

Achieving biomimetic porosity and strength of bone in magnesium scaffolds through binder jet additive manufacturing

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ABSTRACT

Magnesium (Mg) alloys are a promising candidate for synthetic bone tissue substitutes. In bone tissue engineering, achieving a balance between pore characteristics that facilitate biological functions and the essential stiffness required for load-bearing functions is extremely challenging. This study employs binder jet additive manufacturing to fabricate an interconnected porous structure in Mg alloys that mimics the microporosity and mechanical properties of human cortical bone types. Using scanning electron microscopy, micro-computed tomography, and mercury intrusion porosimetry, we found that the binder jet printed and sintered (BJPS) Mg—Zn—Zr alloys possess an interconnected porous structure, featuring an overall porosity of 13.3 %, a median pore size of 12.7 μm , and pore interconnectivity exceeding 95 %. The BJPS Mg—Zn—Zr alloy demonstrated a tensile strength of ~ 130 MPa, a yield strength of ~ 100 MPa, an elastic modulus of ~ 21.5 GPa, and an ultimate compressive strength of ~ 349 MPa. These values align with the ranges observed in human bone types and outperform those of porous Mg alloys produced using the other conventional and additive manufacturing methods. Moreover, the BJPS Mg—Zn—Zr alloy showed level 0 cytotoxicity with a greater MC 3T3-E1 cell viability, attachment, and proliferation when compared to a cast Mg—Zn—Zr counterpart, since the highly interconnected 3D porous structure provides cells with an additional dimension for infiltration. Finally, we provide evidence for the concept of using binder jet additive manufacturing for fabricating Mg implants tailored for applications in hard tissue engineering, including craniomaxillofacial procedures, bone fixation, and substitutes for bone grafts. The results of this study provide a solid foundation for future advancements in digital manufacturing of Mg alloys for biomedical applications.

1. Introduction

An ideal synthetic bone tissue substitute should have five significant features: adequate mechanical properties, desired bioactivity, suitable interconnected porosity, biocompatibility, and degradability [1]. These are the goals of third-generation biomaterials developed through the use of three metals [magnesium (Mg), iron (Fe), and zinc (Zn)]. Among these metals, Fe offers the easiest manufacturability; Zn has the closest corrosion rate to the ideal degradation value, and Mg provides the best biocompatibility and the most comparable mechanical properties to

human bone [2]. Mg is the fourth most prevalent mineral in the human body. More than half of the total Mg content in our body is stored in our bones. Additionally, the unique osteopromotive property of Mg during bone regeneration is well known [3–6]. These characteristics make Mg a key biodegradable metal, especially for orthopedic applications [7,8]. As such, researchers have long studied the technologies behind the use of Mg for biomedical applications, ranging from developing new alloys and surface modification techniques to assessing in vitro and vivo degradation behavior and clinical translation [9–13].

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The design and fabrication of bone scaffolds that closely replicate the biomechanical characteristics of native bones remain among the most critical challenges in regenerative orthopedic medicine. Porous scaffolds are particularly favored due to their customizable porosity and stiffness, which can be adjusted to meet specific requirements. The interconnectivity of pores is crucial for enhancing permeability and diffusion, thereby promoting vascularization within the scaffold. However, increasing porosity compromises mechanical strength, necessitating a delicate balance between porosity and mechanical integrity in bone scaffold design. Traditional manufacturing techniques, such as casting and conventional powder metallurgy, have been extensively explored for fabricating porous Mg structures [14–18]. While these methods have their merits, they also present significant limitations. For example, the use of salts or space-holder materials to create pores can introduce impurities, particularly in highly reactive Mg alloys. Moreover, these conventional techniques are generally limited to producing porous structures with simple geometries, and they offer restricted control over pore characteristics.

Additive manufacturing (AM) presents a promising alternative for fabricating geometrically complex bone scaffolds with intricate porous architectures, addressing many of the challenges associated with traditional methods towards fabricating the ideal synthetic bone substitute with all of the above criteria [1]. However, the technology readiness level of AM for Mg alloys is still relatively immature compared to other metallic systems such as steel, titanium, aluminum, and nickel alloys. This is largely due to the low boiling point and high reactivity of Mg. While laser powder bed fusion dominates the AM of Mg alloys [19], binder jetting offers a less challenging approach by fabricating a green part at near-ambient temperatures, followed by sintering at elevated temperatures. Previous studies have developed a process flow for binder jetting of Mg powder, utilizing capillary-mediated assembly to create a green part [20–22], which is then sintered in conventional or microwave furnaces [23–25].

In prior research on AM of Mg alloys for biomedical applications, lattice structures with dense struts, typically with diameters $>500\text{ }\mu\text{m}$, have been fabricated using laser powder-bed fusion and material extrusion techniques [26–29]. While binder jetting has successfully produced Mg scaffolds with interconnected micron-sized porosity, these structures have often lacked the mechanical integrity, particularly tensile strength, required for practical applications due to the high sensitivity of tensile strength to porosity percentage [21,23]. The present study explores the use of binder jet AM in fabricating a porous structure in Mg alloys that mimics the microporosity and mechanical properties of human cortical bone. We examined the microstructure of a binder jet printed and sintered (BJPS) Mg–Zn–Zr alloy with scanning electron microscopy (SEM), along with its pore characteristics assessed through micro-computed tomography (μCT) and mercury intrusion porosimetry. We also evaluated the mechanical performance of fabricated scaffolds through tensile and compression testing. An *in vitro* biological assessment and metallic ions release of BJPS scaffolds and conventionally cast Mg was conducted, providing evidence for the viability of using this AM technique to digitally manufacture biodegradable Mg implants tailored for hard tissue engineering applications, including craniomaxillofacial procedures, bone fixation, and as substitutes for bone grafts.

2. Materials and methods

2.1. Fabrication of Mg samples

Mg powder with the measured chemical composition of Mg–5.49 wt% Zn–0.17 wt% Zr, was sourced from International Laboratory, USA. The powder was sieved to remove particles smaller than $32\text{ }\mu\text{m}$. Fig. 1a shows the morphology of the sieved powder having a relatively uniform particle size with a spherical shape. The D10, D50, and D90 (percentile) of Mg–Zn–Zr powder were determined to be $46.49 \pm 0.03\text{ }\mu\text{m}$, $62.59 \pm 0.06\text{ }\mu\text{m}$ and $84.95 \pm 0.10\text{ }\mu\text{m}$, respectively (Fig. 1b). The elimination of fine powder in the powder feedstock prevents smaller particles from filling the gaps between larger particles as the feedstock is spread over the powder bed during binder jetting. Furthermore, this results in a more consistent interparticle spacing between neighboring particles (Fig. 1c). The powder density was determined using a gas pycnometer and found to be $1.8252 \pm 0.0008\text{ g}\cdot\text{cm}^{-3}$.

The formulation of binders or solvents (ink materials) is crucial for the success of binder-jet printing, as the ink influences both the printing parameters and the subsequent post-printing processes. An effective ink must exhibit several key characteristics in addition to the correct formulation, including appropriate rheological properties for consistent jettability by the printhead and suitable wettability to ensure proper interaction with the powder bed. For example, commercial binder jetting systems typically employ furan, phenolic, silicate, and aqueous-based binder materials. A more detailed discussion of binder material classifications and the stages of binder development can be found in [30]. In this study, we used a single-phase ink, free of binder or solute, formulated specifically for Mg alloys. This ink was designed to interact with the MgO film on the surface of Mg powder, facilitating the formation of solid interparticle bridges [21].

The fabrication of Mg samples through binder jetting and sintering follows those of previous studies [20–25]. In brief, the powder was fed into a custom binder jet 3D printer. The print head deposited a specially formulated ink on to $100\text{ }\mu\text{m}$ -thick layers of powder. Subsequently, the as-printed objects were retrieved from the powder bed by gently dusting away the unbound powder. A rectangular crucible with a tight lid was used for sintering of as-printed samples. Sintering took place in a tube furnace with the temperature set at $615\text{ }^{\circ}\text{C}$ under an argon atmosphere. A separate set of bulk Mg–Zn–Zr samples with the same composition was obtained by gravity casting to serve as a reference for comparison with BJPS samples. Mg–Zn–Zr ingots were heated to $750\text{ }^{\circ}\text{C}$ before pouring the molten metal into rectangular molds.

2.2. Characterization of the as printed and sintered Mg samples

The density of the sintered Mg samples was determined using the Archimedes method, as per ASTM B962 standards. To ensure reproducibility, the measurement results were collected from five different specimens. Mercury intrusion porosimeter (Micromeritics, AutoPore IV 9500 Series) was utilized to characterize the pore size distribution of the BJPS samples. Mercury was introduced into the specimens at varying pressure from 0.55 to 6878 psia. The pore characteristics of BJPS samples were further analyzed by X-ray computed tomography (Phoenix v|tome|x m). The tomograms had a voxel size of $1\text{ }\mu\text{m}$ and were obtained using a nanofocus tube.

