Cytocompatibility of 3D printed dental materials for temporary restorations on fibroblasts

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Research article

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Abstract

Background Three-dimensional (3D) printing is widely used in the fabrication of dental prostheses; however, the influence of dental materials used for 3D printing on temporary restoration of fibroblasts in tissues is unclear. Thus, the influence of different dental materials on fibroblasts were investigated.

Methods Digital light processing (DLP) type 3D printing was used. Specimens in the control group were fabricated by mixing liquid and powder self-curing resin restoration materials. The temporary resin materials used were Model, Castable, Clear-SG, Tray, and Temporary, and the self-curing resin materials used were Lang dental, Alike, Milky blue, TOKVSO CUREFAST, and UniFast III. Fibroblast cells were cultured on each specimen and subsequently post-treated for analysis. Morphology of the adhered cells were observed using a confocal laser scanning microscope (CLSM) and a scanning electron microscope (SEM).

Results CLSM and SEM cell imaging revealed that the 3D printed material group presented better cell adhesion with well-distributed filopodia compared to that in the conventional resin material group. Cell proliferation was significantly higher in the 3D printing materials.

Conclusion This indicates that using resins fabricated by 3D printing technology rather than the ones fabricated by self-curing technology is recommended for the fabrication of dental temporary restorations.

Background

Temporary restoration materials are widely used in dental clinics and are important for predicting the successful prognosis of endodontic treatment including inlay, onlay, crown, and bridge [1,2]. These materials are also used to protect the invasion of external substances and microorganism and to help the recovery of tooth functions, including mastication and esthetics [3,4].

Recently, the introduction of three-dimensional (3D) printing equipment has enabled quick fabrication of dental restoration through the use of an automatized protocol [5]. Unlike conventional fabrication methods of temporary restorations, such as resin curing or CAD/CAM milling, digital dentistry is dominantly driven by 3D printing technology [6,7].

The resin curing method, which is the conventional fabrication method of temporary restorations, adopts the curing reaction of an acrylic resin system, resulting from the reaction of dibutyl phthalate, a plasticizer, due to the interaction between the powder and liquid when mixed. The powder contains poly-methyl methacrylate (PMMA), a reaction initiator, and the liquid contains methyl methacrylate (MMA) and a small amount of inhibitor [8,9]. This manually driven technique has advantages including the ability to create the desired shape, quick hardening, and excellent handling. However, the conditions of the work environment are strict and the process is, thus, time consuming.

3D printing fabrication is classified into several subtypes, including extrusion, wire, granular, and light polymerized, based on the type of the technology used. Temporary restorative resin materials for dental use are treated with the use of digital light processing (DLP) technology, which adopts light polymerized technique to enable the processing of polymers [10,11]. This technology prints the resins layer by layer as it hardens, by projecting light in the desired shape for photo hardening liquid resins [12]. This technology is advantageous, as it is capable of printing without the use of any supporting beam inside the sculpture, and it produces printing products with excellent details and smooth surfaces and has a high printing speed [13]. On the other hand, there are some limitations, such as the colors of applicable materials are limited and the base materials and printer itself are expensive [14]. In addition, the more exquisite the printed product is, the more complicated is the work in creating the printed product [15].

Temporary restorations that are fabricated through such method undergo polishing and cleansing processes and are used until the placement of the permanent restoration for recovery of the function of the lost teeth [16]. Since temporary restorations are placed inside the mouth, temporary restorative resin materials are used based on analyses of their material properties [17]. There is a lack of studies that assess “what kinds of relationships exist between the negative micro influences of the restorations on intraoral living tissues and the restorations applied after the secondary processing following fabrication using such a method.”

According to a previous study, the monomers leached from the temporary restorative resin materials may cause dental pulp injury, oral mucosal irritation, and allergic reaction [18]. Furthermore, these may also cause hypersensitive asthmatic response, conjunctivitis, neurologic response, and epithelial response in dentists and other dental clinic staff [19]. Toxicity of acrylic resins has been reported from in-vitro cell experiments [20].

