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In vitro biocompatibility testing of 3D printing and conventional resins for occlusal devices



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ARTICLE INFO	A B S T R A C T		
Keywords: Dental resins 3D printing Biocompatibility Cytotoxicity Occlusal devices Bruxism	<i>Objective:</i> To assess and compare the <i>in vitro</i> biocompatibility of new resins (Keysplint Soft (Keystone Industries), NextDent Ortho Rigid (3D System), and Freeprint Splint (Detax)) and traditional resins (Orthocryl (Dentaurum)) used for dental splints. <i>Methods:</i> Standardized discs ($n = 40$) and 1:1, 1:2, and 1:4 extracts of the tested materials were prepared. Human gingival fibroblasts (hGFs) were isolated from gingival tissues. Different biological tests were carried out, including MTT assays to assess cell metabolic activity, cell migration assays, cell cytoskeleton staining, cell apoptosis, generation of intracellular reactive oxygen species (ROS), and scanning electron microscopy (SEM). Statistical analyses were performed using one-way ANOVA and Tukey's post hoc test ($p<0.05$). <i>Results:</i> MTT experiments showed that Freeprint Splint significantly reduces the hGF metabolic activity (*** $p<0.001$), whereas SEM analysis showed almost no cells adhered on its surface. Cell migration was significantly lower after exposure to undiluted extracts of Freeprint Splint at 48 and 72 h (*** $p<0.001$). Cell cytoskeleton staining assays showed that only cells exposed to Keysplint Soft extracts displayed similar cell viability to the control group. Finally, ROS levels detected in undiluted extracts of all resins were significantly enhanced compared to the control group (*** $p<0.001$). <i>Conclusions:</i> The 3D-printed resins and the conventional dental resin showed a similar biocompatibility, except for Freeprint Splint, which was the most cytotoxic on hGFs. <i>Clinical significance:</i> 3D printing has been on the rise in recent years and its use in daily clinical practice is expanding over time. Two of the three 3D-printed resins tested in this study performed as well in the cytotoxicity tests as the conventional one, supporting their use, but caution and further testing are required		

1. Introduction

Occlusal devices are removable, acrylic appliances that fit on the surface of the teeth. These devices have different uses: protect teeth, treat pain or temporomandibular disorders, sleep apnea, or bruxism; among others. These devices are classically made in a conventional manner by mixing a powder and liquid manually. However, in recent years, other manufacturing methods have appeared, such as milling and 3D printing [1–3].

Digital dentistry has been on the rise in recent years. New materials for digital dentistry continue to appear on the market, being a sector in constant evolution [4,5]. Digital workflow helps to reduce costs, the number of appointments, and patient waiting time [6]. 3D printing is an alternative to the use of milled materials, but its clinical indication is still limited. The appearance of low-cost 3D printers, certified for the use of dental resins, is turning it into an option within everyone's reach. Due to the increasing interest of manufacturers and companies in 3D printing, resins and 3D printers are becoming more and more accessible to the consumer [6].

Manufacturing a three-dimensional object layer by layer is called additive manufacturing and it can be classified into 7 categories: vatpolymerization, direct energy deposition, powder-based fusion,

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material extrusion, sheet lamination, material jetting, and binder jetting [7].

3D printing with vat polymerization is a process in which there is a container filled with liquid light-curing resin, and through an ultraviolet light source, the model is generated layer by layer, while a platform moves the object being made downwards after each new layer.

Despite the number of new materials available for Vat polymerization additive manufacturing and their use in the oral cavity, no information about the biocompatibility of these novel materials is available in the literature, and thus their cytotoxicity remains unclear [8].

Dental material biocompatibility has long been described in the dental literature as the effect a material may have on cell survival [9, 10]. Regarding the mechanical properties of new materials for Vat polymerization additive, different studies have been carried out [11,12], but there are only very limited studies describing their biological properties [13,14]. despite the fact that these resins remain in the oral cavity in contact with the oral mucosa during a substantial period of time.

In vitro biological studies are essential to know if these materials can be an alternative to the laboratory materials used classically, since they can provide a preliminary assessment on their biological properties and thus elucidate possible harmful effects, such as irritations of the oral mucosa. This is can be performed by analyzing the biological responses of dental materials towards different oral cells, as has already been done with other materials that are kept for long periods of time in the mouth e.g. denture adhesives or thermoplastic aligners [13,15-17]. Similarly, such studies are clinically essential because they represent a suitable model for screening and correcting different dental material properties and evaluating their potential health risks before clinical use [18]. To our knowledge, this is the first toxicity study conducted with 3D printing resins for splints. There are other available studies on other 3D printing resins that also show their toxicity [13,14].

