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PAPER

Assessing cytotoxicity: a comparative analysis of biodegradable and conventional 3D-printing materials post-steam sterilization for surgical guides

Matthias W Gielisch[1,*], **Daniel G E Thiem**[1], **Ulrike Ritz**[3], **Christoph Bösing**[2], **Bilal Al-Nawas**[1] and **Peer W Kämmerer**[1]

[1] Department of Oral and Maxillofacial Surgery, University Medical Center of the Johannes Gutenberg University, 55131 Mainz, Germany
[2] Bösing Dental GmbH & CO. KG, Franz-Kirsten-Straße 1, 55411 Bingen, Germany
[3] Department of Orthopaedics and Traumatology, Biomatics Group, University Medical Center of the Johannes Gutenberg University, 55131 Mainz, Germany
[*] Author to whom any correspondence should be addressed.

E-mail: matthias.gielisch@uni-mainz.de

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Abstract

Introduction. Ecological concerns and the depletion of petroleum resources have driven the exploration of biodegradable 3D-printing materials derived from bio-renewable sources, such as polylactic acid (PLA) and polyhydroxyalkanoates (PHA). This study aimed to compare the potential cytotoxic effects of a biodegradable PLA/PHA blend filament, a conventional photopolymer (MED610), and a combination of MED610 with a support material (SUP705) before and after steam sterilization in vitro, with a focus on their application in the production of surgical guides. *Materials and Methods.* PLA/PHA, MED610, and SUP705 (both in their pure and steam-sterilized forms; $n = 6$ per group) were assessed for their cytotoxic effects on human fibroblasts using the neutral red uptake assay. Positive controls included zinc diethyldithiocarbamate and zinc dibutyldithiocarbamate, while high-density polyethylene served as a negative control. A stock solution of the extraction medium was used as the vehicle control (VC). *Results.* Significant differences in cell viability were observed between pure PLA/PHA (1.2 ± 0.24) and MED610 (0.94 ± 0.08) ($p = 0.005$). However, both materials exhibited non-cytotoxicity, with cell viability exceeding 70% compared to VCs. SUP705 (0.58 ± 0.42) demonstrated significantly reduced cell viability compared to PLA/PHA ($p = 0.001$) and MED610 ($p = 0.007$). After steam sterilization, no significant difference in cell viability was noted between MED610 (1.0 ± 0.08) and PLA/PHA (1.2 ± 0.25) ($p = 0.111$). While both materials remained non-cytotoxic after sterilization, SUP705 (0.60 ± 0.45) exhibited cytotoxic effects compared to MED610 ($p = 0.006$) and PLA/PHA ($p < 0.001$). Steam sterilization did not induce significant cytotoxic effects in the investigated materials ($p = 0.123$). *Conclusion.* Pure and steam-sterilized PLA/PHA and MED610 were not cytotoxic, supporting their potential use in the production of surgical guides. However, the observed cytotoxicity of SUP705 suggests caution in scenarios requiring sterile conditions, as the removal of support material from complex printed parts may be challenging. The consideration of PLA/PHA is recommended in such settings to ensure biocompatibility.

1. Introduction

The field of oral and maxillofacial surgery has witnessed a paradigm shift with the increasing prominence of 3D printing technology. Particularly noteworthy are applications characterized by a high degree of individualization, such as the fabrication of implants, including joint prostheses, osteosynthesis materials, and reconstruction plates. Additionally, 3D printing has proven to be highly effective in producing surgical accessories, such as cutting or positioning guides, and in generating intricate 3D-printed

models that serve both educational purposes and preoperative planning [1–7]. A wide variety of 3D printing technologies are available for these purposes, including stereolithography, material jetting (MJ), laser melting or sintering, and fused filament fabrication (FFF) [8]. FFF, known for its cost-effectiveness and versatility due to the broad range of available filament materials, is a widely adopted technique in this domain [9]. Notably, newer filaments incorporating materials such as palm fiber or wood flour have significantly expanded the range of possibilities in 3D printing [10–12]. However, the safety and biocompatibility of these materials in medical applications require rigorous evaluation. To address this, the International Organization for Standardization (ISO) has developed a set of standards for biocompatibility testing of medical devices, including in vitro cytotoxicity testing (ISO 10 993–5) [13].

Photopolymers commonly used in 3D printing are known for their potential toxicity [14], often necessitating additional post-processing steps, such as UV light exposure, to mitigate their harmful effects [15]. One such commercially available photopolymer is MED610 (Stratasys, Minnesota, United States), an acrylic-based material with documented biocompatibility under specific conditions, including skin contact for over 30 d and mucous membrane or bone contact for up to 24 h. Importantly, MED610 has been approved for steam sterilization when the prescribed procedural guidelines are strictly followed. When printing complex structures with MED610, support materials like Support 705 (SUP705, Stratasys, Minnesota, United States) are indispensable. The removal of this support material, particularly in medical applications, typically involves water jet and alkaline cleaning processes. Studies evaluating various cleaning protocols for MED610 have highlighted the importance of optimized procedures to enhance cell viability and ensure in vivo biocompatibility [16]. In addition, when comparing four different printing materials, including MED610 and a polylactic acid (PLA) filament in vitro, MED610 was found to exhibit a comparably higher level of cytotoxicity [17].

