Odontology
https://doi.org/10.1007/s10266-026-01432-1

ORIGINAL ARTICLE

# Biocompatibility of printed, milled, and conventionally fabricated indirect resin-based restorative materials: an in vitro comparative study

Ayse Atay[1] · Demet Cagil Ayvalioglu Samiloglu[2] · Soner Sismanoglu[3] · Zafer Yildirim[4] · Aslihan Usumez[5]

Received: 9 January 2026 / Accepted: 13 May 2026
© The Author(s) 2026

## Abstract

The increasing use of digitally fabricated resin-based materials for definitive dental restorations has raised concerns regarding their biological performance and biocompatibility. This in vitro study evaluated the biocompatibility and surface characteristics of different indirect resin-based restorative materials used in definitive dental restorations. A total of 144 disc-shaped specimens (diameter 15 mm, height 2 mm) were fabricated using printed, milled, and conventional resins (48 specimens per material), with 12 specimens allocated to each analysis (n=12). Specimens were sterilized using ultraviolet irradiation. Culture medium extracts were obtained on days 1, 3, and 7 and evaluated using L929 mouse fibroblast cells. Cell viability was assessed using the XTT assay, apoptosis by Annexin V-FITC/PI staining, and interleukin-6 (IL-6) release using a Mouse IL-6 ELISA kit. Cell viability, apoptosis, and IL-6 levels were analyzed using two-way ANOVA; surface roughness was analyzed using one-way ANOVA, followed by Tukey's HSD post hoc tests ($p<0.05$). Significant differences were observed among the manufacturing groups across incubation periods in terms of cell viability, apoptosis rate, and IL-6 release ($p<0.05$). The milled-resin group showed the highest cell viability on day 7, whereas the conventional resin group showed the lowest ($p<0.05$). On days 3 and 7, the conventional resin group exhibited the highest rates of apoptosis and IL-6 release ($p<0.05$). Both printed and milled-resin groups showed no significant impact on fibroblast cell responses, whereas exposure to eluates from the conventional resin group resulted in altered cell viability, IL-6 levels, and apoptosis, suggesting potential cytotoxicity relevant to material selection for definitive prosthodontic restorations.

**Keywords** 3D-printing · CAD/CAM milling · Cell viability · Interleukin-6 · Apoptosis

## Introduction

Digital dental technologies have advanced, leading to the development of production techniques and resin-based materials for definitive indirect restorations, such as crowns, inlays, onlays, and endocrowns, that better mimic natural tooth structure [1]. Composite resins are among these materials, and permanent indirect composite restorations can be fabricated using either conventional techniques or digital workflows with computer-aided design and computer-aided manufacturing (CAD/CAM) technologies, including milling and three-dimensional (3D) printing [2, 3].

In the conventional manufacturing, hand-made, laboratory-processed composites are polymerized using special devices that apply light, heat, pressure, or their combinations to improve durability and longevity [3]. Extended, multi-angle light polymerization increases the degree of

✉ Ayse Atay
ayse.atay@altinbas.edu.tr; ayseatay82@hotmail.com

1 Department of Prosthodontics, Faculty of Dentistry, Altinbas University, Zuhuratbaba Mah. Incirli Cad. No: 11/A, 34147 Bakirkoy Istanbul, Turkey

2 Department of Prosthodontics, Faculty of Dentistry, Istanbul Health and Technology University, Istanbul, Turkey

3 Department of Restorative Dentistry, Faculty of Dentistry, Istanbul University-Cerrahpasa, Istanbul, Turkey

4 Department of Medical Biology, Faculty of Medicine, Ege University, Izmir, Turkey

5 Private Clinic, Istanbul, Turkey

Published online: 20 June 2026

conversion (DC) [1]. The DC in direct composite resins typically ranges between 50 to 60% [4], increases to 70 to 80% [5] in laboratory-processed indirect composites, and reaches approximately 90 to 95% in CAD/CAM resin composites [6]. Compared with direct composites, indirect resin composites achieve higher DC, which is associated with reduced residual monomer release, improved biocompatibility, and lower intraoral elution [7, 8]. Although degree of conversion is influenced by both material composition and processing conditions, differences in fabrication techniques may also affect residual monomer release and subsequent biological behavior [9, 10]. Additional clinical benefits include better proximal contact formation, higher wear resistance, and simplified development of a natural anatomic form in large defects [11, 12].

Recent advances in intraoral imaging, data acquisition, and manufacturing have facilitated the integration of CAD/CAM in dentistry. The CAD/CAM workflow comprises data acquisition, design, and fabrication using either subtractive or additive methods [1]. Subtractive manufacturing mills prefabricated homogeneous blocks [1, 2]. Although efficient and compatible with high-quality materials, it has limitations, including high material waste, difficulty producing complex geometries, and bur wear that depends on material properties [2, 3]. Worn tools can compromise restoration integrity, and the smallest bur diameter limits detail accuracy, which has driven interest in additive manufacturing as an alternative [3]. In contrast to subtractive approaches, additive manufacturing fabricates structures through a layer-by-layer process using liquid, powder, or solid materials, enabling diverse applications such as frameworks, crowns, splints, and complete dentures [2, 3]. For 3D-printed objects, a post-processing protocol must follow the manufacturer's instructions and typically includes detachment from the build platform, washing, drying, and post-polymerization [7, 13, 14]. After detachment, unreacted monomers are removed by rinsing with an organic solvent such as isopropyl alcohol or ethanol [7], and then post-polymerization is performed with LED, UV, or flash units [13]. This final curing stage promotes additional cross-linking of residual monomers. Since DC directly influences performance, higher DC generally reduces residual monomer content, improves mechanical properties, and enhances biocompatibility [8, 15, 16].

CAD/CAM composite resin blocks offer standardized material properties because they are polymerized industrially under controlled light, heat, and pressure, resulting in a high DC (90 to 95%) [6] and minimal porosity that supports mechanical strength, color stability, biocompatibility, and long-term performance [1, 2, 13, 17]. Moreover, CAD/CAM fabrication enables precise milling with excellent reproducibility and can reduce chairside time compared with conventional hand-made techniques [1, 3].

The biocompatibility of definitive indirect restorations is clinically relevant because of their prolonged proximity to oral soft tissues. Several studies have investigated the biocompatibility of laboratory-processed indirect composites and CAD/CAM resin composites [17–19]. The emergence of 3D printing has introduced novel materials for permanent crowns; however, the literature evaluating the biocompatibility of 3D-printed permanent crown materials remains limited [20–22]. Given the growing use of 3D printing in definitive restorations, optimizing the biocompatibility of printed resins is essential. Therefore, the present study aimed to investigate the biocompatibility of indirect resin-based restorative materials produced by three different fabrication methods, focusing on cell viability, interleukin-6 (IL-6) release, and apoptosis induction. In addition, surface roughness was evaluated to confirm comparable surface conditions among the tested materials. The null hypothesis was that there would be no significant differences in biocompatibility among the tested materials.

## Materials and methods

Three indirect resin-based restorative materials fabricated using distinct fabrication techniques (printed, milled, and conventional) were investigated in this in vitro study, as outlined in Table 1. The overall experimental procedure is depicted in Fig. 1.

