Cytotoxic assessment of 3D printed photoinitiated prosthodontic resins versus heat polymerized acrylic resin (In-Vitro Study)

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Abstract

Background: Although 3D printed photoinitiated resins are among the many materials utilized in prosthetic appliances today, biocompatibility for photocuring 3D printing materials for direct and long-term contacting with living body remain scarce. The purpose of this in vitro study was to evaluate the cell viability of human gingival fibroblasts after the exposure to two different 3D printed photoinitiated resins and compare it to the traditionally used heat polymerized acrylic resin for up to 7 days.

Methods: This comparative in vitro study of sample size (n= 96), where the 3D printed resin disc samples (n= 64), were divided into two test groups, test group 1 (TG1) for NextDent Base resin (n= 32) and test group 2 (TG2) for Dental LT clear resin (n= 32), to be compared to Heat polymerized acrylic resin samples (Reference group (RG)) (n= 32). Human gingival fibroblasts were extracted from attached keratinized gingival tissues collected from healthy patient undergoing clinical crown lengthening procedure, cellular viability using MTT assay in response to TG1, TG2 and RG samples was assessed throughout four-time intervals (24, 48, 72 and 168 hours). The One-Way ANOVA test followed by Tukey's post hoc test and Repeated Measures ANOVA test were used for statistical analyses, statistically significant different at P value ≤ 0.05

Results: Throughout time intervals, there was a decrease in cell viability of all groups but with favorable cell viability which was more that 90% denoting non cytotoxicity. It was found to be significant among RG ($P< 0.0001$). The highest cell viability was found after 24 hours among all groups; however, the least viability was found after 48 hours among TG1 and RG, and among TG2 after 72 hours. After 168 hours, there was a non-statistical significant change in cell viability between groups ($P= 0.526$). there was significant increase in optical density for all groups throughout time intervals ($P< 0.0001$).

Conclusion: Photoinitiated resins are comparable to traditionally used heat polymerized acrylic resin with equivalent cytotoxic effect for long term use. 3D printed photoinitiated resins are biocompatible and suggested for long term intraoral use.

Background

Various resins have been introduced into computer aided design/computer aided manufacturing (CAD/CAM) dental treatments and their effectiveness is based on their physical, chemical, and biological properties. Biocompatibility describes the ability of a material to perform with an appropriate host response when applied as intended. The release of chemicals through solubility or corrosion is what determines a material's biocompatibility [1, 2]. Toxic compounds have the potential to damage biological tissues in a variety of ways, including improper biochemical function, organ damage, cell destruction, and death [3, 4]. Biocompatibility is largely determined by cytotoxicity [5].

In the twenty first century, the additive manufacturing technology (AM), commonly known as three dimensional (3D) printing [6], has become one of the most important developments in the dental field [7]. Due to the flexibility of employing a variety of materials and equipment, their passive nature, low cost and

low percentage of lost raw material, additive technology has recently been deemed superior to alternative digital manufacturing approaches [8–12]. The various additive manufacturing materials and technologies, lead to multiple dental applications including appliances which are intended for extended time of use such as interim obturators for maxillary defects, sleep apnea devices, removable partial dentures metal framework, complete dentures and implant supported fixed dental prostheses [13–17].

Biocompatibility and oral soft tissue response to materials used in conventional and subtractive dental materials are well documented; however, the impact of photoinitiated 3D printing materials for direct and long term contacting with living body are currently not entirely clear [18–25]. Only a few commercially available photoinitiated resins have been approved as biocompatible thus far. In a multispecies toxicity test, a resin that had been certified as biocompatible according to USP Class VI was shown to be harmful. This impact was noticed much later than the typical exposure time of 24 hours (according to ISO 10993-5). Even if a resin has already been declared biocompatible, the biocompatibility of commercial resins must be tested for each application independently [26]. Polymers are the most used materials in the 3D printing industry due to their diversity and ease of application. In dentistry, the photoinitiated resins are the most commonly used material [27], however, due to residual monomer and photoinitiator, most photosensitive resin materials are cytotoxic, affecting cell survival and physiological functions, since monomer diffusion impairs gingival cell viability [25, 28, 29].

This study aimed to evaluate the long term cytotoxic effect of two different 3D printed photoinitiated resins on oral Human gingival fibroblasts (HGFs) compared to traditionally used heat polymerized acrylic resin. Null hypothesis was that 3D printed resins are biocompatible and would not show higher cytotoxic effect compared to heat polymerized acrylic resins.

Materials And Methods

Study design

This comparative in vitro study of sample size (n = 96), where the 3D printed resin disc samples (n = 64), were divided into two test groups, test group 1 (TG1) for NextDent Base resin (n = 32) and test group 2 (TG2) for Dental LT clear resin (n = 32), to be compared to Heat polymerized acrylic resin samples (Reference group (RG)) (n = 32) for their cytotoxic effect on HGFs, that were extracted from healthy attached gingiva from donors undergone crown lengthening procedure, using MTT assay after 24, 48, 72 and 168 hours (7 days). The Research Ethics Committee of the Faculty of Dentistry, Alexandria University (IRB NO: 00010556 - IORG 0008839), reviewed and approved the study. This research was conducted in accordance with declarations of Helsinki and gingival tissues were obtained after acquiring informed consent from donors.

