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RESEARCH ARTICLE

TOXICITY TESTING OF SELECTIVE LASER MELTING TECHNOLOGY TOWARDS PRODUCTION OF TITANIUM ALLOYS (Ti6Al4V) ORTHOPAEDIC METAL IMPLANT: PRIMARY ANALYSIS

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Abstract. Selective Laser Melting (SLM) technology has garnered significant attention for producing customisable biomedical implants with complex structures and superior mechanical properties. It is increasingly used for tailoring orthopaedic implants, such as 6-hole plates. Titanium alloys (Ti alloys) implants are prevalent in orthopaedic surgery due to their excellent properties and bioinertness. A successful implant relies steadily on effective interaction with surrounding tissues. This study assesses the *in vitro* and *in vivo* biocompatibility of Ti alloy orthopaedic implants produced via SLM technology at the primary stage by examining cytotoxicity, genotoxicity and pyrogenicity. The results from the MEM elution assay indicated no reactivity (Grade 0) at 100 % concentration when exposed to SLM Ti alloys implant. The AMES test results showed that the number of revertant colonies treated with SLM Ti alloys implant did not exceed twice that of the negative control, regardless of metabolic activation. As for pyrogenicity analysis, the output shows that the absence of pyrogenic substance was noted after being introduced with SLM Ti alloys implant extractions. The positive and negative controls exhibited the expected action. In conclusion, the findings suggest that the production of Ti alloys orthopaedic implants through SLM technology do not induce toxic effects, confirming their biocompatibility and safety for orthopaedic use. This research highlights the promising biological safety of Ti alloys orthopaedic implants manufactured through SLM technology, with no observed toxicity at the primary stage.

Keywords: SLM Ti alloys, cytotoxicity, genotoxicity, pyrogenicity and orthopaedic implant.

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1. INTRODUCTION

The field of orthopaedics continuously faces challenges, particularly in managing bone fractures, which are common in trauma cases. Stabilising fracture fragments require fixator implants to provide mechanical support and ensure optimal alignment [1, 2]. These implants are crucial for maintaining bone function under the physiological loading of both bones and joints [2, 3]. A careful approach to implant design involves considering surface characteristics, bioinertness, and biomechanical and chemical properties to ensure effective bone integration and implant integration [4-6]. Selective Laser Melting (SLM) technology has been extensively explored for manufacturing metallic implants. This advanced digital fabrication technique enables the production of complex, precise structures with excellent mechanical properties [6, 7]. Furthermore, SLM enables the fabrication of personalised medical devices and implants customised to meet the unique anatomical requirements of individual patients. [6-8].

Titanium alloys (Ti alloys), particularly Ti6Al4V, are favoured for orthopaedic implants owing to their mechanical performance, biocompatibility, osteoconductivity, corrosion resistance, and high specific strength [8-10]. These attributes make Ti alloys reliable for producing implants and devices to address bone fractures and defects [6-9]. In the medical industry, orthopaedic implants are predominantly manufactured using machining technology, a conventional method applied across various metallic implant materials [9, 11]. However, this technique presents certain limitations. It is not well-suited for customised implants, and the manufacturing process is expensive. Machining involves shaping implants from solid metal stock, leading to substantial material waste and inefficiencies [11]. As an alternative, the production of orthopaedic implants through SLM technology has been introduced to overcome those issues. In Malaysia, a collaborative project has successfully developed a six-hole Ti alloys metallic implant employing SLM technology. This product comprises Ti alloys grade 23, Ti-6Al-4V ELI, or TAV-ELI. Ti alloys are ideal for production due to the powder bed fusion to create a solid layer, the flexibility of the implant design, good surface finish, and porosity to allow for better bone ingrowth, load-bearing implant, and customisation [6, 9, 10].