**Table 1** The brand names, material type, classifications, compositions, and manufacturers of the materials used in the present study

| Material | Type | Classification | Composition | Manufacturer |
|---|---|---|---|---|
| Saremco print-CROWNTEC | Additive Manufacturing (Printed resin) | 3D printed composite resin | Bis-EMA, dental glass and silica fillers (30–50%), initiators, inhibitors, and color pigments | SAREMCO Dental AG, Rebstein, Switzerland |
| Grandio Blocs | Subtractive Manufacturing (Milled resin) | Nano-hybrid composite resin | 86% nano silica and barium glass fillers in a polymer matrix. 14% UDMA, DMA. | VOCO GmbH, Cuxhaven, Germany |
| Gradia Plus | Conventional Manufacturing (Conventional resin) | Composite resin | Bis-GMA (1–5%), TEGDMA (5–10%), UDMA (1–5%), and ceramic filler | GC Europe, Leuven, Belgium |

*Bis-EMA* Ethoxylated bis phenol A dimethacrylate, *Bis-GMA* Bisphenol A diglycidyl ether dimethacrylate, *DMA* Dodecyl dimethacrylate, *TEGDMA* Triethylene glycol dimethacrylate, UDMA Urethane dimethacrylate.

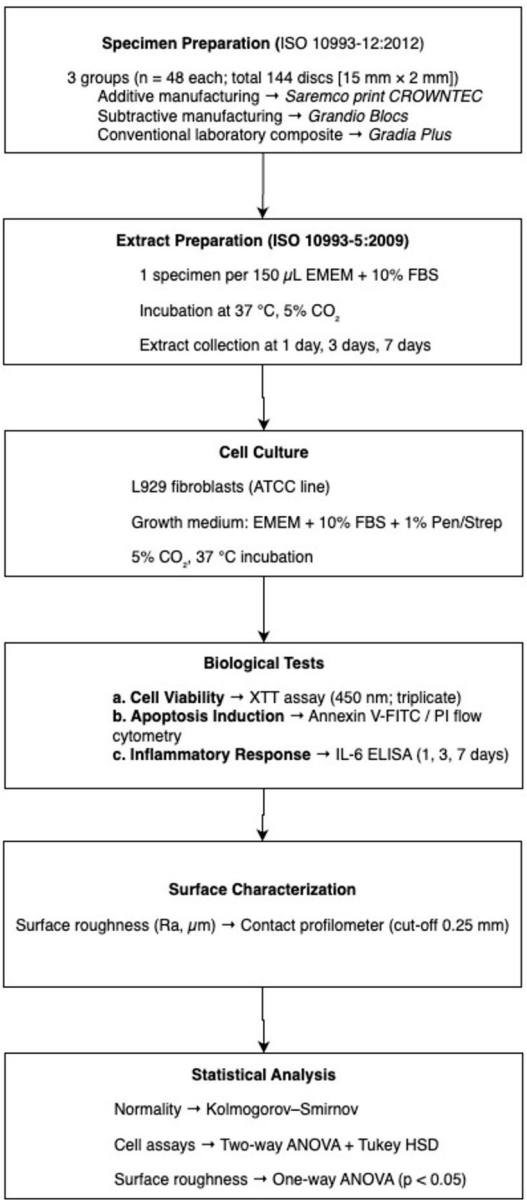


**Fig. 1** Flowchart of study design

## Specimen preparation

A total of 144 disc-shaped specimens (15 mm diameter, 2 mm thickness) of three groups were produced following ISO 10993-12:2012 [23] sample preparation standards and manufacturers' instructions. Each group was randomly divided into four subgroups according to the evaluated parameters (n = 12): cell viability, apoptosis, interleukin-6 (IL-6) release, and surface roughness.

Specimens of the printed resin group (Saremco Print CROWNTEC; SAREMCO Dental AG, Rebstein, Switzerland) were digitally designed using 3D modeling software (TinkerCAD; Autodesk, CA, USA). The digital design of each disc-shaped specimen was then transferred to a 3D printer (MAX UV; ASIGA, Sydney, Australia) for production, and the printing process was performed at a controlled temperature of 35 °C. After printing, the specimens were separated from the build platform using a spatula. Any remaining uncured resin was eliminated by wiping with a cloth moistened in 96% alcohol, followed by ultrasonic cleaning in distilled water for 5 minutes and subsequent air-drying. Final polymerization was performed in a UV-curing unit (Otoflash G171; NK-Optik GmbH, Baierbrunn, Germany) under a nitrogen oxide atmosphere, delivering 4000 light flashes within a wavelength range of 320–500 nm, according to the manufacturer's instructions. Specimens of the milled resin group (Grandio Blocs; VOCO GmbH, Cuxhaven, Germany) were milled using a dental milling machine (Ceramill Motion 2 System; Amann Girrbach AG, Koblach, Austria). To produce the conventional resin group (Gradia Plus; GC Europe, Leuven, Belgium) specimens, a silicone mold was prepared. The Gradia Plus indirect composite resin material was placed into the mold and overlaid with a transparent matrix strip. A glass coverslip was applied to remove any excess material and to achieve the desired shape. Subsequently, the specimens were polymerized using a light-emitting diode (LED) curing unit (VALO; Ultradent, South Jordan, UT, USA) in standard mode for 10 seconds. The irradiance was approximately 1000 mW/cm$^2$ according to the manufacturer's specifications. Additional polymerization was performed in a composite light-curing unit (Labolight Duo; GC Corp., Tokyo, Japan) for 3 minutes.

After fabrication, the specimens were kept in distilled water at 37 ± 1 °C for 24 hours in a light-protected container. Finishing and polishing procedures were performed sequentially under water cooling using aluminum oxide-coated abrasive discs (OptiDisc; Kerr, CA, USA), in accordance with the manufacturer's guidelines. The finishing and polish set consists of 4 different aluminum oxide coated discs. Coarse (80 µm), medium (40 µm), fine (20 µm), and super thin (10 µm) types of discs were used respectively, under water-cooling at a constant speed of 10.000 rpm for 15 seconds.

All specimens were ultrasonically cleaned in distilled water for 5 minutes, then air-dried. Subsequently, the specimens were exposed to ultraviolet irradiation inside a laminar flow cabinet (Airstream Class II BSC; ESCO, South

Yorkshire, UK) for 40 minutes to ensure sterilization prior to cell culture experiments.

## Preparation of material extracts

The sterilized specimens were placed in 24-well culture plates, with each well containing 150 µL of Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin-streptomycin. Wells without specimens served as the control group. Extracts of the materials were prepared following the guidelines of ISO 10993-5:2009 [24]. To obtain the 1 st, 3rd, and 7th day extracts, each material was divided into three subgroups, and separate specimens were used for each time interval; thus, each time point was evaluated using independent specimens (n = 4 per material per time point). All plates were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$ to obtain the medium extracts of the test materials. The extracts were collected at 1, 3, and 7 days of incubation and stored at −20 °C until further cell culture analysis.

## Cell culture preparation

The L929 fibroblast cell line was obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in EMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were cultured at 37 °C in a humidified incubator with 5% $CO_2$, and the medium was refreshed every three days. Cells were passaged following trypsinization.