Beyond biological considerations, economic and ecological aspects play a pivotal role in material selection. PLA, a biodegradable and recyclable filament derived from renewable resources, presents a sustainable alternative [17–20]. PLA, which contains additives such as nucleating agents and co-polyesters, exhibits favorable biological activity, as evidenced by enhanced osteoblast proliferation and viability compared to traditional materials like titanium [21]. Moreover, studies investigating bone marrow stem cells on PLA highlight its non-cytotoxic nature and high cell viability, further emphasizing its potential in medical applications [22].

While freshly printed PLA parts are considered sterile [23, 24], the application of steam sterilization presents challenges due to the material's thermal transition temperature, which can potentially alter its mechanical and dimensional properties [25]. In addition to PLA, other filament materials such as acrylonitrile butadiene styrene (ABS), nylon, PETG (polyethylene terephthalate glycol-modified), and polypropylene have been investigated with respect to their dimensional stability and cytotoxicity both before and after steam sterilization. Due to the deformations observed, the authors of these studies do not recommend the use of steam sterilization or dry heat processes; instead, they suggest hydrogen peroxide sterilization [26, 27]. However, steam sterilization remains the most commonly available and widespread method, necessitating a different strategy to introduce biodegradable and recyclable materials, such as PLA, into broader medical applications. One such strategy involves blending PLA with polyhydroxyalkanoate (PHA) to reinforce printed parts for steam sterilization without compromising material integrity [28]. PHA, produced by bacteria under specific conditions, exhibits no in vitro cytotoxic effects [29]. While FFF-printed PLA/PHA blends have demonstrated no cytotoxic effects on human embryonic kidney cells [30], the impact of steam sterilization on their cytotoxicity remains unexplored, highlighting a critical gap in our understanding.

This study aimed to address this gap in understanding by systematically comparing the impact of steam sterilization on the cytotoxicity of FFF-printed PLA/PHA blends with that of MED610, a material commonly used in MJ. Additionally, the study investigates the impact of steam sterilization on the cell viability of the support material SUP705, which has not been previously explored. These findings are anticipated to contribute significantly to the informed selection of 3D printing materials for surgical guides, considering both biological safety and ecological sustainability.

2. Materials and methods

2.1. Materials

In this study, two distinct printing materials were employed: a PLA/PHA-based filament (PLA/PHA, greenTec pro, Extrudr FD3D GmbH, Austria) with a diameter of 1.75 mm, printed using a Raise3D Pro 2 machine (Raise3D Inc., California, United States), and MED610 (Stratasys, Minnesota, United States), printed using an ObjetEden260 machine (Stratasys, Minnesota, United States). SUP705 (Stratasys, Minnesota, United States) served as the support material for the ObjetEden260 printer.

The study design included three main groups:

- Group A: PLA/PHA.
- Group B: MED610.
- Group C: MED610/SUP705.

Each group was further subdivided into two subgroups:

- Subgroup I: sterilized.
- Subgroup II: non-sterilized.

The printing parameters for the Raise3D Pro 2 printer were as follows: print-head temperature maintained at 225 °C, heated print-bed at 55 °C, and a layer height of 0.05 mm. Printing was executed using a 0.4 mm steel nozzle. For the ObjetEden260 printer, the following settings were applied: high print quality, matte surface finish, and test bodies with a 100% infill rate.

To assess the indirect biocompatibility, cylindrical test bodies with a diameter of 15 mm and a height of 2 mm were designed. The modeling process was carried out using OpenSCAD, an open-source software (version: 2019.05, www.openscad.org/).

2.2. Sterilization process

Each of the three main groups (A, B, and C) was further subdivided into two subgroups: Subgroup I (sterilized) and Subgroup II (non-sterilized), encompassing PLA/PHA (Group A), MED610 (Group B), and MED610/SUP705 (Group C). The sterilization procedure involved steam sterilization at 134 °C for 8 min, maintained at 3.04 bar pressure, using a specialized steam sterilizer (666–1 H-FD-DST, Holzner GmbH, Heidelberg, Germany). This process ensured the effective sterilization of the test bodies in preparation for subsequent biocompatibility assessments.

2.3. Cell culture

Human gingival fibroblasts (HFIB-G) from Provitro (Product ID: 1210 412, Berlin, Germany) were used in this study. The cell culture process utilized Dulbecco's Modified Eagle Medium (DMEM, Life Technologies—Gibco, Carlsbad, USA) and culture flasks (Product ID: 660 175, Greiner Bio-One, Kremsmünster, Austria). Upon reaching approximately 80% confluency after two to three passages, the cells were harvested.

For subsequent experiments, 100 µl of cell suspension at a density of 1 x 10^5 cells ml^{-1} was plated in the inner 60 wells of a 96-well plate (Product ID: 655 160, Greiner Bio-One, Kremsmünster, Austria). The plated cells were then incubated in a Heraeus incubator (BB16, Hanau, Germany) for 24 h at 37 °C in a humidified atmosphere containing 5% CO2 in air. This incubation period facilitated the establishment of an optimal cellular environment for subsequent biocompatibility assessment.