Hence, the biocompatibility of all dental resin materials used in temporary restoratives that are to be placed in the mouth must be thoroughly studied before and after their clinical use. This study aims to investigate the influence of dental materials fabricated either by 3D printing technology or self-curing technology on fibroblasts. We also assessed the cytocompatibility of dental materials used for temporary restoratives by analyzing and comparing cell adhesion and cell proliferation (Fig. 1).

Figure 1 Dental fabrication process of temporary restorations by DLP 3D printing and Self-curing method cytocompatibility of the fibroblast cell.

Methods

Preparation of Specimen

The ten specimens were designed in sizes of 10 × 10 × 3 mm, a size capable of use in cell culture, using the CAD design program (Exocad; GmbH, Darmstadt, Germany) and fabricated with the 3D printer (ZENITH D; Dentis, South Korea) adopting the DLP 3D printing method. Dental materials that are commercially used to fabricate temporary restorations were used and the materials were MODEL (ZMD-

1000B MODEL; Dentis, South Korea), CASTABLE (ZMD-1000B CASTABLE; Dentis, South Korea), CLEAR-SG (ZMD-1000B CLEAR-SG; Dentis, South Korea), TRAY (ZMD-1000B TRAY; Dentis, South Korea), and TEMPORARY (ZMD-1000B TEMPORARY; Dentis, South Korea). Existing temporary resin materials fabricated in self-curing method used were Jet Tooth Shade Acrylic Resin (Lang REF 1430; Lang Dental Mfg, USA), Alike (ALIKE 81; GC America, USA), Milky blue (MILKY BLUE; Nissin Dental, Japan), Tokuso Curefast (CUREGRACE; Tokuyama Dental, Japan), and Uni-Fast (A3; GC Dental Product co, Japan).

In this study, specimens for each material were fabricated and 2 g of the powder of the standard mixing ratio and approximately 1 ml of the liquid that is exclusive for each resin powder were used for the self-curing resin materials. After pouring the liquid into a rubber cup, the powder was added and mixed with a spatula for approximately 10 seconds. The specimens were fabricated when the mixture adopted a dough state, which is free of adhesiveness and allows easy shaping. After the final setting, fabrication of all specimens were completed using the polishing process standard in dental restoration fabrication. The specimens were polished using a carbide bur, silicone point, and finishing wheel and cleansed.

Cell Culture

A fibroblast cell line (L929; derivative of strain L, Mus musculus mouse, ATCC, CRL-2593) was used in this experiment. Fibroblast cells were cultured at 37℃ in a humidified incubator containing 5% CO_2. The culture medium used was minimum essential medium (α-MEM; Welgene Co., Ltd., Seoul, Korea) containing 10% fetal bovine serum (FBS), 1% penicillin streptomycin, 10 mM β-glycerophosphate (Sigma), and 10 μg/mL ascorbic acid. Cell culture maintenance was performed by washing the cells with Dulbecco's phosphate-buffered saline (DPBS) followed by cell detachment using trypsin-EDTA. The detached cells were then suspended in culture medium, centrifuged, counted using trypan blue dye, plated in culture plates (10 mL, 3×10^4 cells/mL), and cultured at 37 ℃.

Cell attachment analysis

The surface and edge of the 10 specimens made in each of the 10 materials were trimmed in the shape of a plate of a size sufficient for cell culture. Cell morphology was compared using confocal laser scanning microscopy (CLSM; C1 Plus, Inverted IX81, Olympus, Japan) and scanning electron microscopy (SEM; JSM-6360; JEOL Techniques, Tokyo, Japan). Fibroblast cells with a density of 3×10^4 cells/mL were cultured for 1 day on each of the specimens sterilized with 70% ethanol. To prepare for CLSM observation, cells were then fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.1% Triton X, and blocked with 1% bovine serum albumin. The specimens were then immersed in phalloidin and 4',6-diamidino-2-phenylindole to stain the cellular actin and nuclei, respectively. Prior to SEM characterization, attached cells were rinsed with DPBS and fixed with 2.5% glutaraldehyde for 10 min. Thereafter, sequential dehydration was conducted by 5 min immersions in 75%, 95%, and 100% ethanol, and the specimens were treated with 1,1,1,3,3,3-hexamethyldisilazane for 10 min.

