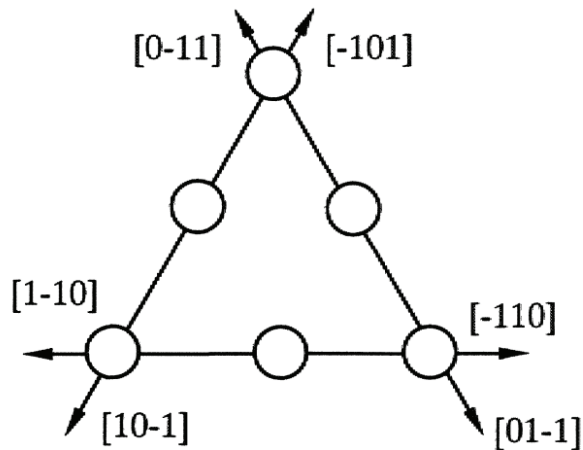


Figure 2.4. FCC structure slip directions
(Source: Jackson 1991)

If the material exhibits a stronger $\langle 110 \rangle$ crystallographic texture, the dislocation movement becomes easier because $\langle 110 \rangle$ directions align with the primary slip directions in FCC materials. As a result, the material's ductility can be enhanced due to the increased activation of slip systems, allowing for greater plastic deformation before failure.^{11,18}

2.3. Microstructure of Additively Manufactured Austenitic Stainless Steel 316L

Additively manufactured 316L is fully austenitic stainless steel types. Therefore, the sample produced with this production method also has an FCC structure. The additive manufacturing process has a high cooling rate with complicated solidification theory. Solidification history based on the thermal gradient (G), growth rate (R), and undercooling (ΔT) in laser powder bed fusion (LPBF).

Constitutional supercooling theory analyzes the relation between G , and R . G/R prescribes the solidification mode, whereas $G \times R$ determines the cooling rate.¹⁹ Planar

solidification occurs at very high G/R ratios, while moderate G/R ratios produce cellular structures, and low G/R ratios result in columnar or equiaxed dendritic structures [51].

The LPBF process, with its exceptionally high cooling rates of 10^3 to 10^7 K/s, typically results in high G/R values, favoring the formation of cellular structures in most LPBF alloys, as shown in Figure 2.5.^{20,21}

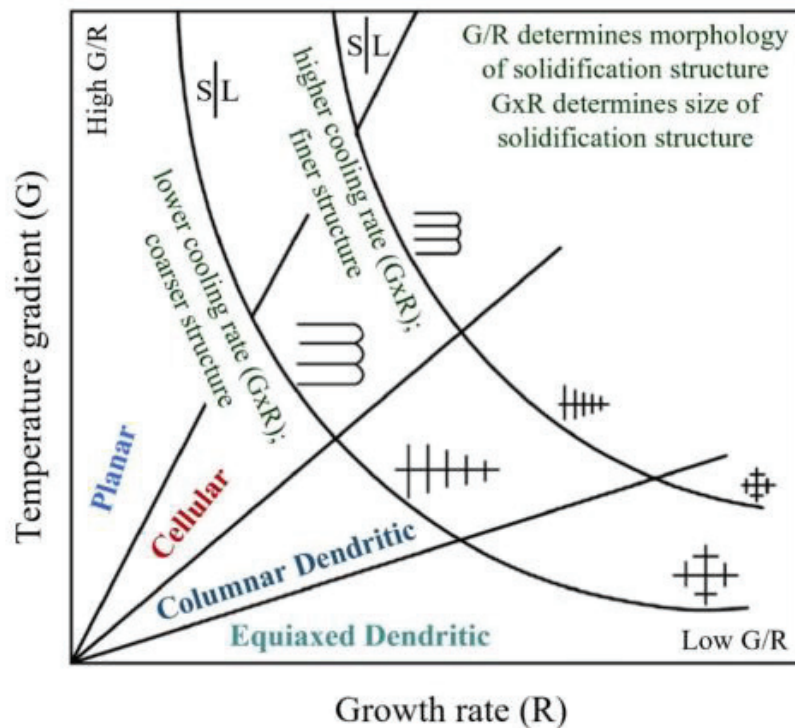


Figure 2.5. The effect of the thermal gradient (G) and growth rate (R) on microstructure
(Source: Dogu et al. 2022)

Cellular structures (honeycomb-like) occur by epitaxial growth due to the nature of the AM process, since it solidifies layer-by-layer. Cellular structures form based on the closest heat flow direction. Normally, the thermal gradient is parallel to the building direction (BD), but it can change based on the processing parameters. Therefore, as illustrated in Figure 2.6a cellular structure grows along the building direction.

According to the solidification theory, $\langle 001 \rangle$ crystallographic direction is the preferred growth direction of cellular structures.²¹ Appearance can vary depending on the

perspective. This is more clearly seen in the image below. Due to changes in the heat direction, cellular structures form at different angles, and their honeycomb shapes exhibit varying aspect ratios (AR). As a result, they appear with different widths (w).²²

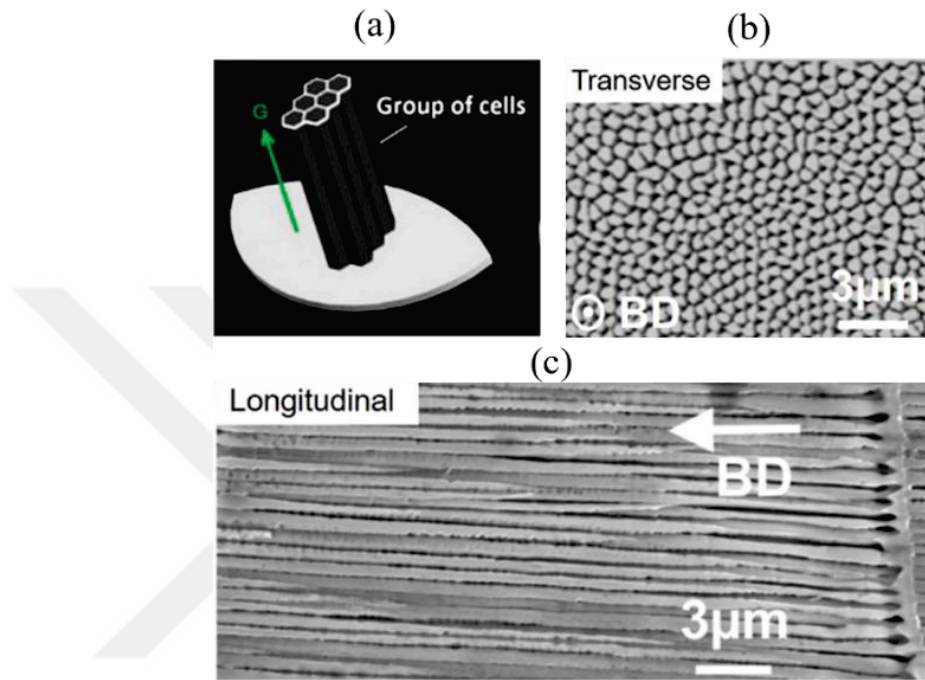


Figure 2.6. (a) Illustration of cellular structures, section view of (b) perpendicular to building direction (c) along building direction
(Source: Z. W. Chen, Phan, and Darvish 2017)

The optical image in Figure 2.8a describes a dense structure with well-overlapped melt pools, characterized by a semi-circular shape in a plane parallel to the build direction. The overlapping melt pools indicate a successful fusion of powder particles and strong interlayer bonding. The SEM image in Figure 2.8b reveals columnar grains that cross the fusion line and continue to grow beyond it. Notably, the grain growth is unaffected by the fusion line and is oriented perpendicular to the curvature of the fusion lines, as shown in Figure 2.8b. The open black arrow shows the fusion line, while the black arrow shows the growth direction along the building direction (parallel plane to the BD). The white arrow shows the columnar structures.²³

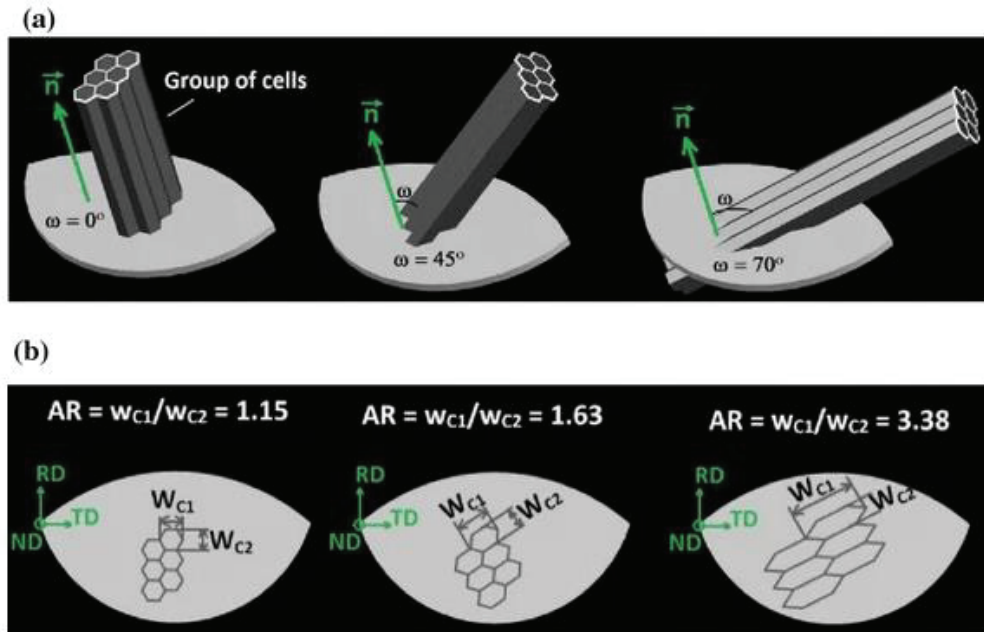


Figure 2.7. 3D illustration of tubular structures in melt pools (a) with different elongated angles (b) section views of normal to the tubular structures
(Source: Z. W. Chen, Phan, and Darvish 2017)

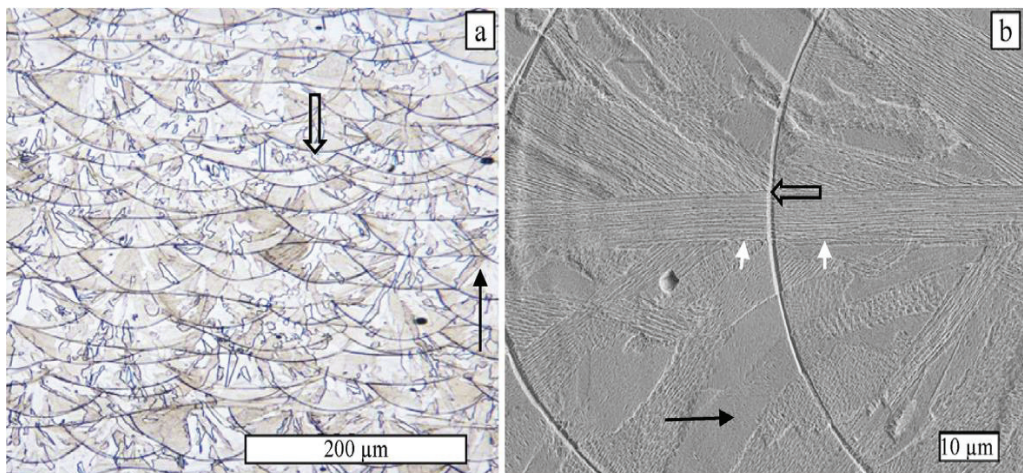


Figure 2.8. a) Optical microscopy image, and b) SEM image: of melt pool morphology along the building direction
(Source: Tucho et al. 2018)

Figure 2.9 shows the optical microscopy images. The left image displays the melt pools from the side view (parallel to the building direction), while the right image shows the view normal to the building direction. In the top view, melt pools are not visible; only laser tracks can be clearly observed. (Qu et al., 2022)

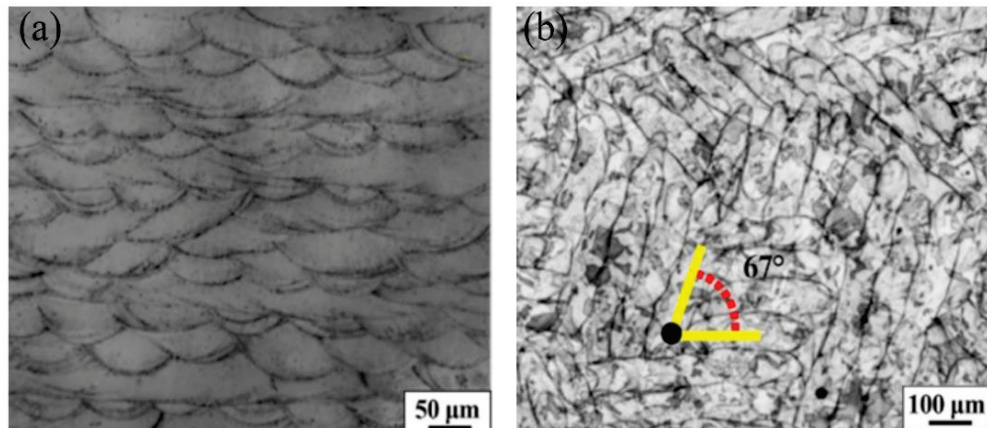


Figure 2.9. Optical microscopy (OM) image showing the (a) melt pools in the side view, (b) laser tracks in the top view

(Source: Qu et al., 2022)

2.4. Additive Manufacturing Methods

Additive manufacturing is an advanced manufacturing process technology.²⁴ The American Society for Testing and Materials (ASTM) group “ASTM F42 – Additive Manufacturing” established a set of standards to classify additive manufacturing (AM) processes into seven categories. These categories include Vat photo polymerization (VPP), material extrusion (ME), material jetting (MJ), binder jetting (BJ), powder bed fusion (PBF), direct energy deposition (DED), and sheet lamination.^{25,26}

Seven different categories can be found in Figure 2.10 and their sub-categories in the right side.²⁷

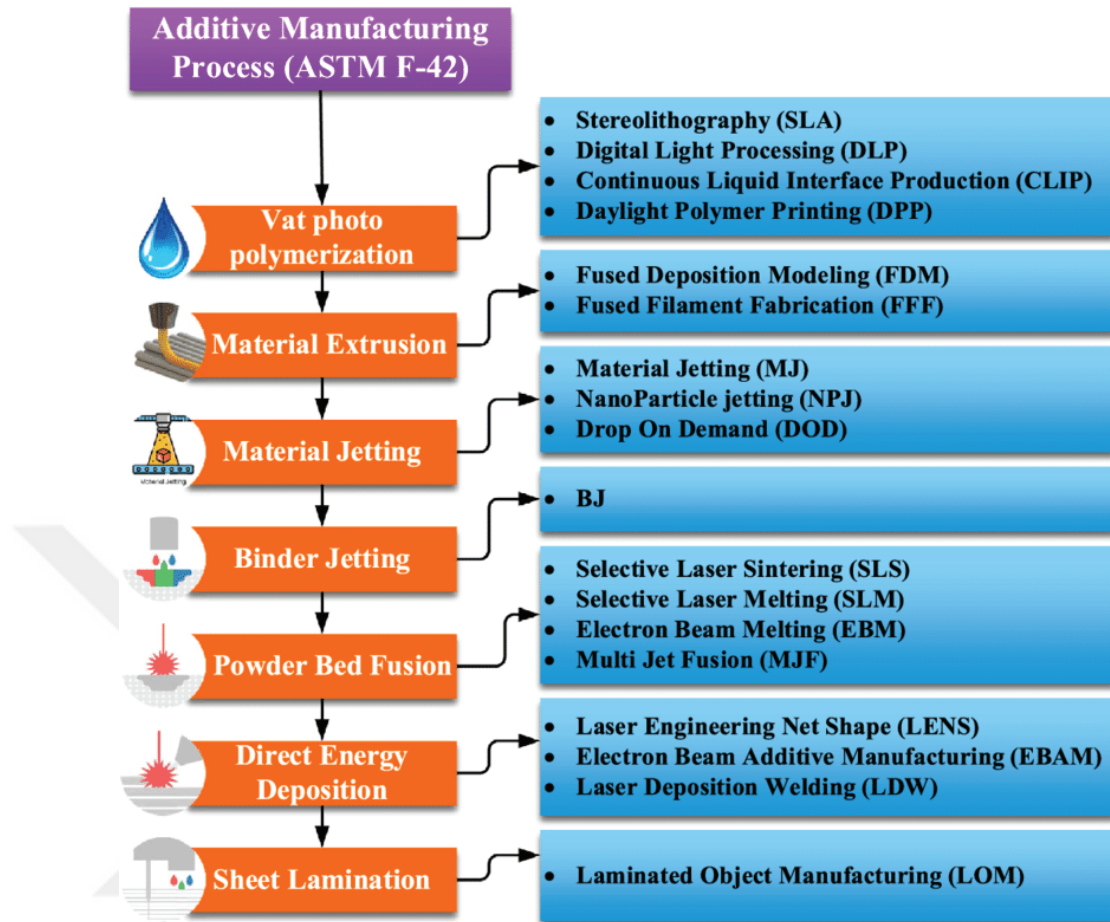
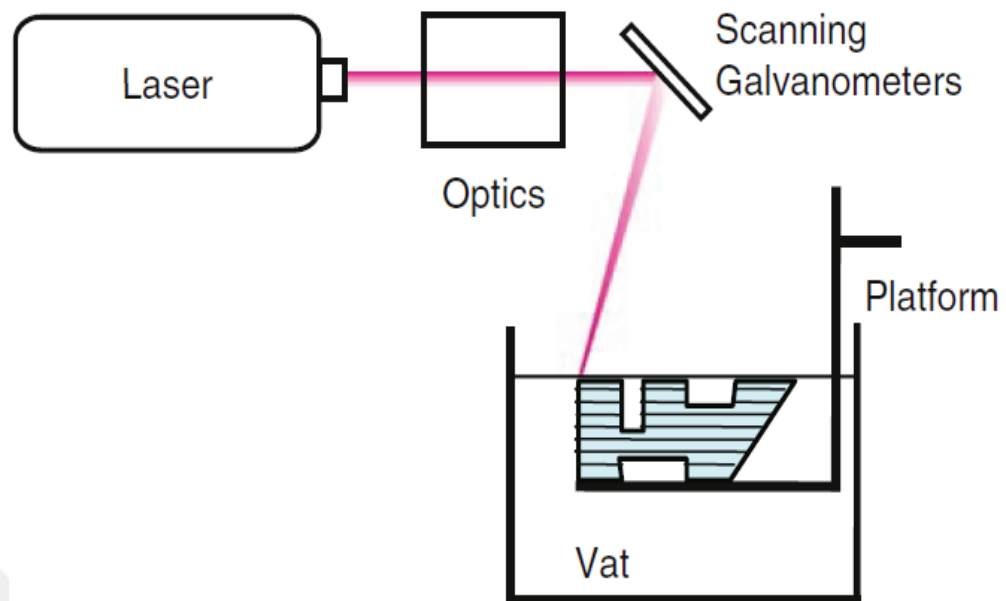


Figure 2.10. Seven categories of additive manufacturing based on the ASTM F-42
(Source: Sefene 2022)

2.4.1. Vat Photopolymerization

Also referred to as stereolithography (SLA) (Revilla-León & Özcan, 2019), this technique utilizes liquid photopolymer resin. The resin reacts upon exposure to ultraviolet (UV) light wavelengths and, in some cases, visible light. Figure 2.11 illustrates a basic commercial stereolithography machine with vector scanning capabilities. Resin materials compatible with this technique include epoxy acrylates (EA), polyester acrylates, and polylactic acid (PLA). There is a limitation about the support material.²⁵



schematic of vector scan SL

Figure 2.11. Commercial vector scan SL machine

(Source: Gibson, Rosen, and Stucker 2010)