Due to the emergence of new impression resins, the aim of this study was to evaluate and to compare the cytotoxicity of four different dental splint resins on human gingival fibroblasts (hGFs), three 3D printing resins, and one conventional acrylic resin: Keysplint Soft, Orthocryl, NextDent Ortho Rigid and Freeprint Splint. The null hypothesis was that there was no significant difference in terms of cytotoxicity between dental splint resins.

2. Material and methods

2.1. Preparation of dental splint resins and extracts

Detailed information of the 3D printing materials used in this study (Keysplint Soft (Keystone Industries), NextDent Ortho Rigid (3D System), Freeprint Splint (Detax) and the traditional resin Orthocryl (Dentaurum), is shown in Table 1, but currently there is no information available about their respective compositions .These materials were chosen due to their availability and for being some of the most widely used among clinicians. Vat polymerization 3D printer (Phrozen Sonic Mini 4k, Phrozen Technology, Hsinchu City, Taiwan; monochrome LCD, Table 2) was used following its manufacturer's instructions. Both the

Tabl	le	1

Γ	'ested	materials	characteristic	s.

MATERIAL	MANUFACTURER	LOT NUMBER
Keysplint Soft	Keystone Industries GmbH, Stockholzstr. 11, 78,224, Singen, Germany	Kg5622
Orthocryl	Dentaurum GmbH & Co. Kg, Turnstr 31, 75,228, Ispringen, Germany	499166A
NextDent Ortho Rigid	3D System. Centurionbaan 190 3769 A V Soesterberg, The Netherlands	WY153N034
Freeprint Splint	Detax GmbH & Co Carl Zeiss str 4, 76,725, Ettlingen, Germany	231,109

2

Table 2

Characteristics of	f tl	ıe	3D	printer	used.
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LED array	405 nm PARALED Matrix 2.0
Technology XY Resolution Wavelength	Vat-Polymerization display (LCD) 35 µm 405 nm 10 100
Min/Max Layer Thickness LCD Pixel Resolution Printing Volumen	10–100 μm 3840 × 2160 130 × 73 × 130 mm

printer and the different printing resins were individually calibrated before printing the studied materials. Each resin needs a specific time of exposure to ultraviolet light to achieve an optimal setting. Once the sample discs were printed, the uncured resin was washed from the surface with an organic solvent, isopropyl alcohol (IPA 99%) for three minutes using a washing machine (Wash & Cure 2.0; Anycubic). Then, a second bath for no more than two minutes was carried out. The total cleaning time did not exceed 5 min. The 3D printed parts where then left for 30 min in a dark room for the evaporation of IPA. The polymerization was completed using a UV-polymerization machine (LC-3DPrint Box) with a wavelength of 405 nm, a light power intensity of 40 mW/cm² and temperature control. During the polymerization process, the specimens were submerged in liquid glycerin to avoid exposure to oxygen. Orthocryl mixture was made in a silicone mixing bowl at a ratio of 2.5: 1 (powder: fluid) for 5 min. The final polymerization of the material was carried out at a pressure vessel (2.2 bar) at a temperature between 40 °C and 46 °C for 20 min. A laboratory technician with previous experience with the tested materials made the samples, following the proportions recommended by their respective manufacturers. The discs made were 6 mm in diameter and 2 mm in height (56.54 mm³ (n = 40)). All tests were performed in accordance with the standard and guidelines of ISO 10,993-5: 2009 "Biological evaluation of medical devices", in order to evaluate the biological responses to these resinous materials. [19]. International Organization for Standardization (ISO) guidelines 10, 993-12 for biological evaluation of medical devices sample preparation and reference materials were also followed. The extraction procedure was as follows: the tested materials were immersed in the culture medium for 24 h at 37 °C in a humid atmosphere containing 5% CO₂. In accordance with the ISO standard, the ratio between the surface of the sample and the volume of the medium was 1.5 cm^2 / ml. The extraction medium was collected at the end of this period and filtered through a 0.22-µm syringe filter (Merck Millipore, Billerica, MA, USA). Then, in order to study the effect of the concentration of each material, various dilutions (1:1, 1:2, and 1:4 v/v) of these extraction media were prepared using fresh complete DMEM medium.