Sample size calculation

Sample sized was based on 5% alpha error, 80% power and a standardized effect size of 0.9215 derived from previous study [6]. The minimum sample size was 8 discs per group (three groups) at each time

interval (four time intervals), giving a total sample of 96 printed resin discs.

Preparation and Manufacturing of samples

Preparation and samples manufacturing were done at Digital laboratory at Prosthodontics Department, Faculty of Dentistry Alexandria University, Egypt. By using CAD software (ExocadGMBH,Germany), the test group disc samples were 3D designed with a diameter of 30 mm and a thickness of 4 mm to cover the majority of the culture area of a six well plate, then were translated to a Standard Tessellation Language file " STL file" to tessellate the 3D shape and slice it into digital layers (Fig. 1) [17]. 3D printing was performed via Stereolithography using the Phrozen shuffle (Phrozen Technology, Hsinchu City, Taiwan) used for printing the NextDent Base material (NextDent B.V., Soesterberg, Netherlands) (Fig. 2). The 3D denture base's precise composition is confidential, however the material safety data sheet shows that it is a "monomer based on methacrylate ester" and contains methacrylate oligomers and phosphine oxides (photoinitiator), or more specifically, the bisacylphosphine oxide (BAPO) phenylbis (2, 4, 6-trimethylbenzoyl)-phosphine oxide (Omnirad 819) [10]. While FormLabs Form 2 machine (Formlabs, Somerville, MA, USA.) with the Dental LT Clear resin (FLDLCL01) (Formlabs, Somerville, MA, USA) (Fig. 3) which is composed of trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bismethacrylate, 2-hydroxyethyl methacrylate, bis(1,2,2,6,6- pentamethyl-4-piperidyl) sebacate and methyl 1,2,2,6,6-pentamethyl-4-piperidyl sebacate, diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide, acrylic acid, monoester with propane-1,2-diol ethylene dimethacrylate, 2-hydroxyethyl acrylate, and mequinol, 4-methoxyphenol, hydroquinone monomethyl ether [30].

Both the TG1 and TG2 samples were removed from the platform by scraping tool, then placed in an ultrasonic bath (CD-4820 Codyson, Misr Sinai, Egypt) filled with pure isopropyl alcohol (IPA) (El Nasr Pharmaceutical Chemicals Co., Egypt) for 15 minutes, then removed and soaked again in fresh IPA for an additional 5 minutes to clean the samples and remove liquid resin before post curing. The samples were allowed to dry completely in the air and the support structures were then removed with a cutter. The printed samples were placed into a UV light curing box as a post cure for 20 minutes for each side, for final polymerization to ensure that materials obtained full polymer conversion and the residual monomer was reduced to a minimal amount with the highest mechanical properties were obtained. This procedure was a necessary step to produce a biocompatible end product as recommended by the manufacturer [10, 11, 28]. For the Heat polymerized acrylic resin samples (RG), (Meliodent, BayerUK, Berkshire, UK.), were fabricated with the same used 3D resins dimensions, by making a print space in stone molds within a dental flask using finished 3D printed sample in the mold, then packing and processing were carried out in accordance with the manufacturer’s instructions (100°C, 30 min). Finally, the acrylic resin samples were finished and polished as the usually done with an actual acrylic resin denture base.

All samples were disinfected by 70% ethanol (October Pharma S.A.E, Egypt) for 5 minutes then washed by phosphate buffer saline (PBS) (Biowest, Business Park Lane, USA). All samples were put separately in sealed sterilization pouches to be sterilized under UV light for 60 minutes in biosafety cabinet (EscoMicroPte.Ltd,Singapore) prior to each experiment to prevent any bacterial contamination. Experiments were designed to assess the in direct effects of each resin after 24 hours, 48 hours, 72 hours,

and 168 hours (7 days) intervals in growth media (GM) through their cellular viability among the corresponding time intervals. To assess the in direct cellular response to the chemical leachate from each resin, samples were placed in six well plates of culture area that were acellular, containing only medium (MEDIA ONLY). Each well was used for the transfer of chemical leachate medium to its corresponding experimental well during each repeat, ensuring that transferred medium is directly representative of either cumulative resin degradation or control at each specific time point (Fig. 4) [6]. The conditioned media were stored at -20°C till the commencement of the cytotoxicity study.

Isolation and Culture of Gingival tissue [31]

A sample of attached keratinized gingival tissue was taken under local anesthesia from donor undergoing crown lengthening procedure at Department of Periodontology, Faculty of Dentistry, Alexandria University, Egypt. The gingival samples were then transported to the laboratory (Center of Excellence for Research in Regenerative Medicine and its Applications (CERRMA, Alexandria University, Faculty of Medicine)) in 15 ml falcon tube containing PBS + 3% penicillin/streptomycin/amphotericin (containing 10,000 IU/ mL penicillin; 10,000 µg/mL streptomycin; and 25 µg/mL amphotericin B, Lonza). The sample was de-epithelialized with a scalpel #11, leaving only the connective tissue. The gingival samples then washed three times in PBS then divided into small fragments 1x1 mm. Tissue fragments were cultured in tissue culture dishes in low glucose Dulbecco's modified Eagle's medium (LG-DMEM) supplemented with 10% fetal bovine serum, 2 mm L-glutamine, 1% penicillin/streptomycin (Biowest, Business Park Lane, USA) and left in humidified incubator with 5% CO2 at 37 ^{0}C (Fig. 5).