2.4. Extraction

Each test sample had a total surface area of 4.48 cm^2, and 15 test bodies were utilized for each group. Following ISO 10 993–12 guidelines [31], the extraction medium was prepared at a ratio of 3 cm^2 ml^{-1}, resulting in a total volume of 22.38 ml.

Extraction procedures (figure 1) were conducted for 24, 48, and 72 h in 50 ml tubes (Product ID: 210 261, Greiner Bio-One, Kremsmünster, Austria). The extraction medium comprised 5% newborn calf serum (Product ID: 12 023 C, Sigma-Aldrich, St. Louis, USA), 4 mM l-glutamine (Product ID: G7513, Sigma-Aldrich, St. Louis, USA), 100 IU/ml penicillin (Product ID: P0781, Sigma-Aldrich, St. Louis, USA), 100 µg/ml streptomycin (Product ID: P0781, Sigma-Aldrich, St. Louis, USA), and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Product ID: 15 630 056, Life Technologies—Gibco, Carlsbad, USA).

Positive control materials, specifically zinc diethyldithiocarbamate (ZDEC, Polyurethane film containing 0.1% zinc diethyldithiocarbamate) and zinc dibutyldithiocarbamate (ZDBC, Polyurethane film containing 0.25% zinc dibutyldithiocarbamate) (Food and Drug Safety Center, Hatano Research Institute, Hadano, Japan), were treated in a manner similar to the test samples. For the positive controls, 15 cm^2 of ZDEC or ZDBC foil was cut into small pieces, followed by the addition of 10 ml extraction medium. The extraction was then conducted for 24, 48, and 72 h.

The negative control materials consisted of high-density polyethylene tubes (Product ID: 210 261, Greiner Bio-One, Kremsmünster, Austria) cut into square pieces to achieve a surface area of approximately 4.48 cm^2. Subsequently, these pieces were incubated in 22.38 ml extraction medium for 24, 48, and 72 h. The entire extraction process for both test and control samples was performed at 37 °C.

2.5. Dilution series

Following the extraction process, a dilution series was prepared as outlined below. Eight 50 ml Falcon tubes (Product ID: 210 261, Greiner Bio-One, Kremsmünster, Austria) were arranged for the dilution series. The first tube received 4 ml of the stock solution, while the other seven tubes were each filled with 1168 µl of the extraction medium. The dilution process involved transferring 3000 µl of the stock solution to the first tube, representing the lowest dilution, followed by vortexing. Subsequently, 3000 µl from this first tube was transferred to the next tube, and this process was repeated sequentially for all tubes up to the eighth tube, thereby establishing eight test concentrations (C1—undiluted to C8—highest dilution). Pure extraction medium was used as the vehicle control (VC) in this dilution series.

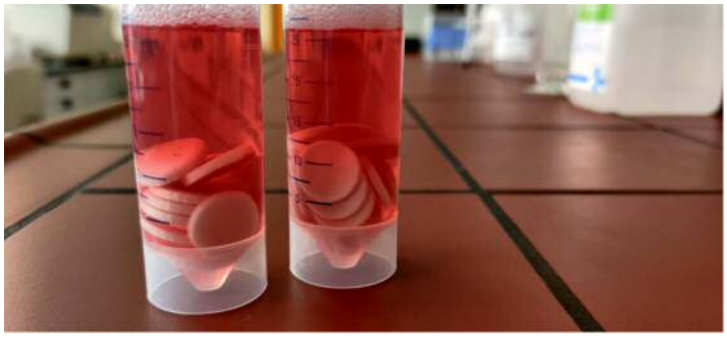

Figure 1. Extraction procedure with cylindric test bodies and extraction medium in 50 ml tubes. The test specimens were transferred into the tubes under sterile conditions and incubated for 24 h, 48 h, or 72 h at 37 °C.

2.6. Neutral red cytotoxicity assay

Cytotoxicity was assessed using a commercially available neutral red cytotoxicity assay kit (PK-CA577-K447, PromoKine, Heidelberg, Germany). Following a 24-hour incubation in the growth medium, the culture medium was carefully removed, and each well was washed with 200 µl of washing solution. The outer rows and columns were filled ytotoxicity was assessed using a commercially available neutral red cytotoxicity assay kit (PK-CA577-K447, PromoKine, Heidelberg, Germany). After a 24-hour incubation in the growth medium, the culture medium was carefully removed, and each well was washed with 200 µl of washing solution. The outer rows and columns were filled with 100 µl of extraction medium, while 100 µl of the respective extraction solution was transferred to six wells, representing one dilution per column (n = 6 with all dilutions on one plate). Additionally, two rows were designated as untreated VCs and filled with 100 µl of extraction medium per well. After an additional 24 h of incubation, a microscopic control was performed. The extraction solution was then removed, and all wells were rinsed with 200 µl of washing solution. Subsequently, 100 µl of neutral red solution was pipetted into each well and incubated for 2 h. After this incubation period, the neutral red solution was removed, and the wells were rinsed with 250 µl of washing solution. Finally, all wells were filled with solubilization solution, and the microplate was placed on a shaker for 20 min. The optical density (O.D.) was then measured using a Spectramax ID5 multi-mode microplate reader (Molecular Devices, San José, California, USA) at 540 nm.