## Cell viability

Cell viability was assessed using the XTT assay (Cell Proliferation Kit; Roche Applied Science, Basel, Switzerland) to quantify the metabolic activity of viable cells based on the reduction of the XTT reagent to formazan by mitochondrial enzymes. L929 cells were seeded at a density of $1 \times 10^4$ cells per well in 24-well culture plates and allowed to adhere for 24 hours. Following the removal of the culture medium, 100 µL of the extract from each material was promptly dispensed into wells containing L929 cells. After an incubation period of 48 hours, cellular metabolic activity was determined by detecting the absorbance of the formazan derivatives at 450 nm using a microplate spectrophotometer (Thermo Scientific, Vantaa, Finland), which reflects the mitochondrial enzymatic activity of viable cells. These experiments were performed in triplicate per specimen. Cell viability assays with eluates were performed according to ISO 10993-12:2012 [23].

## Induction of apoptosis

L929 fibroblast cells were cultured in 96-well plates at a seeding density of $1 \times 10^4$ cells per well and allowed to attach and stabilize during a 24-hour incubation period under standard cell culture conditions (37 °C, 5% $CO_2$, and humidified atmosphere). Following this initial incubation, 100 µL of the existing culture medium was carefully aspirated from each well and replaced with 200 µL of the prepared medium extracts obtained from the tested materials to evaluate their potential cytotoxic effects on the cells. Following 48 hours of treatment, the cells were rinsed with phosphate-buffered saline (PBS), resuspended in 100 µL of binding buffer, and stained with 1 µL propidium iodide (PI) and 1 µL Annexin V. After incubation, the cells were analyzed using an apoptosis detection kit (Annexin V FITC/PI; BD Biosciences, San Jose, CA, USA).

Fluorescence of the cells for Annexin V-FITC (FL1) and PI (FL3) was simultaneously detected and analyzed using a flow cytometer (Accuri C6; BD Biosciences, San Jose, CA, USA). The proportions of viable (Annexin $V^-/PI^-$; lower left quadrant), apoptotic (Annexin $V^+/PI^-$; lower right quadrant), and necrotic (Annexin $V^-/PI^+$ and Annexin $V^+/PI^+$; upper left and upper right quadrants) cells were determined according to the observed fluorescence patterns.

## Inflammatory response

IL-6 concentrations in the cell culture supernatants were determined using a sandwich-type enzyme-linked immunosorbent assay (ELISA). The specimens were placed in a 24-well plate containing 150 µL of EMEM supplemented with 10% FBS and 100 U/mL penicillin-streptomycin at 37 °C for 24 hours. After the initial incubation, the culture media were replaced with the previously prepared material extracts. IL-6 secretion was quantified using a Mouse IL-6 ELISA Kit (Elabscience Biotechnology, Houston, TX, USA) after exposure periods of 1, 3, and 7 days. The absorbance was read at 450 nm using a microplate spectrophotometer. All assays were performed in triplicate for each specimen.

## Surface roughness measurement

Surface roughness measurements were conducted using a contact profilometer (Surtronic 25; Taylor Hobson, Leicester, UK). Prior to data collection, the device was calibrated with a reference standard. Three random linear traces were recorded for each specimen, starting from the center, with a traverse length of 1.75 mm, a cut-off value of 0.25 mm, and a scanning speed of 0.5 mm/s. The mean Ra value (µm) was obtained by averaging the three readings for each sample.

## Statistical analysis

All statistical analyses were performed using SPSS for Windows 22.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Kolmogorov-Smirnov test, and homogeneity of variances was evaluated using Levene's test. Cell viability, apoptosis induction, and IL-6 levels were analyzed using two-way ANOVA with material type and time as independent variables. Surface roughness values were analyzed using one-way ANOVA. Pairwise comparisons were performed using Tukey's HSD post hoc test. Statistical significance was set at $p < 0.05$. The sample size ($n = 12$ per subgroup) was determined based on previous similar in vitro studies [17, 21].

## Results

### Cytotoxic effects of indirect restoration materials

The cell viability results of the tested materials at different time intervals are presented in Fig. 2. The baseline measurements taken from the first-day medium extracts demonstrated statistically significant variations in cell viability among the tested material groups ($p < 0.05$). The milled resin group had significantly higher cell viability values (98.30 ± 1.66) than the conventional resin group (93.16 ± 1.85) and the printed resin group (90.17 ± 0.49) ($p < 0.05$). There were no significant differences between the printed and conventional resin groups ($p > 0.05$).

On the third day of evaluation, no statistically significant differences were observed among the tested material groups with respect to cell viability ($p > 0.05$). The recorded viability values were 93.02 ± 2.90 for the milled resin group, 86.59 ± 6.46 for the printed resin group, and 83.23 ± 4.51 for the conventional resin group, respectively.

On the seventh day, the material type had a significant effect on cell viability ($p < 0.05$). The milled resin group showed the highest cell viability (93.69 ± 1.46), whereas the conventional resin group exhibited the lowest values (78.92 ± 0.65) ($p < 0.05$).

The printed resin group exhibited no statistically significant variation in cell viability across the evaluated time intervals ($p > 0.05$). The recorded viability values for this

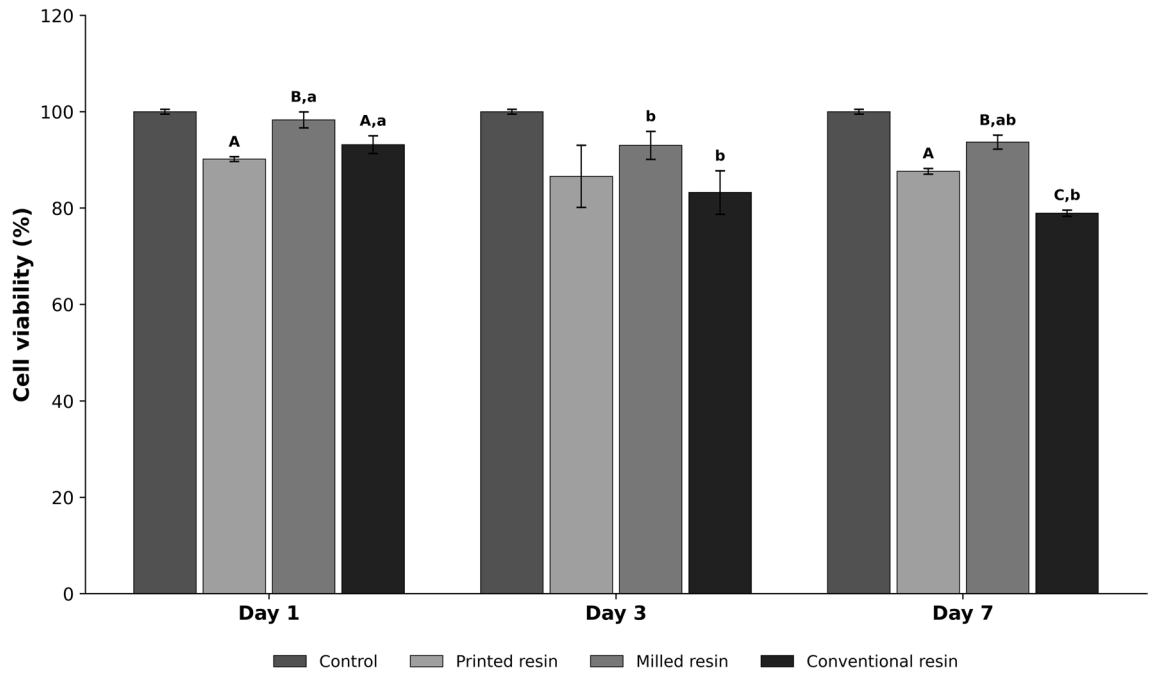


**Fig. 2** Cell viability (%) of L929 fibroblast cells exposed to 1 st, 3rd, and 7th day medium extracts of printed resin, milled resin, and conventional resin groups. Different uppercase letters indicate statistically significant differences among materials within the same time point ($p < 0.05$). Different lowercase letters indicate statistically significant differences among time points within the same material ($p < 0.05$). Bars without letters indicate no statistically significant differences ($p > 0.05$)

group were 90.17 ± 0.49 on day 1, 86.59 ± 6.46 on day 3, and 87.63 ± 0.59 on day 7. In the milled resin group, significant differences in cell viability were observed among the time periods ($p < 0.05$). Cell viability value on day 1 (98.30 ± 1.66) was significantly higher than on day 3 (93.02 ± 2.90). The cell viability values of the conventional resin group showed a significant difference over time periods ($p < 0.05$). The highest cell viability values were observed on the 1 st day (93.16 ± 1.85). However, cell viability did not differ significantly between days 3 (83.23 ± 4.51) and 7 (78.92 ± 0.65) ($p > 0.05$). Throughout the entire test period, the milled resin group consistently exhibited cell viability higher than 90% (Fig. 2).