Over a 14-day period, the GM were changed every 2 to 3 days to permit the growth of the tissue explanted fibroblasts. After reaching 80–85% confluence, the cells were detached from the monolayer by treatment with trypsin-EDTA (0.25% trypsin, 1 mM EDTA) (Biowest, Business Park Lane, USA). After that the cells were sub-cultured in tissue flasks in same conditions till reached passage 4.

Once confluent, Fibroblasts were trypsinized and characterized using fluorescent-labeled monoclonal antibodies (mAb) for CD90, CD105, CD 11b, CD 45, and CD 73 markers and then was analyzed using Becton Dickinson, FACS caliber flow cytometer operated with Cell Quest software (Becton Dickinson, New Jersey, USA) [32].

Cell viability assessment [31]

Cells at passage 4 (Fig. 6) were seeded in 96 well plates at a seeding density of 7 X 10^3 / well and cultured for 24 hours to get adherent and about 70% confluent. Plates were then divided into 4 groups; Control group (CG) where cells were cultured in Complete growth media, RG where cells were cultured in conditioned media of Heat polymerized acrylic, TG1 cells were cultured in conditioned media of NextDent Base and TG2 cells were cultured in Dental LT Clear conditioned media. Growth complete media or conditioned media were transferred to the cells and incubated for 24 hours, 48 hours, 72 hours, and 7 days, according on the time intervals collected from all resins (Fig. 7). The cell viability of HGFs was measured by an MTT [(3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test [31]. After

incubation the cells were washed with PBS, and the medium was changed to one containing 0.5 mg/mL MTT (Trevigen, Helgerman CT,Gaithersburg, MD,USA) in DMEM and left for 4 hours at 37 ^{O}C (Fig. 8). All readings were carried out using an UV reader at 570 nm (Tecan Trading AG, Switzerland). The MTT assay was performed in four independent experiments, eight replicate wells for each experimental point [31]. All the steps were done in the biosafety cabinet class II to prevent contamination and to protect the operator and samples.

Cell viability calculation

The viability percentage was calculated using the formula: Cell viability (%) = (optical density of the test group ÷ optical density of cellular control group) x 100.Cell viability was scored according to the classification of Ahrari et al. were cell viability > 90% means no cytotoxicity, 60%-90% cell viability means slight cytotoxicity, 30%-59% cell viability means moderate cytotoxicity and < 30% cell viability indicates severe cytotoxicity [33].

Statistical analysis

Statistical analysis was performed using SPSS for windows version 2. Normality was checked using Shapiro Wilk test, box plots and descriptives. Data was normally distributed and presented using Mean and Standard deviation (SD). Groups were compared cell viability using One Way ANOVA test followed by Tukey's post hoc test. A change across time was assessed within each group using Repeated Measures ANOVA test. Significance level was set at *P* value of .05. All tests were two tailed.

Results

The cell viability percentage of study group materials were evaluated relative to the periods of the 24, 48, 72 and 168 hours compared with the control (cultured growth media). Throughout time intervals, there was a decrease in cell viability of all groups but with favorable cell viability more than 90% denoting that all used resins were not cytotoxic. However, this decrease was only significant among RG (P = < 0.0001) (Table 1), which found when comparing the cell viability after 24 hours to 48 hours, 72 hours, and 168 hours with P value of < 0.0001, 0.001 and 0.018 respectively (Table 2), while the decrease in cell viability along time found to be insignificant among TG1(P = 0.503) and TG2 (P = 0.216) (Table 1). According to one-way ANOVA, statistically significant difference was only found among study groups after 48 hours (P = 0.006) (Table 1), where RG showed the least cell viability (87.62%) denoting slight cytotoxicity and TG2 showed higher cell viability (97.12%) and it was statistically significant with P value of 0.005 (Table 3).

Table 1
Cell viability at different time intervals among the study groups

Study group / Time intervals	RG (n = 8)	TG1 (n = 8)	TG2 (n = 8)	F Test (*P* value)
	Mean (SD)			
24 hours	99.86 (3.39)^{A}	95.53 (4.23)	99.64 (4.29)	2.989 (0.072)
48 hours	87.62 (4.41)^{a,B}	90.51 (5.36)^{ab}	97.12 (6.22)^{b}	6.552 (0.006*)
72 hours	92.09 (3.73)^{B}	92.19 (3.30)	93.20 (6.63)	0.131 (0.878)
168 hours	92.13 (2.07)^{B}	93.71 (11.90)	96.71 (7.06)	0.662 (0.526)
Repeated Measures ANOVA Test (P value)	22.079 (< 0.0001*)	0.808 (0.503)	1.848 (0.216)	

* Statistically significant different at *P* value ≤ .05, Different lowercase letters denote statistically significant difference between groups, Different uppercase letters denote statistically significant difference within group, RG = Reference group (Heat polymerized acrylic resin), TG1 = Test group1 (NextDent Base), TG2 = Test group2 (Dental LT Clear), > 90% cell viability is no cytotoxicity, 60%-90% cell viability is slight cytotoxicity, 30%-59% cell viability is moderate cytotoxicity, < 30% cell viability indicates severe cytotoxicity.