Analysis of Cell Proliferation

For this experiment, cell proliferation was attempted and observed from the specimens fabricated using the 3D printing method and resin specimens fabricated using the self-curing method. The plates were then placed into the wells and fibroblast cells were cultured. After 3 and 5 days of culturing, the specimens were rinsed with DPBS. For the methoxyphenyl tetrazolium salt (MTS) assay, FBS-absent medium containing 10% of MTS was added to each of the specimens and incubated at 37°C for 2 h. 200 µl of the medium was then placed into a 96-well plate and absorbance was measured at 490 nm using a Micro-reader (Model 550; BioRad, USA).

Statistical Analysis

Tests for normality were performed using a Kolmogorov-Smirnov test and Shapiro-Wilk test. Levene's test was performed to test homogeneity of variances. After performing Kruskal-Wallis test using non-parametric statistics and statistically significant differences were presented. Pair-wise comparison was performed, and inter-group comparison was done within the confidence interval of 95%. Statistical significance was indicated as *p < .05 and **p < .01. Statistical analyses were performed using IBM SPSS (IBM SPSS 25.0 Inc., Chicago, IL, USA).

Results

Cell attachment analysis

The morphology and adhesion of fibroblasts in this in-vitro cell experiment using the specimens fabricated by 3D printing and self-curing resin technology are presented in Fig. 1, 2. A relatively high number of multi-nucleate cells, dyed in blue, and well-stretched cytoplasm, dyed in red, adhering on the specimens fabricated with 3D printing were observed using CLSM (Fig. 2A-E). SEM observation of cells presented well-stretched cytoplasm of fibroblasts and a greater number of cells adhering to the 3D-printed specimens (Fig. 3A-E). On the other hand, relatively fewer adhered cells and poor stretch of cell filopodia were observed on the specimens fabricated by using self-curing resins, due to their porous surface (Fig. 3F-J).

Figure 2 CLSM measurement of specimens made with DLP 3D printing and self-curing resin after 24 h fibroblast culture (3×10^4 cells/mL). A: Model. B: Castable. C: Clear-SG. D: Tray. E: Temporary. F: Lang dental. G: Alike. H: Milky blue. I: Tokuso Curefast. J: Unifast □.

Figure 3 Results of SEM of Cell measurements of DLP 3D printing and self-curing resin specimens after 24 h fibroblast cell culture (3×10^4 cells/mL). A: Model. B: Castable. C: Clear-SG. D: Tray. E: Temporary. F: Lang dental. G: Alike. H: Milky blue. I: Tokuso Curefast. J: Unifast III.

Analysis of Cell Proliferation

Cell proliferation of fibroblasts, an in-vitro experiment, was measured 3 days and 5 days after the cell spreading through MTS assay (Fig. 4). The results of the measurement were statistically presented by stating the inter-group difference of light absorbance between 3D-printed specimen group and self-cured

specimen group within confidence interval of 95% and level of significance as 0.05 (Table 1–3). From the measurement taken after 3 days, among the self-curing resin materials, Milky blue presented average and standard deviation as 0.429 ± 0.10, which is statistically significantly small value; however, Uni-Fast presented average and standard deviation as 0.655 ± 0.07, which is statistically significantly large value (Table 1). Among 3D printing resin materials, Castable presented average and standard deviation as 0.58 ± 0.06, which is statistically significantly small value; however, Temporary presented average and standard deviation as 0.69 ± 0.01, which is statistically significantly large value (Table 1). From the measurement after 5 days, among the self-curing resin materials, Milky blue presented an average and standard deviation as 1.08 ± 0.09, which is statistically significantly small value; however, Uni-Fast presented average and standard deviation as 1.35 ± 0.04, which is statistically significantly large value (Table 2). Among 3D printing resin materials, Castable presented average and standard deviation as 1.32 ± 0.10, which is statistically significantly small value; however, Temporary presented average and standard deviation as 1.51 ± 0.13, which is statistically significantly large value (Table 2). Overall, a greater number of cells were found on the 3D printing resin materials compared to the self-curing resin materials.