For microstructural examinations, BJPS samples were cut, following by systematic surface grinding, and polishing to

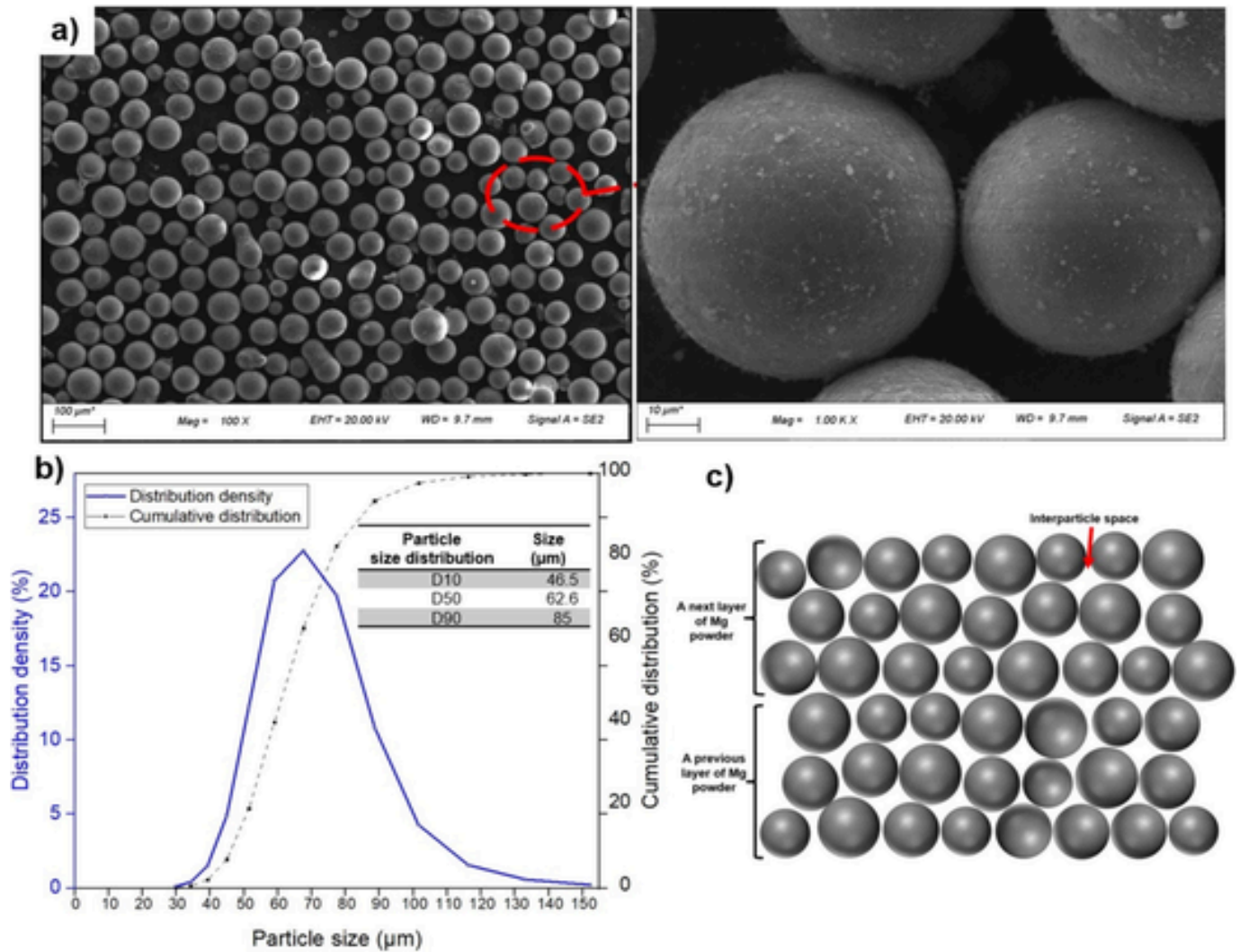


Fig. 1. Characterization of the Mg—Zn—Zr powder used for fabrication of Mg samples. a) Morphology of the Mg powder by SEM, b) particle size of the Mg powder obtained by laser diffraction sizing, and c) a schematic illustration of Mg powder stack-up in binder-jet printed samples.

achieve a surface finish of 0.05 µm while using a non-aqueous based lubricant (DP-Lubricant Yellow, Struers). The cross-sections of samples were characterized using optical microscope (Leica DM 4000 M) and FE-SEM (Zeiss EV O-50).

The tensile (ASTM E8/E8M-22) and compression (ASTM E9-09) testing of the samples were conducted using a universal testing machine (Instron 5982). For tensile testing, four cylindrical specimens with gauge length of 24 mm and diameter 6 mm were prepared via machining from BJPS cylindrical rods. For compression tests, five cylindrical samples of length 12 mm and diameter 8 mm were prepared via machining.

2.3. Assessment of metallic ion release

To evaluate the release of ions from the alloys, samples were submerged in phosphate-buffered saline (PBS) for 1, 3, and 5 days under cell culture conditions of 37 °C and 5 % CO₂, with a surface area-to-volume ratio of 1.25 cm²/mL. The PBS solutions were then analyzed for ion content using an inductively coupled plasma-optical emission spectrometer (ICP-OES). This approach allowed for the accurate determination of the concentrations of ions released by the alloys at each time interval. To calcu-

late the average release rate, the total amount released was divided by the total time period.

2.4. In vitro biological assessment

2.4.1. Cell viability assay

Cell viability was conducted in accordance with ISO 10993 using MC 3T3-E1 Subclone 14 (ATCC, CRL-2594™) preosteoblast cells. The cells were cultured under physiological conditions (37 °C in a humidified atmosphere with 95 % humidity and 5 % CO₂). The culturing media used consisted of an alpha-minimum essential medium (α-MEM) supplemented with 10 % fetal bovine serum (FBS), 100 µg/mL streptomycin, and 100 U/mL penicillin. BJPS and cast pellets of diameter 5 mm and length 2 mm were sterilized by immersion in acetone and ethanol solution followed by UV irradiation. The pellets were then immersed in α-MEM containing 10 % FBS and 1 % penicillin/streptomycin. The extraction medium had a surface area-to-volume ratio of 1.25 cm²/mL. The pellets were incubated under the physiological conditions for 24 h. Cells were incubated in a 96-well culture plate at a density of 5 × 10³ cells/well for 24 h to allow cell attachment. Then, the media were replaced by 100 µL of alloy ex-

tracts. The negative control consisted of culture media with cells, whereas the positive control involved culture media with 10 % dimethyl sulfoxide (DMSO). Following incubation for 1, 3, and 5 days, the extracts were substituted with fresh culture media. Subsequently, 10 μ L of Cell Counting Kit-8 was added to each well and allowed to incubate under physiological conditions for 1 h. The spectrophotometric absorbances of each well were measured using a microplate reader (Dojindo) at a wavelength of 450 nm. Values of the optical density for the well plate reader were then determined to calculate the percentage of viable cells ($p < 0.05$; $n = 3$).

The adhesion, morphology, and distribution of the MC 3T3-E1 cells seeded on pellets incubated for 1, 3 and 5 days were observed with an SEM (JSM-6701F FE G SEM). The samples were rinsed with a phosphate buffer saline. The cells were fixed in 2.5 % glutaraldehyde at 4 °C for 2 h, then treated with 0.1 % osmium tetroxide, and subjected to dehydration in graded concentrations of ethanol (25 %, 50 %, 75 %, 95 %, and 100 %). Finally, the samples underwent gold sputtering to examine under SEM. For cellular live-dead staining test, samples were stained with propidium iodide (for staining dead cells red), and fluorescein diacetate (for staining live cells green). Images were then obtained with a fluorescence microscope (Leica DM RB).

2.4.2. Cytotoxicity assays

The activity of the lactate dehydrogenase enzyme (LDH) served as an indicator to assess cytotoxicity in the culture media. Pure cell culture media containing cells were employed to maximize LDH release. The LDH assay was conducted using the Cytotoxicity One reagent (Promega). 11 mL of assay buffer was added to 1 vial of substrate to form the working solution. MC 3T3-E1 cells were seeded in a 96-well culture plate at a density of 8×10^3 and incubated under the physiological conditions for 24 h to reach desired confluency. After the incubation period, the plates were allowed to cool to room temperature. 100 μ L of Cytotoxicity One reagent was added to all the wells containing the sample extracts. The fluorescence readings were captured using an excitation wavelength of 560 nm and an emission wavelength of 590 nm ($p < 0.05$; $n = 3$). The percent cytotoxicity of each well was calculated using Eq. 1. The results were found to be statistically significant ($p < 0.05$; $n = 3$).

$$\text{Percent cytotoxicity} = \frac{(\text{Experimental value} - \text{Blank control})}{(\text{High control} - \text{Blank control})} \times 100 \quad (1)$$

2.4.3. Cytoskeleton staining

MC 3T3 cells were seeded at a density of 50 K/ml on above prepared samples. After a 48-h incubation period, the cells were fixed with 4 % paraformaldehyde (PFA) (Sigma-Aldrich) and washed with PBS. The cells were then blocked in 1 % bovine serum albumin (BSA) (Sigma-Aldrich) and subsequently stained with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) and phalloidin (Invitrogen) according to manufactures' instructions. Images were captured using a Leica DM i8 microscope (Leica DM RB).

3. Results and discussion

3.1. Density and microstructure

The density of the BJPS Mg sample determined by the Archimedes' principle is $1.58 \pm 0.01 \text{ g cm}^{-3}$. The relative density (i.e., the ratio of the Archimedes density to the density of powder measured by gas pycnometer) and overall porosity (i.e., the total sum of open and closed pores) for the BJPS sample were 86.75 % and 13.25 % respectively. The density determined for

the BJPS Mg sample (1.58 g cm^{-3}) is close to the reported density of human bones, such as those of cortical ($1.8\text{--}2.0 \text{ g cm}^{-3}$) and cancellous bone ($1.0\text{--}1.4 \text{ g cm}^{-3}$) [31]. While the influence of the mass difference between implants and bones is not well understood, a low weight for orthopedic implants is often seen as desirable. It has been shown that in the case of prostheses, a heavier perception of weight can induce psychological dissatisfaction [32]. A lightweight implant is psychologically beneficial to the patient, especially after post-surgery trauma. A lower implant mass also causes a lower load on the muscular system.

Fig. 2a and b shows the SEM micrographs of as-printed and as-sintered Mg samples. The as-printed sample had a relative density of 53.6 ± 1.0 %. This value is close to the atomic packing density of a simple cubic unit cell crystal structure (i.e., 52.4 %). In this unit cell, atoms are situated next to each other analogously in all three dimensions to form large diamond-shaped spaces with an interstitial site size of 0.732 times the atom radius. Free-flowing spherical powder particles can stack up similarly during the binder jetting process (Fig. 1c). Given the particle size distribution of Mg powder in terms of D10, D50, and D90, the sizes of interparticle space in the as-printed sample is expected to be in the range of 17 to 31 μ m. These interparticle spaces and Mg particles shrink during super-solidus liquid phase sintering of as-printed samples, by which sinter necks develop among Mg particles [23,25]. This is also seen in the cross-sectional optical micrograph of sintered sample (Fig. 2c).