The ability of a biomaterial to fulfil its intended function without eliciting detrimental biological responses, termed biocompatibility, is a fundamental requirement for orthopaedic implants [10, 12]. Evaluating biocompatibility is fundamental in developing and regulatory approval of orthopaedic materials for clinical application [6, 9, 10, 12]. To ensure safety, these materials must conform to the biocompatibility requirements stipulated within the ISO 10993 series of standards [12, 13]. Ti alloys are well-established materials in producing orthopaedic implants, compactly integrated into tissues and bones. Since these implants remain in prolonged contact with body tissues, they must promote a favourable cellular response. Biological tests are essential to confirm their suitability for clinical use [12-16]. While it is expected that any foreign material implanted in the human body will elicit a reaction, inert materials typically trigger only the formation of fibrous tissue.

Although the relationship between Ti alloys and Selective Laser Melting (SLM) technology is well-documented, primary biocompatibility studies (toxicity testing) are essential. In this study, toxicity testing is employed to determine its safety before clinical use. SLM, an additive manufacturing process that uses a laser to fuse metal powders, raises concerns about potential residues, such as unmelted particles or contaminants, which could leach into the body. Following ISO 10993 standards for medical devices, toxicity testing was conducted using the MEM Elution Assay, the Bacterial Reverse Mutation Test (Ames Test), and Pyrogenicity Testing. The results were benchmarked against control samples to ensure reliability.

2. MATERIALS AND METHODS

2.1 SLM Ti alloys Plate Extraction Preparation

Prior to extract (suspension) preparation, the SLM Ti alloys plate was sterilised by autoclaving at 121 °C for 20 minutes. The SLM Ti alloys plate was put together with 0.9 % sodium chloride (NaCl) to achieve an ultimate 200 mg/ml concentration. The extraction was then incubated in a water bath at 37 °C for 72 hours and subsequently sterilised via membrane filtration. The SLM Ti alloys plate extracted was prepared according to the established guideline ISO10993-12: 2021 for sample preparation [16]. The extraction was prepared and utilised for all tests in this study. The extraction process was conducted under sterile conditions in a Class II biological safety cabinet (Bioair® Instruments, model Aura 2000).

2.2 Cytotoxicity Test

In this research, the cytotoxicity was conducted as instructed by the established guideline ISO 10993-5: 2009 [13-16]. The growth media (medium only) served as blank control, polyethylene (0.2 g/ml) served as negative control, and zinc diethyldithiocarbamate (0.2 g/ml) served as the positive control. All procedures in this study were conducted under sterilised settings. L-929 mouse subcutaneous connective tissue fibroblast cells were used for cytotoxicity evaluation. The cells were detached from the culture flask through trypsinisation (0.25 % trypsin-EDTA, Sigma-Aldrich®) and subsequently seeded into 96-well plates at a density of approximately 1.0×10^5 cells per well. A subconfluent monolayer was established after 24 hours of incubation at 37 °C with 5 % carbon dioxide (CO_2). The cell monolayer with 75 % subconfluent and morphology of the cultures were verified through microscopic observation before commencing the tests. The culture medium was replaced with SLM Ti alloys plate extracts, with positive, negative, and blank controls in each well. These three critical controls employ: the blank control (quantifies background interference), the negative control (confirms normal cell growth without toxins), and the positive control (validates the assay's toxicity detection capability) [13-15]. The extracts were exposed to the cells for testing purposes and conducted in triplicate. The plates were incubated at 37 °C for 48 hours in a humidified incubator containing 5 % carbon dioxide. After 48 hours, changes in cell morphology were examined microscopically at 100 X magnification using a Nikon Eclipse TS 100 inverted microscope, QICAM Fast 1394 Digital Camera, NIS-Elements Software. The extracts' cytotoxicity degree were observed in triplicate in each well and were graded qualitatively, as shown in Table 1.