A higher optical density, indicating greater uptake of neutral red, suggests higher cell viability. The optical density values from each microplate were normalized to the corresponding VC, allowing for the evaluation of cytotoxicity levels. All reported cell viability values in this article refer to these normalized optical density measurements.

2.7. Statistics

Data collection was conducted using Microsoft Excel (Version 2013, Microsoft, Redmond, USA), and statistical analyses were performed using RStudio (Version 1.2.5033, Integrated Development for R, RStudio, Inc., Boston, USA) and R (Version 3.6.2, R Foundation for Statistical Computing, Vienna, Austria). The calculated parameters included mean and standard deviation.

To assess the assumption of homogeneity of variances, Levene's test was performed. If Levene's test indicated that variances were homogeneous, a two-way analysis of variance (ANOVA) was conducted to evaluate the effects of the independent variables (e.g. material type and sterilization status) on cytotoxicity, as well as their interaction. Following a significant ANOVA result, a Games-Howell post-hoc test was applied to assess pairwise comparisons, particularly in cases where variances were unequal, as indicated by Levene's test. The significance level was set at $p < 0.05$ for all statistical tests. In cases where the data did not meet the assumptions of normality or homogeneity of variances, non-parametric alternatives were considered. Specifically, the Kruskal–Wallis test was used as an alternative to ANOVA for analyzing group differences when data were non-normal or variances were not equal. Post-hoc comparisons for the Kruskal–Wallis test were conducted using Dunn's test with Bonferroni correction.

3. Results

3.1. Cell viability of the printed pure materials

The findings for PLA/PHA and MED610 showed no cytotoxicity at any of the extraction times, with no increase in cytotoxicity observed for lower concentrations of the extraction solution. Pure PLA/PHA samples showed no significant difference in cell viability compared to the negative control ($p = 0.335$), while MED610 differed significantly from the negative control ($p = 0.010$).

Table 1. Means and standard deviations of the normalized O.D. at 540 nm for all materials before and after stream sterilization. Values for MED610/SUP705 and ZDEC (positive control) are below 0.7. SD: standard deviation; ZDEC: diethyldithiocarbamate; ZDBC: dibutyldithiocarbamate; HDPE: high-density polyethylene; PHA/PLA: polyhydroxyalkanoate/polylactide acid.

	PHA/PLA		MED610		MED610/SUP705		ZDEC	ZDBC	HDPE	Overall	
	Non-sterile (N = 27)	Sterile (N = 27)	Non-sterile (N = 27)	Sterile (N = 27)	Non-sterile (N = 27)	Sterile (N = 27)	Non-sterile (N = 27)	Non-sterile (N = 27)	Non-sterile (N = 27)	Non-sterile (N = 162)	Sterile (N = 81)
Normalized absorbance at 540 nm—Mean (SD)	1.2 (± 0.24)	1.2 (± 0.25)	0.94 (± 0.08)	1.0 (± 0.08)	0.58 (± 0.42)	0.60 (± 0.45)	0.48 (± 0.41)	0.87 (± 0.27)	1.0 (± 0.08)	0.84 (± 0.37)	0.92 (± 0.38)

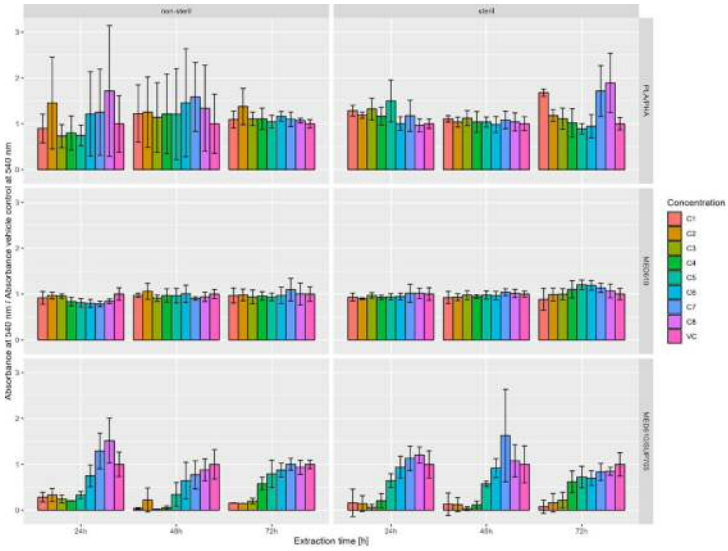

Figure 2. An overview of the normalized* optical density values, measured with 540 nm wavelength, before and after steam sterilisation of PLA/PHA, MED610 and MED610/SUP705. Cell viability of the used human fibroblasts for MED610/SUP705 increased with decreasing extract concentrations whereas no effect on MED610 and PLA/PHA was seen regarding concentrations. Extraction times of 24 h, 48 h and 72 h did not seem to differ (no statistical tests were conducted for concentrations and extraction times).
* *The values correspond to multiples of an equally treated vehicle control.*