## Induction of apoptosis

Significant variations in apoptosis rates were observed among the tested material groups across different incubation periods ($p < 0.05$). However, on the first day, no statistically significant differences were detected among the tested material groups ($p > 0.05$). The apoptosis rates on day 1 were recorded as 2.24 ± 0.39 for the conventional resin group, 1.86 ± 0.32 for the printed resin group, and 1.55 ± 0.18 for the milled resin group, respectively.

On the third and seventh days, significant differences were identified among the tested material groups concerning apoptosis rates ($p < 0.05$). The conventional resin group exhibited significantly higher apoptosis rates on days 3 (3.52 ± 0.28) and 7 (3.40 ± 0.60%) compared with day 1 (2.24 ± 0.39%) ($p < 0.05$). For the printed and milled resin groups, no significant differences were observed in apoptosis rates across the incubation periods ($p > 0.05$) (Figs. 3 and 4).

## Evaluation of inflammatory response

IL-6 levels after exposure to the resin eluates are shown in Fig. 5. The IL-6 release levels of the tested material groups exhibited statistically significant differences on day 1, day 3, and day 7 ($p < 0.05$). The conventional resin group demonstrated the highest IL-6 release across all incubation periods ($p < 0.05$), whereas no significant differences were observed between the printed and milled resin groups ($p > 0.05$). The eluates of the printed resin group showed significant differences across incubation periods ($p < 0.05$). The IL-6 release levels of the printed resin group significantly decreased on the 7th day compared with the values observed on the 1 st and 3rd days ($p < 0.05$).

## Surface roughness measurements

The mean surface roughness values are presented in Table 2. One-way ANOVA revealed no statistically significant differences among the tested material groups ($p > 0.05$).

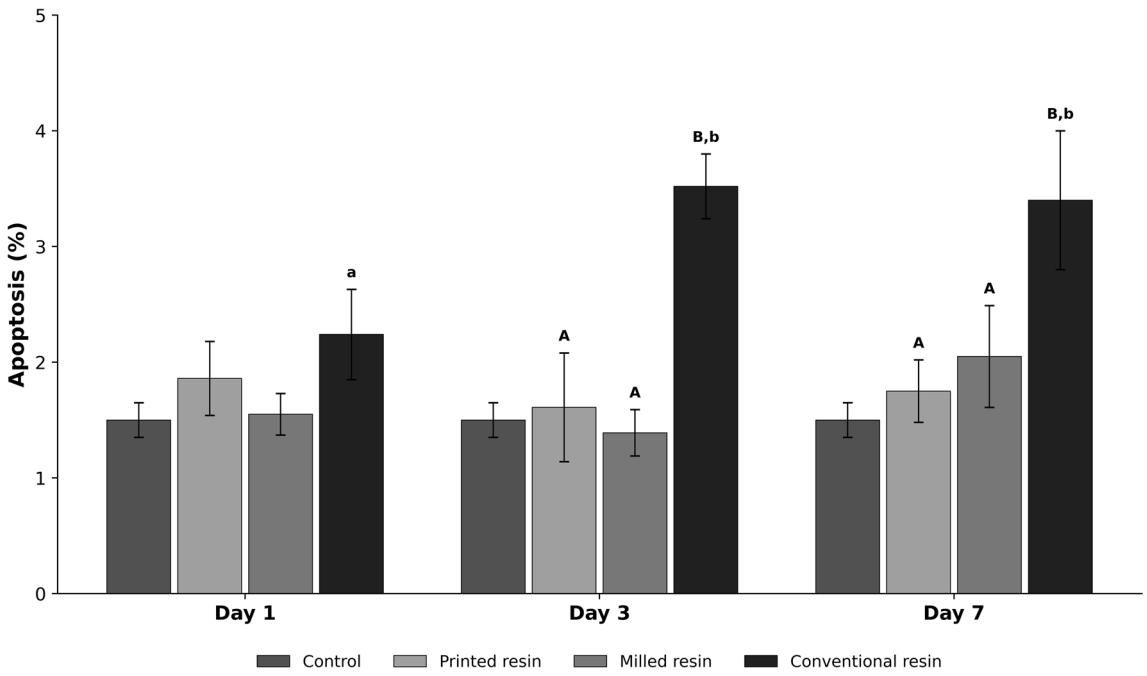


**Fig. 3** Apoptosis rates (%) of L929 fibroblast cells exposed to 1 st, 3rd, and 7th day medium extracts of printed resin, milled resin, and conventional resin groups. Different uppercase letters indicate statistically significant differences among materials within the same time point ($p < 0.05$). Different lowercase letters indicate statistically significant differences among time points within the same material ($p < 0.05$). Bars without letters indicate no statistically significant differences ($p > 0.05$)