Table 1: illustrates the grading scale of cytotoxicity [13]

Grade	Reactivity	Conditions of all cultures
0	None	Monolayer complete, no cell lysis
1	Slight	Cell death not more than 20 %
2	Mild	Cell death not more than 50 %
3	Moderate	Cell death not more than 70 %
4	Severe	Destruction of the cell monolayer

2.3 Cytotoxicity Test

Genotoxicity was evaluated through a bacterial strain: *Salmonella typhimurium* (*S. Typhimurium*) and *Escherichia coli* (*E. coli*). The strains were inoculated, cultured and prepared according to the established guideline ISO10993-3: 2014 [17, 18]. Specifically, *S. typhimurium* strains TA98 and TA1537 served as indicators for frameshift mutations, while the other strains were utilised to detect base-pair substitutions. A range of extract concentrations with ratios 6.25, 12.5, 25, 50 and 100 mg/ml were prepared for this test. Aseptic techniques were strictly followed throughout the extraction process. To ensure assay validity, the controls employed were prepared; without S9 metabolic activation (0.9 % NaCl solution - the negative control and 4-nitro-o-phenylenediamine (CAS No. 99-56-9), 9-aminoacridine (CAS No. 90-45-9), sodium azide (CAS No. 26628-22-8) and methyl

methanesulfonate (CAS No. 66-27-3) – the positive control, with S9 metabolic activation (0.9 % NaCl solution - the negative control and 2-aminoanthracene (CAS No. 613-13-8) – the positive control), all sourced from Sigma-Aldrich, Merck, Germany. As for S9 (enzyme) mix preparation, the minimal glucose agar plates were equipped using Vogel-Bonner E Medium supplemented with 2 % glucose, and 1.5 % agar (15 g/l) was used as media. For the top agar, a mixture containing 0.6 % (w/v) agar and 0.6 % (w/v) NaCl was used, supplemented with histidine for *S. typhimurium* strains or biotin for the *E. coli* strain [17, 18]. This S9 (metabolic activation) homogenate fraction (Sigma-Aldrich, Merck) was equipped following the protocol of Maron and Ames (1983) and was kept on ice throughout the test to maintain metabolic activity.

As for the genotoxicity assay, the bacterial suspensions were prepared and exposed to the alloys extract for approximately 20 minutes, with and without metabolic activation (S9), followed by a 37 °C incubation. The SLM Ti alloys plate extracts (with concentration 6.25 %, 12.5 %, 25 %, 50 % and 100 %) were exposed to the bacterial culture in triplicate. The metabolic activation system utilised a post-mitochondrial (S9) fraction from Aroclor 1254-pretreated Sprague-Dawley rat livers. Each assay tube contained 2 ml molten top agar, 0.1 ml overnight bacterial culture, 0.1 ml test substance/control, 0.2 ml biotin/histidine (*S. typhimurium*) or biotin/tryptophan (*E. coli* WP2 *uvrA*), and 0.5 ml PBS or cold S9 mixture (10 % S9 fraction). The mixture was vortexed, poured onto minimal glucose agar plates, and incubated at 37 °C for 48 hours. Revertant colonies were counted, and mutagenicity was determined by comparing mean colony counts at each test concentration to the negative control (0.9 % NaCl). A twofold or greater increase in revertant colonies relative to the negative control was considered a positive mutagenic response, indicating the induction of frameshift or base-pair substitution mutations in the *S. typhimurium* genome. The protocol was conducted following the guideline by the established guideline ISO 10993-3: 2014 [17, 18]. All procedures in this study were conducted in a sterilised setting. As for mutagenicity assessment, the mean and standard deviation of revertant colonies were calculated and compared in triplicate between SLM Ti alloys plate concentrations and the negative control. A positive mutagenic response was defined as either a concentration-dependent increase in revertant colonies across the tested range or a mean revertant colony count at least twofold greater than the negative control [17, 18]. Positive results indicate that the SLM Ti alloys plate induces frameshift or base-pair substitution mutations in the genomes of *S. typhimurium* and *E. coli*.