2.2. Isolation and culture of human gingival fibroblasts

The Ethics Committee from Universidad de Murcia approved the study protocols (ID: 2199/2018). Human gingival tissue was obtained from ten healthy donors undergoing dental extraction, who previously signed an informed consent form in accordance to the requirements specified in the Helsinki Declaration guidelines. Briefly, gingival tissues were minced mechanically using a scalpel and rinsed with PBS and 1% penicillin/streptomycin (P/S) (Invitrogen, Paisley, Scotland). Then, gingival tissue fragments were enzimatically digested in serum-free DMEM medium (Gibco, Thermo Fisher Scientific, Waltham, MA, United States) containing 0.2% dispase II (Thermo Fischer Scientific) and 0.1% collagenase A (Roche Diagnostics, Basel, Switzerland) at 37 $^\circ\text{C}$ for 2 h. Following digestion, human gingival fibroblasts (hGFs) were washed twice with PBS, filtered through 100-µm nylon cell strainers (BD Biosciences, San Jose, CA, United States) and cultured in DMEM medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, United States), 1% GlutaMAXTM (Thermo Fisher Scientific), and 1% P/S (complete growth medium) at 5% CO₂

and 37 °C. When hGFs reached 70–80% confluence, they were detached with 0.25% TrypLE Express dissociation solution (Gibco, Thermo Fisher Scientific) and subsequently subcultured. Cells in passages 2 up to 5 were used for the subsequent experiments.

2.3. MTT

To evaluate the metabolic activity of hGFs after culturing in presence of the different studied dental splint resins extracts, a 3-(4,5-dimethylthiazol-2-yl)–2,5-diphenyltetrazoliumbromide (MTT) assay were carried out. Briefly, hGFs were resuspended in complete growth medium (w/o red phenol) and plated at 1×10^4 cells/well in 96-well plates (control), or in complete growth medium at different dilutions (1:1, 1:2, 1:4) of dental splint resins and cultured for 24, 48, or 72 h at 37 °C. Then, an MTT reagent (Sigma-Aldrich) at a final concentration of 1 mg/ml was added to the wells, incubated for 4 h at 37 °C, and solubilized with dimethyl sulfoxide (DMSO) (Sigma-Aldrich). Finally, cell proliferation was measured by the determination of absorbance values at 570 nm. Three separate experiments using hGFs isolated from three different donors were performed, each carried out in quintuplicate for each dental splint resin and controls.

2.4. Scanning electron microscopy (SEM) analyses

Eighteen 2.5 mm high and 5 mm diameter disks of the different dental splint resins were randomly divided into three groups (n = 6 samples/group) and used to evaluate hGF attachment to the surface of the resin disks. Briefly, a total of 5×10^4 hGFs were seeded onto each disk and cultured for three days. Then, hGFs were fixed using 4% glutaraldehyde in PBS at 4 °C for 4 h and treated with a series of alcohol solutions in increasing concentrations up to 100% to dehydrate samples. Alcohol was removed by critical point drying. Specimens were mounted on brass stubs and sputter-coated with gold after being placed on a copper grid for 5 min (Bio-RAD Polaron e5400 SEM Sputter Coating System, Kennett Square, PA, United States). Finally, the microscope working distance was 20 mm and images were acquired under 100x and 300x magnifications.

2.5. Cell migration assays

To evaluate the biological effects of the different resins on cell migration, wound healing assays were carried out. HGFs were seeded at 2×10^5 cells/well and cultured at 37 °C for 24 h to obtain confluent cell monolayers. Then, a scratch (wound) was made in each cell monolayer with a sterile 100-µl pipette tip, washed twice with PBS to remove detached cells after scratching, and cultured in complete growth medium alone (control) or in complete growth medium containing the different dental splint resins at 1:1, 1:2, 1:4 dilutions for 24, 48, or 72 h at 37 °C. Finally, ImageJ software (National Institutes of Health, Bethesda, MD, United States) was used to measure the percentage of open wound area at each time point relative to the same wound area at 0 h in the same well. Three independent experiments were performed, each carried out in triplicate for each dental splint resin and dilution.