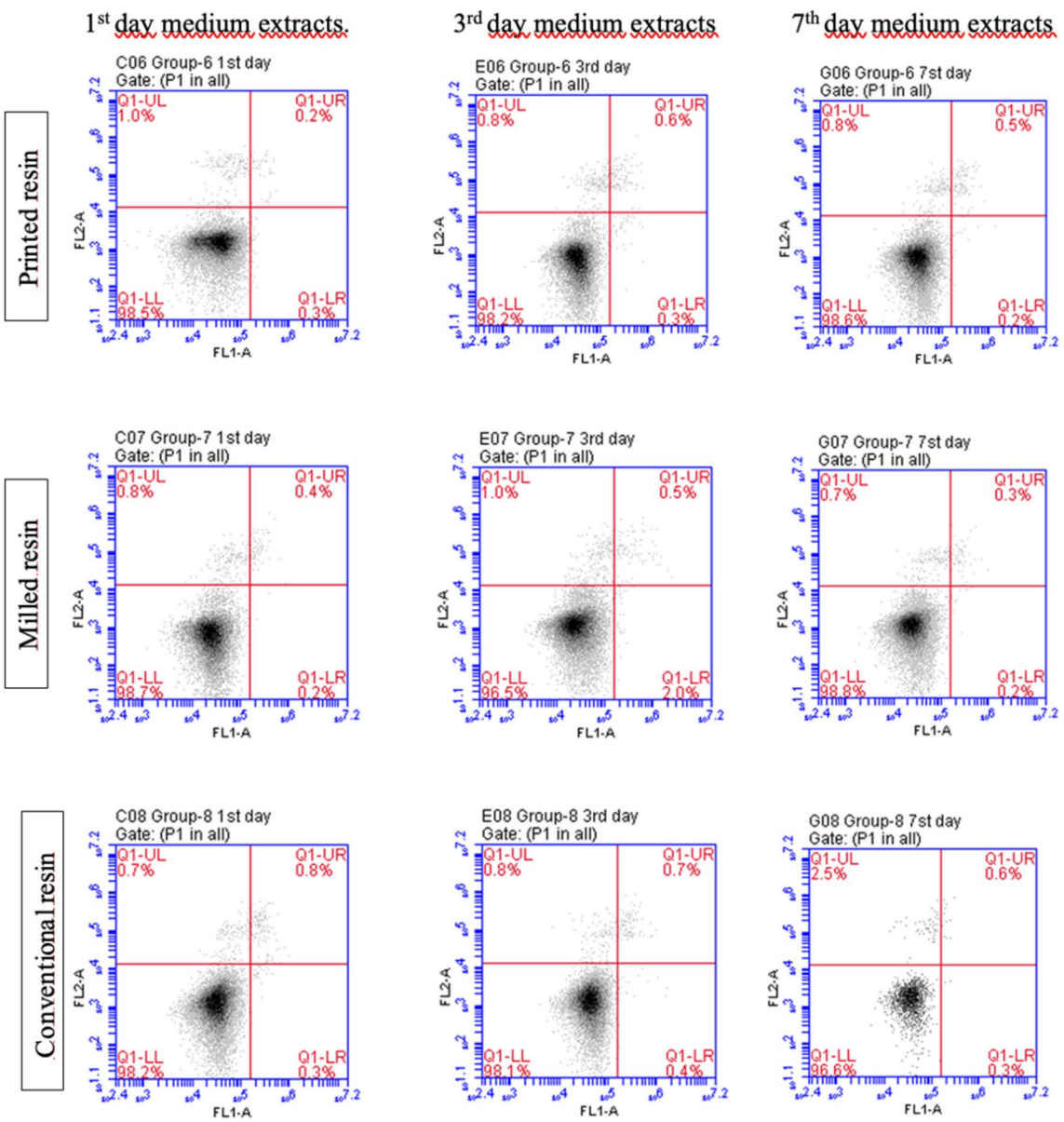


**Fig. 4** Annexin V/PI staining of indicated cell groups. Cells were treated with 1 st, 3rd and 7th day medium extracts of indirect restorative materials for 48 h and then subjected to flow cytometric analysis. *LL* Vital cells, *LR* Early apoptosis, *UR* Late apoptosis, *UL* Necrosis

Mean surface roughness values were 0.39 ± 0.19 µm for the conventional resin group, 0.31 ± 0.09 µm for the printed resin group, and 0.28 ± 0.04 µm for the milled resin group, respectively.

## Discussion

With their increasing performance, additively manufactured resin-based materials are becoming an integral part of the digital dentistry workflow alongside subtractively manufactured resin-based materials. These materials inherit the structural properties of conventional resins; therefore, factors such as filler type, organic matrix composition, and polymerization parameters directly influence the mechanical and biological performance of the definitive restoration, similar to conventional resins. Optimizing the biocompatibility of printed resins is critical given the growing use of 3D printing in dentistry. While the biological properties of conventional and milled resins have been extensively studied, the biocompatibility of 3D-printed resin materials, which are increasingly applied for permanent restorations, remains insufficiently characterized. The present in vitro study investigated the biocompatibility of indirect restoration materials fabricated using three different manufacturing techniques by assessing cell viability, apoptosis induction,

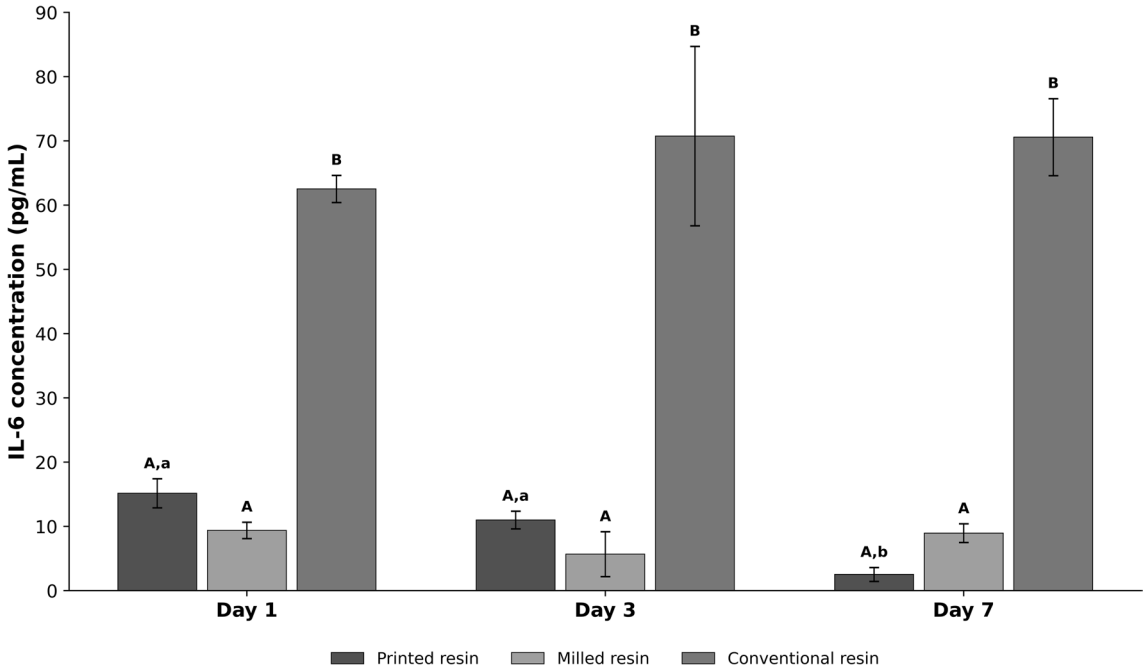


**Fig. 5** IL-6 concentration (pg/mL) released from L929 fibroblast cells exposed to 1 st, 3rd, and 7th day medium extracts of printed resin, milled resin, and conventional resin groups. Different uppercase letters indicate statistically significant differences among materials within the same time point ($p < 0.05$). Different lowercase letters indicate statistically significant differences among time points within the same material ($p < 0.05$). Bars without letters indicate no statistically significant differences ($p > 0.05$)

**Table 2** Mean and standard deviation of surface roughness (Ra ± SD) values of tested materials

| Material | Mean Ra ± SD | *p-value* |
|---|---|---|
| Printed resin | 0.31±0.09 | 0.133 |
| Milled resin | 0.28± 0.04 | |
| Conventional resin | 0.39±0.19 | |

*$p>0.05$

IL-6 release, and surface roughness. According to the results, the printed resin group demonstrated more favorable biological responses, including higher cell viability and lower apoptosis and IL-6 release, compared with the printed resin group, whereas the conventional resin group exhibited the least favorable outcomes. Accordingly, the null hypothesis was rejected, as significant differences were observed in biocompatibility among the tested materials. In addition, surface roughness, evaluated to confirm comparable surface conditions, did not differ significantly among the groups.