In the 3D printing resin materials and self-curing resin materials inter-group comparison, Milky blue presented a statistically significant difference ($p < .05$, $p < .01$) in cell proliferation after 3 days compared to Model and Temporary from the inter-group comparison (Table 3). Tokuso Curefast also presented a statistically significant difference ($p < .05$, $p < .01$) in cell proliferation after 3 days compared to Model and Temporary from the inter-group comparison of cell proliferation (Table 3). Alike presented a statistically significant difference ($p < .05$) compared to Temporary from the inter-group comparison. Milky blue presented a statistically significant difference ($p < .05$, $p < .01$) compared to Tray, Clear-SG, Temporary, and Model from the inter-group comparison after 5 days (Table 3). Tokuso Curefast presented a statistically significant difference ($p < .05$, $p < .01$) compared to Tray, Clear-SG, Temporary, and Model from the inter-group comparison. Alike presented a statistically significant difference ($p < .05$) compared to Temporary and Model from the inter-group comparison.

Figure 4 Fibroblasts MTS assay results measured after 3 and 5 days of cell proliferation. 1: Model. 2: Castable. 3: Clear-SG. 4: Tray. 5: Temporary. 6: Lang dental. 7: Alike. 8: Milky blue. 9: Tokuso Curefast. 10: Unifast III.

Table 1. Statistical results of MTS assay measured after 3 days fibroblast proliferation.

Materials	Absorbance			Mean	SD	Median
Model	0.637	0.648	0.626	0.637	0.011	0.637
Castable	0.597	0.512	0.638	0.582	0.064	0.597
Clear-SG	0.662	0.615	0.608	0.628	0.029	0.615
Tray	0.659	0.634	0.618	0.637	0.021	0.634
Temporary	0.702	0.671	0.703	0.692	0.018	0.702
Lang dental	0.658	0.622	0.627	0.636	0.020	0.627
Alike	0.525	0.516	0.668	0.570	0.085	0.525
Milky blue	0.437	0.527	0.324	0.429	0.102	0.437
Tokuso Curefast	0.518	0.509	0.431	0.486	0.048	0.509
Unifast □	0.721	0.572	0.674	0.656	0.076	0.674

Table 2. After 5 days of fibroblast cell proliferation, the statistical results of MTS assay.

Materials	Absorbance			Mean	SD	Median
Model	1.535	1.463	1.465	1.488	0.041	1.465
Castable	1.311	1.228	1.43	1.323	0.102	1.311
Clear-SG	1.383	1.308	1.533	1.408	0.115	1.383
Tray	1.403	1.328	1.428	1.386	0.052	1.403
Temporary	1.661	1.388	1.505	1.518	0.137	1.505
Lang dental	1.219	1.365	1.474	1.353	0.128	1.365
Alike	1.261	1.155	1.215	1.210	0.053	1.215
Milky blue	1.176	0.994	1.077	1.082	0.091	1.077
Tokuso Curefast	1.144	1.137	1.015	1.099	0.073	1.137
Unifast □	1.389	1.307	1.377	1.358	0.044	1.377

Table 3. Comparison between groups of DLP 3D printers and self-curing resin specimens. (n = 3, p < .05 *, p < .01 **)

Materials		3 days	5days
Self-curing group	DLP 3D printed group	p-value	p-value
Lang dental	Model	.926	.211
	Castable	.404	.853
	Clear-SG	.817	.711
	Tray	.963	.745
	Temporary	.179	.246
Alike	Model	.430	.014*
	Castable	.889	.308
	Clear-SG	.643	.115
	Tray	.458	.126
	Temporary	.041*	.018*
Milky blue	Model	.046*	.002**
	Castable	.286	.095
	Clear-SG	.095	.026*
	Tray	.051	.029*
	Temporary	.001**	.003**
Tokuso Curefast	Model	.046*	.002**
	Castable	.286	.095
	Clear-SG	.095	.026*
	Tray	.051	.029*
	Temporary	.001**	.003**
Unifast □	Model	.610	.179
	Castable	.151	.926
	Clear-SG	.404	.643