2.6. Cell cytoskeleton staining assays

Phalloidin staining was carried out to analyze any possible changes in cell morphology and in F-actin cytoskeleton content and organization. Briefly, 3×10^4 hGFs were added on glass coverslips, allowed to adhere and spread, and cultured in complete growth medium alone (control) or in complete growth medium containing non diluted (1:1), 1:2, or 1:4 dilutions of the different studied resins extracts for 72 h at 37 °C. Then, glass coverslips were rinsed twice with prewarmed PBS at 37 °C, fixed in 4% formaldehyde in PBS for 10 min, permeabilized with 0.25% Triton X-100 solution (Sigma-Aldrich) for 5 min, and rinsed 3 times with PBS. Cell F-actin cytoskeleton and nuclei were then stained with InvitrogenTM AlexaFluor[™]594-labeled phalloidin (ThermoFisher Scientific) and 4,6diamidino-2-phenylindole dihydrochloride (DAPI) (ThermoFisher Scientific), respectively, at r/t in the dark for 30 min. Finally, immunofluorescence images were acquired under a Leica TCS SP2 confocal microscope (Leica, Wetzlar, Germany). Each of the resin elutes and dilutions were evaluated at least in three independent experiments in triplicate.

2.7. Annexin-V/7-AAD staining and evaluation of intracellular ROS production

HGF viability and detection of intracellular reactive oxygen species (ROS) production after exposure to the different dental splint resins were analyzed by annexin-V/7-aminoactinomycin D (7-AAD) and the general oxidative stress indicator CM-H2DCFDA staining, respectively. Briefly, hGFs were cultured in complete culture medium alone (control) or in complete medium containing non diluted (1:1), 1:2, and 1:4 dilutions of the different resins for 72 h at 37 °C. Afterwards, cells were washed and stained with FITC-labeled annexin-V and 7-AAD (BD Biosciences) for 15 min at r/t following its manufacturer's protocol, or with a final concentration of 5 µM CM-H2DCFDA (Molecular Probes, Eugene, OR. United States) for 30 min at 37 °C. Finally, hGFs were acquired in a BD FACS Canto IITM flow cytometer (BD Biosciences), and percentages of live and apoptotic/necrotic cells or CM-H2DCFDA positive cells were analyzed with FlowJo software (FlowJo LLC, Ashland, OR, United States). All experimental conditions were repeated independently in triplicate and analyzed in three separate experiments.

2.8. Statistical analysis

The experimental results are presented as the mean \pm standard deviation (SD) and all experiments were repeated at least three times. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons using GraphPad Prism software version 8.0.2 (Graph-Pad Software, San Diego, CA, United States). *P* <0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Cell metabolic activity determination

HGFs were exposed to different dilutions of Keysplint Soft, Orthocryl, NextDent Ortho Rigid and Freeprint Splint for 24, 48, and 72 h, and cell metabolic activity was measured by the MTT assay (Fig. 1). At 1:4 dilution, no resin affected cell metabolic activity compared to the control conditions. However, at a 1:2 dilution, Freeprint Splint displayed the highest cytotoxicity at 24 and 72 h (***p <0.001), while the other resins did not affect cell biocompatibility at any time. Finally, undiluted Freeprint Splint-treated cells, exhibited a significantly lower viability than the control group (***p<0.001).

3.2. Evaluation of cell adherence and morphology

As shown in Fig. 2, hGF adherence and morphology on the surfaces of the different dental splint resins at 72 h were evaluated by scanning electron microscopy (SEM) analysis. A substantial quantity of welladhered and spread cells were observed on the surfaces of Keysplint Soft and NextDent Ortho Rigid, also showing a typical spindle-shaped fibroblastic morphology. Conversely, fewer attached cells were observed on the surface of Orthocryl, whereas Freeprint Splint evidenced almost no adhered cells with aberrant morphology.

3.3. Migration assays

The analysis of cell migration by in vitro wound healing assays was



Fig. 1. Determination of metabolic activity of hGFs after exposure to different dilutions of dental 3D printed resins by MTT assays. Absorbance values at 570 nm were significantly different from the control group (***p < 0.001) according to one-way ANOVA and Tukey's post hoc test.



Fig. 2. Analysis of cell attachment of hGFs cultured on dental 3D printed resins at 72 h by SEM. Scale bar = 500 µm, 100 µm and 30 µm.