The cell viability of MED610/SUP705 increased with higher dilutions of the extract at every time point, a behavior comparable to that of the positive control (ZDEC). The difference in cell viability between PLA/PHA (1.2 ± 0.24) and MED610 (0.94 ± 0.08) was statistically significant ($p = 0.002$). Low levels of cell viability were observed for MED610/SUP705 (0.58 ± 0.42, table 1), and this result was significantly different compared to PLA/PHA ($p < 0.001$) but not MED610 ($p = 0.052$). The control samples, ZDEC and ZDBC, showed low cell viability similar to MED610/SUP705 ($p = 1.000$ and $p = 0.180$).

3.2. Cell viability after steam sterilization

No significant differences were observed between sterile and non-sterile samples ($p = 0.123$). Sterilized PLA/PHA (1.2 ± 0.25) and MED610 (1.0 ± 0.08) did not exhibit toxicity compared to the negative control ($p = 0.345$ and $p = 0.977$, respectively). However, MED610/SUP705 exhibited behavior similar to that of ZDEC, with significantly lower cell viability compared to the negative control ($p = 0.003$). No difference in cell viability after steam sterilization was noted between MED610 (1.0 ± 0.08) and PLA/PHA (1.2 ± 0.25) ($p = 0.223$). The plots for these materials before and after sterilization were similar, indicating no cytotoxicity regardless of the extract dilution (figure 2).

Furthermore, no significant effect of steam sterilization was observed for MED610 ($p = 0.337$), PLA/PHA ($p = 1.000$), or MED610/SUP705 ($p = 1.000$) compared with the respective non-sterile materials. However, consistent with the findings before sterilization, a cytotoxic effect was observed for MED610/SUP705 after sterilization (0.60 ± 0.45) compared to MED610 ($p = 0.026$) and PLA/PHA ($p < 0.001$).

4. Discussion

The non-cytotoxic behavior of both PLA/PHA and MED610, as demonstrated by cell viability rates of 85.3% and 72.5%, respectively, indicates their potential as biocompatible materials for 3D-printed surgical guides and anatomical models. PLA/PHA, in particular, showed superior cell viability compared to MED610, suggesting its greater suitability for applications where high biocompatibility is critical.

In contrast, MED610/SUP705 exhibited markedly lower cell viability (58.2%), indicating a cytotoxic effect that persists regardless of the sterilization process. This finding raises concerns about the use of SUP705 as a support material in medical 3D printing, especially in applications requiring direct contact with human tissues. The potential reasons for SUP705's cytotoxicity include the presence of residual monomers, incomplete removal during post-processing, and possible alterations in chemical structure during steam sterilization. These factors underscore the importance of thorough post-processing and validation to ensure the biocompatibility of 3D-printed objects.

Evidence of the cytotoxicity of MED610 and SUP705 remains limited. According to the manufacturer, MED610 is biocompatible and holds

medical approval. These findings align with the manufacturer's claims, as pure MED610 did not significantly affect cell viability compared to the negative control, maintaining viability above 70% relative to the VC. This result is consistent with the findings of Schmelzer *et al* who reported no material-induced cell death following indirect contact with MED610. However, cell death and reduced proliferation were observed in vitro with direct contact. The study also explored the effects of MED610 on human adult keratinocytes, revealing no adverse impact on viability. Additionally, indications of leached estrogen-like substances, such as plasticizers, were noted after contact with MED610 [17]. In another study by Ngan *et al* the cytotoxicity of MED610 printed with SUP706 post-processed using different protocols was tested. After three days, no significant differences in cell viability were observed. However, after ten days, cell death significantly increased when following the manufacturer's protocol [16]. These results parallel the findings of no short-term cytotoxic effect of pure MED610 in the current study, highlighting the importance of considering incubation time and contact conditions.

In contrast, while similar printing materials demonstrated diminished biocompatibility, attributed in part to microscopic particles in the extraction medium [32], such concerns did not arise for the materials investigated in this study.

The investigation of SUP705 as a support material revealed cytotoxicity comparable to that of the positive control ZDEC. While literature on SUP705 is lacking, Ngan *et al* used SUP706, a similar support material, and reported no cytotoxic effects after complete removal and additional cleaning steps [16]. These findings underscore the necessity for further research on the cytotoxicity of support materials, particularly regarding their potential residues in printed objects.

To the best of current knowledge, no study has addressed the cytotoxicity of MED610 or SUP705 in combination with steam sterilization. Only one study explored changes in printed MED610 dimensions for surgical instruments after steam sterilization at 121 °C for 15 min without testing cytotoxicity [33]. The findings suggest that steam sterilization of MED610 induces no significant changes in cytotoxicity compared to non-sterile samples of the same material. Sterilized MED610 also did not exhibit cytotoxicity. However, SUP705 demonstrates cytotoxicity after the sterilization process, with no significant differences detected before and after sterilization. In scenarios involving the printing of surgical guides or anatomical models under sterile conditions, the use of MED610 appears viable. However, thorough removal of all support materials is essential, particularly in cases of complex anatomical models or surgical guides, as incomplete removal may lead to cytotoxic effects that could compromise wound healing [34].