2.4 Pyrogenicity Test

This study investigated the presence of endotoxin and non-endotoxin pyrogens of medical devices in New Zealand White Rabbits (NZWR). A total of three healthy NZWR (weights between 2.6 to 3.7 kg) were acclimatized and used in this study. All animals were handled and prepared according to the ISO10993-2: 2022 guideline. The study conducted at the Advanced Orthopaedics Research Laboratory, Department of Orthopaedics, Traumatology and Rehabilitation, Kulliyyah of Medicine, International Islamic University Malaysia (IIUM), Kuantan, Pahang. Approval of the animal study was endorsed by the Institutional Animal Care and Use Committee (I-ACUC) at IIUM, with the reference number IACUC 2022-022. As for pyrogenicity evaluation, SLM Ti alloys plate extraction was pre-warmed to 37 °C before injection. All rabbits' Control Temperature (CT) was measured in this study. The baseline body (rectal) temperature, which served as control, was measured in all animals by using the digital thermometer within 30 minutes before being injected with SLM Ti alloys plate extraction. This CT was also the base for the determination of any increment in temperature following injection. Animals were restrained by hand when body temperature was measured. The SLM Ti alloys plate extraction was then injected intravenously via the lateral vein of the marginal ear of the rabbit with the volume of 10 ml/kg body weight within 10 minutes. After the administration of the SLM Ti alloy plate extraction, the body temperature of all animals was recorded at 30-minute intervals for up to 3 hours. The body temperature measurements were taken in triplicate in all rabbits. As for evaluation, the differences between the control temperature (Delta T or ΔT) reading and the Maximum Temperature (MT) recorded were observed. According to the guideline, each rabbit must be monitored for changes in body temperature. If no rabbit exhibits a temperature increase of ≥ 0.5 °C above its baseline (control) temperature after three hours, this confirms the absence of pyrogens. If the result is negative (body

temperature changes is lower than body control temperature), the result is considered zero response. This testing was conducted according to ISO 10993-11: 2017 guideline [19]. The mean and standard deviation data of the animal body temperature also were calculated.

3. RESULTS AND DISCUSSION

3.1 Cytotoxicity Test

The cytotoxicity of SLM Ti alloy plates was assessed by incubating L-929 fibroblast cells for 48 hours with 100 % concentration extracts and controls. Microscopic examination revealed the cells maintained a healthy monolayer with proliferation in blank and negative controls (Figures 1(a) & 1(b)) as expected. In contrast, the cytotoxic effects were observed in the positive control (Figure 1(c)). No cell lysis or morphological abnormalities were detected after being exposed to the SLM Ti alloys plate derived extract (Figure 1(d)), which is comparable to the blank and negative controls. These findings confirm the cytocompatibility of SLM-fabricated Ti alloys plate, demonstrating no adverse effects on cell adhesion or viability. This study supports the feasibility of SLM for manufacturing orthopaedic implants, as the material exhibited biocompatibility equivalent to conventionally produced implants [3, 6–7, 11]. The MEM elution assay assessed morphological changes, a critical requirement for evaluating medical device safety under ISO 10993-5 [13–16]. Since prolonged interaction between implant and tissue can lead to leachable and induced cytotoxicity [4–6], this assay provides preliminary insight into clinical applicability. Complementary MTT assay data (was reported separately) further validated these results, showing sustained metabolic activity in treated cells. While these findings suggest that the production of Ti alloys orthopaedic implants through SLM technology for the fabrication of orthopaedic implants is bioinert, additional studies using human osteoblasts and osteoclasts are recommended to confirm suitability for clinical use.