Toxins may affect not only the oral mucosa but also the periodontal ligament, alveolar bone, and salivary glands due to resin components released into gingival fluid and saliva. Fibroblast cells, which are abundant in gingival tissues and play a pivotal role in biological functions [20], were selected to evaluate the biocompatibility of the investigated materials. Fibroblasts play a key role in tissue architecture, producing the extracellular matrix, and are essential in the healing process [25]. Their involvement includes degrading fibrin clots, synthesizing collagen-rich extracellular matrix, and contributing to immune defense mechanisms [25, 26]. Continuous exposure to toxins may impair cell health, potentially leading to compromised wound healing, increased risk of periodontitis, and reduced control of inflammation cascades [27]. ISO 10993-5:2009 describes multiple cell culture test models for evaluating the cytotoxicity of dental materials, including direct contact, indirect contact with a barrier, and extract-based methods [24]. Some authors have suggested that the extract method is more sensitive in detecting subtle cytotoxic effects of dental composites [28]. Therefore, the extract test method was selected in the present study.

Resin-based dental materials are typically formulated with monomers like Bis-GMA, UDMA, and TEGDMA. The monomers, such as Bis-GMA and UDMA, have high molecular weight and viscosity. Thus, low-viscosity resins, which may include ethoxylated Bis-EMA, TEGDMA, and other diluents, are commonly favored for 3D printing to improve accuracy [29]. In a prior investigation, researchers explored the mechanical and biological characteristics of 3D printing resins designed for ultraviolet digital light processing (UV-DLP) 3D printers, utilizing different monomer formulations [30]. They found that resins, incorporating Bis-EMA, UDMA, and TEGDMA, were effectively formulated for 3D printing. These resins enabled the production of objects with diverse shapes and sizes, demonstrating satisfactory accuracy and no cytotoxicity. TEGDMA played

a role as a diluent, reducing viscosity and increasing the degree of conversion. In contrast, Bis-EMA and UDMA enhanced mechanical strength, as demonstrated by mechanical testing [30].

Monomers present in resin-based dental materials are known to exhibit cytotoxicity [31]. A previous study reported that the subtractive manufacturing–based materials demonstrated a cell response similar to lithium disilicate glass ceramic, suggesting no direct cytotoxic effect despite the resin content [17, 32]. In another comparison with the conventional resin group, the milled resin group showed superior biocompatibility based on factors such as proliferation, cell morphology, and inflammatory response [33]. The improved biocompatibility of the SM group was attributed to its polymerization method under high pressure and temperature and the exclusion of photoinitiators from its composition. In the present study, the highest cell viability was observed in cells exposed to eluates from the milled resin group, followed by the printed resin group, while the lowest cell viability rates were observed in fibroblast cells exposed to the conventional resin group. A recent study comparing the effects of two printed resins (Saremco print CROWNTEC and Permanent Crown), two milled resins (Vita Enamic and Brilliant Crios), and a conventionally fabricated resin on cell viability found that all materials showed no cytotoxic effect [29]. After 72 hours, the highest cell viability was observed in cells exposed to Vita Enamic, a subtractively manufactured resin-based material, with no significant difference between the other materials [29]. This may be attributed to polymerization under high-pressure and high-temperature conditions, which results in a high degree of conversion. The level of monomer conversion has been identified to influence cell response, supporting previous findings that resin polymerization affects cytotoxicity [34, 35]. Unlike many resin-based restorative materials, Vita Enamic lacks Bis-GMA, a monomer associated with higher in vitro cytotoxicity to fibroblasts than other methacrylate monomers [34]. In the present study, the milled resin group (UDMA/DMA-based) [36] showed a slight reduction in cell viability, whereas the conventional resin group exhibited the lowest cell viability, consistent with previous findings [29]. Notably, the comparable cell viability observed between the printed and milled resin groups, despite the higher organic content of the printed resin group containing Bis-EMA (Table 1), is a promising finding.

The notable decrease in cell viability observed after exposure to conventional resin group eluates, along with the increase in IL-6 cytokine, may be indicative of apoptotic or necrotic processes [37]. IL-6 is a crucial cytokine in the regulation of host defense and inflammation [38]. It is released by cells combating infectious microorganisms, products, and damaged tissues [39]. IL-6 exhibits multiple biological functions, such as promoting B cell differentiation, stimulating T cell proliferation, inducing acute-phase protein production, and enhancing phospholipase A2 activity [40]. Additionally, it serves as a key mediator in gingival and periodontal inflammatory processes [41]. Ideally, dental materials should not promote IL-6 production. According to the findings of the present study, a significant increase in IL-6 levels was observed after 72 hours in cells exposed to the conventional resin group eluates, accompanied by apoptosis induction. However, IL-6 was also increased in cells exposed to eluates from printed and milled resin groups; however, this increase was not comparable to that observed in the conventional resin group (Fig. 4). Interestingly, in a recent study on additively manufactured resin-based materials, monomer elution was associated with transient increases in cytokine release, which declined to non-significant levels after 7 days [42]. The increased release of IL-6, which peaks in the first 24 hours and gradually decreases from 72 hours to day 7 in cells incubated in the printed and milled resin groups, may also reflect processes of terminal differentiation, a phenomenon observed in healthy gingiva [43], rather than purely inflammatory activation. The elevated IL-6 release in cells exposed to eluates from the conventional resin group, coupled with reduced cell viability, may suggest a cellular defense mechanism involving the removal of damaged cells through terminal differentiation and apoptosis [44]. This finding aligns with the knowledge that IL-6-induced apoptosis occurs in a dose-dependent manner [45].

Unpolymerized methacrylate monomers, such as Bis-GMA, TEGDMA, and UDMA, have been reported to exhibit cytotoxic effects even at relatively low concentrations, leading to reduced cell metabolism, oxidative stress, and induction of apoptosis in cultured cells [46, 47]. Given that the printed resin material has a much higher organic matrix content than the other tested materials, it is more likely to be toxic. Additionally, when comparing the structures of Bis-EMA and Bis-GMA, which constitute the organic matrix of the examined printed resin, they may exhibit a higher tendency to elute. This phenomenon is attributed to the lack of hydrogen bonding within the polymer network, which allows fluids to penetrate and expand the structure, thereby releasing entrapped residual monomers [7, 48, 49]. A recent review emphasized that robust post-processing steps (washing + UV post-curing) dramatically reduce residual monomer leaching and cytotoxicity in 3D-printed dental materials [50]. To ensure biological safety in additive manufacturing, it is crucial to use certified materials, select appropriate manufacturing parameters, and implement effective post-processing techniques, given the potential toxicity of the monomers used [20, 51]. Previous research reported that printed resin specimens without

post-polymerization exhibit noticeable cytotoxicity, indicating that post-curing is an essential step [1, 14]. On the other hand, there are a limited number of studies that measured the residual monomer release of printed resin materials, and these studies examined occlusal splints [2], complete dentures [13], or temporary restoratives [3]. A recent study reported that residual monomer release from indirect resin-containing restorative materials, including printed and milled resins, was not clinically significant [21]. However, the monomer release of printed resins used as permanent restorative materials should be examined in future studies. Considering that complete polymerization does not occur on the restoration surface due to the oxygen-inhibition layer, finishing and polishing processes might contribute to reducing residual monomer release. Based on the results of the present study, it is observed that all tested specimens exhibit surface roughness values within a similar range. Surface roughness and finishing and polishing processes are essential not only for aesthetics but also for biological considerations. Studies show that resin-based restorative materials processed with optimal finishing and polishing are more biocompatible [15]. Recent evidence also confirms that the surface microtopography and hydrophobicity of 3D-printed hybrid materials substantially influence bacterial adhesion and cell response, even in the absence of cytotoxicity. A recent investigation reported that materials exhibiting the lowest surface roughness and highest hydrophobicity demonstrated minimal bacterial adhesion without compromising fibroblast viability [52]. These findings highlight that surface optimization and post-processing are as critical as polymer chemistry for ensuring the long-term biological compatibility of printed restorations.