3.2. Pore characteristics

The pore characteristics of the BJPS sample were further analyzed by using μ CT and mercury porosimetry. Fig. 3a shows its representative tomogram along the build direction, and Fig. 3b displays the representative tomogram perpendicular to the build direction. The tomograms indicated a uniform porous microstructure throughout the BJPS sample without inter- and intra-layers zones in both directions. This homogeneity originates from an optimized permeation of binder materials in both the lateral and vertical directions during the build process and the nature of binder jetting to stack up powder layers without using compaction or heat. This isotropic as-printed Mg sample is then transformed into a homogeneous structure during the sintering process, as discussed in previous studies on developing binder jetting and sintering processes for Mg alloys [21,23–25]. Achieving a microstructure in Mg alloys as homogeneous as that obtained through binder jet AM presents a greater challenge when employing fusion-based additive manufacturing processes. In these processes, microstructure inhomogeneities within fabricated parts are caused by distinct gradients of localized heating and cooling resulting from the multiple steps of melting and solidification. For example, in wire arc AM, the continuous exposure of Mg substrates to a complex thermal cycle during the layer-by-layer deposition leads to structural segregations [33]. Similarly, the formation of melt zone and heat-affected zone during laser powder-bed fusion of Mg alloys results in substantially segregated and precipitated phases and, therefore, microstructural variations [34]. These microstructural differences can negatively influence the obtained properties of fusion-based AM fabricated Mg specimens, especially their corrosion performance [34].

In the computational analysis using software to compute the overall porosity and the interconnectivity of the pore channels of the BJPS sample, the interconnected open pores were differentiated from isolated/closed ones, as visualized by the red and blue colors in the 3D reconstructed μ CT tomogram (Fig. 3c). The ratio of the volume of the open pores to the total pore volume pro-

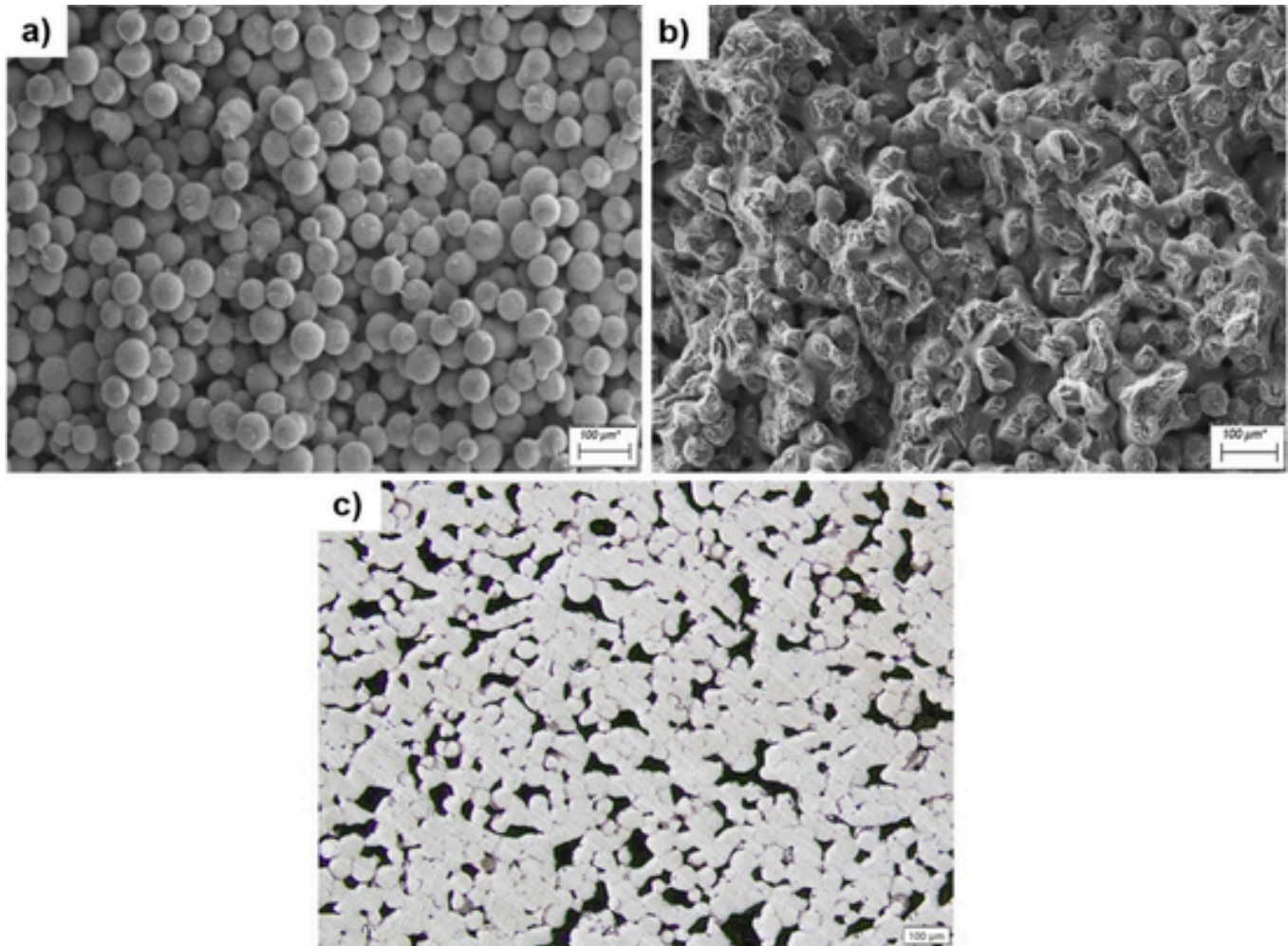


Fig. 2. Micrographs of binder-jet printed Mg powder in various condition, a) a SEM image of the as-printed condition, b) a SEM image of the as-sintered condition, and c) an optical image of the as-sintered condition.

vides the pore interconnectivity or pore openness index. This value for the BJPS sample was determined to be 95.3 %. Furthermore, the software calculated the overall porosity of the BJPS sample to be 11.5 %, which is in close agreement with the porosity value obtained from the Archimedes method. This porosity value in the BJPS Mg sample lies within the range found in human bones: The frontal (10.24 ± 3.72 %) and parietal bone (13.94 ± 4.02 %) in the cranium [35], bones found in the upper (proximal humerus (2 %–20 %) [36], distal radius (2 %–20 %) [37]) and lower limbs (femoral diaphysis (5 %–30 %) [38]). Moreover, open porosity is known to facilitate the movement of nutrients and waste products [31]. Orthopedic implants with open porosity are highly desirable for promoting bone regeneration and osseointegration. The pore interconnectivity of scaffolds enables the diffusion of growth factors and enhances the ingrowth of blood vessels and tissues. The presence of open porosity also increases the exposed surface area, allowing for proliferated cell adhesion, which is thoroughly discussed in Section 3.4.

Fig. 3d shows the pore size distribution of the BJPS sample from the mercury porosimetry test. The porosity percentage was measured at 12.78 %. While Archimedes and μ CT methods determine both open and closed pore types, mercury intrusion technique only characterizes open pores within the sample. This means that the pore openness index can be obtained by

taking the ratio of porosity determined via mercury porosimetry to that of Archimedes principle. The pore interconnectivity of the BJPS sample determined from the mercury porosimetry analysis is 96.4 %, which is in close agreement with the previous value obtained from the μ CT results (i.e., 95.3 %). Additionally, the median pore size of the BJPS sample obtained from mercury porosimetry was $12.73 \mu\text{m}$, which lies within the size range of osteoblast cells (i.e., 10–15 μm) [39].

Bones are interconnected open porous structures with distinct porosities in different regions to support a variety of functions. Examples of such a variation in porosity sizes in human cortical bones include the Haversian and Volkmann vascular canals region (40–100 μm), osteocyte lacunae region (10–30 μm), and canaliculi region with pores in the tens of nanometers range [31]. The results of the mercury test in terms of the relative distribution of pore sizes (Table 1) show that the percentage of open pores with sizes <1 μm is 9.4 %, for the pores sizes between 1 and 10 μm is 24.2 %, for the pores with sizes between 10 and 30 μm is 53.7 %, and eventually the pores with sizes >30 μm is represented by the remaining 12.5 %. All pores in the BJPS sample had curved surfaces (Fig. 2b). Studies confirm that scaffolds with smaller pores provide more surface areas for bone ingrowth [40]. Additionally, scaffolds with surface features such as roughness and curvature promote tissue regeneration at a greater rate than those with a planar surface because of