2.4.2. Material Extrusion

Fused Deposition Modeling (FDM), developed by Stratasys, is the most widely used extrusion-based additive manufacturing technology. It works by melting a thermoplastic polymer (such as acrylonitrile butadiene styrene (ABS)) filament in a heated chamber. The material is fed through a nozzle, heated, and deposited layer by layer. The nozzle moves horizontally, while the platform adjusts vertically after each layer is added, as can be seen in Figure 2.12.²⁶

This technique comes with drawbacks such as slower build speeds, limited accuracy, and lower material density. Achieving finer layer thicknesses, such as 0.078 mm, requires expensive equipment and results in extended production times.²⁵

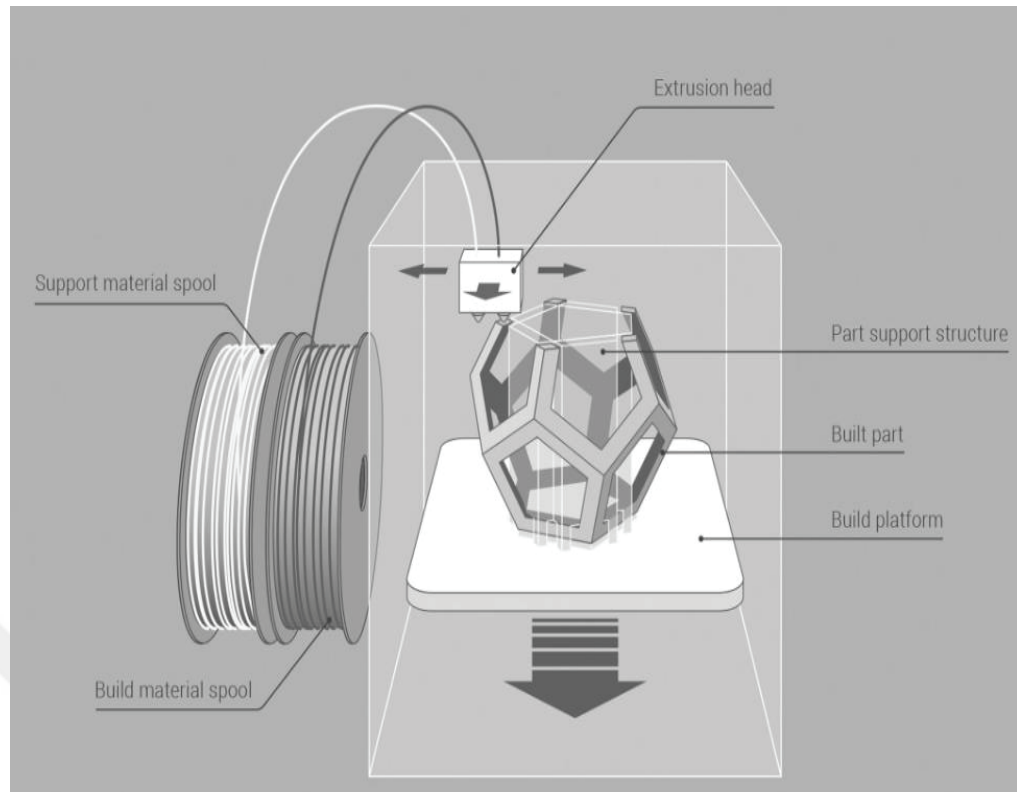


Figure 2.12. FDM additive manufacturing machine
(Source: Revilla-León and Özcan 2019)

2.4.3. Material Jetting

Material Jetting (MJ) is an additive manufacturing technique that builds parts by selectively depositing droplets of build material, which are then solidified, typically using ultraviolet (UV) light, schematically shown in Figure 2.13. This process enables the creation of complex geometries with high dimensional accuracy and smooth surface finishes, both thermosets and thermoplastics can be used.

MJ is particularly advantageous for producing multi-material and full-color components, as it allows for the simultaneous deposition of different materials. However, limitations such as material properties and production costs should be considered when selecting MJ for specific applications.²⁸

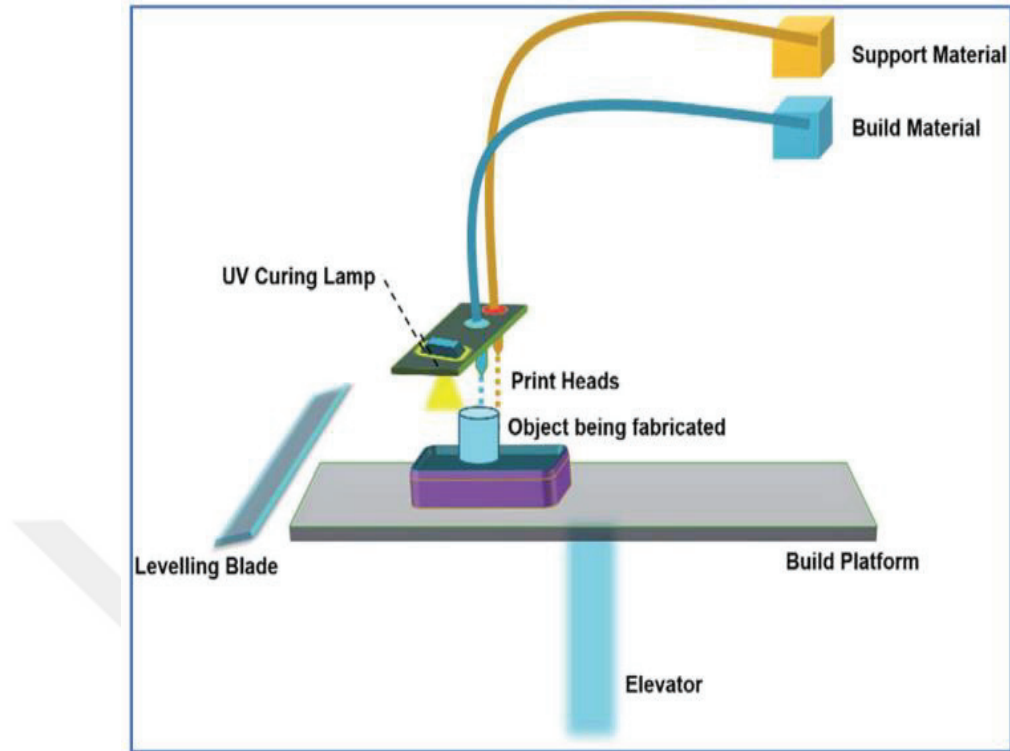


Figure 2.13. Illustration of the MJ technique
(Source: Gülcan, Günaydın, and Tamer 2021)

2.4.4. Binder Jetting

Binder jetting forms parts by gluing powder particles layer by layer using a binder, eliminating the need for heat during fabrication. Polyvenyls, polyacrylic acid (PAA), and polysiloxanes are commonly used liquid binder materials. A binder is jetted onto a powder bed to create each layer, with the build platform lowering after every pass, schematically shown in Figure 2.14.²⁹

While fast, the printed parts are fragile and require post-processing to enhance mechanical properties.³⁰ The use of metal binder jetting (BJ) technology is still challenging, primarily because of the hazardous properties and the complexity of removing the commonly utilized phenolic solvent-based binder.³¹

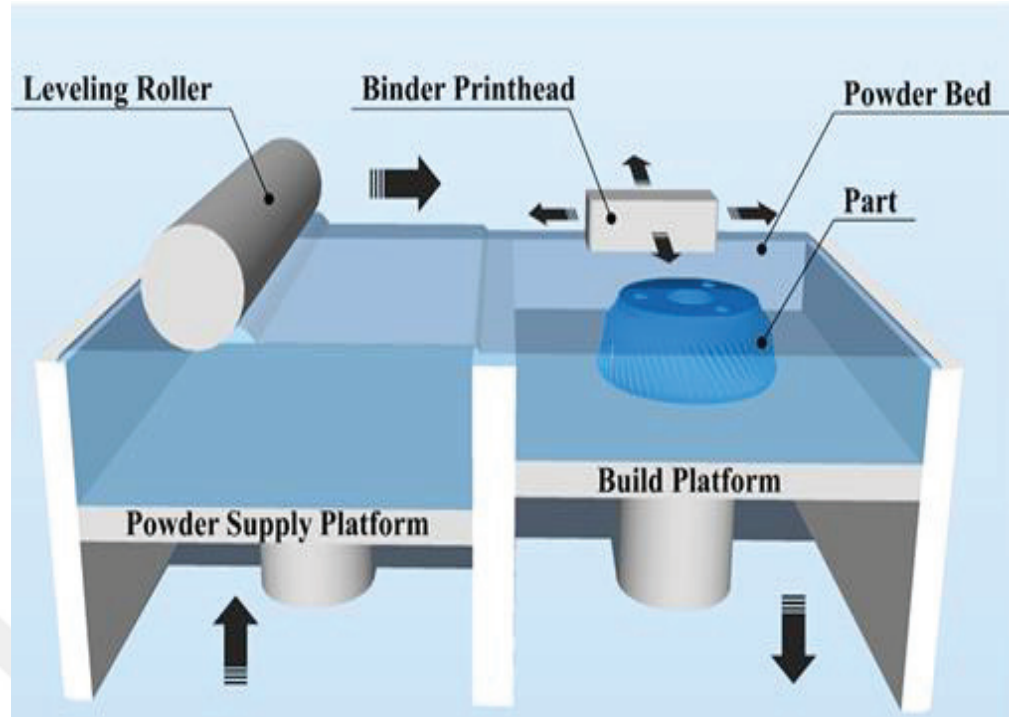


Figure 2.14. Binder jetting process
(Source: M. Li et al. 2020)

2.4.5. Powder Bed Fusion

Laser-Powder Bed Fusion (L-PBF) is a widely known technique in additive manufacturing processes. The first commercialized type of this process is Selective Laser Sintering (SLS), while other variations include Selective Laser Melting (SLM) and Electron Beam Melting (EBM).

These methods enable the production of nearly fully dense materials. SLM utilizes a laser, and EBM employs an electron beam to melt and fuse powdered materials. While SLM offers higher dimensional accuracy and lower surface roughness, EBM often requires post-processing to achieve precise dimensions due to the limitations of electron beam focus. PBF processes can be used to manufacture metals, ceramics, polymers, and composites.^{25,32} Figure 2.15 illustrates the selective laser melting process.³³

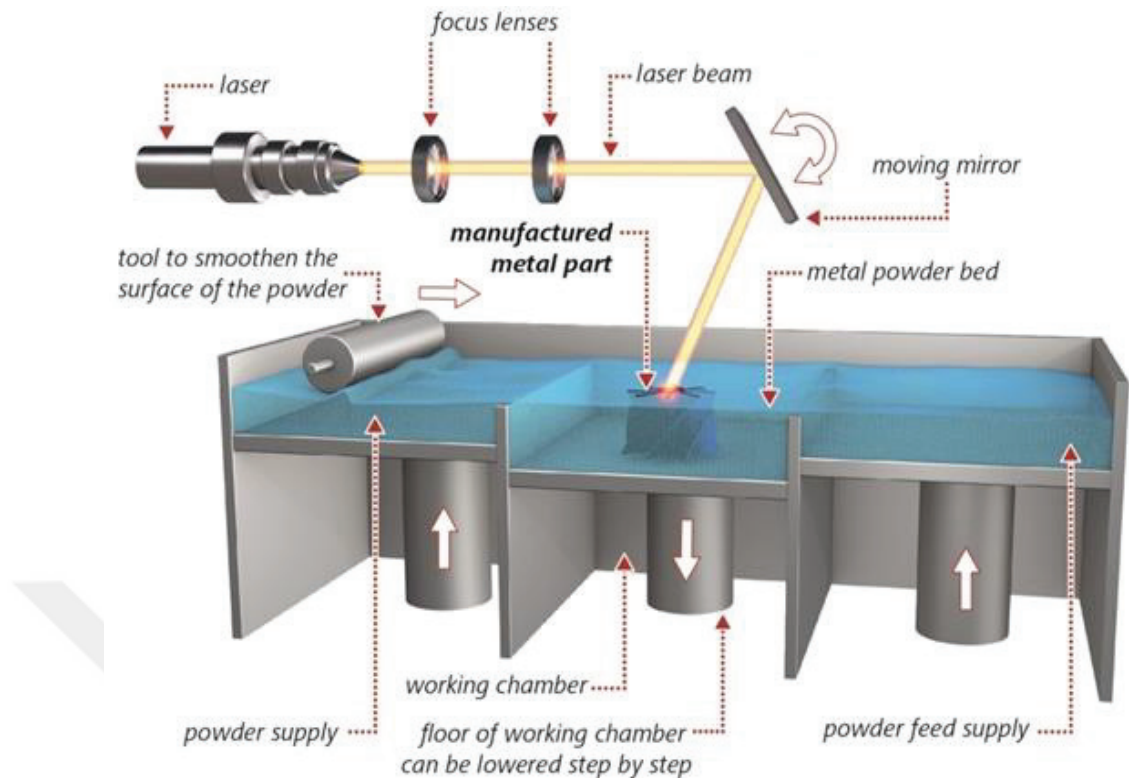


Figure 2.15. Illustration of SLM process

(Source: “(SLM) - 906 - Coating Competence Center - Empa,” n.d.)

2.4.6. Direct Energy Deposition

Beam Deposition (BD) processes, commonly used for metallic materials, involve melting and depositing material from powder or wire feedstock. Powder feedstock allows greater flexibility for creating complex geometries, while wire feedstock ensures high material efficiency and lower porosity but is less suitable for complex shapes and often requires post-processing. An illustration of these processes is shown in Figure 2.8.³⁴ Direct Energy Deposition (DED) processes are categorized into three main types: Laser Engineered Net Shaping (LENS) and Electron Beam Additive Manufacturing (EBAM), which use powder feedstock, and Wire and Arc Additive Manufacturing (WAAM), which utilizes wire feedstock.^{25,32}

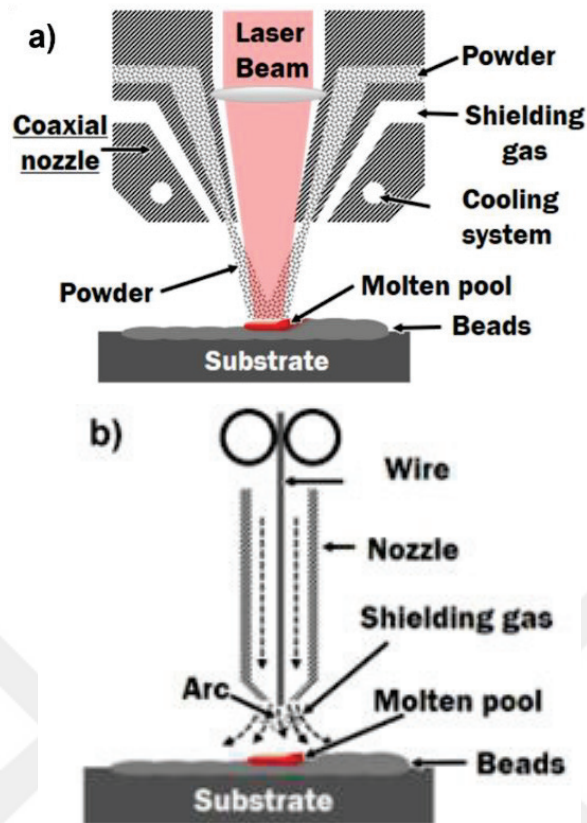


Figure 2.16. DED processes a) powder feedstock, and b) wire feedstock

(Source: Ahn 2021)

2.4.7. Sheet Lamination

Laminated Object Manufacturing (LOM) involves cutting paper sheets using a CO₂ laser and bonding them layer-by-layer to create final parts. For polymeric materials, adhesive bonding is commonly used, while thermal bonding is preferred for metallic materials.

This method provides unique benefits, including the flexibility to incorporate different materials or alter fiber composite orientations in each layer. These features make LOM particularly promising for structural applications and multi-material designs, even though it is less prevalent compared to other additive manufacturing technologies.^{25,35}

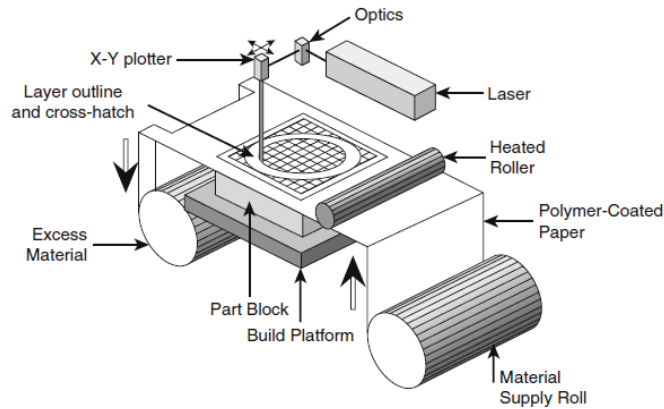


Figure 2.17. Illustration of LOM process
(Source: Gibson, Rosen, and Stucker 2010)

2.5. Process Parameters of SLM Process

In Selective Laser Melting (SLM), the interaction between the laser beam and the powder layer is critical, as it governs the formation and evolution of the melt pool, which directly impacts the quality and properties of the final part. It has different cooling rates when compared to the conventional casting method. The casting cooling rate is 5-100 K/s, but SLM is in the range of 10^3 - 10^7 K/s due to the localized heating and melting.^{36,37} Key process parameters such as laser power, scanning speed, and interaction time must be carefully optimized to ensure sufficient energy for melting the powder, forming a stable melt pool with controlled geometry. Phenomena like recoil pressure, surface tension, and the Marangoni effect influence the hydrodynamics within the melt pool, determining its size, shape, and solidification behavior. The precise control of these factors is essential to achieve defect-free layers and consistent part performance.³⁵

Laser Power (P) is related to the amount of energy delivered to the material powder. Scanning speed (v) refers to the movement speed of the laser across the surface. Layer thickness (t) is the height of each deposited powder layer. Hatching space (h), is the distance between successive adjacent laser tracks. Point distance is the space between two laser successive laser spots in the same laser track.^{36,38}

$$\text{Volumetric Energy Density (VED)} \left(\frac{J}{mm^3} \right) = \frac{P}{v \cdot h \cdot t} \quad (2.1)$$

Volumetric energy density is related to the abovementioned process parameters. It provides a quantitative evaluation of these parameters on the printed final part. Optimized VED values provide proper melting with minimizing defects, and enhanced mechanical properties.³⁹ Scanning strategy is another important parameter that significantly influences the defects in the manufactured part. It minimizes the defects in the final part. Common scanning strategies were given in the Figure 2.18^{27,40} In addition, there are three types of building orientation in the LPBF process. Anisotropic mechanical properties are highly influenced by the building orientation. It can be vertical, inclined, or horizontal, as shown in the Figure 2.19.^{27,41}

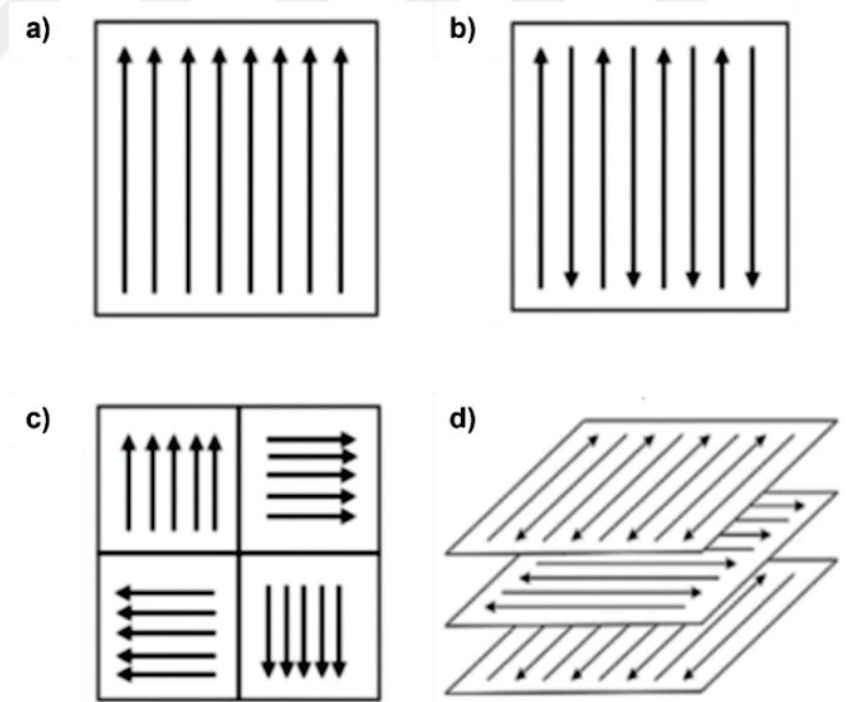


Figure 2.18. Common types of a) unidirectional b) bidirectional c) island
d) bidirectional 90° rotation scanning strategies