The main limitation of the present study is that the residual monomers released from each material were not precisely quantified using methods such as gas chromatography-mass spectrometry. In this respect, only the information reported by the manufacturers was used to draw the study's conclusions. Cell-level responses obtained by knowing which monomers were released from each test material and at what level could have been interpreted more precisely. Another limitation is the fabrication characteristics of printed resin-based materials, which vary across manufacturers because they are relatively new. Since there was only one type of printed resin-based material in the current study, only one fabrication protocol was included. The validity of the findings of this study should be confirmed through studies involving different printed resin-based materials and different parameters and printing protocols recommended by their manufacturers. The current study addresses specific aspects of fibroblast cell response; the findings suggest that the influence of printed resin-based materials was minimal under the conditions presented. While it has been demonstrated that monomer elution from printed resin-based materials can occur, post-polymerization and rinsing procedures have a positive effect on cell viability and response compared with the conventional resin group. Similarly, standardized manufacturing of milled resin-based materials can lead to higher monomer conversion and, consequently, lower monomer elution [8, 53].

## Conclusion

Within the limits of this in vitro extract model, printed and milled indirect resin-based materials did not induce biologically relevant adverse effects on L929 fibroblasts, whereas the conventional resin showed a clear adverse response, with lower cell viability, higher IL-6 release, and greater apoptosis. Overall, biocompatibility followed the trend milled resin ≥ printed resin » conventional resin, leading to rejection of the null hypothesis. The biological response to the printed material improved over time (day-7 IL-6 attenuation), supporting the importance of rigorous post-processing (thorough rinsing + post-curing). Surface roughness after standardized finishing/polishing was similar among groups, suggesting that manufacturing method and polymerization efficiency, rather than surface topography, primarily drove the biological response in this setup.

**Author contributions** A.A.: Study and experimental design, sample preparation, data collection, analysis and interpretation of results, manuscript writing. D.C.A.S.: Sample preparation, performing surface roughness test. S.S.: Analysis and interpretation of results, manuscript writing. Z.Y.: Performing cytotoxicity tests, data collection. A.U.: Study and experimental design, manuscript revision.

**Funding** Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK).

## Declarations

**Conflict of interest** The authors declare that they do not have any financial interest in the companies whose materials are included in this article.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

## References


1. Kim D, Shim JS, Lee D, Shin SH, Nam NE, Park KH, et al. Effects of post-curing time on the mechanical and color properties of three-dimensional printed crown and bridge materials. Polymers. 2020;12(11):2762.
2. Wedekind L, Güth JF, Schweiger J, Kollmuss M, Reichl FX, Edelhoff D, et al. Elution behavior of a 3D-printed, milled and conventional resin-based occlusal splint material. Dent Mater. 2021;37(4):701–10.
3. Berghaus E, Klocke T, Maletz R, Petersen S. Degree of conversion and residual monomer elution of 3D-printed, milled and self-cured resin-based composite materials for temporary dental crowns and bridges. J Mater Sci Mater Med. 2023;34(5):23.
4. Knobloch LA, Kerby RE, Seghi R, van Putten M. Two-body wear resistance and degree of conversion of laboratory-processed composite materials. Int J Prosthodont. 1999;12(5):432–8.
5. Souza RO, Ozcan M, Michida SM, et al. Conversion degree of indirect resin composites and effect of thermocycling on their physical properties. J Prosthodont. 2010;19(3):218–25.
6. Lambert H, Durand JC, Jacquot B, Fages M. Dental biomaterials for chairside CAD/CAM: state of the art. J Adv Prosthodont. 2017;9(6):486–95. https://doi.org/10.4047/jap.2017.9.6.486.
7. Li P, Lambart AL, Stawarczyk B, Reymus M, Spintzyk S. Post-polymerization of a 3D-printed denture base polymer: impact of post-curing methods on surface characteristics, flexural strength, and cytotoxicity. J Dent. 2021;115:103856.
8. Jennes ME, Tomakidi P, Husari A, Hellwig E, Polydorou O, Schulz SD. Response of human gingival keratinocytes to hybrid CAD/CAM material eluates. Dent Mater. 2022;38(9):1532–46.
9. Bagis YH, Rueggeberg FA. The effect of post-cure heating on residual, unreacted monomer in a commercial resin composite. Dent Mater. 2000;16(4):244–7.
10. Putzeys E, Vercruyssen C, Duca RC, et al. Monomer release from direct and indirect adhesive restorations: a comparative in vitro study. Dent Mater. 2020;36(10):1275–81.
11. Tribst JPM, Veerman A, Pereira GKR, Kleverlaan CJ, Dal Piva AMO. Comparative strength study of indirect permanent restorations: 3D-printed, milled, and conventional dental composites. Clin Pract. 2024;14(5):1940–52.
12. Türkmen C, Durkan M, Cimilli H, Öksüz M. Tensile bond strength of indirect composites luted with three new self-adhesive resin cements to dentin. J Appl Oral Sci. 2011;19(4):363–9.
13. Srinivasan M, Chien EC, Kalberer N, Alambiaga Caravaca AM, Castelleno AL, Kamnoedboon P, et al. Analysis of the residual monomer content in milled and 3D-printed removable CAD-CAM complete dentures: an in vitro study. J Dent. 2022;120:104094.
14. Xu Y, Xepapadeas AB, Koos B, Geis-Gerstorfer J, Li P, Spintzyk S. Effect of post-rinsing time on the mechanical strength and cytotoxicity of a 3D printed orthodontic splint material. Dent Mater. 2021;37(5):e314-27.
15. Çimen C, Demirsoy FF, Özdemir A, Ark M, Özalp N. Effect of finishing-polishing procedures on cytotoxicity of resin-based restorative materials via real-time cell analysis. J Clin Pediatr Dent. 2022;46(1):24–9.
16. dos Santos RL, de Sampaio GA, de Carvalho FG, Pithon MM, Guênes GM, Alves PM. Influence of degree of conversion on the biocompatibility of different composites in vivo. J Adhes Dent. 2014;16(1):15–20.
17. Atay A, Gürdal I, Bozok Çetıntas V, Üşümez A, Cal E. Effects of new generation all-ceramic and provisional materials on fibroblast cells. J Prosthodont. 2019;28(1):e383-94.
18. Barutcigil K, Dündar A, Batmaz SG, Yıldırım K, Barutçugil Ç. Do resin-based composite CAD/CAM blocks release monomers? Clin Oral Investig. 2021;25(1):329–36.
19. Beltrami R, Colombo M, Rizzo K, Di Cristofaro A, Poggio C, Pietrocola G. Cytotoxicity of different composite resins on human gingival fibroblast cell lines. Biomimetics. 2021;6(2):26.
20. Wuersching SN, Hickel R, Edelhoff D, Kollmuss M. Initial biocompatibility of novel resins for 3D printed fixed dental prostheses. Dent Mater. 2022;38(10):1587–97.
21. Süsgün Yıldırım Z, Batmaz SG. Monomer release, cell adhesion, and cell viability of indirect restorative materials manufactured with additive, subtractive, and conventional methods. J Oral Sci. 2024;66(1):9–14.
22. Jin G, Gu H, Jang M, Bayarsaikhan E, Lim JH, Shim JS, et al. Influence of postwashing process on the elution of residual monomers, degree of conversion, and mechanical properties of a 3D printed crown and bridge materials. Dent Mater. 2022;38(11):1812–25.
23. International Organization for Standardization. ISO 10993-12:2012. Biological evaluation of medical devices—Part 12: Sample preparation and reference materials. Geneva: ISO; 2012.
24. International Organization for Standardization. ISO 10993-5:2009. Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity. Geneva: ISO; 2009.
25. Darby IA, Hewitson TD. Fibroblast differentiation in wound healing and fibrosis. Int Rev Cytol. 2007;257:143–79.
26. Li B, Wang JH. Fibroblasts and myofibroblasts in wound healing: force generation and measurement. J Tissue Viabil. 2011;20(4):108–20.
27. Boström EA, Kindstedt E, Sulniute R, Palmqvist P, Majster M, Holm CK, et al. Increased eotaxin and MCP-1 levels in serum from individuals with periodontitis and in human gingival fibroblasts exposed to pro-inflammatory cytokines. PLoS One. 2015;10(8):e0134608.
28. Cabrol A, Chuy V, Fron-Chabouis H, Naveau A. Effectiveness of postprocessing on 3D printed resin biocompatibility in prosthodontics: a systematic review. J Prosthet Dent. 2024;S0022–3913(24):00588–2.
29. Aydın N, Karaoğlanoğlu S, Kılıç-Süloğlu A, Öztürk S. Comparison of the cytotoxic effect of 3D-printed resins, resin-based CAD/CAM blocks and composite resin. J Clin Exp Dent. 2023;15(12):e984–90.
30. Lin CH, Lin YM, Lai YL, Lee SY. Mechanical properties, accuracy, and cytotoxicity of UV-polymerized 3D printing resins composed of Bis-EMA, UDMA, and TEGDMA. J Prosthet Dent. 2020;123(2):349–54.
31. Şişmanoğlu S, Demirci M, Schweikl H, Ozen-Eroglu G, Cetin-Aktas E, Kuruca S, et al. Cytotoxic effects of different self-adhesive resin cements: cell viability and induction of apoptosis. J Adv Prosthodont. 2020;12(2):89–99.
32. Grenade C, De Pauw-Gillet MC, Pirard C, Bertrand V, Charlier C, Vanheusden A, et al. Biocompatibility of polymer-infiltrated-ceramic-network (PICN) materials with human gingival keratinocytes (HGKs). Dent Mater. 2017;33(3):333–43.
33. Tassin M, Bonte E, Loison-Robert LS, Nassif A, Berbar T, Le Goff S, et al. Effects of high-temperature-pressure polymerized resin-infiltrated ceramic networks on oral stem cells. PLoS One. 2016;11(5):e0155450.
34. Gupta SK, Saxena P, Pant VA, Pant AB. Release and toxicity of dental resin composite. Toxicol Int. 2012;19(3):225–34.
35. Lin-Gibson S, Sung L, Forster AM, Hu H, Cheng Y, Lin NJ. Effects of filler type and content on mechanical properties of photopolymerizable composites measured across two-dimensional combinatorial arrays. Acta Biomater. 2009;5(6):2084–94.
36. Albrecht M, Schmidt F, Menzel F, Yassine J, Beuer F, Unkovskiy A. Comparative analysis of modern 3D-printed hybrid resin-ceramic materials for indirect restorations: an in vitro study. Polymers (Basel). 2024;16(22):3161.