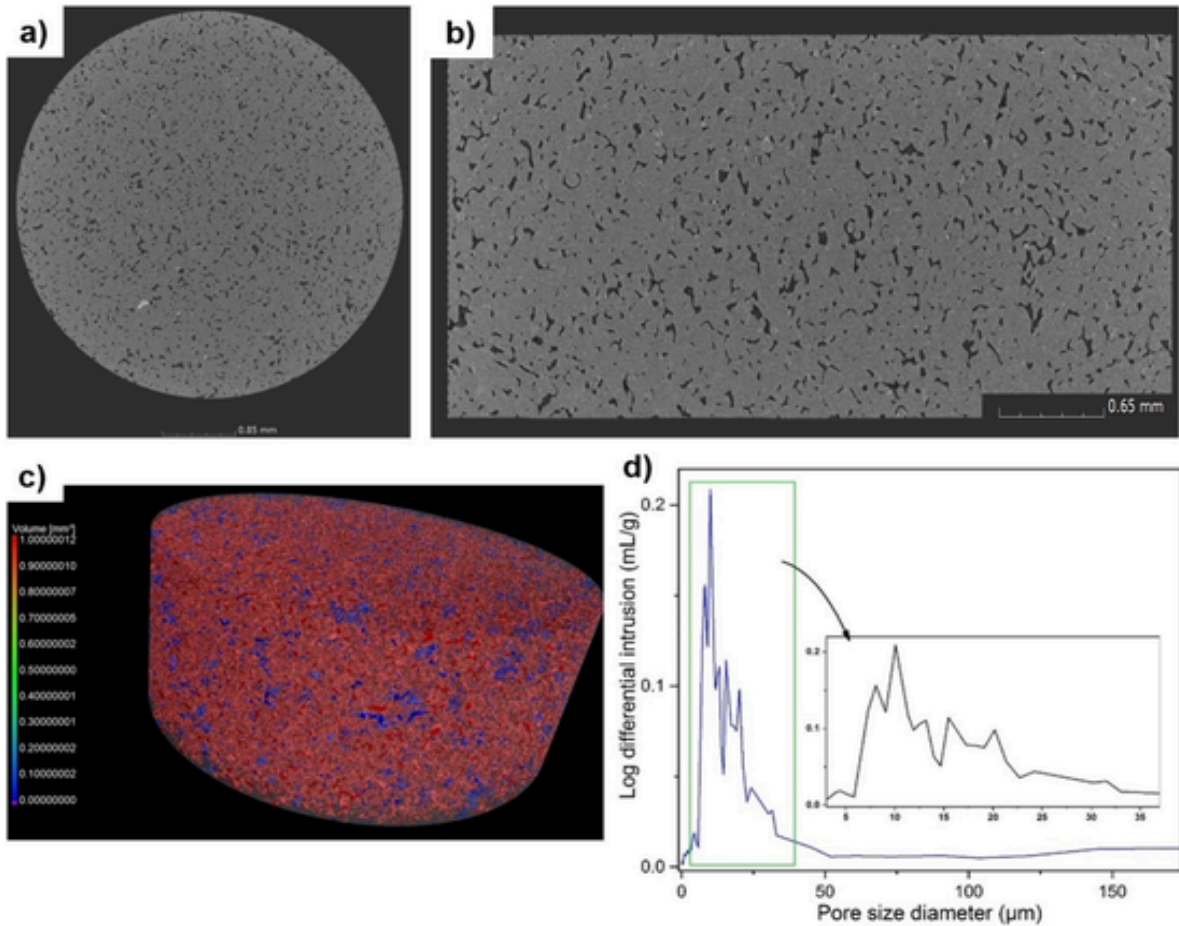


Fig. 3. Characterizations of porous structures of binder-jet printed and sintered Mg—Zn—Zr alloy, a) a representative μ CT tomogram along the build direction, b) a representative μ CT tomogram perpendicular to the build direction, c) a 3D representation of μ CT tomograms reconstructed for analyzing the interconnectivity of pore channels in which open pores highlighted in red and closed pores in blue color, and d) the mercury intrusion porosimetry analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

The statistical analysis of the mercury intrusion results for the BJPS samples along with the reported values in the literature.

Specimen name	Relative density (%)	Pore connectivity (%)	Median pore size diameter (μ m)	Relative volume of overall pores (%)				Ref
				Pore size diameter <1 μ m	1 μ m <Pore size diameter <10 μ m	10 μ m <Pore size diameter <30 μ m	Pore size diameter >30 μ m	
Mg -5.49 Zn -0.17Zr alloy, binder jet printed and sintered	86.8	96.4	12.7	9.4	24.4	53.7	12.5	This study [45]
Binder-jet printed and sintered Mg samples	87	65	6.9	9	66.9	10.9	13.2	
Binder-jet printed and sintered Mg samples, sintered in a conventional furnace	71.5	95	14.7	10	16.3	69.3	4.4	[23]
Binder-jet printed and sintered Mg samples, sintered in a microwave furnace	71.7	98	17.9	4.4	10	69.9	15.7	[23]

greater sites for attachment, proliferation, and differentiation of new cells [41,42].

Comparing pore sizes and the level of pore interconnectivity determined in this study with those previously reported for traditional manufacturing techniques and binder-jet printed and sintered Mg samples and offers intriguing insights. The BJPS sample exhibits an exceptionally high level of interconnectivity exceeding 95 %, even for significantly smaller pore dimensions ($\sim$ 13 μ m), in contrast to the outcomes obtained through traditional manufacturing techniques. For instance, a pore intercon-

nectivity of $\sim$ 99 % was reported in Mg alloys produced through a space holder infiltration method, with pore sizes ranging from 250 μ m to 400 μ m [43]. As another example, a pore interconnectivity of $\sim$ 94 % was obtained from a space holder powder technique for the pore size between 70 and 380 μ m [44].

In comparison to differently sintered binder-jet printed Mg samples reported in the literature, the trend observed in the statistical analysis of pore characteristics indicates that increasing relative density shifts the median pore size towards smaller values and significantly reduces pore interconnectivity. Specifi-

cally, increasing the relative density from approximately 71.5 % to 87 % reduces the median pore size from 17.9 μm to 6.9 μm and decreases pore interconnectivity from 98 % to 65 % [45]. Interestingly, the BJPS Mg samples in this study exhibit pore interconnectivity exceeding 95 % at a relative density of 86.8 %. A comparison of the statistical results in Table 1 also demonstrates that the proportions of submicron pores (i.e., $<1\mu\text{m}$), median-sized pores (i.e., 1–30 μm), and larger pores (i.e., $>30\mu\text{m}$) remain relatively consistent across relative density ranges. These pores originate from the spaces between Mg particles in the green state, with their sizes determined by the particle size distribution of the Mg powder, particularly in terms of D10, D50, and D90, as discussed in Section 3.1. Thus, if the raw powder has a broad particle size distribution, the gap-filling effect of finer particles results in reduced interconnectivity. Sieving fine powder from the BJPS Mg feedstock prevents smaller particles from filling these diamond-shaped gaps between larger particles, thereby maintaining high interconnectivity and density. These comparisons illustrate the critical role of controlling the particle size distribution of the Mg powder feedstock and fine-tuning the sintering profile in regulating microporosity size.

The micron-sized porosity types in the BJPS sample are governed by binder jetting process parameters such as powder specifications, printing variables, and sintering profiles. In addition to these pores created through process, larger pore channels can be designed in CAD files (pores by design) to fine-tune the pore size distribution and overall porosity percentage in binder jet AM of Mg scaffolds. The advancement of an AM technique enabling the fabrication of a broad range of porosity sizes is of paramount importance for Mg scaffolds. Structural designs developed for nondegradable porous materials may not apply to Mg scaffolds. The time-dependent characteristic is a vital determining factor and is currently the biggest obstacle in designing a porous structure for Mg scaffolds in line with clinical requirements. The presence of porosity greatly diminishes mechanical properties and significantly increases the degradation rate of porous Mg alloys [46,47]. During the initial stages of bone tissue regeneration, the mechanical integrity of the scaffold is entirely dependent on the Mg substitute, while its gradual degradation allows the regenerated bone to progressively regain strength [48]. Consequently, the porous structure of Mg scaffolds must be carefully designed and fabricated to achieve a balance between pore characteristics that support biological functions and the

necessary stiffness for load-bearing applications. A detailed review of various aspects of designing porous structures for Mg alloys is provided here [49]. Binder jet AM, as demonstrated here as well as Section 3.6 of this study, possesses the capability to fabricate a broad range of pore sizes, enabling the production of a reproducible micro and macro porous architecture for Mg scaffolds. This versatility makes binder jet AM a viable option for developing tailored products for applications in this domain.

3.3. Mechanical properties

Fig. 4 displays tensile and compressive stress-strain curves of the BJPS Mg sample, with their corresponding values tabulated in Table 2. The BJPS Mg sample exhibited a tensile strength of $\sim 130\text{ MPa}$, a yield strength of $\sim 100\text{ MPa}$, an elastic modulus of $\sim 21.5\text{ GPa}$, and an ultimate compressive strength of $\sim 349\text{ MPa}$, falling within the ranges of mechanical properties reported for different types of human cortical bones, including the femur, tibia, and the carpal bones (Table 2). Load-bearing bone scaffolds should resemble the mechanical properties of replacement bones. Scaffolds with weaker mechanical properties than those of natural bones fail to provide sufficient support, while those with mechanical properties that are too strong can lead to the stress shielding phenomenon.

Relative density is the most influential factor in determining the mechanical properties of porous materials. Structural pores not only reduce the load-bearing area but also act as stress concentration points that facilitate cracking. The strength of a porous material inversely correlates with its relative density [50], creating a trade-off between the two. Among the various models in the literature that relate mechanical properties to relative density, the Gibson-Ashby model is widely used to describe the relationship between strength and relative density [51]:

$$\sigma = \sigma_s (\rho/\rho_s)^M \quad (2)$$

where σ is a porous material strength, σ_s is a fully dense material strength, ρ is a porous material density, ρ_s is a fully dense material density, and M is a proportionality constant. In this study, the tensile and compressive strengths of the BJPS Mg sample were examined relative to the strength of the fully dense Mg—Zn—Zr alloy (Table 2). The value of M was found to be 5.4 for tensile strength and 0.1 for compressive strength. This indicates that the tensile strength of the BJPS porous Mg structure is

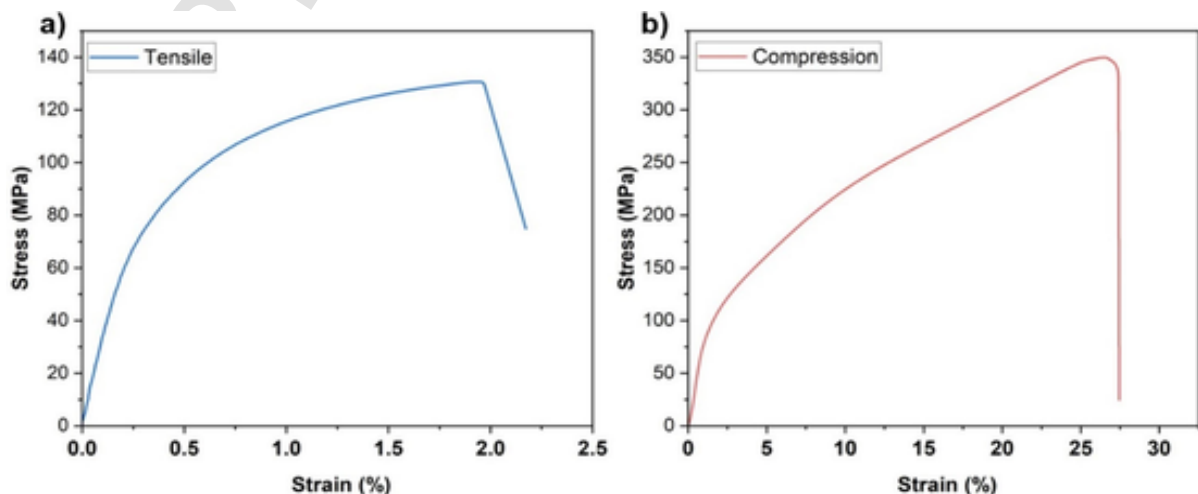


Fig. 4. Stress-strain curves of the binder-jet printed and sintered Mg—Zn—Zr alloy under a) tensile and b) compression loading.