(Source: Jia et al. 2021; Sefene 2022)

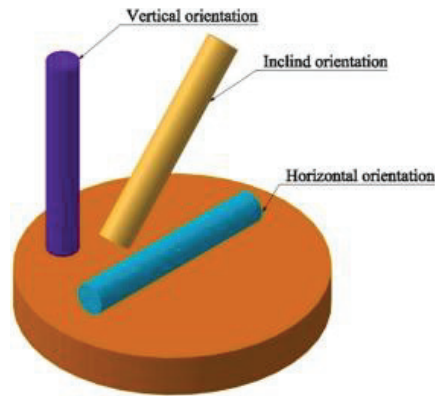


Figure 2.19. Building orientation types of LPBF process
(Source: Kurzynowski et al. 2018; Sefene 2022)

A schematic illustration of all the aforementioned parameters is provided in Figure 2.20. Hatch distance, laser spot, and point distance are depicted in the related figure. The building direction is vertical and represented along the z-axis. ³⁶

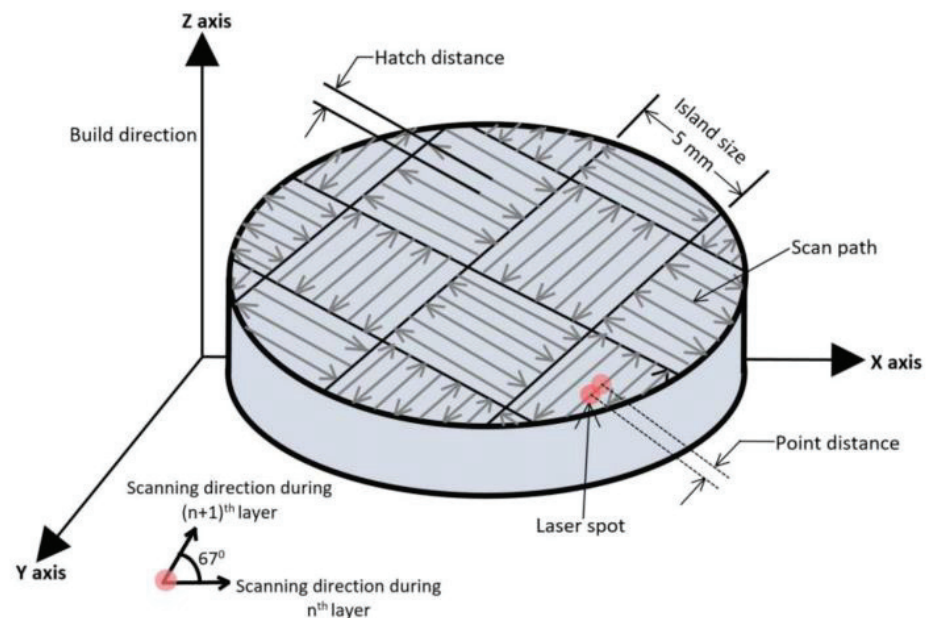


Figure 2.20. Fabrication strategy and process parameters of the SLM process
(Source: Al-Mamun et al. 2020)

2.6. Influence of Process Parameters on Microstructure and Mechanical Properties of Stainless Steel 316L

The effects of SLM process parameters on material properties have been widely investigated. In the literature, there is a lot of research investigating the effect of volumetric energy density on both porosity and hardness values. Liverani et al.⁴² studied in the range of 500-900 mm/s scanning speed, and 90-150 W laser power. They concluded that there is an optimum range of VED value for nearly fully dense materials. The other research studied three different VED values, which are 41.8, 104.5, and 209.3 J/mm³. Porosity is high for low and high VED values, however, nearly zero porosity percentage was obtained for the medium VED value (104.5 J/mm³).⁴³

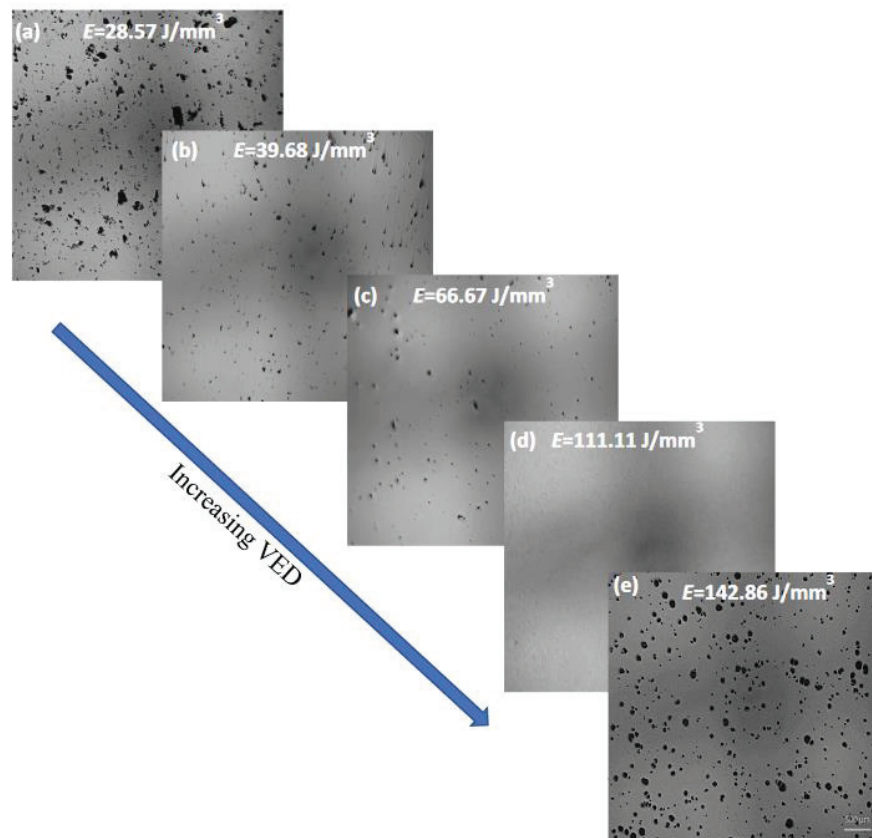


Figure 2.21. Relationship between increasing VED and porosity
(Source: Eliasu, Czekanski, and Boakye-Yiadom 2021)

Figure 2.21 shows the increasing volumetric energy density and surface porosity relation. As can be seen in the figure, the lowest and highest values for the VED give the highest porosity level than others. Lack of fusion pores can be formed for the low energy input, besides that, keyhole pores can be formed for the high energy input. ³⁹

In additively manufactured 316L stainless steel, part density is related to the melting mode of the SLM process. Lack of fusion means insufficient melting of metal powders due to the lower laser power values, or the usage of high scanning speed. If high laser power and low scanning speed are used, keyhole pores may occur. If low laser power and high scanning speed is used, a lack of fusion pores may form. The laser affects very deep of the formed melt pools, and a deep U-shaped melt pool may form. All of these were found in Figure 2.22. ^{35,39}

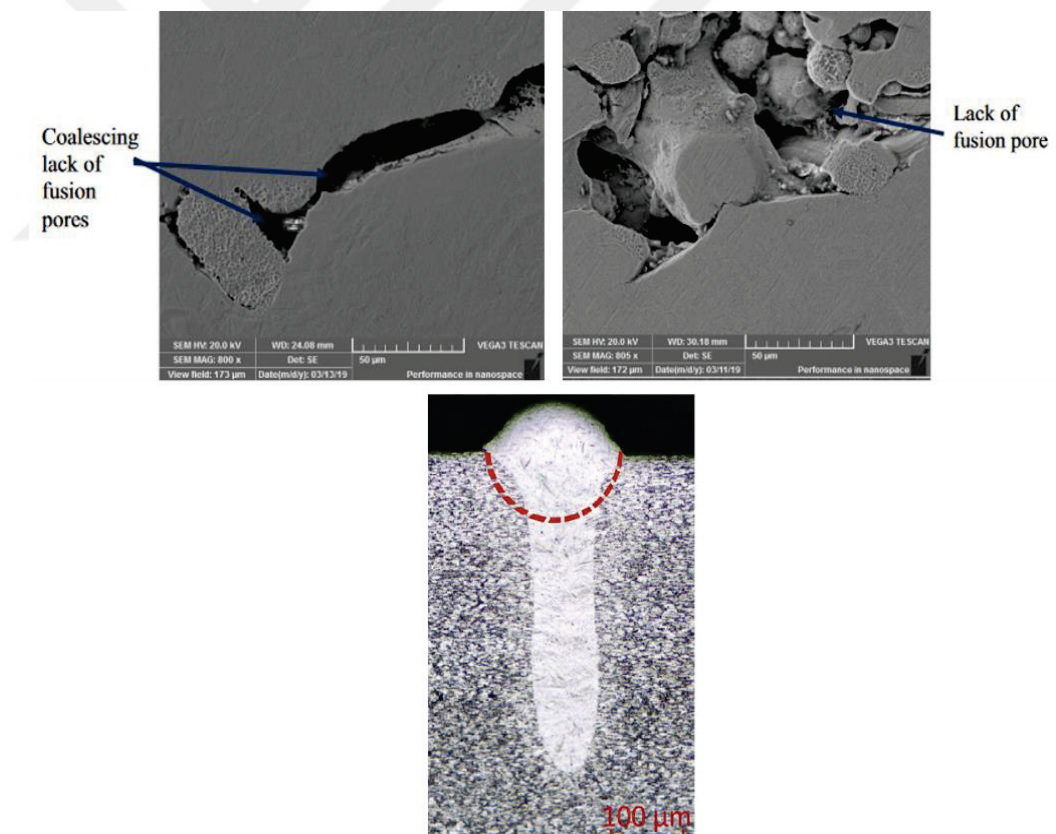


Figure 2.22. Lack of fusion pores and keyhole melting mode (lower image)
(Source: Eliasu, Czekanski, and Boakye-Yiadom 2021)

According to literature results for the SLM-ed 316L stainless steel, there is an optimum range of energy density value for bulk materials. Dense parts can be obtained in the range of 45 and 125 J/mm³, but process parameters should be considered together. 38,41,44

Hatch spacing influences the surface quality, and overlap ratios of the melt pools. Dong et. al. investigated the effect of hatch spacing when other VED parameters were kept constant. The overlap rate is in percentage value as shown in Figure 2.23. Increasing hatch spacing from 75 µm to 200 µm overlap ratio decreased from 65.1% to nearly 0%. Therefore, increased hatch space resulted in insufficient melting. ⁴⁵

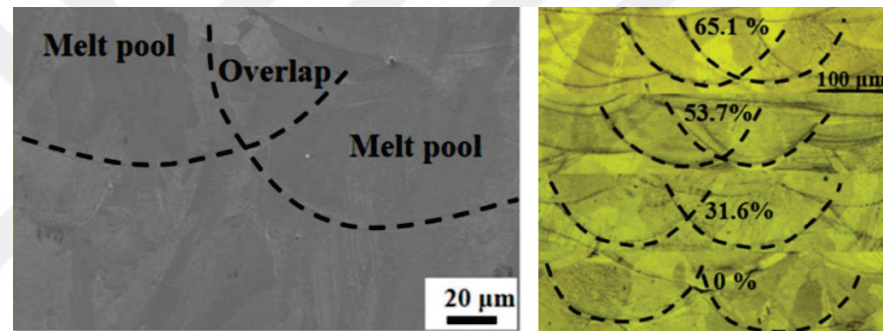


Figure 2.23. SEM image showing the overlapped melt pools
(Source: Dong et al. 2018)

At low hatch spacing values, the temperature gradient (G) is high, therefore the cooling rate is also high. As the cooling rate increases finer cellular structures can be obtained as given in Figure 2.24. ⁴⁵

There are three texture options in additively manufactured stainless steel 316L which are <100>, <110>, and <111>. In literature, several studies ^{46–48} have revealed that all of these textured materials have different mechanical properties. These crystallographic textures can be determined by using stereographic projection. It uses an angular relationship between crystallographic planes, which is called a Wulff net. Figure 2.25 shows the stereographic projection (complete inverse pole figure) for the FCC crystal

structure. The blue triangle refers to the main region of the inverse pole figure (IPF), shows the main three crystallographic directions as mentioned above.⁴⁹

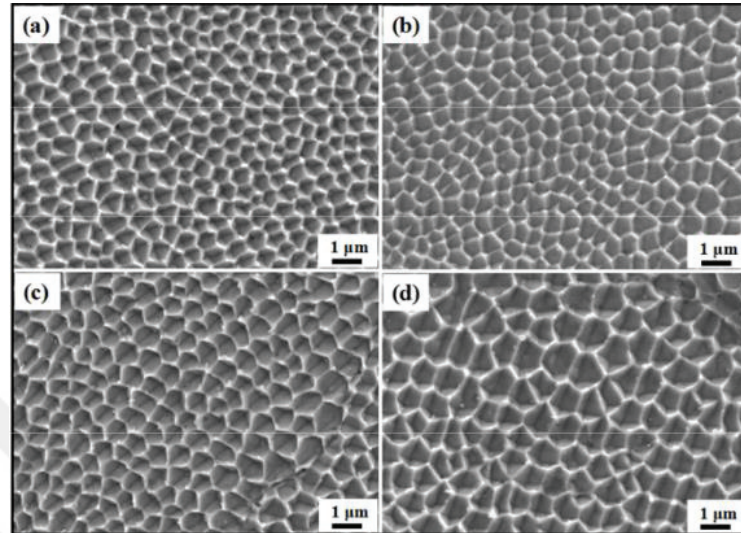


Figure 2.24. SEM images of sub-grain structures of (a) 75 μm , (b) 100 μm , (c) 150 μm , and (d) 200 μm
(Source: Dong et al. 2018)

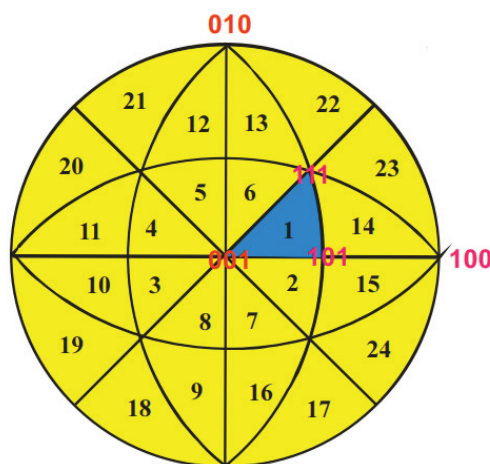


Figure 2.25. Stereogram of cubic crystal structure
(Source: Kelly and Knowles 2012)

Marattukalam et al.⁵⁰ investigated the effect of laser scanning strategies on mechanical properties related to crystallographic textures. Laser power, scanning speed, layer thickness, and hatch spacing were constant, only difference between the samples was scanning strategies. These strategies shown schematically in Figure 2.26.

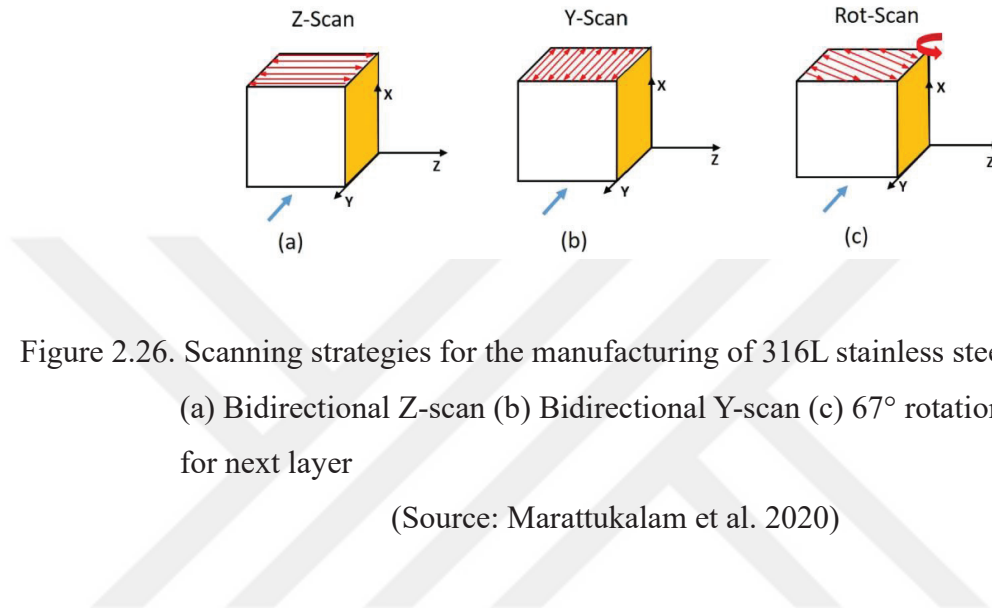


Figure 2.26. Scanning strategies for the manufacturing of 316L stainless steel samples
(a) Bidirectional Z-scan (b) Bidirectional Y-scan (c) 67° rotation strategy
for next layer

(Source: Marattukalam et al. 2020)

In Figure 2.27, EBSD results showed that the Z-scan strategy has strong $\langle 100 \rangle$ texture due to the laser scan strategy. Y-scan strategy has a strong $\langle 110 \rangle$ direction in the XY plane (along Z-axis). In the rotation scan strategy, the XY plane shows a different morphology than others. Since the rotation disrupts the single-crystalline like texture. However, in the YZ plane of Figure 2.27f, strong $\langle 110 \rangle$ texture can be observed in the pole figures. Tensile test results showed that $\langle 110 \rangle$ crystallographic texture gives the highest ductility due to the slip system nature of FCC structures.⁵⁰

In another research done by Güden et al.^{51,52}, 90° rotation was used between each layer. They revealed that $\langle 110 \rangle$ strong fiber texture, with an anisotropic mechanical property, can be obtained by using a rotational scanning strategy along the building direction. Residual stresses can also be decreased with rotational scanning. The dominant deformation mechanism for this texture is twinning, especially nano-twinning due to the cellular structures of SLM samples. Twinning plays a crucial role because of its ability to store dislocations, contributing to increased ductility and improved work-hardening capability.