Table 2
Pair wise comparisons within each group across time intervals regarding cell viability

Time interval	Groups	Compared to	(*P* value)
RG	24 hours	48 hours	< 0.0001*
		72 hours	0.001*
		168 hours	0.018*
	48 hours	72 hours	0.112
		168 hours	0.375
	72 hours	168 hours	1.00

* Statistically significant different at P value ≤ .05, RG = Reference group (Heat polymerized acrylic resin), TG1 = Test group1 (NextDent Base), TG2 = Test group2 (Dental LT Clear).

Table 3
Pair wise comparisons between groups regarding cell viability (ONE WAY ANOVA)

Time interval	Groups	Compared to	(*P* value)
48 hours	RG	TG1	0.540
		TG2	0.005*
	TG1	TG2	0.057

* Statistically significant different at *P* value ≤ .05, RG = Reference group (Heat polymerized acrylic resin), TG1 = Test group1 (NextDent Base)

The results showed that the highest cell viability was found after 24 hours among all groups; however, the least viability was found after 48 hours among RG and TG1, and among TG2 after 72 hours. By comparing the cell viability percentages of photoinitiated resins with the ones of heat polymerized acrylic resin for long term use (168 hours), it was found that photoinitiated resins were less cytotoxic than heat polymerized acrylic resin but with no statistically significant difference (Table 1).

The results showed significant increase in the optical density (OD) of cells throughout time with *P* value of < 0.0001 for all study groups (Table 4). According to ONE-WAY ANOVA, statistically significant difference was found among all study groups across 24, 48 and 72 hours, with *P* value of 0.001, < 0.0001 and .001 respectively (Table 4).

Table 4
Optical density at different time intervals among the study groups

Study group	RG (n = 8)	TG1 (n = 8)	TG2 (n = 8)	CG (n = 8)	F Test (*P* value)
Time intervals	Mean (SD)				
24 hours	0.32 (0.002)	0.31 (0.009)	0.32 (0.005)	0.32 (0.009)	7.364 (0.001*)
48 hours	0.56 (0.03)	0.57 (0.02)	0.62 (0.02)	0.64 (0.04)	11.240 (< 0.0001*)
72 hours	0.68 (0.04)	0.68 (0.01)	0.69 (0.03)	0.74 (0.02)	7.331 (0.001*)
168 hours	1.16 (0.03)	1.18 (0.15)	1.22 (0.09)	1.26 (0.00)	1.970 (0.141)
Repeated Measures ANOVA Test (P value)	989.455 (< 0.0001*)	186.517 (< 0.0001*)	424.501 (< 0.0001*)	465.489 (< 0.0001*)	

* Statistically significant different at P value ≤ .05, RG = Reference group (Heat polymerized acrylic resin), TG1 = Test group1 (NextDent Base), TG2 = Test group2 (Dental LT Clear), CG = Control group where (Cells cultured in growth media only)

Discussion

The null hypothesis was accepted based on the results of the experiment, as the study revealed that there were no statistically significant differences of HGFs cytocompatibility between photoinitiated resins both types and heat polymerized acrylic resin on long term interval. Despite the decrease in cell viability of all specimens, but they still falling in accepted cytotoxicity level except some of heat polymerized acrylic resin specimens.

In the present study, HGFs were selected for viability assessment as they are dominant resident cells in gingival connective tissue, easily isolated, grow fast in normal culture medium, and have a high sensitivity in cytotoxicity assays [20–22]. The MTT assay was used to assess the viability and proliferation of the cells exposed to test materials because it is the most commonly used test to assess the cytotoxicity of dental materials as it is a quick and inexpensive method, as documented by many authors [11, 22, 28]. The cytotoxic effect the 3D printed photoinitiated resins materials were compared to conventional heat polymerized acrylic resin material, as it is the traditionally preferred denture base

acrylic resins, as well as it includes less residual monomers than those that are chemically cured [22–24]. NextDent Base resin with Class IIa biocompatible standards was the first 3D photoinitiated resin chosen, that is a suitable for 3D printing of all types of dentures base [10]. The other was Dental LT Clear, which is ideal for hard splints, occlusal guards, and other direct printed long term orthodontic appliances, with LT standing for long term FDA Class IIa biocompatibility [11, 26, 28].

Since adequate contact between cells and test material is very important in biological evaluation of materials [2], a modified technique of cell contact through extracts and elutes method (in direct) was used in the present study. The cells were incubated in conditioned media for duration corresponding to that resin was left in the growth media, to keep the cells in contact with the elutes and give more accurate cell response.

The results of this study revealed that, photoinitiated resin materials exhibited non-significant cytotoxicity on HGFs with a visible trend of an in significant decrease in cell viability from 24 hours to 168 hours. While for the heat polymerized acrylic resin there was slight cytotoxic effect after 48 hours. All resins showed their least cytotoxic effect on HGFs was after 24 hours, while the highest cytotoxic effect for heat polymerized acrylic resin and NextDent Base resin was found after 48 hours, while for Dental LT resin it was increased after 72 hours but without significant value. After 168 hours (long term interval) there was slight reduction in cell viability and consequently slight increase in cell cytotoxicity. The results of some cytotoxic effect of dental resins may be attributed to their content of polymethylmethacrylate which was previously reported to be the reason of resin cytotoxicity as found by Fayyaz et al [28] who reported that photoinitiated resins contains polymethylmethacrylates and the release of methacrylate monomer might be the reason for the cytotoxicity of the resins.