Table 2

Mechanical properties of the binder-jet printed and sintered Mg—Zn—Zr alloy along with human bone, cast Mg—Zn—Zr alloy, and additive manufactured Mg scaffolds.

Specimen	Relative density (%)	Pore structure characteristics	Compressive properties			Tensile properties				Ref
			Ultimate compressive strength (MPa)	Compressive yield strength (MPa)	Fracture strain (%)	Elastic modulus (GPa)	Ultimate tensile strength (MPa)	Yield stress (offset 0.2 %) (MPa)	Ductility (%)	
Mg—5.49 Zn—0.17Zr alloy, binder jet printed and sintered	86.8	Average pore diameter of 12.7 μ m	349.3 $\pm$ 5.1	—	27.3 $\pm$ 0.9	21.5 $\pm$ 0.4	130.3 $\pm$ 3.2	99.9 $\pm$ 0.6	2.1 $\pm$ 0.1	This study
Cortical bones (longitudinal direction)	—	—	205 $\pm$ 17.3	—	—	17.9 $\pm$ 3.9	135 $\pm$ 15.6	—	—	[58]
Cortical bones (transverse direction)	—	—	131 $\pm$ 20.7	—	—	10.1 $\pm$ 2.4	53 $\pm$ 10.7	—	—	[58]
Cranial bones	86.9 $\pm$ 4.23	—	96.5 $\pm$ 35.9	—	5.1 $\pm$ 3.2	11.5–32.5	20.4–124.6	—	0.34–1.09	[35,59,60]
Tibia	—	—	159	—	—	14.1–18.1	120.9–175.9	123.7–178.2	—	[61,62]
Femur	90.6 $\pm$ 3.0	—	167	—	—	9.55–17.3	121–172.5	115.7–154.4	—	[61–63]
Mg—Zn—Zr, binder jet printed and sintered	71.5	Average pore diameter of 14.7 μ m	173	40.3	34.5	18.6	—	—	—	[23]
Mg—Zn—Zr, binder jet printed and sintered	71.7	Average pore diameter of 17.9 μ m	176.6	39	28	20.5	—	—	—	[23]
Mg—Zn—Zr, binder jet printed and sintered	—	—	260	41	—	—	71	—	0.7	[21]
Mg—5.8Zn—0.61Zr, commercially casted ingot	—	—	354	118	19	—	278	140	14	[64]
WE 43, laser powder bed fusion	40	Gyroid scaffolds with pore size of 500–1400 μ m	66.8–78.5	—	—	1.03–1.22	—	—	—	[52]
WE 43, laser powder bed fusion	36	Average strut size of 420 $\pm$ 4 μ m	< 30	24	10	0.8	—	—	—	[65]
WE 43, laser powder bed fusion	24–42	Tunable strut size of 250–750 μ m	55	—	—	—	—	—	—	[66]
WE 43, laser powder bed fusion	~20	300–500 μ m	4.37–21.21	—	—	—	—	—	—	[67]
Pure Mg, 3D gel printed and sintered	23.1–53.4	—	13.18–20.05	—	—	—	—	—	—	[68]
Mg—Zn, solvent-cast 3D printed and sintered	49.7	516 μ m	95	14.5	30	0.5	—	—	—	[69]

proportional to its relative density raised to the power of 5.4, demonstrating a significant dependency of tensile strength on porosity. In contrast, the compressive strength is less sensitive to porosity. The larger M value observed under tension compared to compression in binder jet printed Mg samples is attributed to the densification that occurs during the compression of porous structures [20].

The mechanical properties reported in the literature for Mg scaffolds fabricated by using various AM processes were summarized in Table 2. Comparing the properties of BJPS Mg samples with varying relative density, as summarized in Table 2, highlights the significant potential of binder jet AM in realizing tunable mechanical properties. It is evident that the BJPS Mg samples in this study provides twice the compressive strength of the binder-jet printed Mg—Zn—Zr alloys with a relative density of 71.5 %. When comparing these results with Mg alloys fabricated using the laser powder bed fusion method, it is evident

that pore sizes ranging from 250 μ m to 1400 μ m were introduced into Mg structures, resulting in porosity percentages between 60 % and 80 %. As anticipated, the pore size directly influenced the strength and elastic modulus of the fabricated scaffolds. For example, gyroid scaffolds made of WE 43 alloy with a pore size of 500 μ m exhibited a compressive strength of 78.5 MPa and an elastic modulus of 1.22 GPa [52].

Similarly, when comparing the mechanical properties of BJPS Mg samples to those of porous Mg alloys fabricated using material extrusion techniques (as presented in Table 2), it is clear that BJPS alloys offer superior properties. The large pore sizes in Mg structures significantly reduced the mechanical properties of the fabricated Mg scaffolds (i.e., 4 MPa < compressive strength < 95 MPa), making them considerably weaker than both the BJPS Mg sample (i.e., 173–349 MPa) and human compact bone (i.e., 96–205 MPa). The relatively low mechanical properties exhibited by these Mg scaffolds may compromise

their ability to safely bear loads, particularly when replacing load-bearing cortical structures, thereby potentially limiting their clinical applications. Moreover, the current mechanical strength of Mg scaffolds reported in the literature does not even match that of biodegradable polymers such as poly(lactic acid) (PLA), which has a tensile strength of 66 MPa [53], or polycaprolactone (PCL), with a tensile strength of 38.8 MPa [54]. This discrepancy in strength could deter the adoption of Mg scaffolds, especially given the widespread use of biodegradable polymers for non-load-bearing applications [55].

Improving the mechanical properties of Mg scaffolds produced through laser powder bed fusion and material extrusion methods would require a substantial reduction in strut diameter—a task that would be extremely challenging, if not impossible, to achieve. For instance, the minimum achievable diameter in the laser powder bed fusion process is constrained by the laser spot size (e.g., 80 μm) and the melt pool size (typically two to three times the laser spot size) [56,57]. In this study, we have demonstrated that binder jet additive manufacturing can produce Mg scaffolds with an open porous structure characterized by significantly refined pore sizes (Fig. 3d). This capability allows Mg scaffolds to achieve an exceptional and adjustable balance between mechanical properties and porosity, effectively addressing the long-standing challenge of the strength-porosity trade-off.

3.4. In vitro biological evaluation

3.4.1. Cytocompatibility

Fig. 5a shows the cell viability of MC 3T3-E1 mouse pre osteoblast precursor cells cultured with the BJPS and casted samples. The viability percentages of the MC 3T3-E1 mouse pre osteoblast precursor cells cultured with the BJPS samples are higher compared to those cultured with the cast and control counterparts at the end of every incubation period. At the end of Day 1, a robust level of cell viability was observed with a 121 % for the viability percentage of the BJPS sample and 114 % for the cast sample. The cell viability increased at the end of Day 3, with that for the BJPS sample being higher (151 %) than that of the cast counterpart (131 %). At the end of Day 5, the cell viability decreased, with a 114 % for the viability percentage of the BJPS sample and 97 % for the cast

sample. Nonetheless these values for cell viability are above the threshold for cytotoxicity based on ISO standard (70 %) [70]. The BJPS sample showed lower cytotoxicity percentage (8 %) than that of the cast sample (14 %; Fig. 5b), classifying under the level 0 cytotoxicity (<25 %) in accordance with ISO 10993-5 [70]. The greater cell viability and lower cytotoxicity for the BJPS sample than in the cast counterpart indicates its superior cytocompatibility. This superiority is attributed to the presence of 3D interconnected porous structures that provide more locations and a higher surface area for the interactions between the culture medium and cells (Section 3.2).

The decrease in the cell viability percentage at the end of Day 5 stems from the contact inhibition as a result of cell confluence. This decrease in cell viability was also demonstrated by Mostofi et al. [71], in which the MC 3T3-E1 cell viability in Mg and Mg alloys decreases with increased cultured duration. The cell viability has been demonstrated to decrease steadily with an increase in Mg^{2+} concentration above the reported critical limit (0.3 g L^{-1}). Similarly, Lin et al. [68] reported the reduced cell viability of MC 3T3-E1 due to the increase in alkalinity of the solution caused by the accumulation of hydroxide ions as a byproduct of Mg degradation. Cell viability has additionally been proposed to be impeded by the formation of crystallized corrosion products on the surface [71]. This reduction in viability does not necessarily mean that Mg-based materials are unsuitable for implants. Under in vitro cell culture conditions, the ions released into the culture medium by the corroding Mg sample accumulates, while the dynamic in vivo environment has mechanisms to reduce Mg ion concentration through material transport. To illustrate this point, the in vivo degradation of Mg—Zn alloy was found to have no detrimental effects on neighboring bone tissues [72]. This is attributed to the ability of the body to excrete excess Mg^{2+} through the renal system, while Zn^{2+} is absorbed by adjacent tissues and excreted via both the gastrointestinal tract and the renal system. Both the BJPS and cast samples showed great cytocompatibility (Fig. 5) based on the criteria set by the above-mentioned ISO standard. This is largely due to the presence of Mg, which has been demonstrated to stimulate the adhesion of osteoblastic cells and maintaining genomic stability [73]. The presence of Zn in the Mg—Zn—Zr alloy also enhances osteoblast cells' viability, proliferation, and migration [74]. In particular, Zn ions have been found to promote anabolic effect in