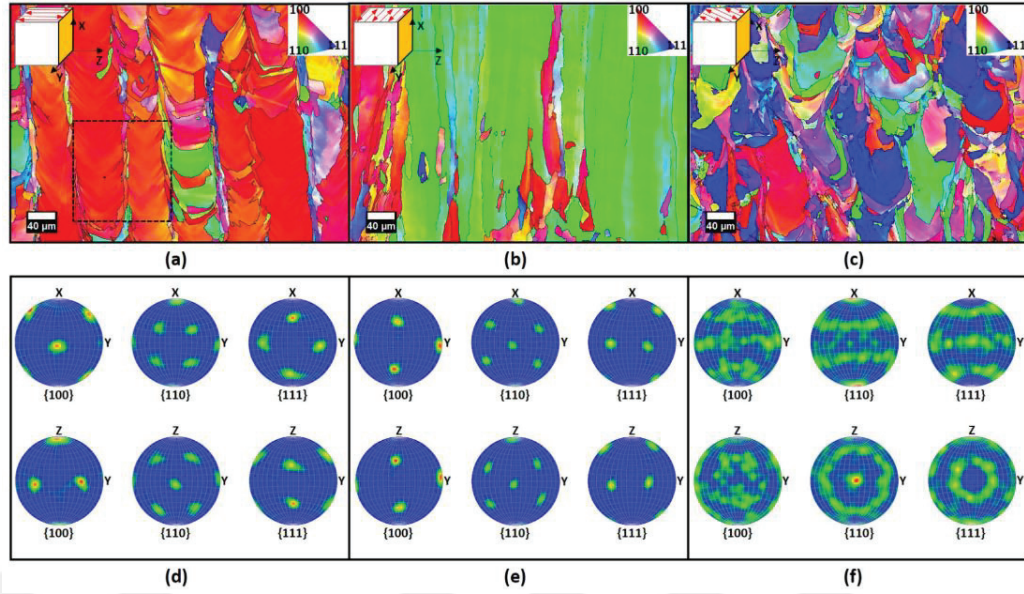


Figure 2.27. EBSD maps (XY plane) and pole figures (XY and YZ planes) illustrating the crystallographic orientations in 316L produced via selective laser melting. The scanning strategies were (a, d) Z-scan, (b, e) Y-scan, and (c, f) with a 67° rotation (Source: Marattukalam et al. 2020)

2.7. Cell Studies of Conventional and SLM samples

2.7.1. Cell Studies of Conventional Stainless Steel 316L

In order to enhance the cellular activity of conventional stainless steel 316L, mechanical processes, and severe plastic deformation methods have been studied to date. These methods can produce textured materials and nano-sized grain structures in bulk materials. Obtaining nano-sized grains improves both mechanical properties and cell attachment; however, it reduces ductility. Strength-high ductility are crucial criteria for ensuring long-term stability in implant applications.

Misra et al.⁵³ investigated the effect of the rolling-annealing sequence method to obtain different grain sizes and to identify the optimum grain size for both mechanical properties and cell attachment behavior. They obtained five different grain sizes ranges

from 320 nm to 22 μm . They obtained the highest cell viability for nano-grained (NG) structures compared to coarse-grained (CS) structures as indicated in the Figure 2.28. High strength-ductility is achieved for nano-size grains since wettability increases with decreasing grain sizes.

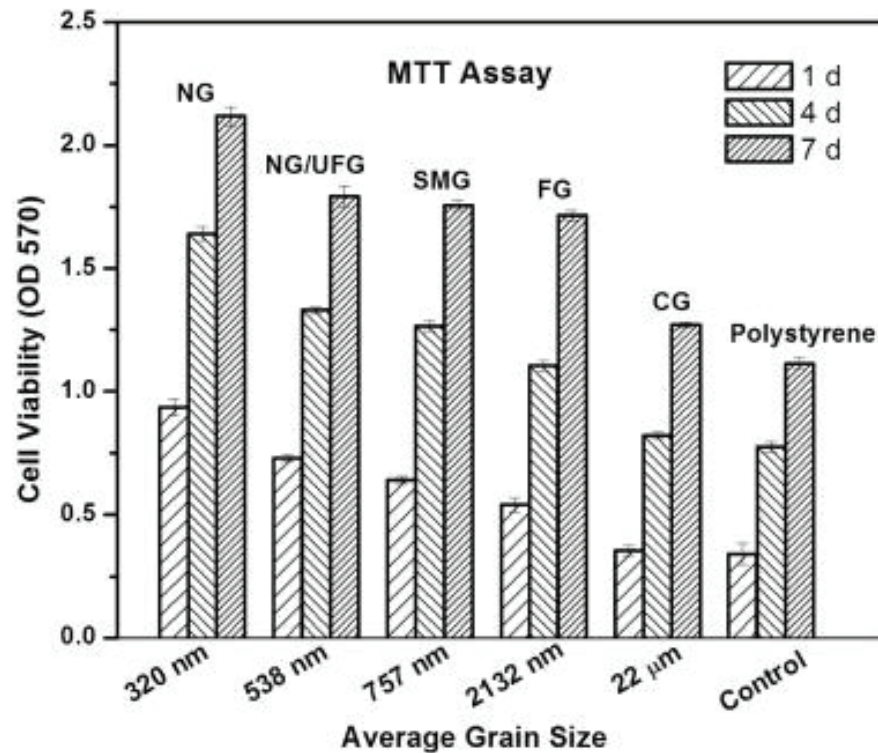


Figure 2.28. Cell Viability of austenitic stainless steels with different grain sizes
(Source: Misra et al. 2013)

Uzer et al.⁵⁴ examined the effects of plastic deformation on cell adhesion. Austenitic stainless steel 316L samples were subjected to five different plastic strain values: 5%, 15%, 25%, 35%, and 60%. They obtained textured material as increasing strains by reorienting of grains along the loading direction of the tensile test. Cell attachment increases as strain increases as shown in the Figure 2.29.

Higher cell attachment behavior was correlated with the increase in roughness value. This can be explained by micro-deformation mechanisms like twinning.

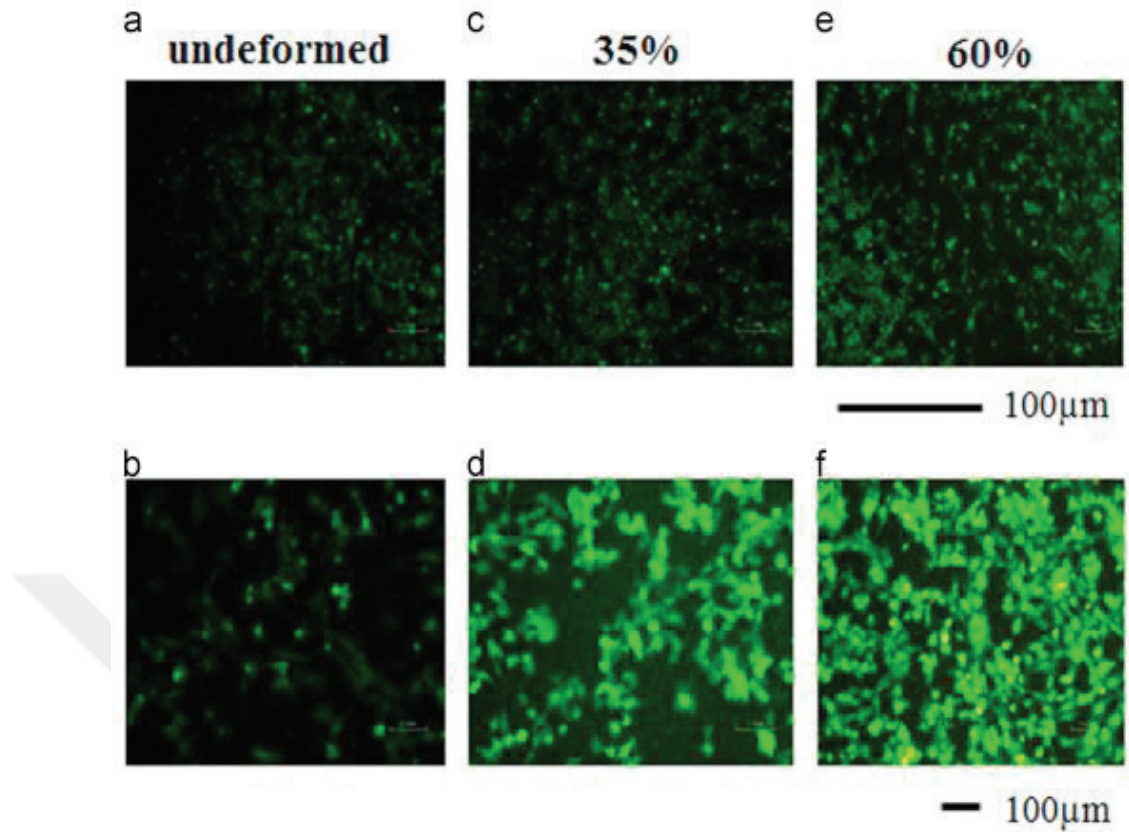


Figure 2.29. Fluorescent microscopy images of a), b) undeformed samples, c), d) 35% strain samples, and e), f) 60% strain samples
(Source: B. Uzer et al. 2016)

Severe plastic deformation (SPD) methods also have been used for both bulk and surface modification technique. Severe shot peening (SSP) is one of the SPD methods for surface modification. SSP uses compressive stresses to change surface properties.⁵⁵ One research⁵⁶ investigated the effect of both conventional shot peening and severe shot peening methods.

Figure 2.30 shows the cell proliferation results for the not peened (NP), conventionally shot peened (CSP), and severely shot peened (SSP) samples, and increasing osteoblast cell densities were obtained for SSP samples. Since the SSP method creates higher surface roughness, it increases wettability. Enhancing wettability positively affects cell attachment behavior.

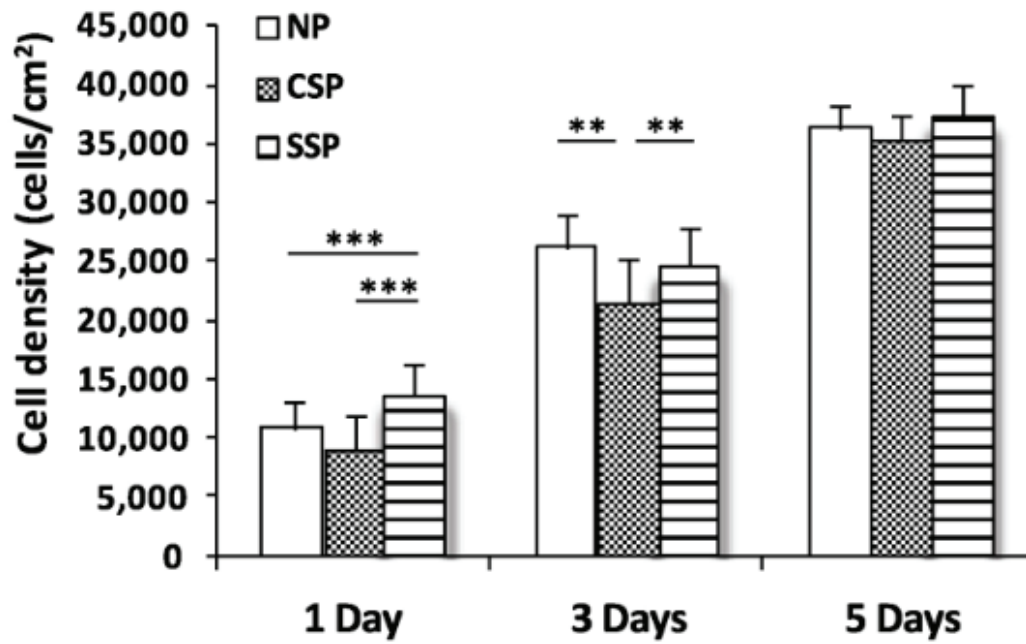


Figure 2.30. Cell proliferation of not peened (NP), conventionally shot peened (CSP), and severely shot peened (SSP) samples (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

(Source: Bagherifard et al. 2015)

2.7.2. Cell Studies of SLM Stainless Steel 316L

Microstructural and mechanical properties of SLM 316L stainless steel samples have been investigated up to now. Different studies can be found in references.^{57–59} However, there are not many studies in the literature on biocompatibility analyses of stainless steel 316L produced by selective laser melting method.

Kong et al.⁶⁰ investigated the effect of different laser power on cell proliferation and compared it with quenched one. 120 W, 150 W, 195 W, and 220 W laser powers were used with the 67° rotation scanning strategy. MC3T3 cells were used in cytotoxicity tests. Cell proliferation was observed in all samples for different days. 195 W and 220 W samples shows higher proliferation than quenched samples as seen in the Figure 2.31.

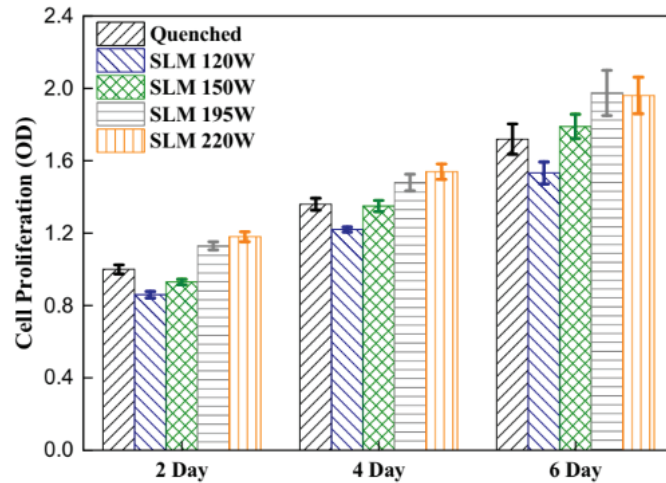


Figure 2.31. Cell proliferation results for 2,4, and 6 days
(Source: Kong et al. 2018)

Another research done by Shang et al.⁶¹, the effects of varying scanning speed on biocompatibility were investigated. 195 W laser power, 90 μm hatch spacing, 20 μm layer thickness were used with 800, 900, and 1083 mm/s scanning speed values. The highest cell proliferation was obtained for 900 mm/s speed as shown in the Figure 2.32.

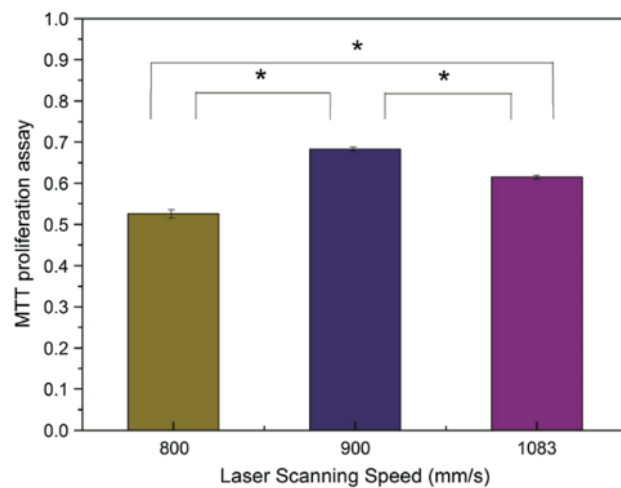


Figure 2.32. Cell proliferation for 24 h extract from cell culture for 800, 900, and 1083 mm/s scanning speed

(Source: Shang et al. 2017)

They suggested that the variation in results at different scanning speeds could be attributed to differences in the microstructure. Therefore, they revealed that scanning speed significantly affects in vitro cell viability when other processing parameters are kept constant.

2.8. Motivation of the Thesis

As mentioned in '2.7.1. Cell Studies of Conventional Stainless Steel 316L' additional processes are needed for the enhancement of cell-implant behavior of conventional stainless steel 316L. However, using additive manufacturing methods like selective laser melting, direct enhancement can be achieved due to different microstructural features. In addition to that, textured materials can be directly obtained with this manufacturing method. For all these reasons, the motivation of this thesis is to produce 316L stainless steel with anisotropic properties (textured materials) using the selective laser melting method and to investigate the effects of this textured material on biocompatibility in comparison to the conventional one.

CHAPTER 3

MATERIALS AND METHODS

3.1. Manufacturing of Materials

Three different sets of parameters were selected from the literature based on the material properties as mentioned in Section 2.6. Table 3.1 shows the details of parameters, and samples named as 1B, 2B, and 3B, respectively. Bi-directional rotational scanning strategies were used for 1B and 3B samples, however, 2B sample was scanned using X-scan strategy.^{50,62,63} Illustrations for scan strategies were given in the Figure 3.1.

Table 3.1. SLM process parameters for the fabrication of 316L cuboid samples
(Source: Marattukalam et al. 2020; Tsutsumi et al. 2021; Zhao et al. 2021)

Sample Name	Laser Power (W)	Scan Speed (mm/s)	Layer Thickness (μm)	Hatch Spacing (μm)	Energy Density (J/mm^3)	Scanning Strategy
1B	195	1000	20	90	108.3	Bi-directional 90° rotation
2B	250	1000	40	80	78.1	Bi-directional X-scan
3B	107	800	20	70	95.5	Bi-directional 67° rotation

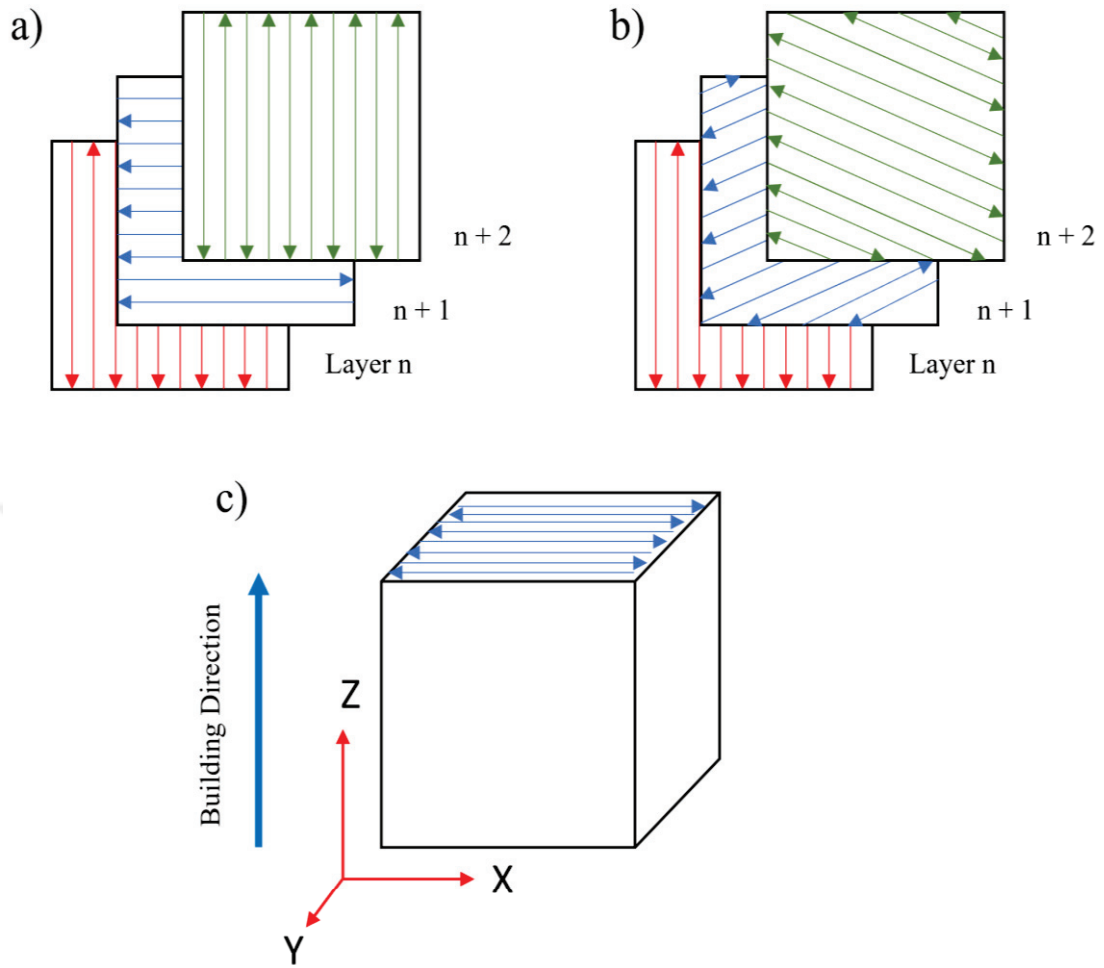


Figure 3.1. Scanning strategy for a) 90° rotation, b) 67° rotation, and c) X-scan

Gas-atomized stainless steel 316L powder (m4p material solutions GmbH) was used for the manufacturing of three samples by LPBF. As shown in Figure 3.2, 316L powders mostly consist of spherical particles, and the particle size distribution ranges from 15-45 μ . 316L stainless steel samples were manufactured using Selective Laser Melting (SLM) with an SLM Solutions device equipped with a 400 W Nd:YAG Gaussian profile laser with a wavelength of 1064 nm.