3D prints made from PLA exhibited no cytotoxicity following various sterilization procedures [35]. Gonzalez *et al* compared the microscopic growth of human embryonic kidney cells (HEK293) and a fibroblast cell line from lung tissue (WI-38) on printed PLA/PHA. Although WI-38 showed reduced growth, no toxicity was detected. In another study, no negative impact on biocompatibility with human gingival keratinocytes was observed for printed and sterilized PLA/PHA [36]. The current study supports these findings, as both pure and sterilized FFF-printed PLA/PHA displayed no cytotoxic effects on human fibroblasts. Additionally, no significant difference was observed between sterile and non-sterile PLA/PHA samples [30].

Studies on PHA alone have demonstrated better cell survival than PLA, indicating a potential positive influence on cell proliferation [37, 38]. This effect is possibly due to PHA and its degradation products [39]. The composition of the PLA/PHA blend also appears to influence cellular viability [40]; however, as the filament used is commercial and the exact composition is unknown, the specific effects of the composition in the researched filament cannot be fully classified. Beyond good cell viability, PHA does not appear to have carcinogenic effects [41]. The findings from this investigation align with this trend, suggesting that the PLA/PHA blend in the studied filaments may promote higher cell proliferation. While no significant difference was observed between PLA/PHA and MED610 after sterilization, PLA/PHA was significantly superior before sterilization. Furthermore, SUP705 exhibited significantly worse cell viability than PLA/PHA. Considering the impracticality of printing MED610 without a support material such as SUP705, PLA/PHA emerges as the preferred choice in terms of cytotoxicity.

The non-cytotoxic nature of both PLA/PHA and MED610, as revealed by this study, underscores a pivotal advancement in the selection of materials for 3D printing in medical applications. The superior cell viability exhibited by PLA/PHA not only emphasizes its potential in biocompatible applications but also aligns with the growing emphasis on sustainability in the medical field. This is particularly relevant given the urgent need to reduce medical waste and the environmental footprint of healthcare practices. The use of biodegradable materials like PLA and PHA, derived from renewable resources, represents a critical step towards achieving these sustainability goals [42].

Furthermore, the contrast in cytotoxicity between the studied materials and SUP705 highlights the complexity of material selection for 3D printing applications. The cytotoxic potential of SUP705, independent of steam sterilization, raises important

considerations for its use in medical settings. This finding is consistent with recent studies suggesting that the presence of residues from materials could compromise the biocompatibility of 3D-printed objects [17]. This underscores the importance of thorough post-processing, including cleaning and support removal, and the validation of all materials intended for clinical use, especially those in direct contact with human tissues, such as surgical guides or wound dressings.

Moreover, the ecological implications of selecting biodegradable materials such as PLA/PHA over conventional plastics cannot be overstated. With the healthcare sector increasingly recognized as a significant contributor to global environmental degradation, the shift towards more sustainable practices is both a moral and practical necessity. The adoption of biodegradable materials in 3D printing aligns with these goals, offering a path to reduce the environmental impact of medical waste without compromising patient care.

5. Conclusion

In conclusion, both sterile and non-sterile PLA/PHA and MED610 demonstrated non-cytotoxic behavior in accordance with ISO 10 993–5, supporting their potential use in medical applications, particularly in the production of 3D-printed surgical guides and anatomical models. However, this study suggests the need for enhanced post-processing protocols that ensure the complete removal of support materials like SUP705. This could include the development of more effective cleaning methods or the use of alternative sterilization techniques that minimize the risk of cytotoxicity.

Data availability statement

The data cannot be made publicly available upon publication because no suitable repository exists for hosting data in this field of study. The data that support the findings of this study are available upon reasonable request from the authors.

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Matthias Gielisch, Peer W. Kämmerer

Data Curation: Matthias Gielisch

Investigation: Matthias Gielisch, Peer W. Kämmerer

Methodology: Matthias Gielisch, Ulrike Ritz, Peer W. Kämmerer.

Visualization: Matthias Gielisch

Supervision: Peer W. Kämmerer, B. Al-Nawas

Validation: Matthias Gielisch, Ulrike Ritz, Christoph Bösing, Peer W. Kämmerer

Writing—original draft: Matthias Gielisch, Peer W. Kämmerer

Writing—review & editing: Matthias Gielisch, Daniel G.E. Thiem, Christoph Bösing, Ulrike Ritz, Peer W. Kämmerer, B. Al-Nawas

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ORCID iDs

Matthias W Gielisch https://orcid.org/0000-0002-9229-8662
Daniel G E Thiem https://orcid.org/0000-0002-4081-1487
Ulrike Ritz https://orcid.org/0000-0001-8936-3227
Bilal Al-Nawas https://orcid.org/0000-0002-8665-5803
Peer W Kämmerer https://orcid.org/0000-0002-1671-3764