Although both types of photoinitiated 3D resins have previously been classified as biocompatible, the contradictions with the current study's finding of slight cytotoxicity could be due to different incubation periods for cytotoxicity testing; for instance, the longer incubation period used in the study could lead to gradual long term effect accumulation, implying that a longer incubation period could lead to further reduced cellular viability, as reported Kreb et al. [26], that support the results of the present of reduced cell viability through time intervals. The same observation was found by Moreno et al. [12], who assessed the biocompatibility of Dental LT resin, where the biocompatibility test showed the decrease in number of live cells throughout time intervals which may be related to a slow release of chemicals. These results support our observation of decreased cell viability which can be related to methylmethacrylate release.

While at 2020, Fayyaz et al. [28] used the MTT assay to assess the in vitro cytotoxicity of the direct printed aligner using Dental LT clear resin on 3T3 mice fibroblast cells at varied time intervals, where the extraction medium was changed at the 1st, 3rd, 5th, and 7th day. From day one to day seven, the authors observed a significant increase in cell viability. The contrary to our results could be related to the fact that only one sample was utilized for the used resin, and the medium were changed and evaluated at each time interval. Another aspect that could have influenced the outcome was the different cell line used.

On the reverse, Kumar at 2019 [11] observed that Dental LT clear resin exhibited decreased toxicity from day 1 to day 5 but there was slight increase in toxicity on day 7, indicating that the increase in toxicity emerged after longer time intervals, and their findings were in accordance to the current study.

Regarding NextDent Base, Ulmer [10] evaluated NextDent Base's biocompatibility and compare it to conventional polymethyl methacrylate (PMMA) denture bases. An MTT assay was employed, and specimens were aged for 1, 3, 7, 10, and 14 days. There was an increase in optical density through groups but after day 7, there were no statistically significant differences among the groups which matches and supports our research findings of increased optical density with time intervals, particularly after long term use.

Regarding to heat polymerized acrylic resin, Çakırbay et al. [22] examined the cytotoxic effect of heat polymerized resins and came to the same conclusion as the current study. The specimens were stored in water for 24 hours or 15 days. Cytotoxicity was evaluated with MTT assay using L929 cells after 72 hours cell incubation. Cell viability was reported to be high after 24 hours of water storage and then decreased after 15 days.

This study has numerous limitations in terms of direct correlation with clinical situations and grown cells for longer periods than 7 days, and the use of only one type of cell line, given the research restrictions. Further experimental and clinical evaluation is required.

Conclusions

The 3D photoinitiated resin was found to be comparable to traditionally used heat polymerized acrylic resin with equivalent cytotoxic effect for long term use. The gradual increasing of cytotoxicity, signifying that the slow release of chemicals with longer incubation intervals will lead to a gradual long term effect on reducing cellular viability but within acceptable range. Within the research's limitations, 3D printed photoinitiated resins are suggested for long term use.

Clinical trials with larger sample size and longer time to validate the outcome reached by this study are recommended.

Abbreviations

3D
Three dimensional
(CAD/CAM)
Computer Aided Design/Computer Aided Manufacturing
AM
Additive manufacturing
BAPO
Bisacylphosphine oxide

BPO
Dibenzoyl peroxide
CG
Control group (Cells cultured in growth media only)
EGDMA
Ethylene glycol dimethacrylate
FBS
Fetal bovine serum
GM
Growth media
HGFs
Human gingival fibroblasts
IPA
Isopropyl alcohol
LG-DMEM
Low glucose Dulbecco's modified Eagle's medium
mAb
Monoclonal antibodies
MMA
Methyl methacrylate
MSCs
Human mesenchymal stem cells
MTT
3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OD
Optical density
PBS
Phosphate buffer saline
PMMA
Polymer polymethyl methacrylate
RG
Resin group (Heat-polymerized acrylic resin)
SLA
Stereolithography
STL
Standard Tessellation Language
TG1
Test group1 (NextDent Base)
TG2
Test group2 (Dental LT Clear)

UV
Ultraviolet

Declarations

- **Ethics approval and consent to participate**

he Research Ethics Committee of the Faculty of Dentistry, Alexandria University (IRB NO: 00010556 - IORG 0008839), reviewed and approved the study. This research was conducted in accordance with declarations of Helsinki and gingival tissues were obtained after acquiring informed consent from donors.

- **Consent for publication**

Not Applicable.

- **Availability of data and materials**

The data and materials used to support the findings of this study are available from the corresponding authors upon request

- **Competing interests**

The authors declare that there is no conflict of interest.

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- **Authors' contributions**

MF contributed to the conception, design of the work and drafted the study under supervision of FM, RM and MA. HA contributed to statistical analysis and interpretation of data. All authors read and approved the final manuscript.

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Not applicable.