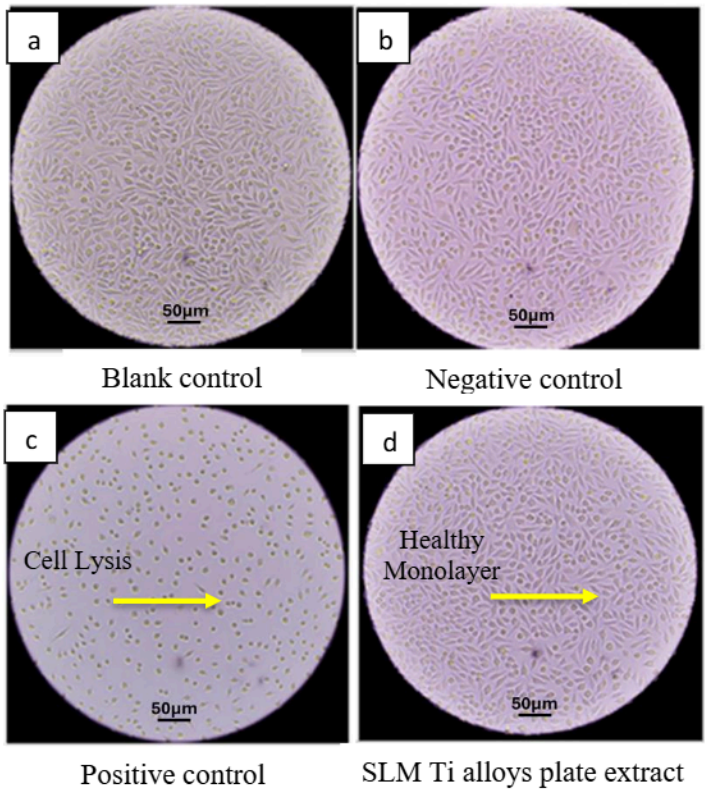

Figure 1: Micrographs depicting L-929 cell morphology (1000 x magnification) following treatment with (a) blank control, (b) negative control, (c) positive control and (d) SLM Ti alloys plate extract

3.2 Genotoxicity Test

The mutagenic potential of SLM Ti alloys plate was assessed via the bacterial reverse mutation assay (Ames test) using the pre-incubation method. As summarized in Table 2, exposure to SLM Ti alloys plate extracts did not produce did not increase in revertant colonies across all tested strains of *S. typhimurium* and *E. coli*, with or without metabolic activation (S9 mix). Mutagenicity ratios for all SLM Ti alloys plate concentrations remained below the threshold of twofold were comparable to negative controls. This outcome confirming the absence of mutagenic effects. In contrast, positive controls demonstrated the expected significant increase in revertant colonies, validating assay sensitivity. Genotoxicity testing is critical for implantable materials, as device degradation or residual manufacturing by products may release substances capable of causing DNA damage, a precursor to carcinogenesis or reproductive toxicity [10, 17, 18]. To address this, evaluations were conducted under both non activated and metabolically activated (S9) conditions. The former detects direct DNA damaging agents, while the latter identifies pro-mutagens requiring metabolic conversion. This dual approach ensures physiologically relevant risk assessment, bridging *in vitro* and *in vivo* conditions to support clinical safety conclusions [6, 7, 17, 18].

This study utilised the *Salmonella* Reverse Mutation Assay to evaluate the genotoxic potential induced by SLM Ti alloys extract in compliance with ISO10993-3: 2014 regulatory [18]. This well-established testing system serves as a sensitive indicator for detecting point mutations, frameshift mutations, and DNA damage through the evaluation of reverse mutations in histidine dependent of *Salmonella typhimurium* strains [10, 18]. The experimental protocol involved exposing bacterial cultures to SLM Ti alloys extracts at concentrations ranging 6.25 to 100 %. Testing was performed under two metabolic conditions direct exposure without metabolic activation and exposure with S9 liver homogenate that functioned to simulate mammalian metabolic conversion. Following incubation at 37 °C for 48-72 hours, revertant colony formation was quantified and compared against negative (vehicle) and positive (known mutagen) controls [12, 17, 18]. The results demonstrated there is no increase in revertant colonies numbers across all tested strains after being exposed with SLM Ti alloys concentration.

In addition, mutagenicity ratios remained below the critical threshold of twofolds which shows comparable to the negative controls with consistent negative responses in both metabolic conditions (with and without S9 activation). These findings confirmed the absence of mutagenic potential in SLM processed Ti alloys for orthopaedic implant, supporting its biocompatibility for medical device applications [6-8]. Importantly, the results indicate that the SLM manufacturing process does not introduce genotoxic hazards which is comparable to conventional titanium implant production methods as reported [4-9]. This data is particularly relevant for orthopaedic applications where long term of implant and tissue interaction is critical [10, 12]. While these results are promising, comprehensive genotoxicity assessment such as mammalian cell-based assays that function to evaluate chromosomal damage is advisable. This approach would provide a safety profile for SLM-manufactured orthopedic implants and support regulatory approval processes. The study demonstrates that SLM technology can produce titanium alloys implants with equivalent genotoxicological safety to conventionally manufactured devices, while offering the advantages of design flexibility and patient-specific customization inherent to additive manufacturing.