Table 3. Comparison between groups of DLP 3D printers and self-curing resin specimens. (n = 3, p < .05 *, p < .01 **)			
	Tray	.578	.676
	Temporary	.458	.211

Discussion

In this study, the biocompatibility of dental temporary restoratives fabricated by 3D printing or by conventional self-curing methods through polishing process was compared by analyzing adhesion, morphology, and proliferation of fibroblast cells. Most previous studies on biocompatibility have conducted bacteria-related in-vitro cell experiments on temporary restorative material [21,22]. However, only few studies have assessed the effect of temporary restorative materials fabricated through polishing process on fibroblasts.

The polishing process during the fabrication of dental restorations requires professional expertise and a deep understanding of dental materials, as this knowledge is crucial for fulfilling the functional and esthetic requirements for restoration of lost teeth according to the patient's demands [23,24].

In this experiment, DLP type 3D printing materials including Model, Castable, Clear-SG, Tray, and Temporary were studied. These materials were developed by Dentis, a professional dental device company in Korea. Self-curing, which is an existing temporary restorative fabrication method, was completed using Lang dental, Alike, Milky blue, Tokuso Curefast, and UniFast □. The methods of resin curing included heat-curing, self-curing, and light-curing [25]. Among these, the self-curing method is the most commonly used [26]. Excessive amount of liquid during the resin curing was reported to decrease the physical and chemical properties due to high water absorbance resulting in a decrease of the bonding strength [27,28]. Therefore, the ratio of powder and liquid were set to be as accurate as possible by using a scale while curing the resin.

In this study, the post-treatment process was a challenging step as it may interfere with cell adhesion due to errors occurring during the experiment examining cell adhesion and morphologic patterns or due to environmental factors. SEM measurement revealed that unlike the 3D printed specimens, the self-curing specimens had a porous surface (Fig. 3). This was thought to be caused by the difference in fabrication methods between DLP 3D printing and self-curing [29,30]. Therefore, research on biocompatibility regarding micro leakage of temporary restorative materials are recommended for future research.

Among the components examined 24 h after the leaching of the resin materials, 80–90% contained phthalate esters in plasticizer [31]. This decreases the level of the hormone estrogen and is produced after the resin curing, and may cause severe problems [32]. Despite the rareness of studies on the biocompatibility of plasticizer and remnant monomers in temporary restorative resin materials, such

challenges in this study can be considered to be because of the lack of detailed information of the components of temporary restorative resin materials provided by manufacturers.

Hence, it should be notable that the in-vitro cell experiment investigating biocompatibility in this study was done using specimens that are fabricated in the same manner as are the actual dental restorations, in order to model the temporary restorative resin, which is placed inside the mouth. In addition, additional experiments using fibroblasts are expected in the future with the development of 3D printing technology by studying biocompatible dental materials and complementing the limitation of the components of new materials.

Conclusions

This study analyzed the cytocompatibility of dental materials for temporary restorations that were fabricated using DLP type 3D printing and self-curing technologies with regard to cell adhesion, morphology, and proliferation using fibroblasts involved in tissue cells and obtained the following conclusions. Increased cell adhesion and well-extended filopodia were found when using the specimens fabricated by 3D printing, and inter-group comparison showed superior biocompatibility of 3D printed specimens compared to self-curing resins. This indicates that using resins fabricated by 3D printing technology rather than the ones fabricated by self-curing technology is recommended for the fabrication of dental temporary restorations.

Abbreviations

DLP = Digital Light Processing; CAD / CAM = Computer Aided Design / Computer Aided Manufacturing; CLSM = Confocal Laser Scanning Microscopy; MTS = Methoxyphenyl Tetrazolium Salt.