Three cuboid samples ($10 \times 10 \times 10 \text{ mm}^3$) were fabricated using different sets of parameters to achieve a specified crystallographic texture.

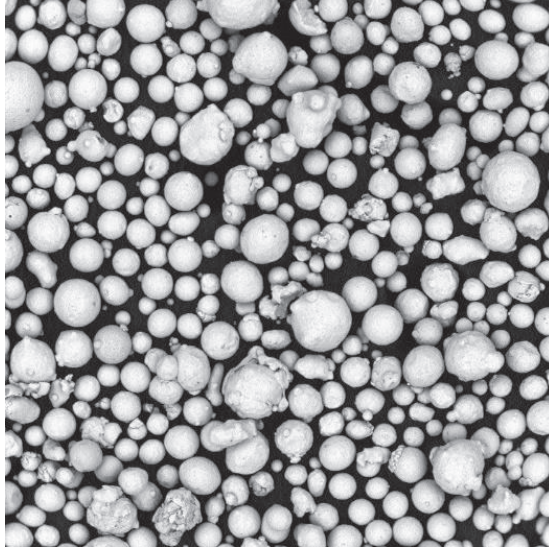


Figure 3.2. SEM image of the gas-atomized 316L powder

3.2. Microstructural Characterization

After manufacturing, all samples were cut using a diamond saw, and the investigation plane was established as the YZ plane, which is parallel to the building direction. Each sample was ground using 120, 240, 320, 400, 600, 800, 1000, 1200, and 2500 grit SiC sandpaper to eliminate any cutting effects. After that, the samples were polished sequentially with 6 μm , 3 μm , and 1 μm diamond suspensions until a mirror-like surface was achieved. The surfaces were then cleaned in an ultrasonic bath to remove any residues from the processes, using a sequence of 10 minutes in acetone, 10 minutes in ethanol, and 10 minutes in pure water. To reveal the microstructural features, samples were etched using diluted aqua regia solution.

Microstructural observations were carried out using a Leica optical microscope (OM) and an FEI QUANTA 250 FEG scanning electron microscope (SEM) with a secondary electron (SE) detector to analyze surface morphology. 15 kV voltage value was applied in SEM analysis. Additionally, a ZEISS EVO10 SEM was employed to analyze surface defects with the use of a backscatter electron (BSE) detector.

The electron backscatter diffraction (EBSD) technique attached to a scanning electron microscope was used for the crystallographic texture characterization of the samples. Before analysis, in addition to the previous sample preparation steps, the materials were subjected to a final polishing process using an OPS solution containing 0.05 μm colloidal silica particles for 15 minutes with a fully automatic Struers device. Orientation microscopy was carried out using the EDAX/TSL EBSD system attached to the Zeiss Sigma 360 FEG-SEM scanning electron microscope, operating at an acceleration voltage of 15 kV. The raw EBSD data was processed using the TSL OIM Analysis v7.3.1 software.

All analysis was performed with respect to (wrt) the building direction (parallel section) for SLM samples and with respect to the normal direction for the conventional 316L sample, as illustrated in the Figure 3.3.

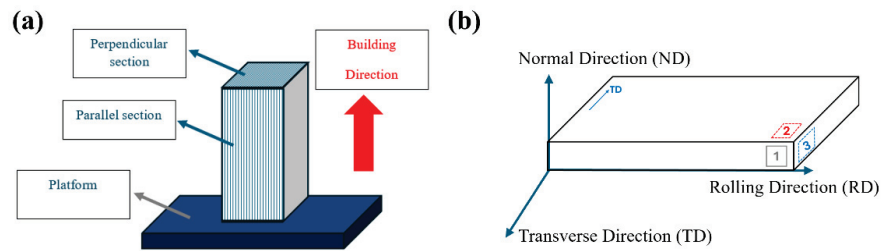


Figure 3.3. Schematic representation of the sections examined by EBSD (a) for SLM samples (b) for conventional sample

3.3. Hardness Measurements

Hardness measurement was performed with a microhardness tester to examine the mechanical properties of the materials. The samples 1B, 2B, 3B, and conventional 316L were prepared by sequentially applying 120, 240, 320, 400, 600, 800, 1000, 1200, and 2500 grit SiC sandpaper before the analyses. This was followed by polishing with 6 μm ,

3 μm , and 1 μm diamond suspensions until a mirror-like surface was obtained. The surface of the material was cleaned in an ultrasonic bath to remove residues formed during the preparation process, using a 10-minute acetone bath, followed by 10 minutes in ethanol, and finally 10 minutes in pure water.

The Vickers hardness (HV) measurement was performed on the polished surface. This was done using a Shimadzu Vickers Microhardness tester, applying a load of 19.61 N with a dwell time of 10 seconds. One sample was used for each parameter set, and the test was repeated 8 times for each set. A sample indentation calculation is provided in Equation 3.1.¹¹ The applied load is denoted as F , and d is the average of the indentation diagonal.

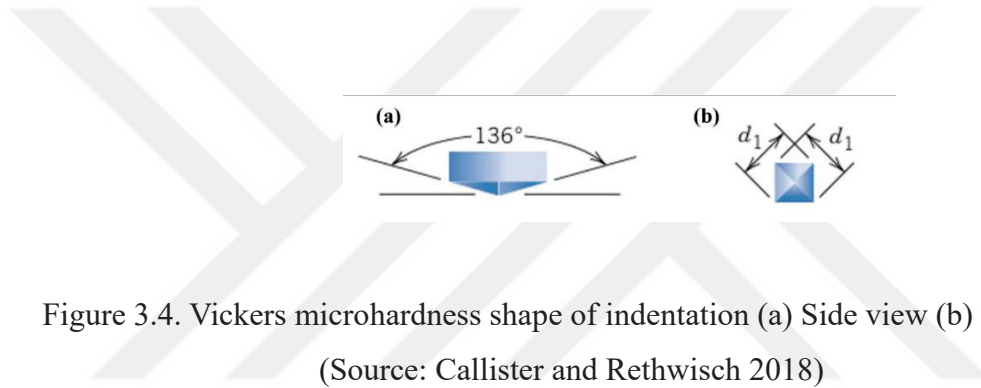


Figure 3.4. Vickers microhardness shape of indentation (a) Side view (b) Top view
(Source: Callister and Rethwisch 2018)

$$HV = 1.854 \left(\frac{F}{d^2} \right) \quad (3.1)$$

3.4. Wettability Analysis

Before the analyses, the 1B, 2B, 3B, and conventional samples were sequentially polished with 120, 240, 320, 400, 600, 800, 1000, 1200, and 2500 grit SiC sandpaper. This was followed by polishing with 6 μm , 3 μm , and 1 μm diamond suspensions until a mirror-like surface was obtained. To remove any oil, dirt, or residues that might form on the surface after the processes, the samples were cleaned in an ultrasonic bath. The cleaning process involved 10 minutes in acetone, followed by 10 minutes in ethanol, and finally 10 minutes in pure water. Contact angle measurements were applied to the polished

and cleaned samples. A 5 μ L droplet of pure water was placed on the surface of the material at room temperature and left for 10 seconds. Three samples from each group were examined, and five different measurements were taken for each sample. Between each measurement, the samples were wiped with a non-abrasive soft tissue (Kimwipes).

3.5. Cytotoxicity Test

MC3T3-E1 subclone 4 (ATCC CRL-2593) mouse pre-osteoblast cell line was used in cell culture studies. Alpha-MEM growth medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin was used as a growth medium throughout the study.

Cell proliferation assay

MC3T3-E1 subclone 4 (ATCC CRL-2593) pre-osteoblast cells were seeded on material surfaces with a seeding density of 9000 cells/cm². Adherent cell metabolic activity was measured with an alamarBlue cell proliferation assay at days 1, 3, and 7. Cells were incubated with 10% alamarBlue solution in a complete growth medium for 3.5 hours. Reduced solutions were transferred into 96-well plates, and their absorbances were read at a wavelength of 570 nm with a reference wavelength of 600 nm. Metabolic activity was calculated according to the manufacturer's guidelines.

Actin cytoskeleton and nuclei staining

Adherent cells on material surfaces were fixed on day 7 of culture with 4% paraformaldehyde in phosphate buffered saline (PBS) for 15 minutes at room temperature. Cells were permeabilized with 0.1% Triton X-100 in PBS and blocked with 1% bovine serum albumin (Biosera, France) in PBS. Actin cytoskeleton was stained with AlexaFluor 488 conjugated phalloidin (Invitrogen, USA) and nuclei were counterstained with DAPI (Invitrogen, USA). Cytoskeleton and nuclei were imaged with their respective filters under a fluorescence microscope.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Microstructural Analysis of SLM and Conventionally Produced 316L Stainless Steel

The optical microscopy images of SLM stainless steel 316L samples were given in the Figure 4.1, showing the microstructural features of the additive manufacturing process of 1B, 2B, and 3B samples, respectively. Melt pool shapes can only be observed on side views. In sample 1B, a large columnar grain is highlighted with black dashed lines. Epitaxial grain growth is observed due to the remelting zone, which suppresses nucleation during solidification. As a result, grain growth occurs perpendicular to the fusion line boundary (melt pool boundary).^{23,58}

Semi-circular (arc-shaped) melt pool boundaries can be seen for all samples. Shapes vary depending on the differences in scanning strategies. In sample 1B, strip-shaped melt tracks are visible due to the observation plane. These melt pools typically appear arc-shaped, but due to the bidirectional rotation strategy applied, they are observed as strip-shaped tracks when viewed from a plane perpendicular to the melt pools.⁵⁰

Irregular melt pool shapes are observed in sample 3B (Figure 4.1c) due to the scanning strategy was 67° rotation between each layer. The width and height of the melt pools are disordered due to the laser tracks coincidence with the same path after every 360 layers.^{50,63,64} In sample 2B, regular melt pool shapes (lamellar microstructure) are formed due to the X-scan strategy, as shown in Figure 4.1b.

The open black arrows indicate individual laser tracks, with the hatch spacing value (80 µm) clearly visible between the centerlines of adjacent melt pools.^{47,62}

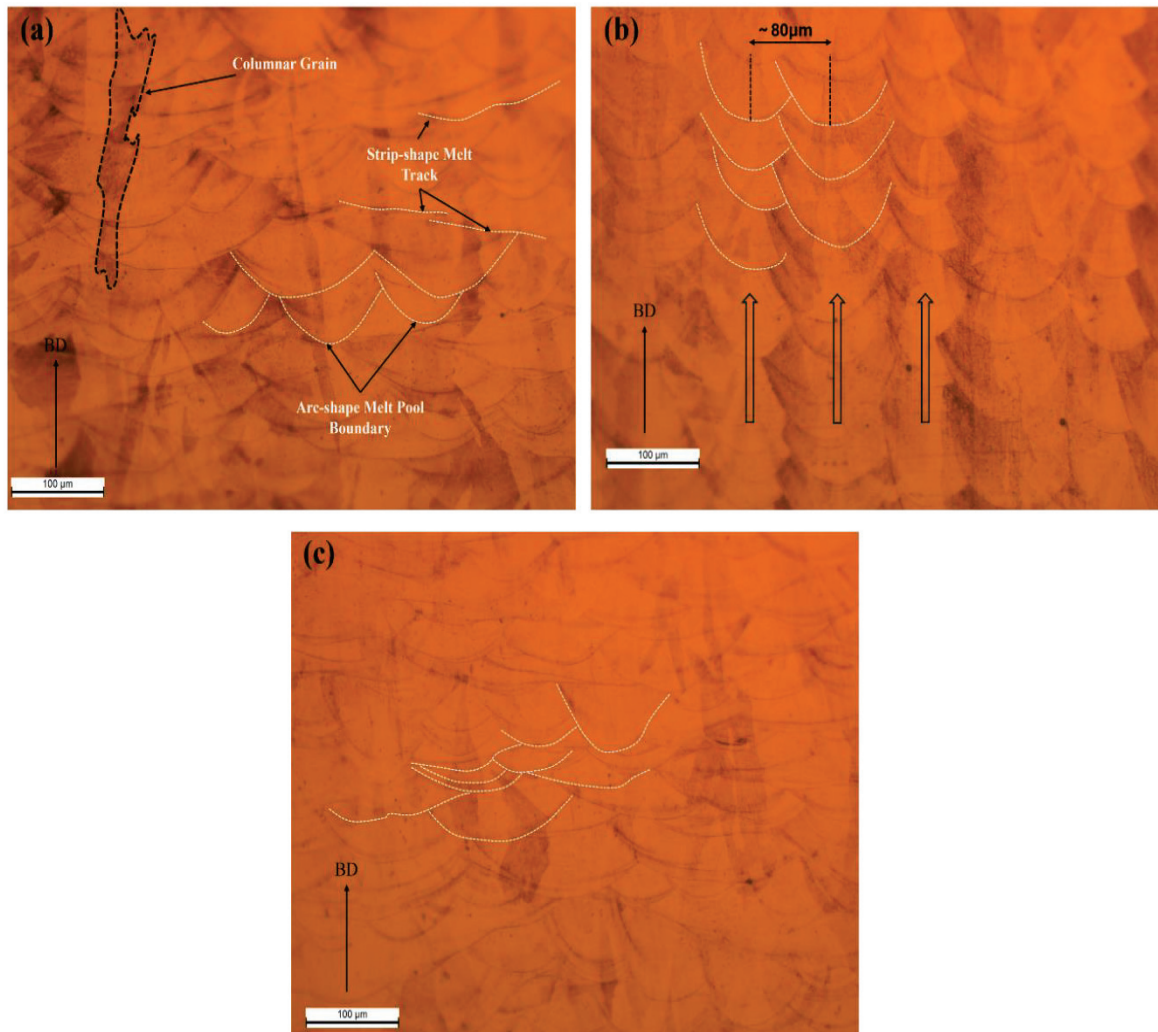


Figure 4.1. Optical microscopy images of (a) 1B sample with 90° rotation (b) 2B sample with X-scan and (c) 3B sample with 67° rotation (black arrows indicate the direction parallel to the building direction)

The microstructures of conventional 316L, shown in Figure 4.2, exhibit distinct differences compared to the SLM samples due to the cooling rate differences. As discussed earlier in Chapter 2, conventional 316L features have isotropic mechanical properties due to its manufacturing processes. Additionally, annealing twins are visible in Figure 4.2 for both optical microscopy and scanning electron microscopy images. Two different imaging analysis results are the same for conventional steel due to the manufacturing process.

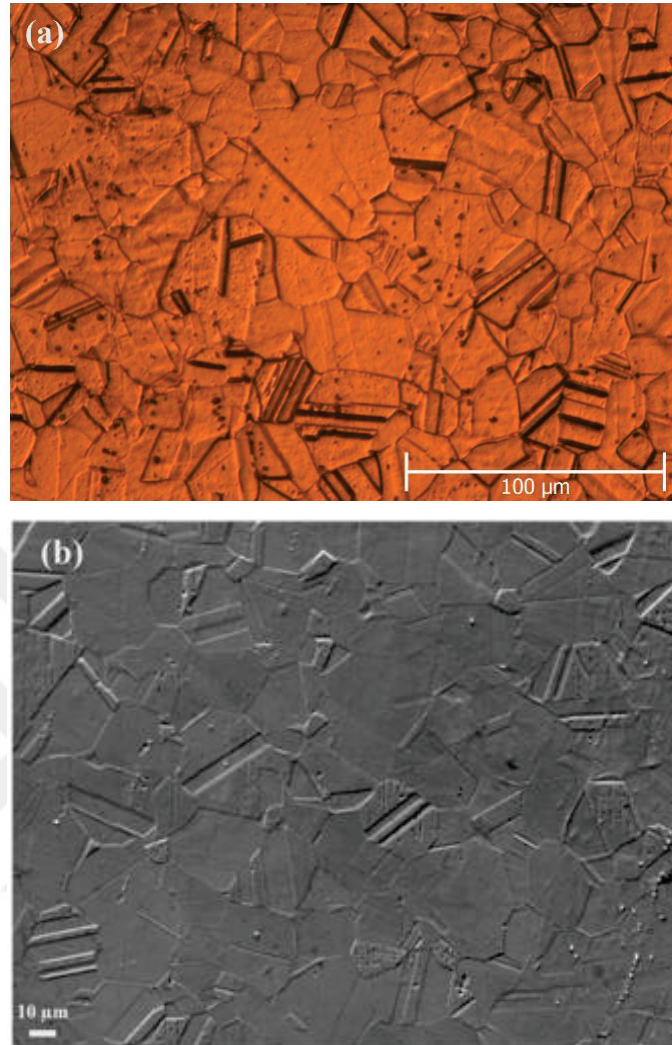


Figure 4.2. Conventional 316L (a) optical microscopy and (b) SEM image

Figure 4.3 presents the scanning electron microscopy (SEM) image of the 1B sample. Yellow dashed lines highlight the cellular sub-structures, while red dashed lines indicate the columnar sub-structures (epitaxial grain growth, as previously discussed).

As mentioned in Section 2.3. Microstructure of Additively Manufactured Austenitic Stainless

Steel 316L), additively manufactured samples contain only cylindrical sub-structures, which appear differently depending on the investigation plane. For instance, columnar grains observed in the YZ plane (as shown in Figure 4.3) look like cellular sub-grains when viewed in the XY plane (perpendicular to the building direction).⁶³

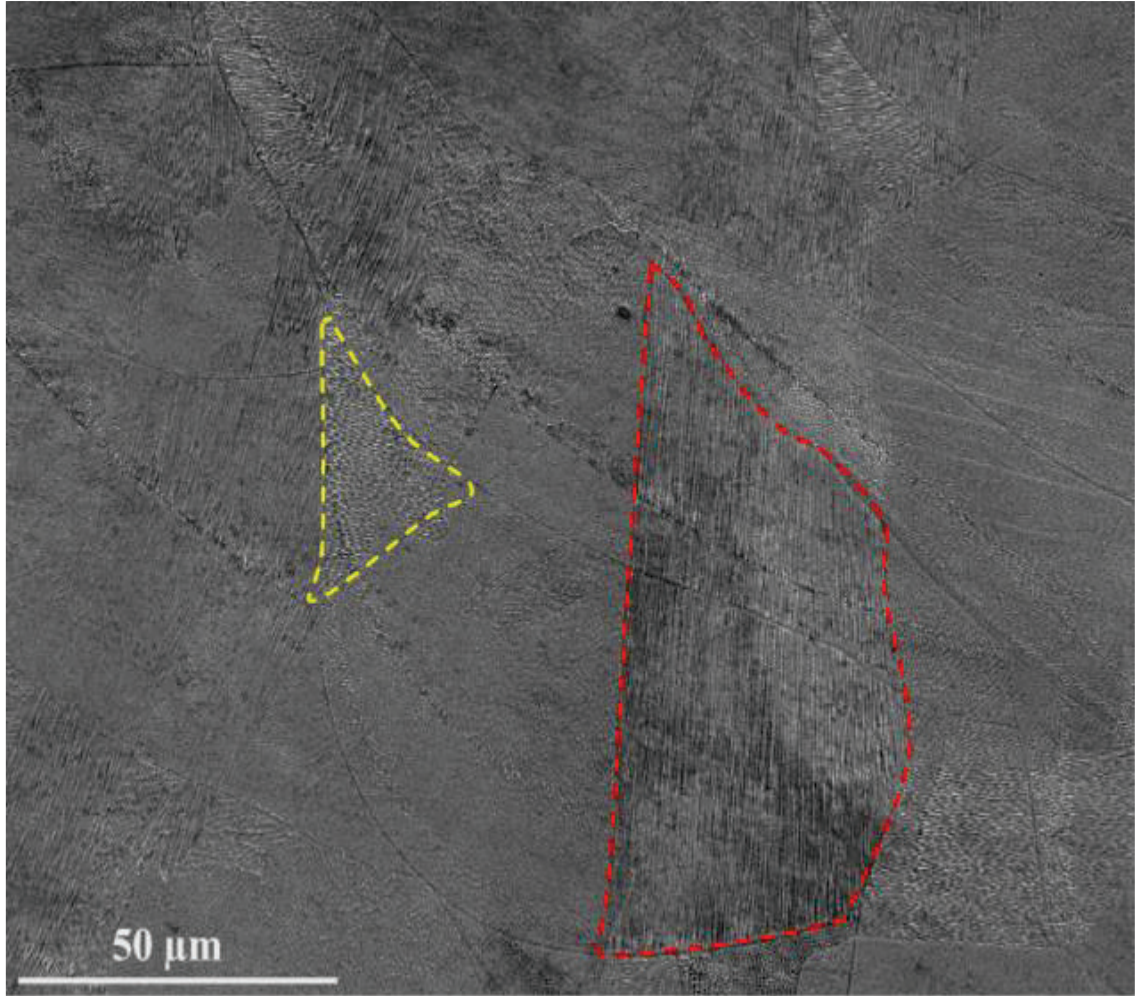


Figure 4.3. SEM image of 1B sample

Size of cellular sub-structures are given in Figure 4.4. The scale bar is 10 μm for all three samples. Based on this, the finest cellular grains were observed in the 3B sample, while the 2B sample exhibited larger cellular grain sizes compared to both the 1B and 3B samples.