References

[1] Goetze E, Kämmerer P W, Al-Nawas B and Moergel M 2020 Integration of perforator vessels in CAD/CAM free fibula graft planning: a clinical feasibility study *J. Maxillofac. Surg.* **19** 61–66
[2] Goetze E, Gielisch M, Moergel M and Al-Nawas B 2017 Accelerated workflow for primary jaw reconstruction with microvascular fibula graft *3D Print. Med.* **3** 3
[3] Goetze E, Moergel M, Gielisch M and Kämmerer P W 2019 Safety of resection margins in CAD/CAM-guided primarily reconstructed oral squamous cell carcinoma-a retrospective case series *Oral Maxillofac. Surg.* **23** 459–64
[4] Goetze E, Thiem D G E, Gielisch M, Al-Nawas B and Kämmerer P W 2020 Digitalization and use of artificial intelligence in microvascular reconstructive facial surgery *Chirurg* **91** 216–21
[5] Hanisch M, Kroeger E, Dekiff M, Timme M, Kleinheinz J and Dirksen D 2020 3D-printed surgical training model based on real patient situations for dental education *Int. J. Environ. Res. Public Health* **17** 2901
[6] Hatz C R, Msallem B, Aghlmandi S, Brantner P and Thieringer F M 2020 Can an entry-level 3D printer create high-quality anatomical models? Accuracy assessment of mandibular models printed by a desktop 3D printer and a professional device *Int. J. Oral Maxillofac. Surg.* **49** 143–8
[7] Dimitroulis G, Austin S, Sin Lee P V and Ackland D 2018 A new three-dimensional, print-on-demand temporomandibular prosthetic total joint replacement system: preliminary outcomes *J. Craniomaxillofac. Surg.* **46** 1192–8
[8] Al-Nawas B and Goetze E 2017 3-D-Druck in der MKG-Chirurgie *MKG Chir.* **10** 234–43