37. Moe B, Gabos S, Li XF. Real-time cell-microelectronic sensing of nanoparticle-induced cytotoxic effects. Anal Chim Acta. 2013;789:83–90.
38. Barton BE. IL-6: insights into novel biological activities. Clin Immunol Immunopathol. 1997;85(1):16–20.
39. Takahashi K, Takashiba S, Nagai A, Takigawa M, Myoukai F, Kurihara H, et al. Assessment of interleukin-6 in the pathogenesis of periodontal disease. J Periodontol. 1994;65(2):147–53.
40. Dongari-Bagtzoglou AI, Ebersole JL. Production of inflammatory mediators and cytokines by human gingival fibroblasts following bacterial challenge. J Periodontal Res. 1996;31(2):90–8.
41. Murakami S, Hino E, Shimabukuro Y, Nozaki T, Kusumoto Y, Saho T, et al. Direct interaction between gingival fibroblasts and lymphoid cells induces inflammatory cytokine mRNA expression in gingival fibroblasts. J Dent Res. 1999;78(1):69–76.
42. Turkalj M, Pully D, Li Q, Sun W, Ghosh M, Čokić SM, et al. Monomer elution and cytotoxicity of 3D-printed resin-based composites. Dent Mater. 2025;41(9):1140–50.
43. Wang X, Steinberg T, Dieterle MP, Ramminger I, Husari A, Tomakidi P. FAK shutdown: consequences on epithelial morphogenesis and biomarker expression involving an innovative biomaterial for tissue regeneration. Int J Mol Sci. 2021;22(18):9774.
44. Schulz SD, Laquai T, Kümmerer K, Bolek R, Mersch-Sundermann V, Polydorou O. Elution of monomers from provisional composite materials. Int J Polym Sci. 2015;5(1):617407.
45. Morse L, Chen D, Franklin D, Xiong Y, Chen-Kiang S. Induction of cell cycle arrest and B cell terminal differentiation by CDK inhibitor p18(INK4c) and IL-6. Immunity. 1997;6(1):47–56.
46. Ansteinsson V, Kopperud HB, Morisbak E, Samuelsen JT. Cell toxicity of methacrylate monomers-the role of glutathione adduct formation. J Biomed Mater Res A. 2013;101(12):3504–10.
47. Schweikl H, Spagnuolo G, Schmalz G. Genetic and cellular toxicology of dental resin monomers. J Dent Res. 2006;85(10):870–7.
48. Decker C. Kinetic study and new applications of UV radiation curing. Macromol Rapid Commun. 2002;23(18):1067–93.
49. Schmalz G, Arenholt-Bindslev D. Biocompatibility of dental materials, vol. Vol. 1. Berlin: Springer; 2009.
50. Arossi GA, Abdou NA, Hung B, Garcia IM, Zimmer R, Melo MA. Safety of 3D-printed acrylic resins for prosthodontic appliances: a comprehensive cytotoxicity review. Appl Sci Basel. 2024;14(18):8322.
51. Alifui-Segbaya F, Bowman J, White AR, Varma S, Lieschke GJ, George R. Toxicological assessment of additively manufactured methacrylates for medical devices in dentistry. Acta Biomater. 2018;78:64–77.
52. Wang S, Dai J, Xu S, Li P, Fouda AM, Yilmaz B, et al. Surface characteristics, cytotoxicity, and microbial adhesion of 3D-printed hybrid resin-ceramic materials for definitive restoration. J Dent. 2025;152:105436.
53. Phan AC, Tang ML, Nguyen JF, Ruse ND, Sadoun M. High-temperature high-pressure polymerized urethane dimethacrylate-mechanical properties and monomer release. Dent Mater. 2014;30(3):350–6.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.