Higher laser power in SLM resulted in deeper melt pools, reducing the cooling rate and leading to the formation of coarser cellular sub-structures.

The 2B sample, with the highest laser power value (250 W), exhibited the coarsest structures, while the 3B sample, with the lowest laser power (107 W), formed finer cellular sub-structures due to shallower melt pools and higher cooling rates. These findings align with the results reported in the literature.^{39,52,60}

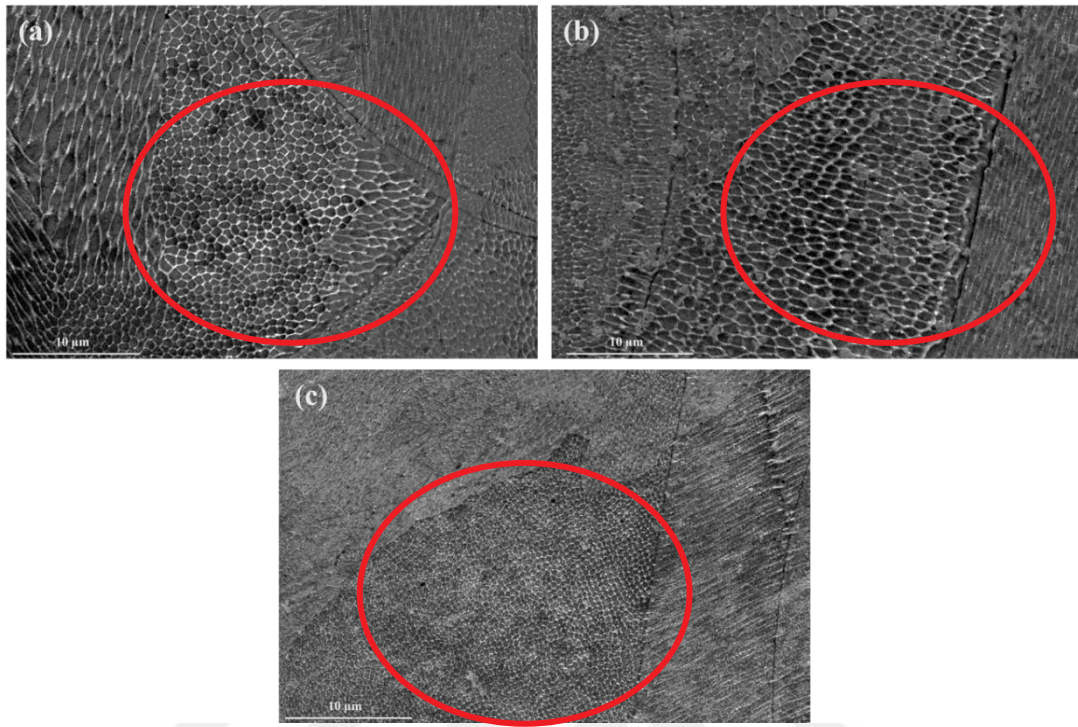


Figure 4.4. Cellular sub-grains comparison of (a) 1B (b) 2B, and (c) 3B samples

The surfaces of the produced samples were examined using a scanning electron microscope (SEM) equipped with a backscatter electron (BSE) detector, and a secondary electron (SE) detector. Mostly lack of fusion (LOF) defects were observed in the analysis. There are two types of lack of fusion defects: (1) poor bonding defects could occur between the layers, (due to the insufficient melting of metal during solidification) and (2) unmelted metal powders.

Figure 4.5 presents the SEM images of the 1B sample, revealing a smoother and better surface quality compared to the 2B and 3B samples. The volumetric energy density (VED) value of sample 1B is 108.3 J/mm^3 , which falls within the optimal VED range for minimizing porosity.^{39,43} In Figure 4.6a, elongated lack-of-fusion (LOF) defects in the 2B sample are clearly visible along the building direction, near the boundary of the manufactured sample (black arrows indicate the building direction).

Red arrows in Figure 4.6b highlight poor bonding defects as previously explained. The measured powder sizes, 21.6 μm and 34.6 μm , fall within the typical range for metal powders used in the SLM process.

Figure 4.7 shows SEM images of the 3B sample, where elongated LOF defects (Figure 4.7a) are observed between layers, more densely than in the 2B sample due to the 3B sample's lower laser power (107 W). In Figure 4.7b, unmelted metal powders are indicated by red circles.^{35,45,65,66}

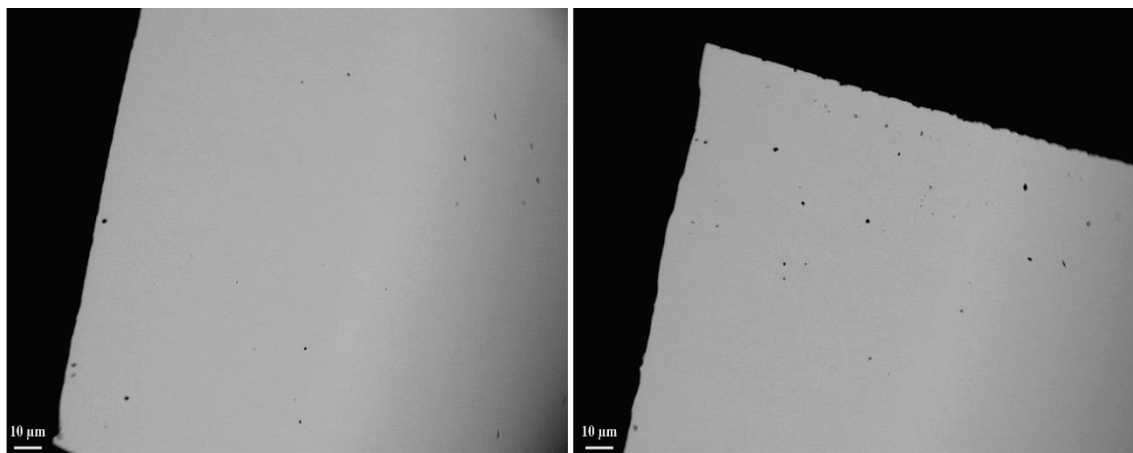


Figure 4.5. Backscattered electron (BSE) images obtained from sample 1B, showing surface “defects”

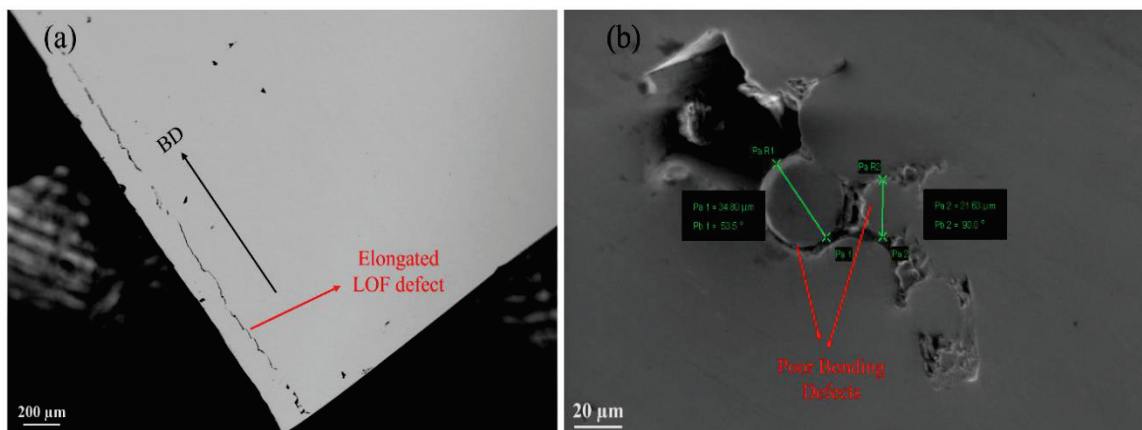


Figure 4.6. SEM images of sample 2B showing (a) Elongated lack of fusion (LOF) defects (b) Poor bonding defects

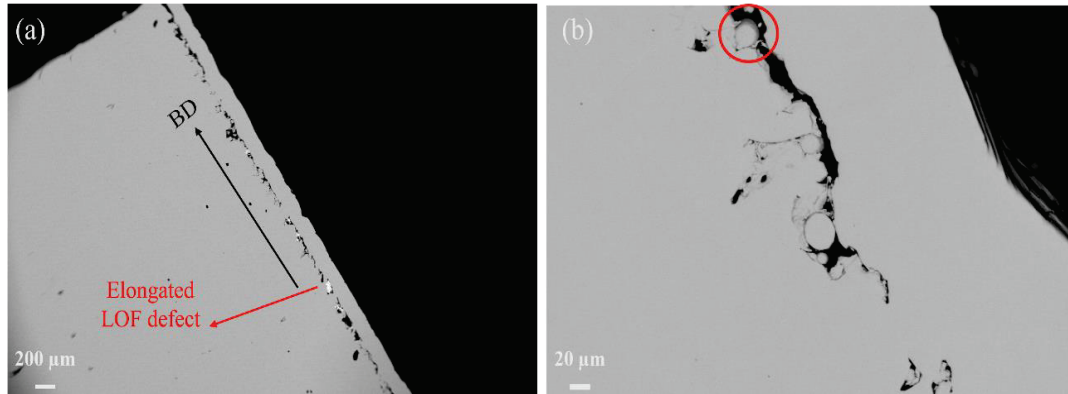


Figure 4.7. SEM images 3B showing (a) Elongated lack of fusion (LOF) defects
(a) Unmelted metal powder

4.2. Electron Backscatter Diffraction (EBSD) Analysis

The microstructure was characterized by examining the EBSD pattern quality maps, inverse pole figures (IPF), and the misorientation angles of grain boundaries. Inverse pole figure (IPF) is a crystallographic texture visualization technique to represent the grain's orientations in a specific direction.⁶⁷

In below Figure 4.8 shows the IPF maps for both conventional and SLM stainless steel 316L samples. In these maps, grains parallel to the building direction (BD) $\langle 001 \rangle$ direction of the face-centered cubic (FCC) structure are shown in red, grains parallel to the $\langle 110 \rangle$ direction in green, and grains parallel to the $\langle 111 \rangle$ direction in blue, as illustrated in the right side of the figure.

For the conventional sample ((a)), a $\langle 110 \rangle // \text{ND}$ orientation is observed, as indicated by the green-colored grains, although grains in other orientations are also present.

In sample 1B, the structure is predominantly composed of grains oriented in the $\langle 110 \rangle // \text{build direction}$. Therefore, sample 1B exhibits anisotropic properties due to its strong $\langle 110 \rangle$ crystallographic texture aligned with the building direction.

Sample 2B shows a random texture due to different color regions. Between melt pool boundaries grains are oriented in the $\langle 001 \rangle$ direction due to the nature of X-scan

strategy. Sample 3B shows again random texture, however, different oriented grains are much smaller than 2B due to the 67° rotation scanning strategy.

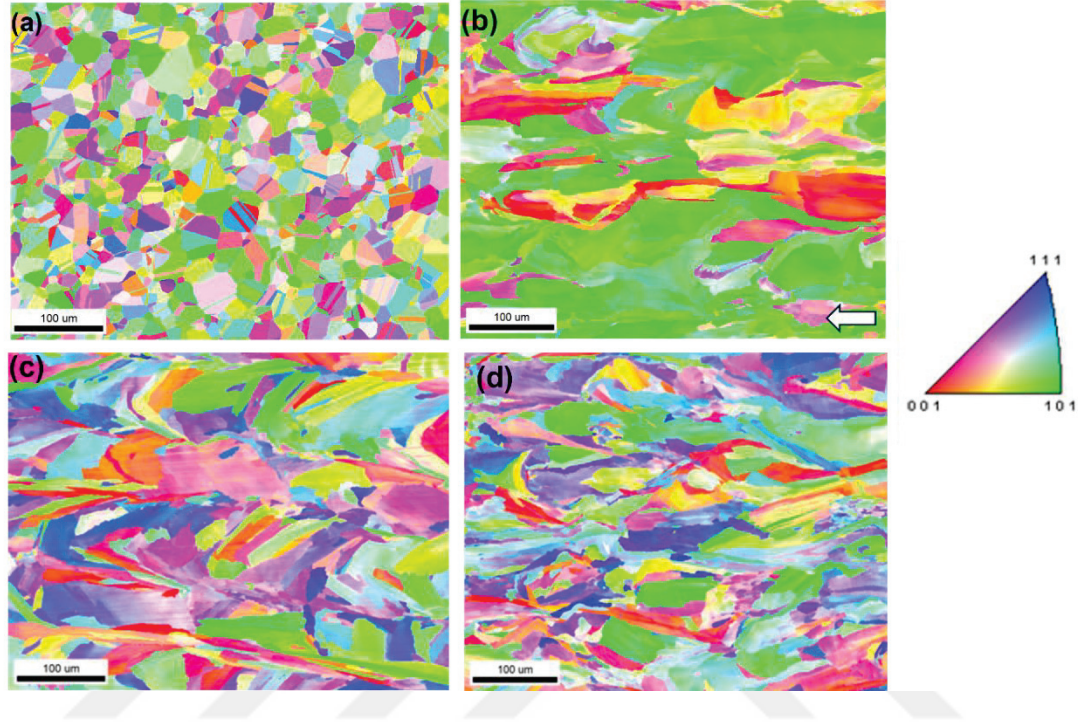


Figure 4.8. EBSD IPF maps wrt normal direction for **(a)** conventional and wrt built direction for **(b)** 1B **(c)** 2B **(d)** 3B; white arrow indicates build direction for AM samples

The EBSD phase maps of the samples are shown in Figure 4.9. In all samples, austenite was detected at a rate of 99.9% or higher. Grain boundaries are divided into three categories: the coincident site lattice (CSL) boundaries, the low-angle grain boundaries (LAGB), and the high-angle grain boundaries (HAGB). These maps also show HAGBs (15° - 63°), LAGBs (5° - 15°), and $\Sigma 3$ twin boundaries (stands for 60° rotation between lattice)⁶⁸. In addition, 2° - 5° shows ultra-low angle grain boundaries (ULAGBs). As shown in Figure 4.9a the conventional sample exhibits a higher degree of twin boundaries (red lines within the grains) as a result of the recrystallization process. In contrast, the SLM samples have significantly fewer twin boundaries. However, when comparing their low-angle grain boundaries (LAGBs), the SLM samples display a higher

proportion due to the repeated heating and cooling cycles inherent in the additive manufacturing process.⁶⁹ In Figure 4.9, ULAGBs are shown in light gray lines. For sample 3B, Figure 4.9c, there is BCC phase (red color in phase map), but this can be neglected since it has very low fraction compared to the austenite phase.

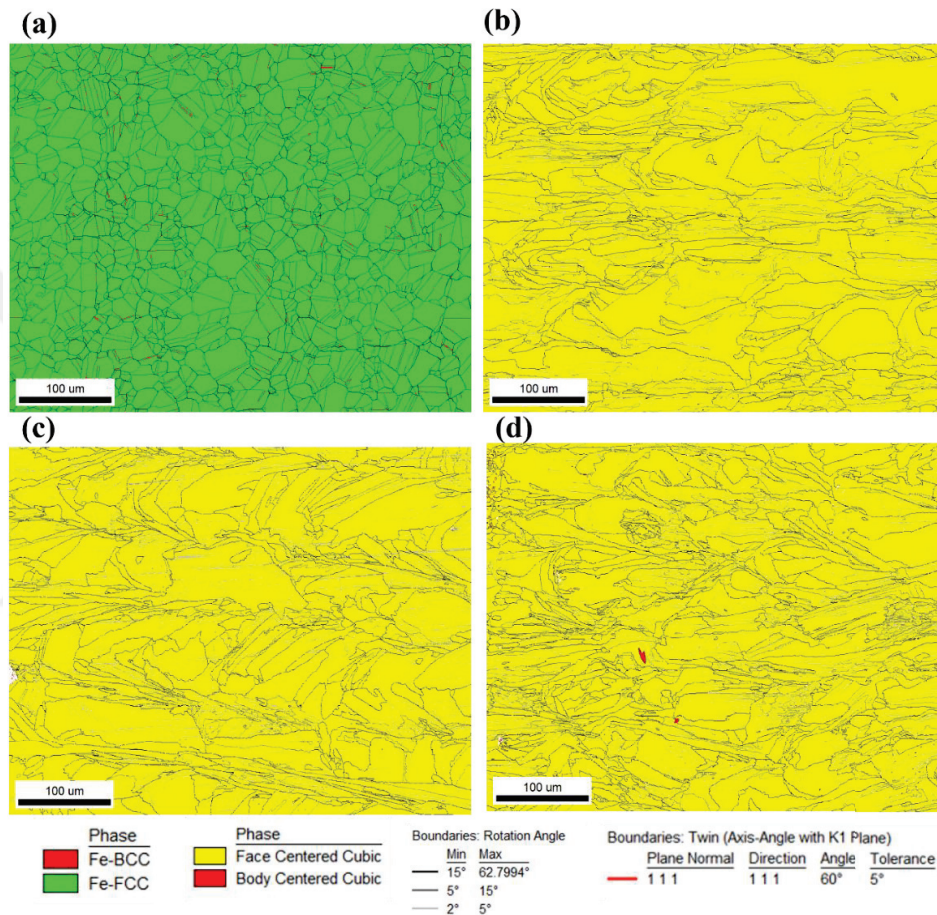


Figure 4.9. EBSD phase maps and grain boundary characteristics for (a) Conventional (b) 1B (c) 2B (d) 3B sample, respectively

Figure 4.10 shows the grain boundary lengths (in mm/mm²) based on the grain boundary types. As explained above, the total twin boundary length is 22.5 mm which is higher than SLM samples. These boundaries are formed due to the high cooling rates during the SLM process. Therefore, SLM samples showed higher values of ULAGBs.