Declarations

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Author Contributions

JHP and JHK: contributed to conception and designed the experiments, JHP and HL: performed the experiments and drafted manuscript, JHP and JWK, HL: contributed to analysis and interpretation, JHK: revised the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

No Ethics Committee approval or consent to participate was requested, as the present was an in vitro study.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Dental fabrication process of temporary restorations by DLP 3D printing and Self-curing method cytocompatibility of the fibroblast cell.

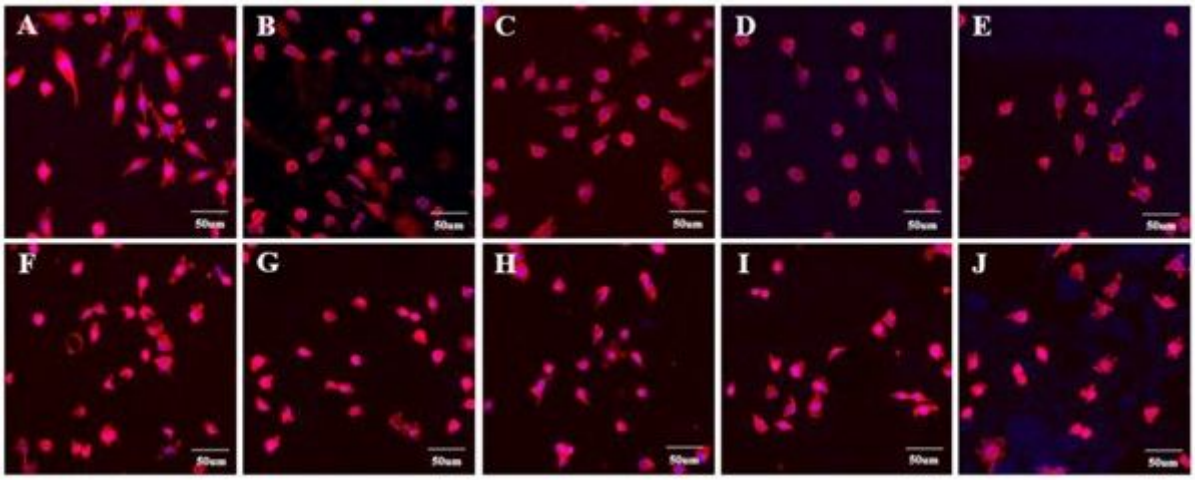

Figure 2

CLSM measurement of specimens made with DLP 3D printing and self-curing resin after 24 h fibroblast culture (3 × 104 cells/mL). A: Model. B: Castable. C: Clear-SG. D: Tray. E: Temporary. F: Lang dental. G: Alike. H: Milky blue. I: Tokuso Curefast. J: Unifast .