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Figures

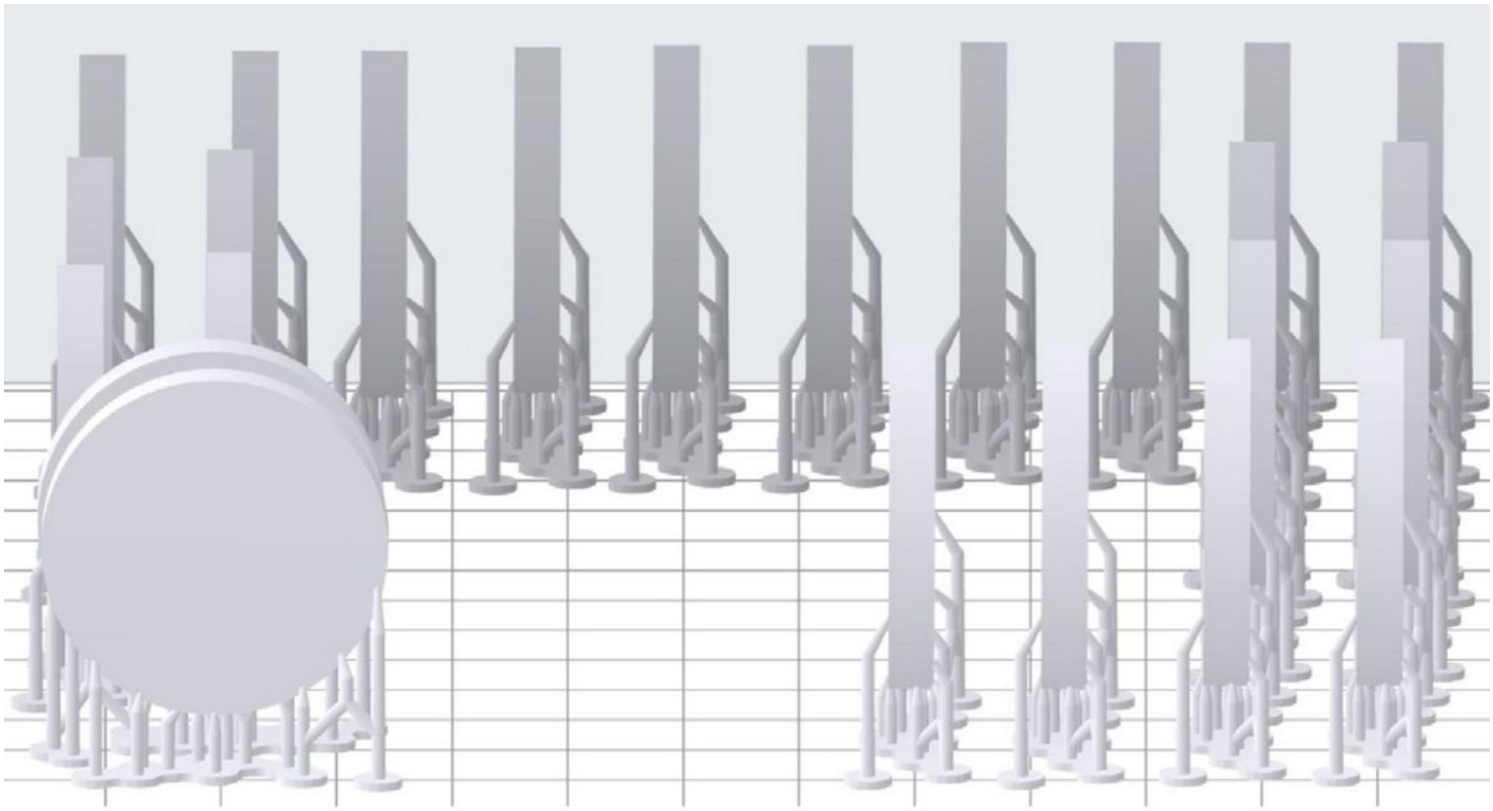

Figure 1

STL file of 3D printed samples of a diameter of 30 mm and thickness of 4 mm.

Figure 2

Printing NextDent base resin using phrozen shuffle printer.

Figure 3

Printing Dental LT clear resin using Form2 formlabs printer.