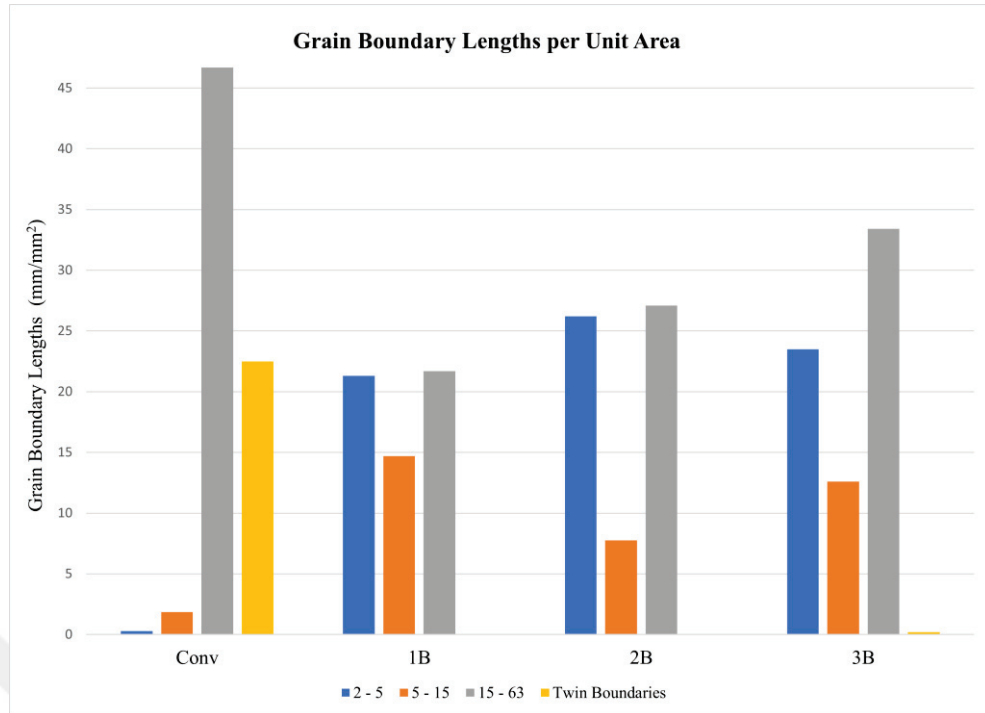


Figure 4.10. Histogram of grain boundary lengths for each sample (based on grain boundary types)

The Kernel average misorientation (KAM) value is calculated separately for each point in the EBSD map. After determining the misorientation angle of each point with its neighbors, the average of these angles is assigned to the calculated point. A threshold value is set to distinguish the angular differences at grain boundaries when calculating the KAM value.⁷² For the current data sets, this threshold value was taken as 5°.

Figure 4.11 shows misorientations lower than 5°. If the misorientation angle between two neighboring points exceeds 5°, it indicates the presence of a grain boundary, and this angular difference is not included in the KAM calculation. The KAM value is affected by crystallographic misorientations, such as dislocation density, as well as by metallographic sample preparation. The KAM values are observed to increase in SLM samples, particularly in the regions near grain boundaries between columnar grains.

In conventional 316L, Figure 4.11a, the microstructure predominantly exhibits zero misorientations, with only a small fraction of ULAGBs present, as previously mentioned above.

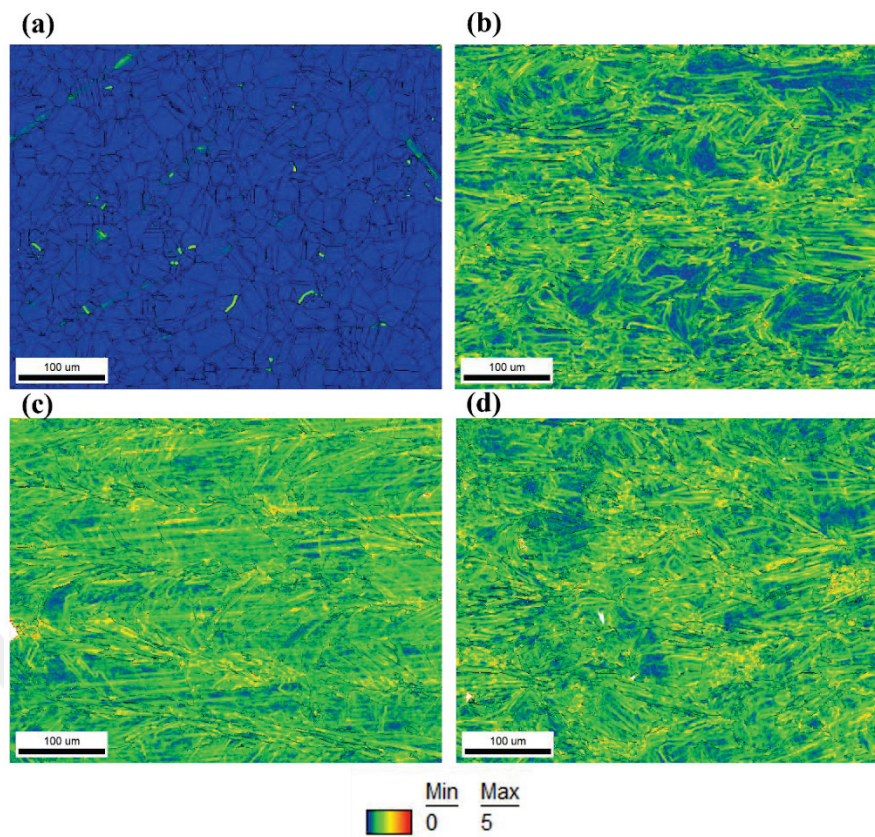


Figure 4.11. EBSD maps showing Kernel average misorientation (KAM) (a) Conventional (b) 1B (c) 2B (d) 3B sample, respectively

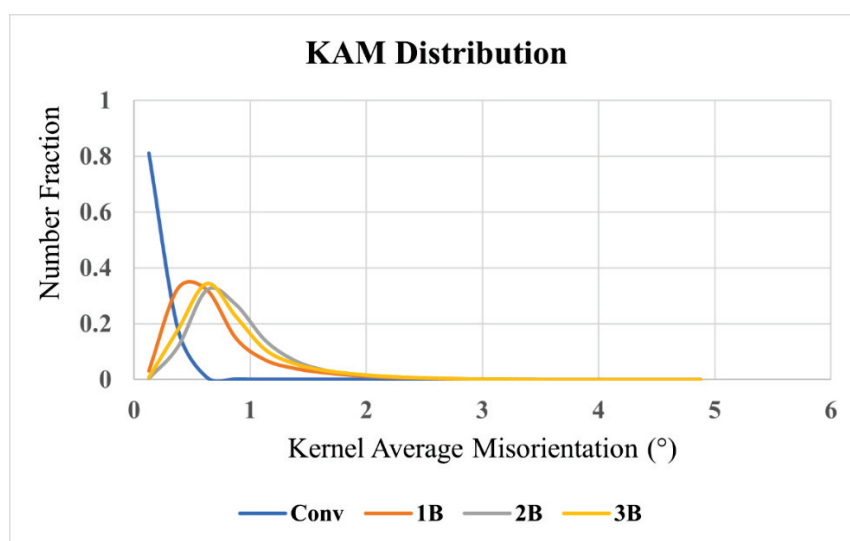


Figure 4.12. Kernel Average Misorientation (KAM) Distribution for Conv., 1B, 2B, and 3B samples

Figure 4.12 shows the KAM distribution plot against the number fraction. For the conventional sample, 81% of the misorientation angles are concentrated at 0.125° , which explains the prominent peak in the plot. For the SLM samples, more than 70% of the misorientation angles are less than 1° due to a high number of sub-grains coming from high cooling rates.

In order to calculate the grain orientation spread (GOS) value, the average orientation of the grain in the map is first determined. Subsequently, the difference between the orientation of each pixel within the grain and the average orientation of the grain is calculated. The average of these differences is then determined to create the mapping.

The EBSD maps showing GOS and high-angle grain boundaries of the samples are presented in Figure 4.13. Each color in the image represents a distinct grain. Due to rapid solidification, grains with high defect density or deformation exhibit high GOS values, whereas recrystallized grains with low defect density display lower GOS values. For conventional 316L, blue color grains represent the recrystallized grains during the annealing processes. Therefore, most of GOS values are close to zero as indicated in Figure part (a).

In the examined SLM samples, the fine and equiaxed grains located between the columnar grains have GOS values close to zero; these grains are represented in blue in Figure 14*b, c, d*. During the additive manufacturing process, heat input is applied to the lower layers as the upper layers are deposited, partially causing recrystallization in the lower layers due to heat affected zones. The blue-colored grains in the GOS maps are considered to have formed through this mechanism.

As shown in Figure 4.13*b*, the GOS value of 2B sample can reach up to 9 degrees, but most grains have lower GOS values compared to 1B and 3B samples. Using a rotational scanning strategy (as in the 1B and 3B samples) generates multiple thermal cycles, leading to overlapping melt pools and the epitaxial growth of grains. As a result, the deformation rates in the grains increase, which subsequently raises the GOS value. Therefore, most of grains for 1B, and 3B samples have higher GOS values than sample 1B. ^{73–75}

In addition, the blue areas in GOS maps represent dislocation-free regions, while the red areas indicate regions with high dislocation density. ⁷⁶

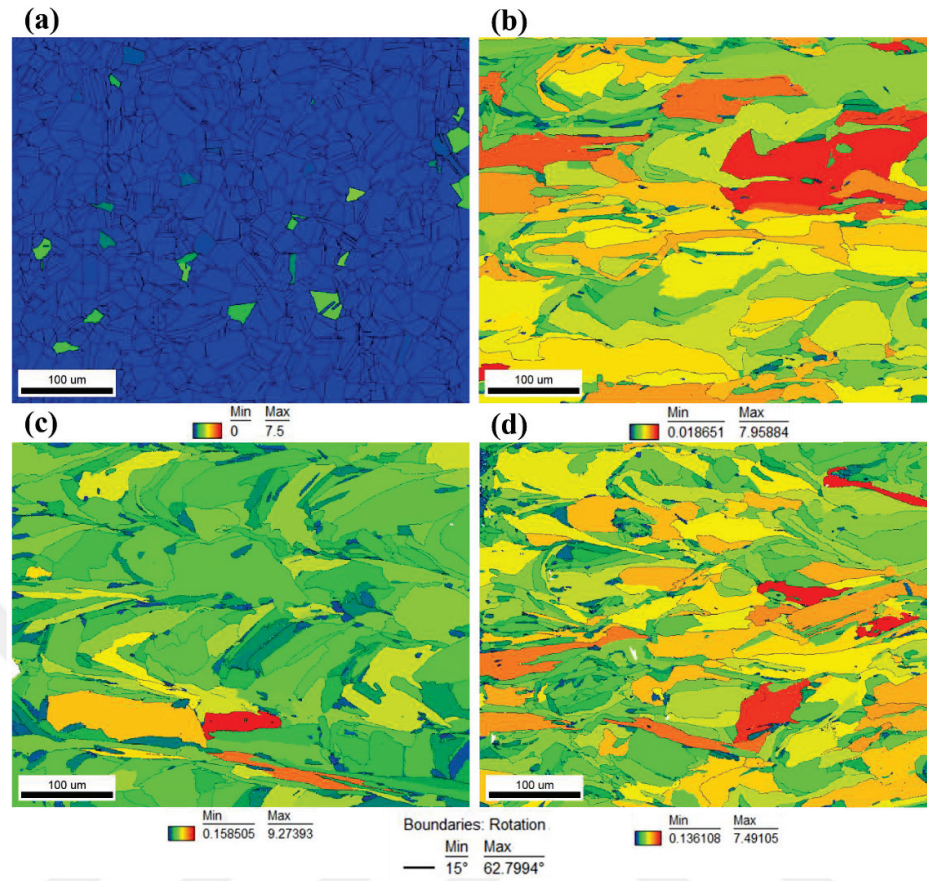


Figure 4.13. EBSD maps showing grain orientation spread (GOS) and high-angle grain boundaries (a) Conventional (b) 1B (c) 2B (d) 3B sample, respectively

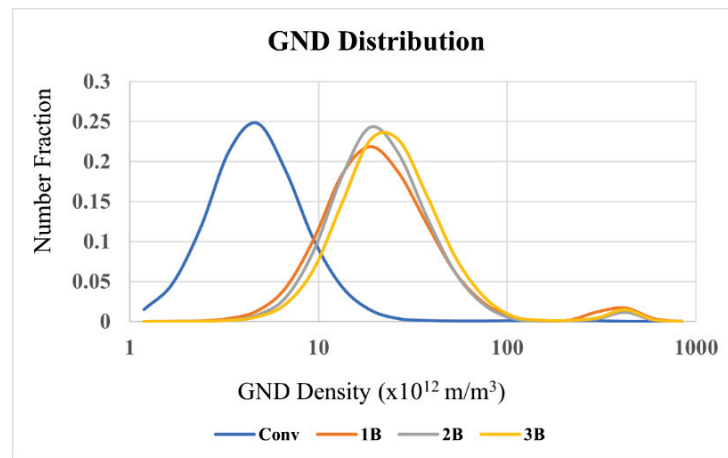


Figure 4.14. Geometrically necessary dislocation (GND) distribution for Conv., 1B, 2B, and 3B Samples

Crystalline materials have two primary types of dislocations: geometrically necessary dislocations (GNDs) and statistically stored dislocations (SSDs). SSDs are stored during plastic deformation without causing lattice rotation. In contrast, GNDs can be quantitatively measured using the EBSD technique.^{67,68} Geometrically necessary dislocations contribute to the work-hardening mechanism, enhancing the material's strength while preventing cracking.⁷⁷ GND distributions were shown in Figure 4.14. SLM samples demonstrate similar distributions with smaller differences compared to conventional ones. The cellular structures (sub-grains) formed during the additive manufacturing process play a role in trapping dislocations at the walls of these sub-grains. Additionally, these ultra-low angle grain boundaries (sub-grains) exhibit high local misorientation angles, resulting in a higher density of geometrically necessary dislocations (GNDs) in SLM samples.⁷⁸ As shown in the Figure 4.11a KAM angles lower resulted in lower GNDs values for the conventional 316L sample.

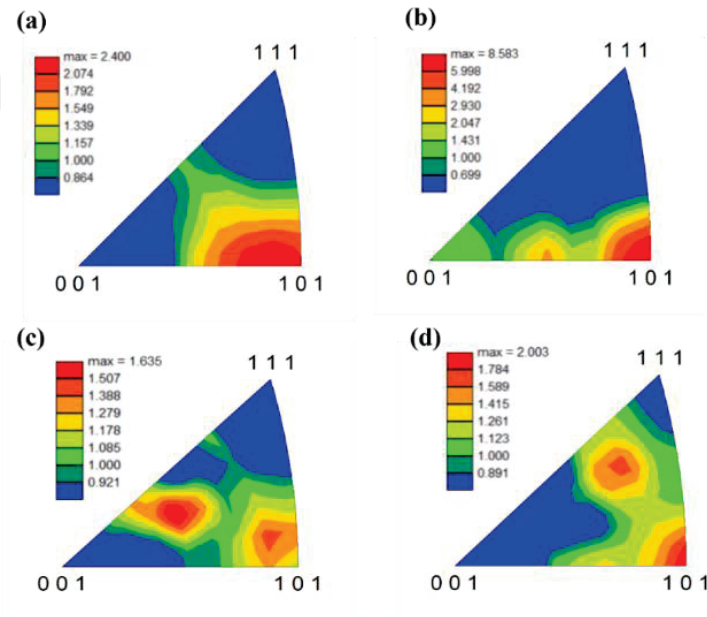


Figure 4.15. Inverse pole figures of (a) Conventional (b) 1B (c) 2B (d) 3B sample

As explained in Chapter 2, to determine the crystallographic texture, inverse pole figure (IPF) can be used to reveal the crystallographic directions based on the reference

plane.⁶⁷ The intensity of texture can be calculated based on multiples of random distribution (MRD) value obtained from IPF.⁷⁹ The reference directions are the normal direction for conventional samples and the building direction for SLM samples, as shown in Figure 3.3.

IPF for each sample is shown in Figure 4.15. In part (a), for the conventional 316L sample, a $\langle 110 \rangle // \text{ND}$ texture was observed, with an MRD value of 2.40. In the 1B sample, the $\langle 110 \rangle // \text{BD}$ texture has the highest texture intensity among all samples, with an MRD value of 8.5. In the 3B sample, although the $\langle 110 \rangle // \text{BD}$ texture is apparent, its texture intensity is lower than that of the 1B sample, with an MRD value of 2.0. However, for the 2B sample, the texture is more random compared to the other samples.

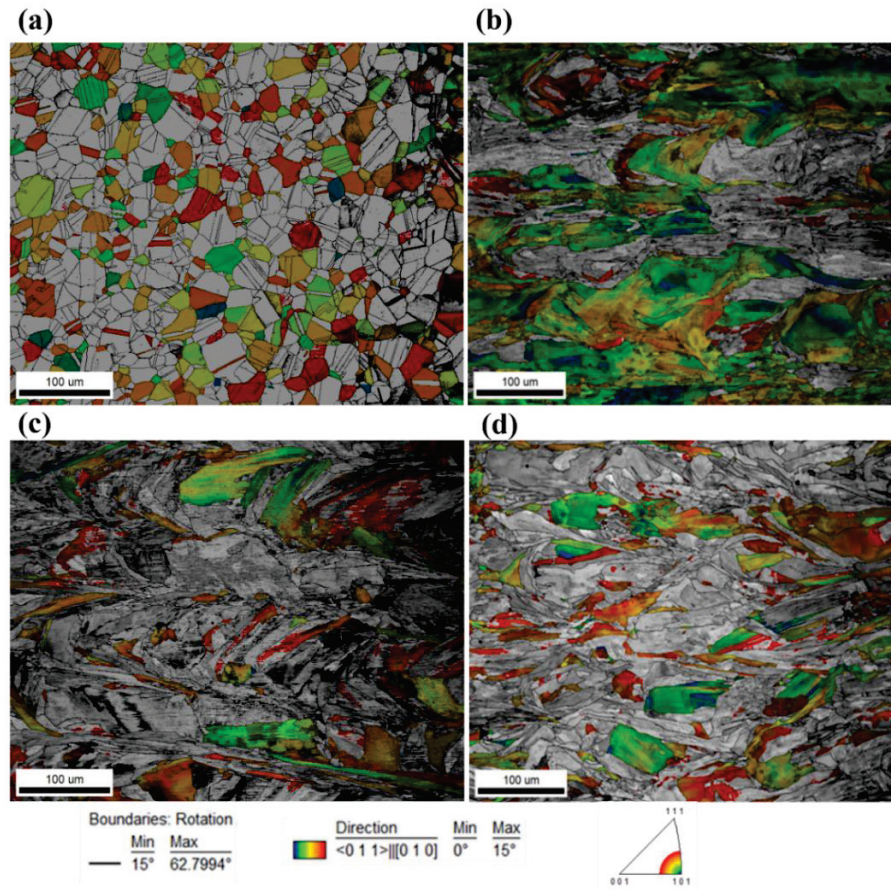


Figure 4.16. EBSD texture component maps showing $\langle 110 \rangle // \text{ND}$ for (a) Conv. and $\langle 110 \rangle // \text{BD}$ for (b) 1B (c) 2B (d) 3B samples. Maximum 15° from ideal orientation is shown, where rainbow colors indicate the degree of deviation from ideal

The EBSD maps in Figure 4.16 show the grains with $\langle 110 \rangle$ orientation parallel to the normal direction (ND) (for part (a) and building direction (BD) (for part (b), (c), and (d)) within a tolerance of 15° and the HAGBs of the produced samples. In these maps, the rainbow colors within the grains indicate the angular deviation from the ideal orientation, while non-colored grains do not exhibit the specified $\langle 110 \rangle$ orientation.

The $\langle 110 \rangle // \text{ND}$ texture was observed at 37.8% of the mapped area of the conventional sample. For SLM samples, the area fraction of this orientation was determined to be 62.7% in the 1B sample, 21.6% in the 2B sample, and 27.4% in the 3B sample. These results are consistent with the inverse pole figures.

4.3. Hardness Results of SLM and Conventionally Produced 316L Stainless Steel

Vickers hardness results were given in Figure 4.17. Conventional 316L stainless steel has an average hardness of 145.6 ± 4.4 . The average hardness for the 1B sample was 240.6 ± 19.3 HV, while the 3B sample had a slightly lower value of 238.7 ± 6.6 HV. The sample with the lowest average hardness value, among SLM samples, was 225.8 ± 10.5 HV for the 2B sample.