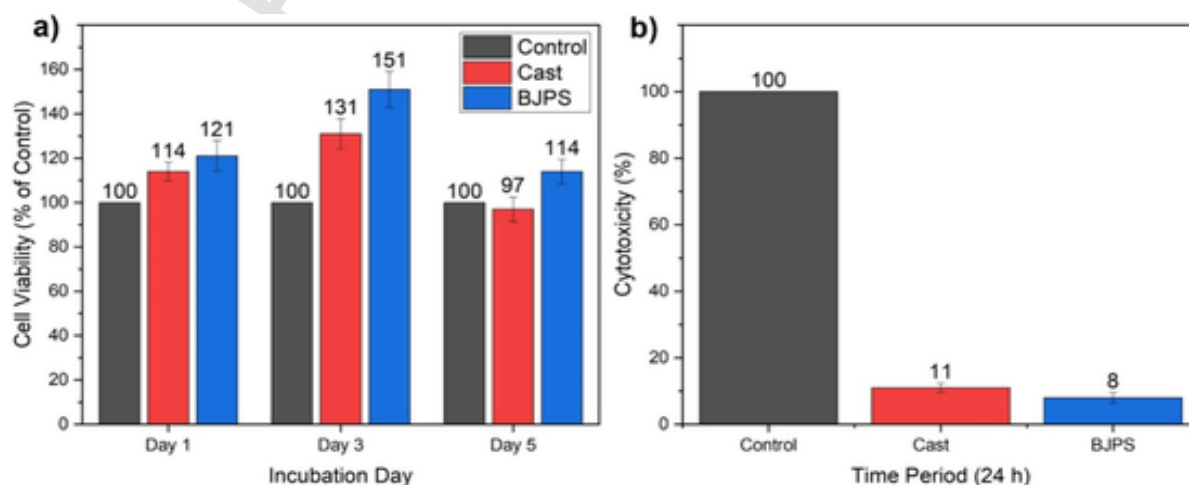


Fig. 5. Cell viability of MC 3T3-E1 mouse pre osteoblast precursor cells cultured with cast and binder-jet printed and sintered alloys, a) cell viability of MC 3T3-E1 cells cultured with 100 % extracts for period up to 5 days, and b) cytotoxicity percentage LDH enzyme release after 24 h incubation.

osteoblastic MC 3T3-E1 cells [75]. In pure Mg alloy, the MC 3T3-E1 cells' viability percentages at the end of Culture Days 1, 3, and 5 were reported to be 117 %, 91 %, and 85 %, respectively [76]. Comparing among the values for pure Mg, the BJPS, and cast samples verifies the superior cytocompatibility seen here. In vivo experiments have demonstrated that the Mg—Zn alloy has excellent biocompatibility, with its degradation products being biocompatible with the spleen, kidney, liver, and heart [77].

3.4.2. Cells morphology and cytoskeleton organization

Cell attachment is crucial in cells' viability and their proliferation, and functionality. Factors including the substrate materials' characteristics, cell-cell interactions, and cell-substrate interactions determine the cell morphology and cytoskeleton. The images of the cytoskeletal structure and cellular morphology of MC 3T3-E1 cells following a 24 h culture period with BJPS and cast samples show the elongate cells extending on to the sample surface (Fig. 6). The cells adhering to the BJPS surface showed more spreading (depicted by the area of cytoplasm in red) and more extensive filopodia extensions with cell-cell contacts, when compared to the cells adhering to the cast counterpart.

The MC 3T3-E1 cells morphology after incubation for 1, 3 and 5 days on BJPS and cast samples (Fig. 7) support the trend observed in Fig. 5a. At the end of Day 1, the cells showed initial attachment and spreading for both samples, with the cells showing an elongate morphology and being well spread out. At the end of Day 3 and Day 5, there was a noticeable increase in the coverage of cells concerning both cell attachment and proliferation on the surface of the BJPS sample. The cells exhibited a polygonal morphology and achieved nearly 100 % confluency. The results indicate good cell attachments and cell viability for both samples. In addition, we observed the formation of crystalline corrosion products on the surface of both samples, being more extensive in the cast sample. As previously stated, this formation of crystalline corrosion products has been proposed to impede cell viability and might be the reason for reduced cell viability in both samples after Day 3 [71].

The enhanced cell morphology observed in the BJPS sample can be attributed to the larger bioactive surface area available for cell attachment as well as increased permeability (Section 3.2). A porous structure is known to promote better adhesion of osteoblast cells, and its higher surface area may also have increased the nutrient release rate, increasing cell proliferation [69]. High interconnectivity and permeability of porous structures allow for cell infiltration. An interconnected open architecture in porous

scaffolds provides the cells with the passage to penetrate through the length of scaffolds without binding between the cells and the surface-adsorbed proteins [78]. Furthermore, porous scaffolds with great permeability enhance bone ingrowth while suppressing the development of cartilaginous tissue at the regeneration site [78]. With a high pore interconnectivity of > 95 % (Section 3.2), the open porous structure of the BJPS sample allows for three-dimensional cell attachment, whereas in the cast sample, cells can only adhere in a two-dimensional fashion. This potential of the BJPS samples to enhance osteogenic cell differentiation is a promising avenue for future research.

Live and dead cell staining was conducted to assess the proliferation of MC 3T3-E1 cells after 1, 3 and 5 days of culture using both BJPS and cast samples. In the fluorescent microscopy images (Fig. 8), viable cells are represented in green and dead cells are indicated in red. Merged images depict both live and dead cell populations. The cells were widely distributed with fewer dead cells. The density of live cells increased to confluence after 3 days. As the living cells occupy almost all the available surface, the space available for cell proliferation is reduced. This led to a few dead cells seen with the cast sample at the end of Day 3, attributed to cell confluency contact inhibition. More live cells and fewer dead cells were observed in the BJPS sample after Day 3, largely due to the presence of pores that provides cells with a third dimension for infiltration. While both samples showed favorable cytocompatibility, BJPS had a slightly better performance. The increased proliferation of the cells suggests better biocompatibility for the BJPS sample compared with the cast counterpart. These observations corroborate the previous results.

3.5. Metallic ion release

Table 3 summarizes the release profiles of metallic ions from the BJPS scaffolds and cast samples at various time points. The BJPS samples exhibited an initial burst release phase, followed by a more gradual release of Mg, Zn, and Zr ions. The BJPS sample demonstrated higher average release rates for these ions compared to the cast sample. The increased release in the BJPS sample is attributed to its larger exposed surface area in physiological media, which accelerates biodegradation and, consequently, leads to a higher ion release. It has been established that the degradation rate of porous Mg scaffolds correlates with pore size [52]. Notably, the corrosion rate of porous materials is not linearly proportional to the porosity percentage but is signifi-

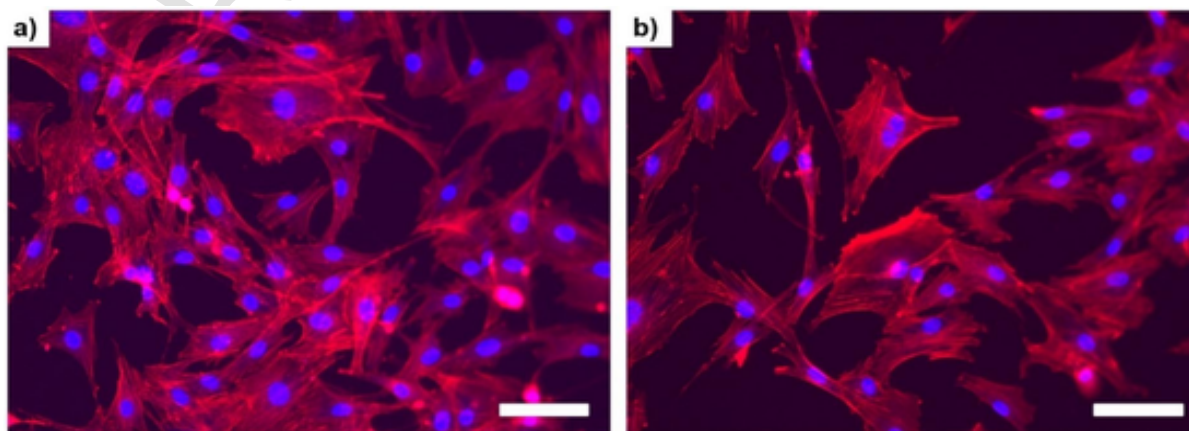


Fig. 6. MC 3T3-E1 cells morphology and cytoskeleton organization after incubation for 24 h, a) binder-jet printed and sintered alloy, b) cast alloy. The scale bar indicates 50 μm .