Fig. 3. Analysis of migration of hGFs after treatment with different dilutions of dental 3D printed resins by wound healing assays. Confluent hGF monolayers were cultured with complete growth medium (control) or different extract dilutions (non-diluted (1:1), 1:2 and 1:4) of the indicated resins for 72 h. Cell migration was expressed as the percentage of open wound area at each time point relative to the same wound area at 0 h (100%). Migration was significantly reduced compared to control (***p < 0.001) according to one-way ANOVA and Tukey's post hoc test.

performed at 24, 48, and 72 h (Fig. 3). Predominantly, cell migration rates of hGFs cultured with Keysplint Soft, Orthocryl, and NextDent Ortho Rigid were comparable to those observed in the control conditions at any time point and dilution. 1:1 Freeprint Splint-treated cells, however, significantly increased the number of migrated hGFs compared to that of the control (*p<0.05), with a 42% and 14% open wound area at 48 and 72 h, respectively.

3.4. Cell cytoskeleton staining

Phalloidin staining showed that Keysplint Soft, Orthocryl, and NextDent Ortho Rigid-treated cells, showed a fibroblastic morphology and well-organized cytoskeleton F-actin fibers at all dilutions, similar to the control group, whereas 1:1 and 1:2 Freeprint Splint-treated cells evidenced an aberrant morphology, suggesting cytotoxicity (Fig. 4).

3.5. Induction of cell apoptosis and necrosis and intracellular ROS production

Representative density dot plots of the distribution of live (Annexin- $V^-/7$ -AAD⁻; bottom left quadrant), early apoptotic (Annexin- $V^+/7$ -AAD⁻; bottom right quadrant), and late apoptotic/necrotic (Annexin- $V^+/7$ -AAD⁺ and Annexin- $V^-/7$ -AAD⁺, top left and right quadrants) cells in control hGFs or treated with non-diluted (1:1), 1:2, and 1:4 dilutions of the different resin extracts are shown in Fig. 5. Percent of viable HGFs exposed to all dilutions of Keysplint Soft, and 1:2 and 1:4 dilutions of Ortocryl, Nexdent, and Freeprint Splint was similar (> 96%) compared to the control conditions. Conversely, non-diluted (1:1) Ortocryl and Freeprint Splint extracts showed an increased percent of early and late apoptotic and necrotic hGFs (> 6%).

As shown in Fig. 6, intracellular ROS levels measured in HGFs cultured in the presence of Freeprint Splint and Ortocryl extracts (1:1 and 1:2 dilutions) or Keysplint Soft and NextDent Ortho Rigid (1:1 dilution) were significantly enhanced compared to the detected ROS

levels in the control cells (***p<0.001). However, when hGFs were cultured with Freeprint Splint or Ortocryl (dilution 1:4), there were no differences from those observed on control cells. Finally, Keysplint Soft and NextDent Ortho Rigid (dilution 1:4) were only slightly augmented compared to the control, although this difference was statistically significant (*p<0.05).

4. Discussion

The aim of this study was to analyze the cytotoxicity of four materials to make splints devices: three 3D printing materials (Freeprint Splint, Keysplint Soft and NextDent Ortho Rigid), and an analog material commonly used by laboratory technicians (Orthocryl).

To our knowledge, there are few studies on the cytotoxicity of 3D printing materials, such as on provisional restorations [20], or on printed materials for splint devices [13,14]. Similar to our results regarding the toxicity of Freepring Splint, other 3D printing resins showed toxicity despite possessing an ISO certification of biocompatibility [14]. This justifies the need for this type of studies.

Wedekind et al. [13] described the number of free monomers that the occlusal splints present according to the manufacturing system, showing that the printed splints presented the highest proportion of free monomers. Therefore, the fact that they have free monomers could be a reason for their increased cytotoxicity. Regarding our study, the results exhibited by Freeprint Splint would be in line with that study. However, the results obtained with the rest of the printed materials differ.

Hunter et al. [14] showed that dental resins used in 3D printing technologies release ovo-toxic leachates. These findings cannot be compared with ours, although they show that a commercialized resin could be toxic, as we have elucidated.

One explanation for the apparently conflicting results among the different cytotoxicity studies could be that most other studies are conducted with animal cells or cell lines of various origins, rather than primary human oral cells, as used in this study. Regarding the biological



Fig. 4. Analysis of cell morphology changes and cytoskeleton F-actin fibers organization on hGFs cultures exposed to the indicated dental resins extracts by confocal fluorescence microscopy. F-actin fibers were stained with AlexaFluorTM 594-labeled phalloidin (red), whereas cell nuclei were counterstained with DAPI (blue). Images shown are representative from three independent experiments performed in triplicate for each material. Scale bar: 100 μ m.

properties of Orthocryl, various studies have been performed, showing that it is a safe material and not cytotoxic [21,22].