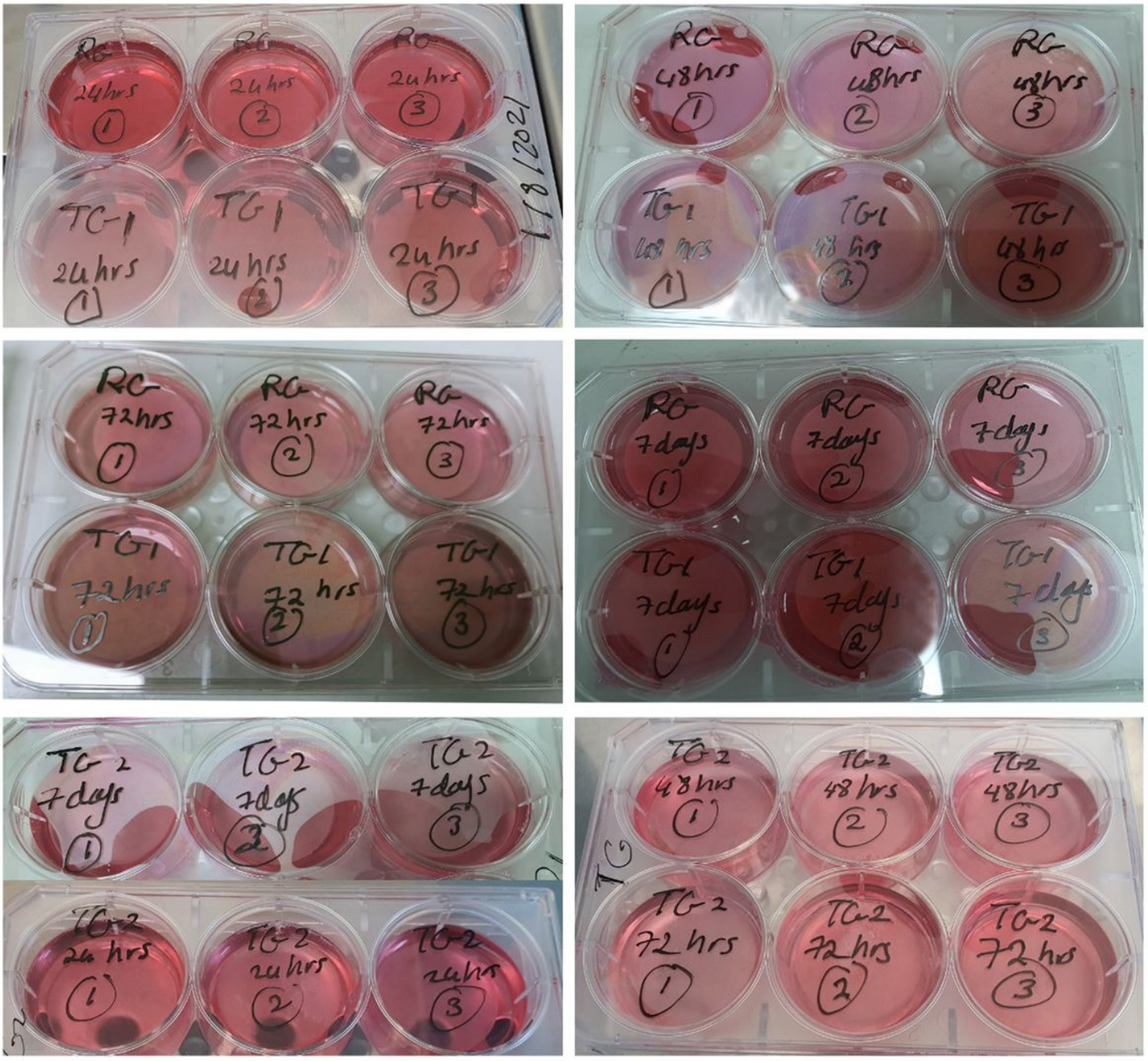

Figure 4

Study groups samples in six well plates containing a cellular culture media.