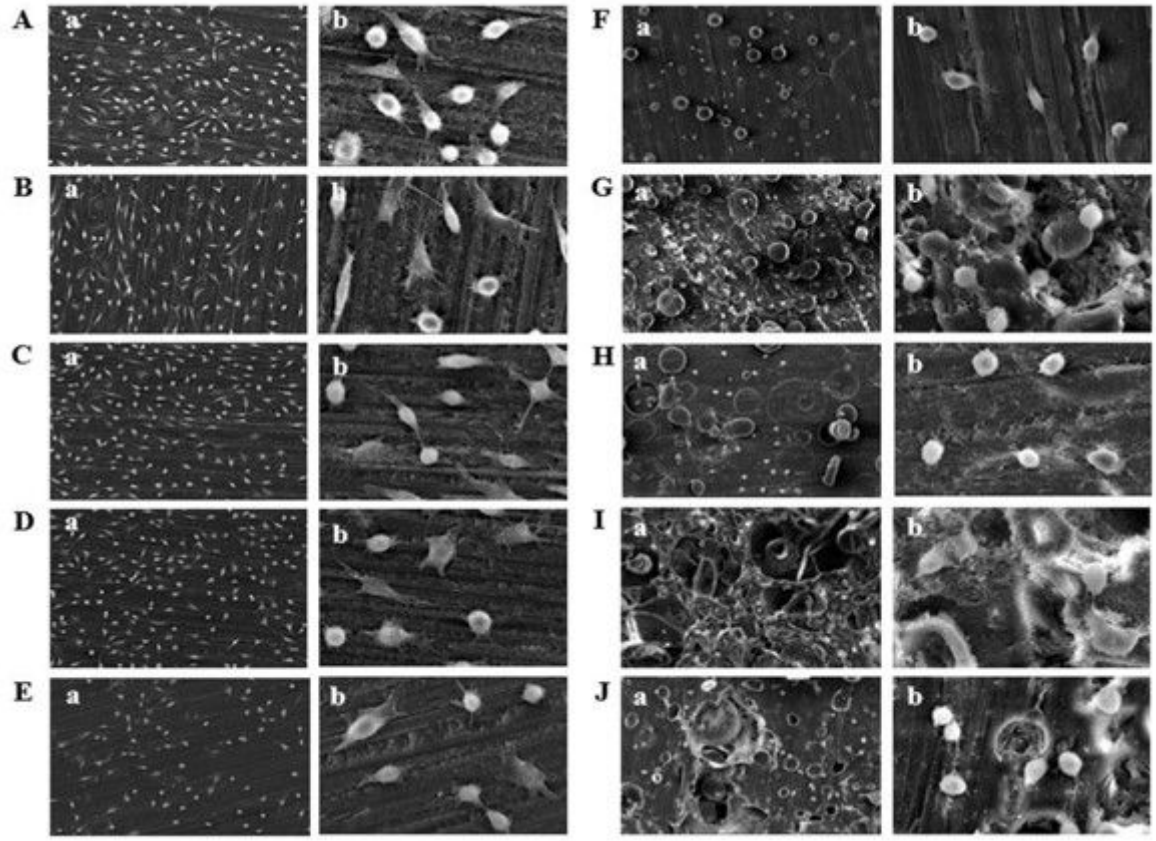

Figure 3

Results of SEM of Cell measurements of DLP 3D printing and self-curing resin specimens after 24 h fibroblast cell culture (3 × 104 cells/mL). A: Model. B: Castable. C: Clear-SG. D: Tray. E: Temporary. F: Lang dental. G: Alike. H: Milky blue. I: Tokuso Curefast. J: Unifast III.

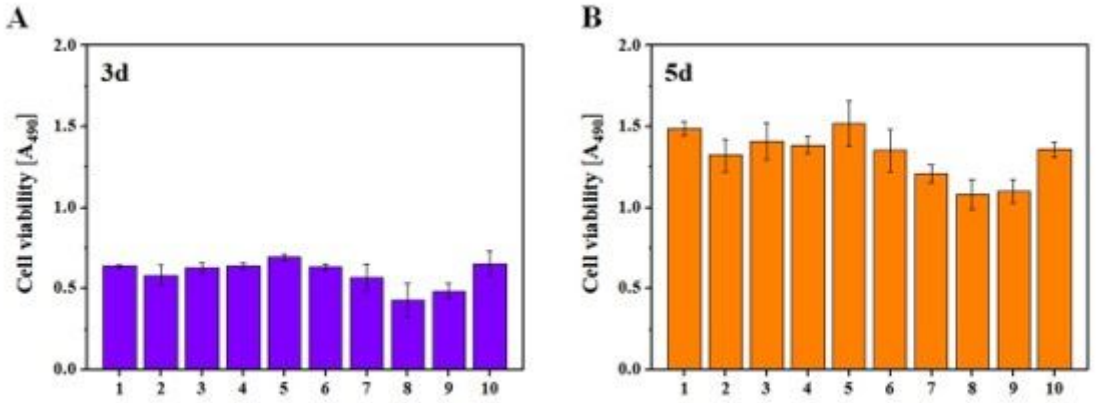

Figure 4

Fibroblasts MTS assay results measured after 3 and 5 days of cell proliferation. 1: Model. 2: Castable. 3: Clear-SG. 4: Tray. 5: Temporary. 6: Lang dental. 7: Alike. 8: Milky blue. 9: Tokuso Curefast. 10: Unifast III.