[9] Wu H, Fahy W, Kim S, TZhao N, Pilato L, Kafi A, Kafi A, Bateman S and Koo J H 2020 Recent developments in polymers/polymer nanocomposites for additive manufacturing *Prog. Mater. Sci.* **111** 100638
[10] Wu C S, Liao H T and Cai Y X 2017 Characterisation, biodegradability and application of palm fibre-reinforced polyhydroxyalkanoate composites *Polym. Degrad. Stab.* **140** 55–63
[11] Wu C S and Liao H T 2017 Fabrication, characterization, and application of polyester/wood flour composites *J. Polym. Eng.* **37** 689–98
[12] Faruk O, Bledzki A K, Fink H P and Sain M 2012 Biocomposites reinforced with natural fibers: 2000–2010 *Prog. Polym. Sci.* **37** 1552–9
[13] ISO 10993–5 2018 *Tests For In Vitro Cytotoxicity* (International Organisation for Standardization) Standardization IOf
[14] Macdonald N P, Zhu F, Hall C J, Reboud J, Crosier P S, Patton E E, Wlodkowic D and Cooper J M 2016 Assessment of biocompatibility of 3D printed photopolymers using zebrafish embryo toxicity assays *Lab Chip* **16** 291–7
[15] Oskui S M, Diamante G, Liao C, Shi W, Gan J, Schlenk D and Grover W H 2016 Assessing and reducing the toxicity of 3D-printed parts *Environ. Technol. Lett.* **3** 1–6
[16] Ngan C G Y, O'Connell C D, Blanchard R, Boyd-Moss M, Williams R J, Bourke J, Quigley A, McKelvie P, Kapsa R M I and Choong P F M 2019 Optimising the biocompatibility of 3D printed photopolymer constructs in vitro and in vivo *Biomed. Mater.* **14** 035007
[17] Schmelzer E, Over P, Gridelli B and Gerlach J C 2016 Response of primary human bone marrow mesenchymal stromal cells and dermal keratinocytes to thermal printer materials in vitro *J. Med. Biol. Eng.* **36** 153–67
[18] Bodnarova S *et al* 2019 3D printed polylactid acid based porous scaffold for bone tissue engineering: an in vitro study *Acta Bioeng. Biomech.* **21** 101–10
[19] Fairag R, Rosenzweig D H, Ramirez-Garcialuna J L, Weber M H and Haglund L 2019 Three-dimensional printed polylactic acid scaffolds promote bone-like matrix deposition in vitro *ACS Appl. Mater. Interfaces* **11** 15306–15
[20] Naser A Z, Deiab I and Darras B M 2021 Poly(lactic acid) (PLA) and polyhydroxyalkanoates (PHAs), green alternatives to petroleum-based plastics: a review *RSC Adv.* **11** 17151–96
[21] Wurm M C, Möst T, Bergauer B, Rietzel D, Neukam F W, Cifuentes S C and Wilmowsky C V 2017 In-vitro evaluation of polylactic acid (PLA) manufactured by fused deposition modeling *J. Biol. Eng.* **11** 29
[22] Gremare A, Guduric V, Bareille R, Heroguez V, Latour S, L'heureux N, Fricain JC, Catros S and Le Nihouannen D 2018 Characterization of printed PLA scaffolds for bone tissue engineering *J. Biomed. Mater. Res.* A **106** 887–94
[23] Rankin T M, Giovinco N A, Cucher D J, Watts G, Hurwitz B and Armstrong D G 2014 Three-dimensional printing surgical instruments: are we there yet? *J. Surg. Res.* **189** 193–7
[24] Neches R Y, Flynn K J, Zaman L, Tung E and Pudlo N 2016 On the intrinsic sterility of 3D printing *PeerJ* **4** e2661
[25] Athanasiou K A, Niederauer G G and Agrawal C M 1996 Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers *Biomaterials* **17** 93–102
[26] Valls-Esteve A *et al* 2023 A state-of-the-art guide about the effects of sterilization processes on 3D-printed materials for surgical planning and medical applications: a comparative study *Int. J. Bioprinting* **9** 756
[27] Rynio P, Galant K, Wojcik L, Grygorcewicz B, Kazimierczak A, Falkowski A, Gutowski P, Dołęgowska B and Kawa M 2022 Effects of sterilization methods on different 3D printable materials for templates of physician-modified aortic stent grafts used in vascular surgery-a preliminary study *Int. J. Mol. Sci.* **23** 3539
[28] Kaygusuz B and Özerinç S 2019 Improving the ductility of polylactic acid parts produced by fused deposition modeling through polyhydroxyalkanoate additions *J. Appl. Polym. Sci.* **136** 48154
[29] Murueva A V, Shishatskaya E I, Kuzmina A M, Volova T G and Sinskey A J 2013 Microparticles prepared from biodegradable polyhydroxyalkanoates as matrix for encapsulation of cytostatic drug *J. Mater. Sci., Mater. Med.* **24** 1905–15
[30] Gonzalez Ausejo J *et al* 2018 A comparative study of three-dimensional printing directions: the degradation and toxicological profile of a PLA/PHA blend *Polym. Degrad. Stab.* **152** 191–207
[31] ISO 10993–12 2012 *Sample Preparation and Reference Materials* (International Organisation for Standardization) Standardization IOf
[32] Winkler S, Meyer K V, Heuer C, Kortmann C, Dehne M and Bahnemann J 2022 In vitro biocompatibility evaluation of a heat-resistant 3D printing material for use in customized cell culture devices *Eng. Life Sci.* **22** 699–708
[33] Hooper J, Schwarzkopf R, Fernandez E, Buckland A, Werner J, Einhorn T and Walker P S 2019 Feasibility of single-use 3D-printed instruments for total knee arthroplasty *Bone Joint J* **101** 115–20
[34] Totoraitis K, Cohen J L and Friedman A 2017 Topical approaches to improve surgical outcomes and wound healing: a review of efficacy and safety *J. Drugs Dermatol.* **16** 209–12
[35] Perez-Davila S, Gonzalez-Rodriguez L, Lama R, Lopez-Alvarez M, Oliveira A L, Serra J, Novoa B, Figueras A and González P 2022 3D-printed PLA medical devices: physicochemical changes and biological response after sterilisation treatments *Polymers* **14** 4117
[36] Burkhardt F, Spies B C, Wesemann C, Schirmeister C G, Licht E H, Beuer F, Steinberg T and Pieralli S 2022 Cytotoxicity of polymers intended for the extrusion-based additive manufacturing of surgical guides *Sci. Rep.* **12** 7391
[37] Liu Q and Chen G Q 2008 In vitro biocompatibility and degradation of terpolyester 3HB-co-4HB-co-3HHx, consisting of 3-hydroxybutyrate, 4-hydroxybutyrate and 3-hydroxyhexanoate *J. Biomater. Sci. Polym. Ed.* **19** 1521–33
[38] Yang X, Zhao K and Chen GQ 2002 Effect of surface treatment on the biocompatibility of microbial polyhydroxyalkanoates *Biomaterials* **23** 1391–7
[39] Xiao X Q, Zhao Y and Chen G Q 2007 The effect of 3-hydroxybutyrate and its derivatives on the growth of glial cells *Biomaterials* **28** 3608–16
[40] Santos A R Jr, Ferreira B M, Duek E A, Dolder H, Wada R S and Wada M L 2004 Differentiation pattern of Vero cells cultured on poly(L-lactic acid)/poly(hydroxybutyrate-co-hydroxyvalerate) blends *Artif. Organs* **28** 381–9
[41] Peng S W, Guo X Y, Shang G G, Li J, Xu X Y, You M L, Li P and Chen GQ 2011 An assessment of the risks of carcinogenicity associated with polyhydroxyalkanoates through an analysis of DNA aneuploid and telomerase activity *Biomaterials* **32** 2546–55
[42] Guggenbiller G, Brooks S, King O, Constant E, Merckle D and Weems A C 2023 3D printing of green and renewable polymeric materials: toward greener additive manufacturing *ACS Appl. Polym. Mater.* **5** 3201–29