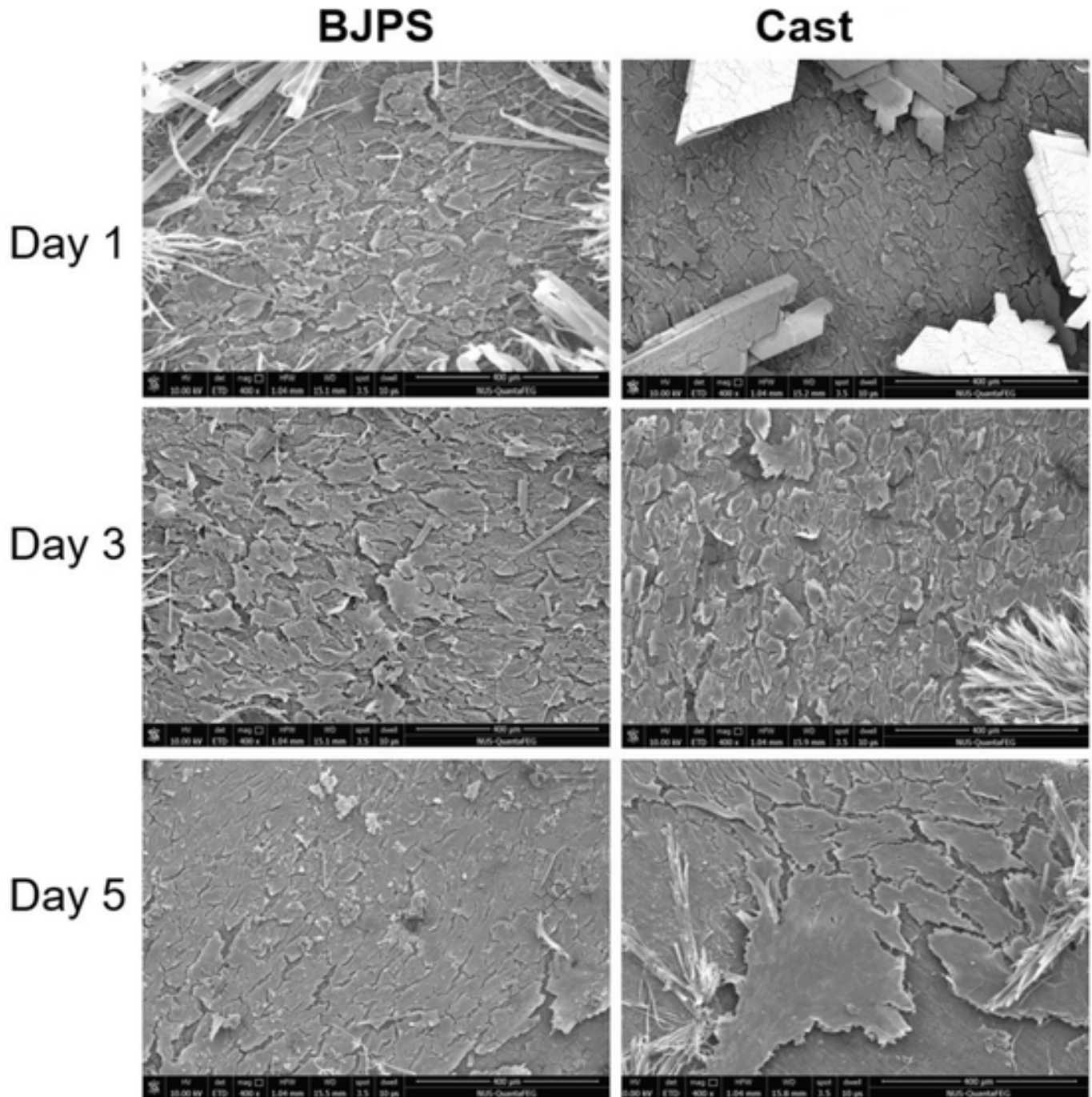


Fig. 7. SEM micrographs of the MC 3T3-E1 cell attachment on the surfaces of cast and binder-jet printed and sintered alloys after 1, 3 and 5 days of incubation.

cantly higher than that of their non-porous counterparts [46]. Therefore, applying a surface coating is essential for controlling the degradation of porous Mg scaffolds [10,29] to maintain Mg concentrations within safe limits for MC 3T3-E1 cells, reported to be around 400 ppm [79].

During the anodic dissolution of Mg—Zn—Zr alloys, both Mg and Zn undergo oxidation, with Zn oxides forming within the inner layer of the corrosion product film [80]. As reported in a previous study [46], the higher degradation rate observed in the BJPS samples is accompanied by increased surface hydrogen evolution, which causes more frequent perturbations and dis-

ruptions of the surface film, thereby releasing more ions into the solution. The Zn ion concentration in the BJPS samples remains well below cytocompatibility limits. The Zn ions released from the cast sample appear to be near the detection limit of the ICP instrument (approximately 0.01 ppm), indicating that Zn ion release was minimal.

3.6. Towards binder jet additive manufacturing of mg implants

BJPS Mg—Zn—Zr parts can be manufactured in different sizes and geometries, demonstrating the tremendous potential

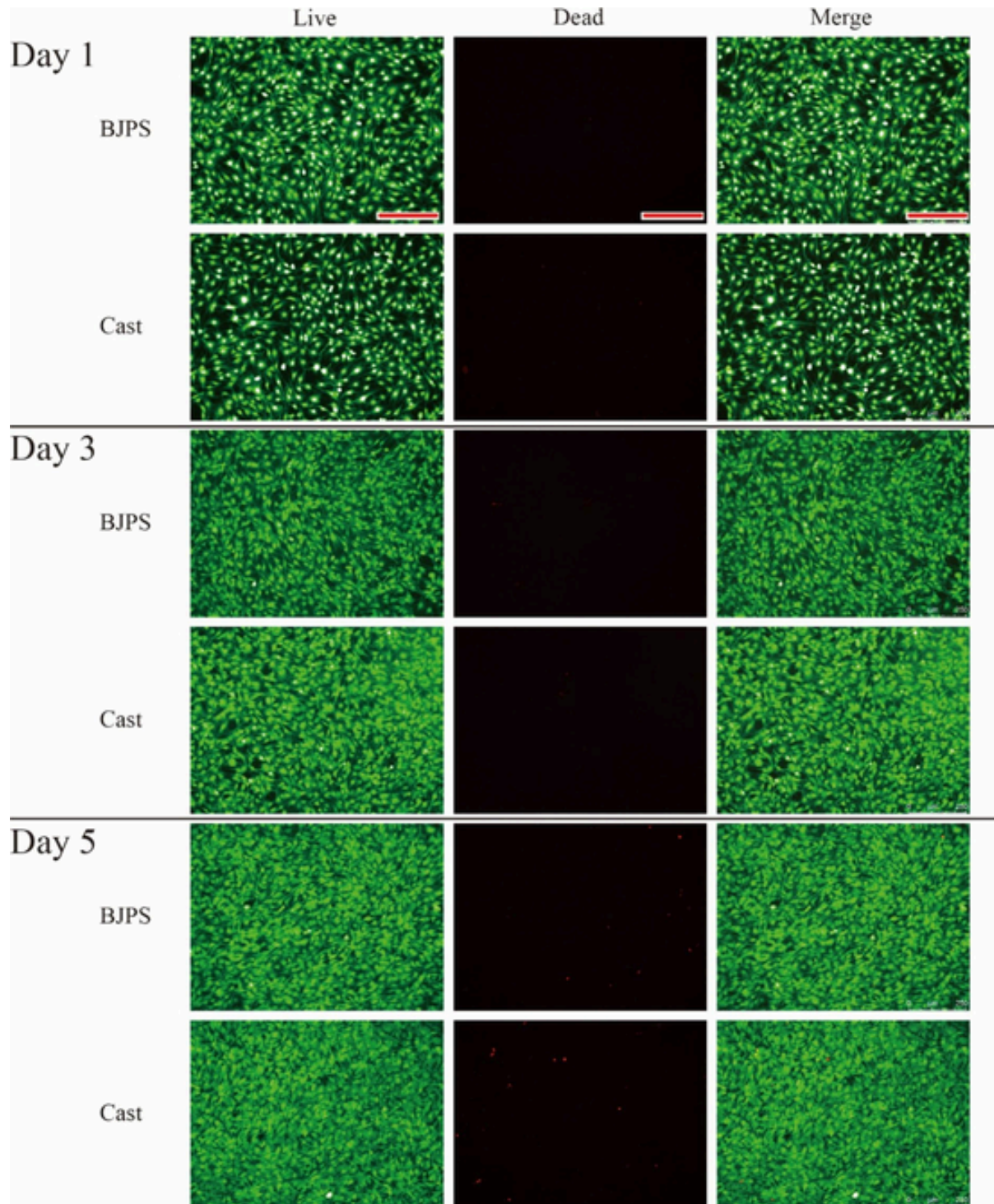


Fig. 8. Live and dead cell staining of MC 3T3-E1 on cast and binder-jet printed and sintered alloys after 1-, 3-, and 5-days incubation. The scale bar indicates 250 μm .

of binder jetting for the digital manufacturing of Mg implants (Fig. 9). In particular, Fig. 9a shows BJPS Mg implants for craniomaxillofacial implants; Fig. 9b represents BJPS Mg bone plates as bone fixation implants; and Fig. 9c-e displays BJPS Mg screws targeting hard tissue engineering applications such as teeth, bone substitutes, femoral interference, tendon grafts, and cranial fixation parts. BJPS Mg parts can be post processed (e.g., by

machining) to meet precise dimensional accuracies required for specific applications (Fig. 9e).

Fig. 10a shows BJPS Mg cranial implants in the as-printed condition and after sintering at two different temperatures. The as-printed BJPS Mg cranial implant demonstrates an isotropic shrinkage during both sintering temperatures in all directions (Fig. 10a–d). Image analyses of optical micrographs taken from different locations throughout the cross-section of the sintered

Table 3

Accumulative metallic ion release from the cast and binder-jet printed and sintered alloys.

Du ration	Samp le	Mg (ppm)	Zn (ppm)	Zr (ppm)
Day 1	BJPS	799	1.58	0.18
	Cast	271	No t detected	0.1
Day 3	BJPS	1309	1.84	0.28
	Cast	617	No t detected	0.16
Day 5	BJPS	1873	2.48	0.48
	Cast	925	No t detected	0.23
Average release rate (ppm /day)	BJPS	265.5	0.98	0.08
	Cast	163.5	–	0.03

implant indicated a uniform microstructure (Fig. 10e). The image analysis of the micrographs indicates a consistent pore and density distribution throughout the cross-section, with the differences among the analyses not being statistically significant (i.e., $\pm 111\%$). The entire 3D porous structure of the sintered implant was further verified by using μ CT (not presented here) that indicated a uniform microstructure and pore distribution throughout the implant.

During the fabrication of an as-printed part in binder jetting, layers of powder in the absence of compaction and heat stack up to form a given green shape, which then transforms into a homogeneous part during the sintering process. Binder jetting can thus replicate a porous structure in a given geometry for any given patient-specific 3D shape. In addition to the micropores controlled by binder jetting processes (i.e., feedstock, printing, and sintering parameters), macropores can be incorporated by design to fulfill any porosity requirement (Fig. 9a). These pore sizes can serve as a tool to tune mechanical properties and customize macro- and micro-architectural features of scaffolds, ac-

commodating differences in an implantation location [81,82]. All of these factors are crucial in constructing biodegradable scaffolds for bone tissue engineering. For instance, large bone defects, also regarded as critical-sized bone defects, are one such critical example that requires the need of fabricating reproducible structures for patient-specific scaffolds, irrespective of scaffolds' size and geometry. BJPS of Mg alloys lends itself to be a promising candidate to address this need. It offers the potential to create a porous synthetic bone substitute tailored to individual patient-specific 3D shapes with tunable mechanical properties. With the capability for precise control over pore size and distribution, BJPS Mg can replicate the macro- and micro-architecture found in human bone tissue.