Table 2: Mean revertant colony counts from the Ames test, showing the effects of various concentrations (6.25 %, 12.5 %, 25 %, 50 %, and 100 %) of SLM Ti alloys plate extract, negative control, and positive control, with and without metabolic activation

Type of Colony &Treatment	Concentration (%)	Without metabolic activation	Reference limit	With metabolic activation (S9)	Reference limit
Revertant Colonies of *S.typhimurium* TA98	6.25	21 ± 3	<38	26 ± 1	<58
	12.5	23 ± 5		28 ± 3	
	25	18 ± 3		27 ± 1	
	50	18 ± 2		29 ± 2	
	100	21 ± 4		28 ± 1	
Negative Control (NaCl)	-	19 ± 1	-	29 ± 3	-
Positive Control (4-Nitro-o-phenylenediamine)	25 µg/ml	296 ± 11	>38	ND	-
Positive Control (2-Aminoanthracene)	5 µg/ml	ND	-	415±11	>58
Revertant Colonies of TA100	6.25	184 ± 1	<364	18'1 ± 2	<360
	12.5	184 ± 3		182 ± 6	
	25	181 ± 1		184 ± 5	
	50	183 ± 3		180 ± 6	
	100	186 ± 2		187 ± 5	
Negative Control (NaCl)	-	182 ± 3	-	180 ± 1	-
Positive Control (Sodium azide)	50 µg/ml	1067 ± 16	>364	ND	-
Positive Control (2-Aminoanthracene)	10 µg/ml	ND	-	795 ± 14	>360
Revertant Colonies of TA1535	6.25	12 ± 1	<28	17 ± 1	<34
	12.5	15 ± 2		19 ± 2	
	25	14 ± 2		17 ± 1	
	50	14 ± 2		18 ± 1	
	100	15 ± 3		18 ± 1	
Negative Control (NaCl)	-	14 ± 1	-	17 ± 2	-
Positive Control (Sodium azide)	5 µg/ml	434 ± 9	>28	ND	-
Positive Control (2-Aminoanthracene)	20 µg/ml	ND	-	232 ± 7	>34
Revertant Colonies of TA1537	6.25	12 ± 1	<26	16 ± 2	<27
	12.5	11 ± 1		13 ± 1	
	25	11 ± 1		13 ± 0	
	50	11 ± 1		13 ± 1	
	100	11 ± 1		13 ± 1	
Negative Control (NaCl)	-	13 ± 1	-	14 ± 1	-
Positive Control (9-Aminoacridine)	500 µg/ml	214 ± 16	>26	ND	-
Positive Control (2-Aminoanthracene)	20 µg/ml	ND	-	269 ± 9	>27
Revertant Colonies of *E. coli* strain WP2 *uvrA*	6.25	80 ± 9	<158	82 ± 3	<164
	12.5	78 ± 3		80 ± 1	
	25	76 ± 7		82 ± 3	
	50	77 ± 7		83 ± 4	
	100	84 ± 5		82 ± 1	
Negative Control (NaCl)	-	79 ± 9	-	82 ± 3	-
Positive Control (Methyl methanesulfonate)	25 µI/ml	1174 ± 46	>158	ND	-
Positive Control (2-Aminoanthracene)	100 µg/ml	ND	-	321 ± 2	>164