These devices are placed in the mouth for long periods of time, in contact with the oral mucosa and gingival tissues (jugal mucosa or papilla area). For this reason, human gingival fibroblasts were selected to perform the different *in vitro* tests, as has been previously achieved with other materials that are kept in contact with the oral mucosa [15, 23]. This study concluded that there were no significant differences between the conventional resin Orthocryl and the 3D-printed materials Keysplint Soft and NextDent Ortho Rigid. In all the experiments, Freeprint Splint displayed the worst result in terms of biocompatibility.

One aspect to consider when assessing the toxicity of these materials, that require a previous curing before their use and are exposed to different handling processes, is that the manufacturer's instructions are followed whenever possible, although it has been shown that following the manufacturer's instructions will not always achieve the best results [24]. In addition, post-processing procedures of the printed materials

are crucial. It is important that when the cleaning step is carried out, new isopropyl alcohol is used and not reused, since we could be contaminating the surface of our devices with traces of old prints [25–27]. This factor has been seen to change the mechanical properties of the resins, so it could also alter their biological properties [20,25,28].

Another fundamental additional aspect is material setting/curing, which must be performed with suitable curing lamps and sufficient power to achieve a complete polymerization of the material [25,26,28, 29]. Dohyum et al. [20] studied the properties of 3D impression materials for provisional printed crowns and bridges, and showed that using a longer curing time they obtained better biological properties due to the reduction in the presence of residual monomers. In another study, Kessler et al. [30] found that the amount of eluted monomers in printed surgical guides was comparable to that of conventional resins, although they did not perform cytotoxicity tests.

Other authors have determined that depending on the postpolymerization unit, mechanical properties of the materials can be



Fig. 5. Flow cytometry analysis of cell apoptosis and necrosis induced by the different dental 3D printed resins extracts on hGFs by Annexin-V-FITC and 7-AAD staining. Numbers inside density plots represent percentages of live (Q4), early apoptotic (Q3), and late apoptotic necrotic cells (Q1 and Q2) and are representative from three independent experiments performed in triplicate for each material.

altered. However, they achieve positive results with the LC-3DPrint box that was used in the present study [31]. It would be interesting to carry out toxicity studies with different post-processing units to assess whether it is essential to follow the protocols established by the manufacturers, although Mostafavi et al. have shown that it is not necessary [24]. are other important issues. It has been observed the parameters used in printing may alter mechanical properties of the 3D-printed resins, which could also change their subsequent biological properties [32]. In our study, all the post-processing procedures were performed i.e. cleaning, post-polymerization... and the same printer was used (Phrozen sonic Mini 4k), thus preventing any of these processes could alter our results.

On the other hand, the type of printer and the printing parameters

The main limitation of this study is the lack of similar studies with





Fig. 6. Analysis of intracellular reactive oxygen production (ROS) after treatment with different dilutions of the indicated resins by CM-H2DCFDA staining. Representative histograms obtained in each experimental condition are shown. Dotted lines represent mean fluorescence intensity obtained in the control. Bar graph show quantification of positive CM-H2DCFDA cells obtained with each resin extract dilution and is represented as mean \pm SD from three independent experiments performed in triplicate. Percentages of CM-H2DCFDA-positive cells were significantly increased compared to the control, *p<0.05; ***p<0.001, respectively, according to one-way ANOVA and Tukey's post hoc test.

these materials to compare our findings and the lack of more detailed information regarding the composition of the printing resins due to the patents and priority rights. Thus, when the patents are released and the exact composition of these resins be known in detail, further studies are needed. Another limitation is the absence of a standardized postprocessing protocol for biocompatible resins, which hinders the comparison of the results with different post-processing methodologies.

5. Conclusions

The new dental resins for 3D printing and the conventional dental resins assessed in this study showed similar biocompatibility, except for Freeprint Splint, which was the most cytotoxic of the four dental resins studied on hGFs. Despite being ISO certified, independent cytotoxicity studies are necessary to verify the safety of these materials.

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CRediT authorship contribution statement

Julia Guerrero-Gironés: Conceptualization, Formal analysis, Project administration. Sergio López-García: Investigation, Methodology. Miguel R. Pecci-Lloret: Conceptualization, Data curation. María P. Pecci-Lloret: Writing – original draft, Supervision, Validation, Writing – review & editing. Francisco Javier Rodríguez Lozano: Supervision, Visualization. David García-Bernal: Investigation, Methodology, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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