4. Co nc lusi on s

We successfully fabricated a porous interconnect Mg structure that mimics the microporosity and mechanical properties of human cortical bone types by employing the binder jet AM technique for the first time. This accomplishment enables Mg alloys to employ a high and tunable correlation between mechanical properties and porosity in ensuring a delicate balance between pore characteristics conducive to biological functions and the necessary stiffness for load-bearing purposes. The following are our conclusions from our study.

1. The BJPS Mg—Zn—Zr alloy displays a uniform microstructure across the interconnected porous structure, featuring an overall porosity of 13.3 %, a median pore size of 12.7 μm , and pore interconnectivity exceeding 95 %. These values align with the ranges observed in human bone types. Importantly, the distinctive pore characteristics in BJPS Mg alloys are unattainable through conventional manufacturing methods.

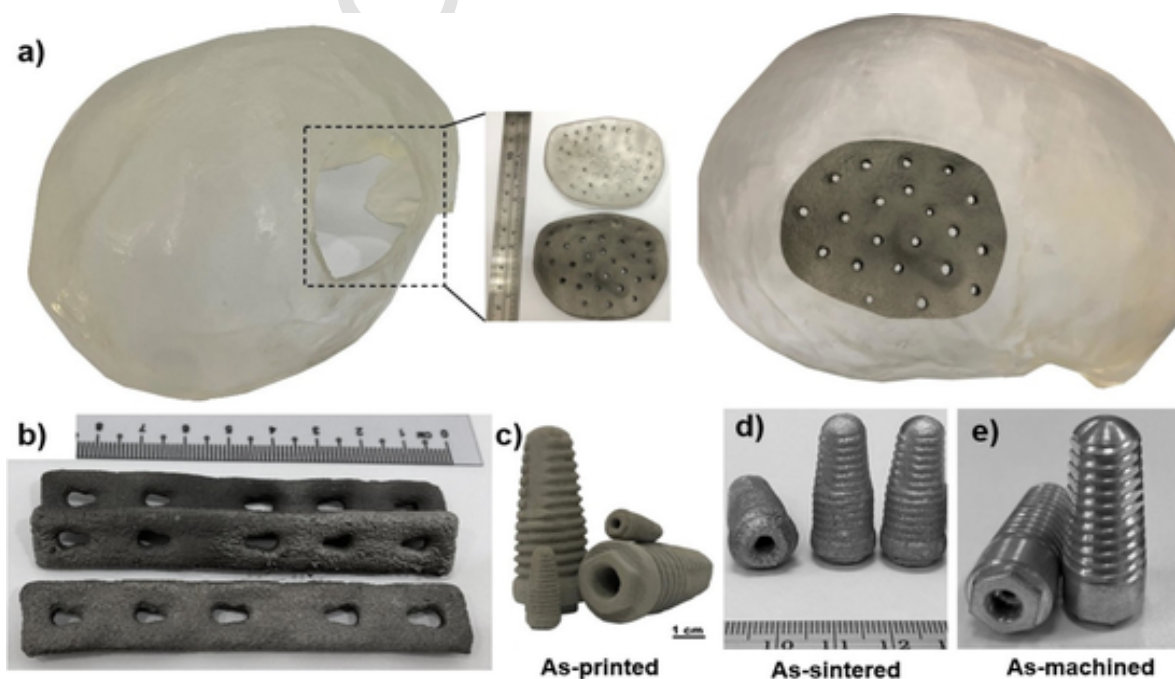


Fig. 9. Binder-jet printed and sintered Mg—Zn—Zr parts for biomedical applications, a) craniomaxillofacial, b) bone plate fixation, and c–e) screws that can be used in hard tissue engineering applications such as teeth, bone substitutes, femoral interference, tendon grafts, and cranial fixation parts.

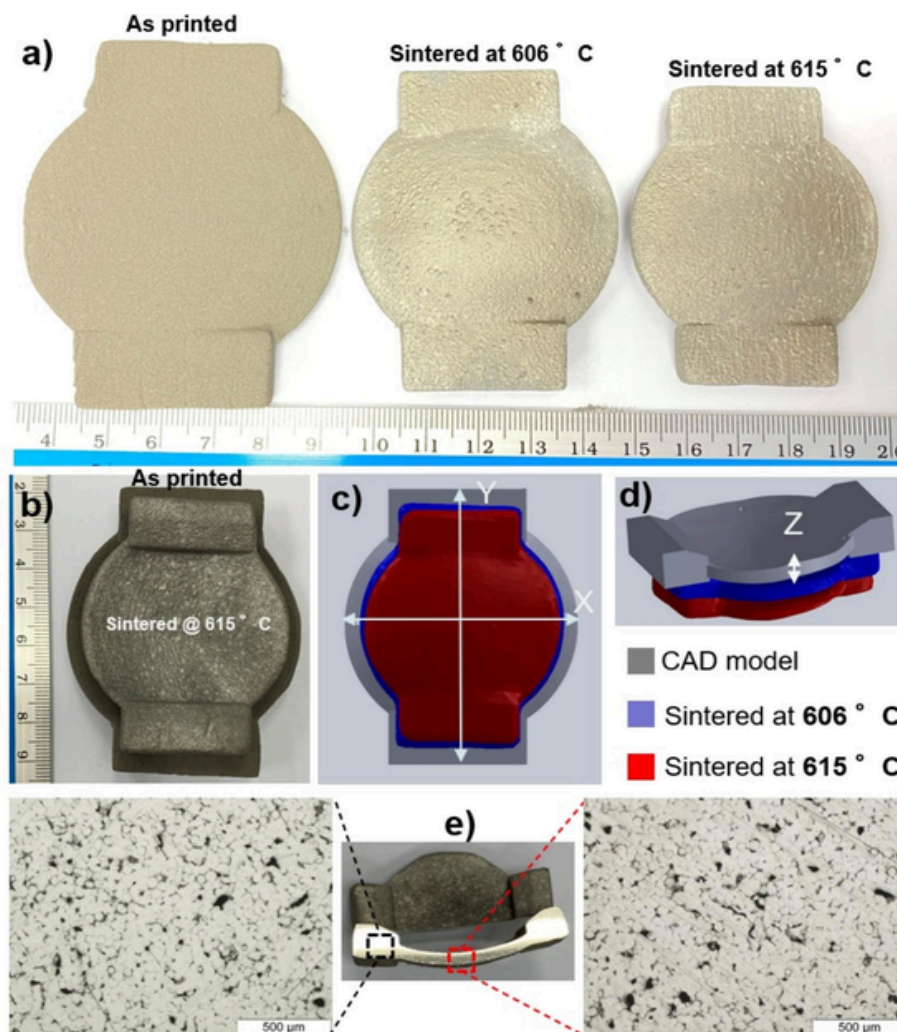


Fig. 10. Binder-jet printed and sintered spherical cap-like implant made from Mg—Zn—Zr powder for cranial applications, a) spherical-cap like Mg implants before and after sintering, b–d) comparisons of the spherical-cap like Mg implants in the as-sintered condition with the as-printed condition and the initial CAD model, showing isotropic shrinkage occurring during sintering of the as-printed shape, e) the cross-sectional view of the spherical cap-like Mg implant after sintering at 615 °C along with representative optical microscope images taken from the polished surface.

- The Mg—Zn—Zr alloy produced through BJPS exhibited a tensile strength of ~ 130 MPa, a yield strength of ~ 100 MPa, an elastic modulus of ~ 21.5 GPa, and an ultimate compressive strength of ~ 349 MPa. These values align with the typical mechanical properties of various human cortical bone types. Significantly, the mechanical properties of BJPS Mg scaffolds are adjustable and outperform those of Mg scaffolds created through laser powder bed fusion and extrusion-based AM methods.
- The BJPS Mg—Zn—Zr alloy exhibits superior MC 3T3-E1 cell viability and a lower cytotoxicity percentage (8 %) compared to cast Mg—Zn—Zr alloy (14 %), demonstrating level 0 cytotoxicity (i.e., no cytotoxic effects) at the same time. Furthermore, the MC 3T3-E1 cells adhering to the surface of the BJPS alloy display greater cell spreading, attachment, and proliferation in comparison to their counterparts on the cast Mg—Zn—Zr surface. This enhanced biocompatibility of the BJPS alloy is attributed to its highly interconnected 3D porous structure, which provides cells with an additional dimension for infiltration (i.e., a larger bioactive surface area) compared to the cast

alloy in which interaction with cells are limited to a two-dimensional manner.

- Binder jet AM offers the capability to reproduce a desired porous structure of Mg alloys, encompassing both micro- and macro-sized pores, in any desired geometry for patient-specific 3D shapes. This adaptability also addresses the diverse porosity requirements necessary for the time-dependent characteristics of biodegradable Mg alloys. We highlight the concept of binder jet AM for the digital manufacturing of biodegradable Mg implants tailored for applications in hard tissue engineering, including craniomaxillofacial procedures, bone fixation, and substitutes for bone grafts.

CR edited authorship contribution statement

Mojtaba Salehi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kaixiang Kuah:** Writing – original draft, Investigation, Data curation. **Somasundaram Prasadh:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Yuehua Li:**

Data curation, Formal analysis, Investigation. **Su Xi a Zh ang**: Formal analysis, Data curation. **Ha ng Li Se et**: Project administration, Methodology, Formal analysis. **Raymond Ch ung We n Wo ng**: Writing – review & editing, Supervision, Methodology, Conceptualization. **Mu i Li ng Sh aron Na i**: Writing – review & editing, Supervision, Funding acquisition.

De clarat ion of comp et ing in te re st

The authors assert that they do not have any known financial interests or personal relationships that could be perceived as influencing the work presented in this paper.

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Da ta avai labi lity

Data will be made available on request.

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