*ND= Not Done

3.3 Pyrogenicity Test

Results showed that for Rabbit 1 (AR022M), the CT is 39.4 °C and the MT is 39.4 °C; for Rabbit 2 (AR030M), the CT is 39.1 °C and the MT is 39.3 °C temperature increment); and Rabbit 3 (AR031M), the CT is 38.8 °C, and the MT is 39.1 °C. Overall, data showed that body temperature in all animals did not increase by 0.5 °C after the introduction of SLM Ti alloys plate extraction, as illustrated in Table 3. No adverse effect was noted during administration and observation. The negative pyrogen test results indicate that extracts from SLM Ti alloy plates did not elicit any pyrogenic response in vivo, confirming the material's safety in this regard. The mean and standard deviation data were calculated to compare the scores for the seven intervals (including before dosing) in all rabbits. The data showed that there is no significant difference between intervals; AR022M (Mean = 39.2 °C, SD = 0.08, ΔT = 0 °C), AR030M (Mean = 39.1 °C, SD = 0.07, ΔT = 0.2 °C) and AR031M (Mean = 38.8 °C, SD = 0.10, ΔT = 0.3 °C). These findings indicate that there is the release of the SLM Ti alloys plate extraction at each time point of intervals did not have a pyrogenicity effect on animal testing. The study revealed no significant difference in rise in body temperature after being treated with SLM Ti alloys plate extract in all rabbits.

Following the protocol outlined in ISO 10993-11:2017 for pyrogenicity assessment, three rabbits were subjected to the SLM Ti alloys plate extract. The resulting data, summarised in Table 3, revealed no individual temperature increases of ≥ 0.5 °C in any of the test subjects. This outcome satisfies the standard's acceptance criteria, indicating the absence of pyrogenic substances and negating the requirement for the expanded testing phase involving five additional animals [19, 20]. In the context of medical device development, pyrogenicity testing is a critical requirement [19, 20], ensuring the implant material is free from pyrogenic contaminants. The results obtained indicate that the SLM Ti alloys plate extract did not induce any detectable pyrogenic effects or adverse reactions.

Table 3: The response of rabbit body temperatures before and after injection with SLM Ti alloys plate extraction

Time (Minutes)	CT	30	60	90	120	150	180	ΔT
Rabbit Coding	**Mean Temperature (°C)**							
AR022M	39.4	39.2	39.2	39.3	39.4	39.2	39.2	0.0
AR030M	39.1	39.1	39.3	39.2	39.1	39.1	39.1	0.2
AR031M	38.8	38.8	38.9	38.8	38.9	39.0	39.1	0.3

*CT = Control Temperature or temperature before procedure

Internal fixation devices serve three primary biomechanical functions: fracture stabilization through rigid fixation, maintenance of anatomical alignment during healing, and preservation of physiological load transfer [1-3]. Recently, SLM technology has increasingly emerged as a transformative approach for orthopaedic implant manufacturing, offering patient specific implant customisation, cost-effective for small batch production with complex geometric fabrication unachievable through conventional methods [7-9]. Based on a literature reviewed, the development of a Ti alloys (grade 23) plate implant fabricated by SLM technology is being introduced to the Malaysian medical industry. To achieve clinical approval, the production of SLM Ti alloys plate must demonstrate a full compliance with ISO 10993-6:2016 for implant evaluation [6-9, 12, 13]. The positive results of this data may be used to develop other orthopaedic implants (Ti alloys material) via SLM technology. Although all data shows that the production of SLM Ti alloys plate may be considered biocompatible, a comprehensive testing including irritation testing, maximisation skin sensitisation assays, and acute systemic toxicity testing (polar and non-polar) are recommended. These further analyses are suggested to establish a robust toxicology profile for Malaysian-made SLM implants, ensure the safety of orthopaedic applications and support the technology transfer to regional markets in future.

4. CONCLUSIONS

The data presented herein demonstrate that the production of SLM Ti alloys plates produces materials with acceptable safety profiles, as evidenced by the results that are non-cytotoxic, non-genotoxic, and the absence of pyrogenic substances. Overall, these findings support the future application of SLM technology in creating personalised orthopaedic implants.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards. The ethical approval was obtained from the IIUM Animal Care and Use Committee (I-ACUC), with approval letter reference number: IACUC 2022-022.

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