Compared to conventional one the hardness values for materials produced via selective laser melting (SLM) are higher. Although the grain sizes were smaller in the conventional sample, higher HV values were observed in the samples produced through additive manufacturing. This is attributed to the presence of sub-grain structures in SLM samples, formed due to the high cooling rates, with sizes ranging from 400 nm to 1.5 μm , which enhance the hardness values.²³

Porosity, microstructure, and laser energy density are factors affecting hardness value in additive manufacturing methods. It was observed that as the energy density increases, the hardness value also rises, aligning with findings in the literature.^{23,80,81} Within the energy density range of 50-120 J/mm³, a direct proportionality was identified. However, studies indicate that outside this range, an increase in energy density does not consistently lead to higher hardness.

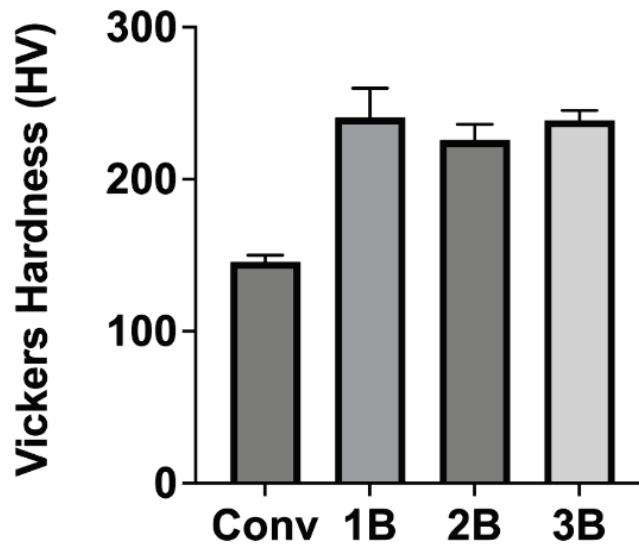


Figure 4.17. Vickers hardness (HV) results for all samples

For materials produced at low power values, such as the 3B sample at 107 W, insufficient melting may occur, resulting in a highly porous structure and reduced hardness. Conversely, at power values above 200 W, as seen in the 2B sample at 250 W, keyhole gas porosity may develop, further decreasing hardness.³⁹ As consistent with the literature, the 1B sample, with a laser energy density of 100-120 J/mm³, exhibited the highest hardness value.

In the microhardness test, surface relief marks around the indentations are shown in Figure 4.18. The sliding of atoms during plastic deformation is called slip.¹¹ There are few slip lines for the conventional sample (indicated by blue arrows).

As mentioned before, the dislocation density value lowest for conv. sample, therefore fewer slip lines occurred. In parts (b), and (d), the intersection of slip lines in different orientations is visible (indicated by red arrows), and 1B and 3B sample hardness values are higher than other samples. In part (c), the distances between the slip bands are smaller than those in the other examples. This may be due to easier local plastic deformation caused by the lower hardness value for the 2B sample, also GND value was lower than both 1B and 3B samples.

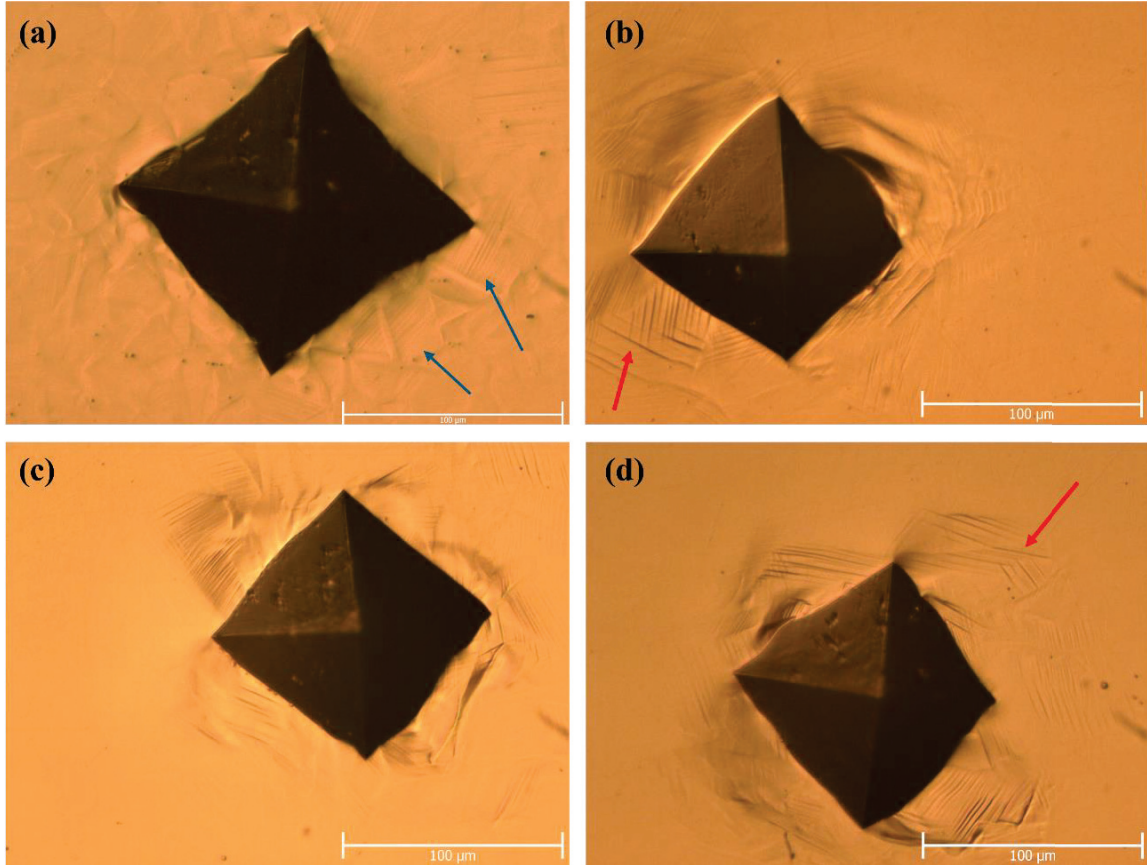


Figure 4.18. Optical microscopy indentation images of (a) Conventional (b) 1B (c) 2B (d) 3B sample

4.4. Contact Angle Results of SLM and Conventionally Produced 316L Stainless Steel

The sessile drop contact angle method was used in this part. The contact angles were measured by fitting tangents to both the right and left sides of drop⁸². Contact angle measurements are shown in Figure 4.19. The conventional sample had an average contact angle of $85.8 \pm 4.1^\circ$. For the 1B sample, the average contact angle was $74.2 \pm 6.7^\circ$, for the 2B sample, it was $82.9 \pm 4.4^\circ$, and for the 3B sample, it was $63.9 \pm 4.9^\circ$. These results indicate that all the materials exhibit hydrophilic properties. Among the three samples, the 3B sample has the highest wettability. The microstructural properties of the materials significantly affect the contact angle values.⁸³

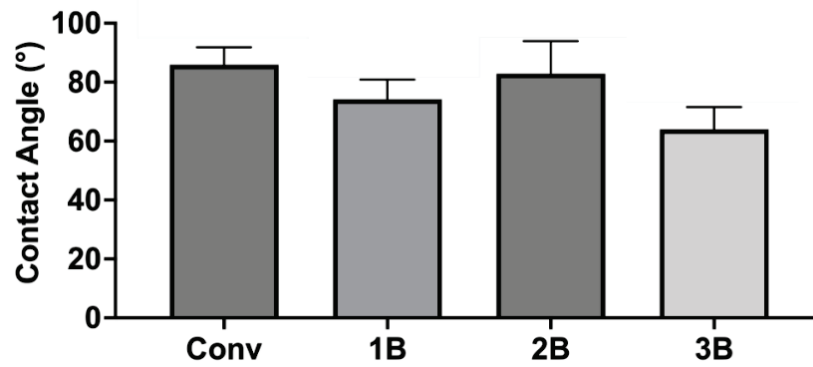


Figure 4.19. Contact angle results for all samples

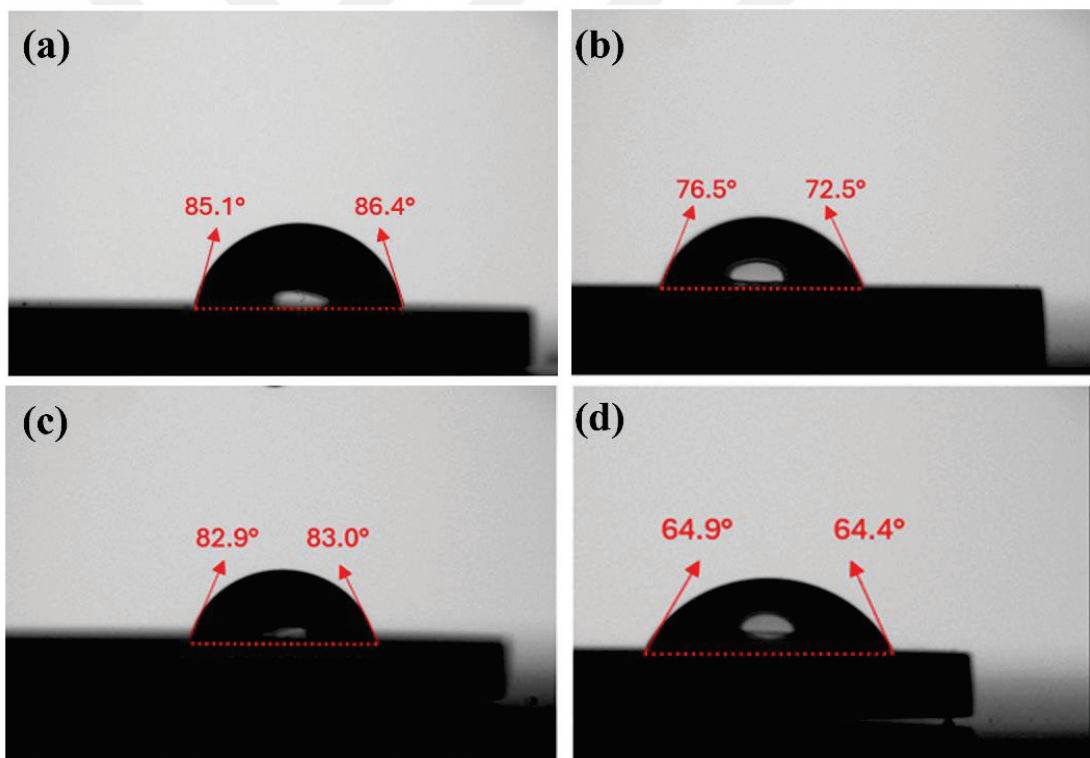


Figure 4.20. Average contact angle droplet images of (a) Conventional (b) 1B (c) 2B (d) 3B sample

In additive manufacturing, properties such as grain shape and grain size of the materials vary depending on the production parameters. Therefore, the fact that these

three materials were produced from the same powder but with different production values may be one of the factors causing this difference. Based on the microstructural examinations, the defects found in the 2B and 3B samples (unmelted powders and formed cracks) should also be considered for their potential impact on these results.

4.5 Cell Attachment Results of SLM and Conventionally Produced 316L Stainless Steel

The Figure 4.21 shows the cell viability of MC3T3 pre-osteoblast cells on days 1, 3, and 7. Cell viability increased even during the initial days. Although a slight decrease was observed in the 3B sample, no significant differences were found among the SLM samples between days 3 and 7 (based on the t-test). This decrease in the 3B sample may be attributed to the defects present in the material.

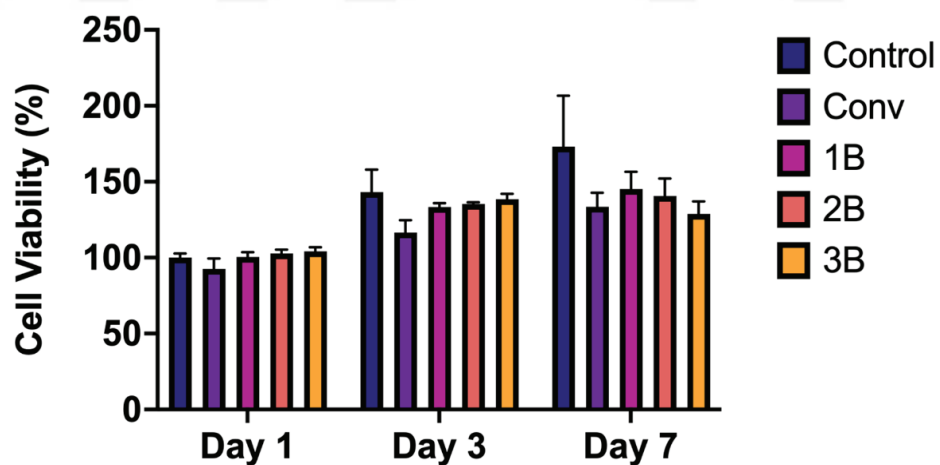


Figure 4.21. Cell viability results by days (Normalized to day 1 control)

The fluorescence microscopy image Figure 4.22 shows the spreading of actin filaments and nuclei. F-actin is one of the main components of the cytoskeleton and is

responsible for cell spreading and movement. Phalloidin stains the actin filaments, highlighting their spreading, while DAPI stains the nuclei, indicating the presence of cells. When comparing cellular spreading and nucleus densities, no significant differences were observed. Compared to the control, all samples were found to be biocompatible.⁸⁴

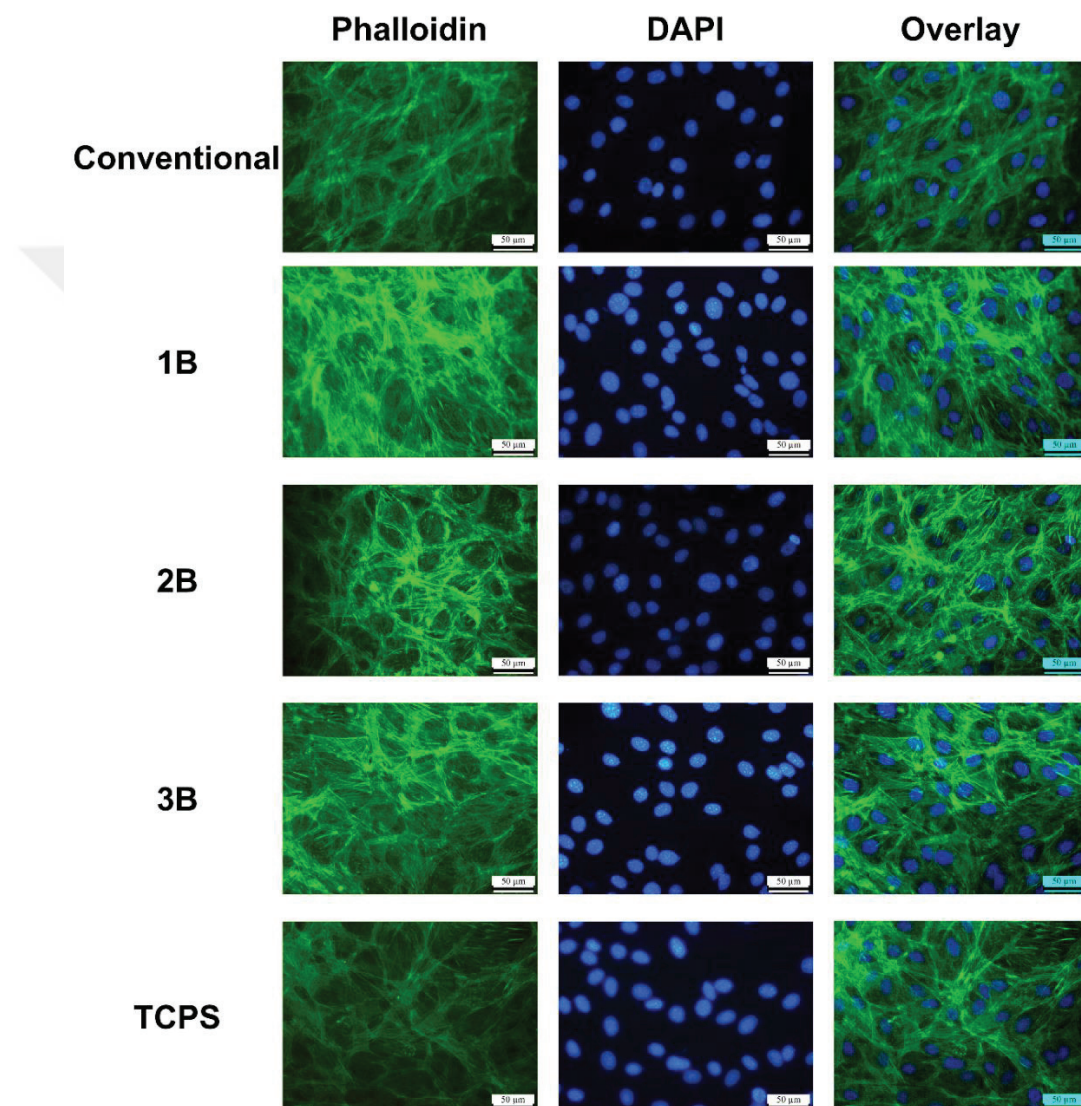


Figure 4.22. Fluorescence microscopy images showing F-actin (Phalloidin) and nuclei (DAPI) staining, along with their merged images, on day 7

CHAPTER 5

CONCLUSION

In this thesis, the effects of processing parameters on the material properties and biocompatibility of 316L stainless steel produced using the selective laser melting (SLM) method were thoroughly investigated. The microstructure, mechanical properties, surface wettability, and biocompatibility performance of the samples produced by the SLM method were evaluated and compared with 316L stainless steel produced by the conventional method. The differences among the samples produced using three different scanning strategies were also examined, particularly concerning the effects of volumetric energy density (VED) and scanning strategies.

Optical microscopy and SEM images revealed that there were significant differences between the microstructures of conventional and SLM samples. Due to the high cooling rates of the SLM process, the samples exhibited sub-grain structures, which significantly enhanced hardness values. The highest hardness value was observed in the 1B sample produced by the SLM method, with a hardness of 240.6 ± 19.3 HV. The results showed that the microstructure is significantly influenced by the scanning strategy. Epitaxial grain growth was observed due to remelting zones. Variations in production parameters also affected the microstructural properties of the SLM samples, such as differences in melt pool sizes and sub-grain sizes. In the 3B sample, which had the lowest laser power (107 W), unmelted powder particles were observed within the structure compared to the other samples.

According to EBSD analyses, a high amount of twin boundaries was observed in the conventional sample due to the recrystallization process. In contrast, a significant proportion of ULAGBs (ultra-low angle grain boundaries) was observed in the SLM samples. Based on the IPF results, Area fraction of grains having $\langle 110 \rangle // \text{ND}$ orientation were 37.8% for the conventional sample, while the 1B sample demonstrated the highest texture intensity, with a 62.7% $\langle 110 \rangle // \text{BD}$, indicating its anisotropic properties. On the other hand, the 2B and 3B samples exhibited a more random texture, area fraction of grains having $\langle 110 \rangle // \text{BD}$ were 21.6% and 27.4 %, respectively. Since $\langle 110 \rangle$ is the

primary slip direction in FCC materials, it contributes to ductility, suggesting the potential for improved mechanical properties.

Surface wettability tests indicated that samples produced by the SLM method exhibited higher hydrophilic properties, positively influencing biocompatibility. Cell culture test results showed that cell proliferation was higher in the samples produced by the SLM method compared to those produced by conventional methods. However, no significant differences were observed among the SLM samples themselves. The direct effect of crystallographic texture could not be determined at this stage, but the absence of any negative impact is also a critical factor.

In conclusion, it was determined that 316L stainless steel produced by the SLM method offers good mechanical and biocompatible properties compared to those produced by the conventional method, making it more suitable for biomedical implant applications. This study provides an essential foundation for optimizing implant materials produced using the SLM method. Future studies could explore the effects of different combinations of laser power, scanning strategies, and energy density to expand the use of this method in biomedical applications. In this context, SLM technology shows significant potential for utilizing anisotropic material properties in customized implant designs.

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