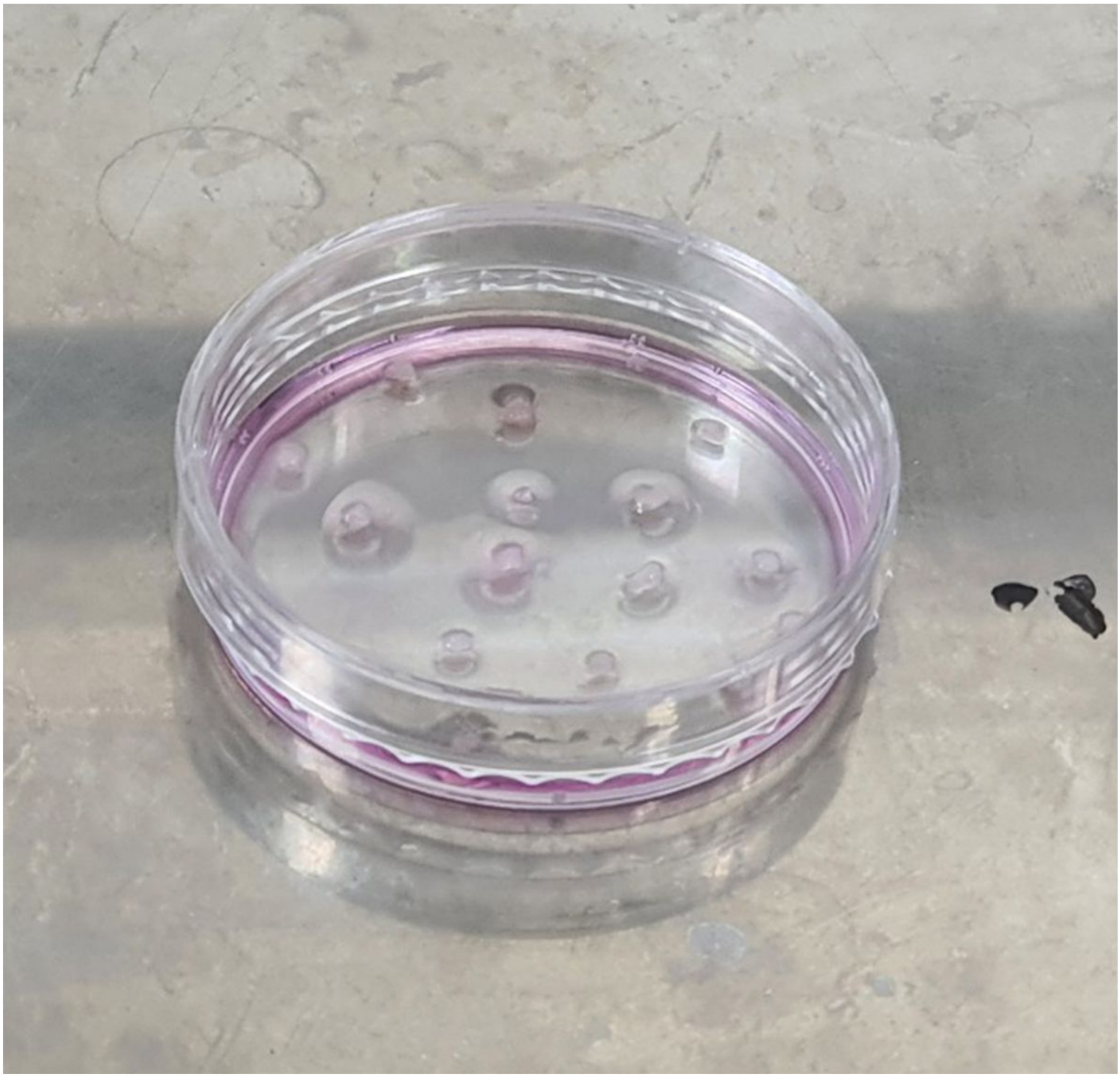

Figure 5

Gingival samples divided into small fragments and cultured in tissue culture dishes LG-DMEM.

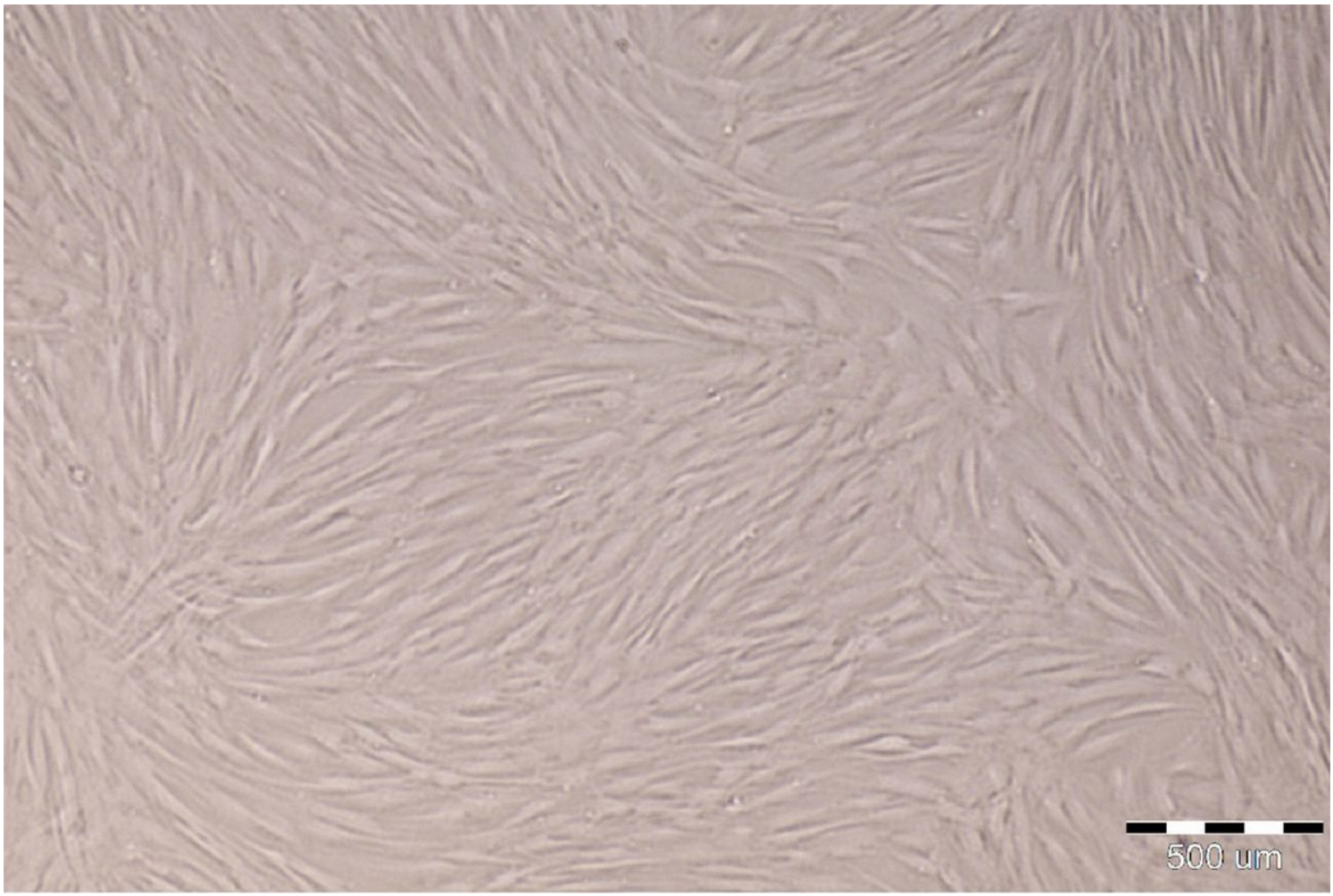

Figure 6

Explanted fibroblasts after reaching passage 4.

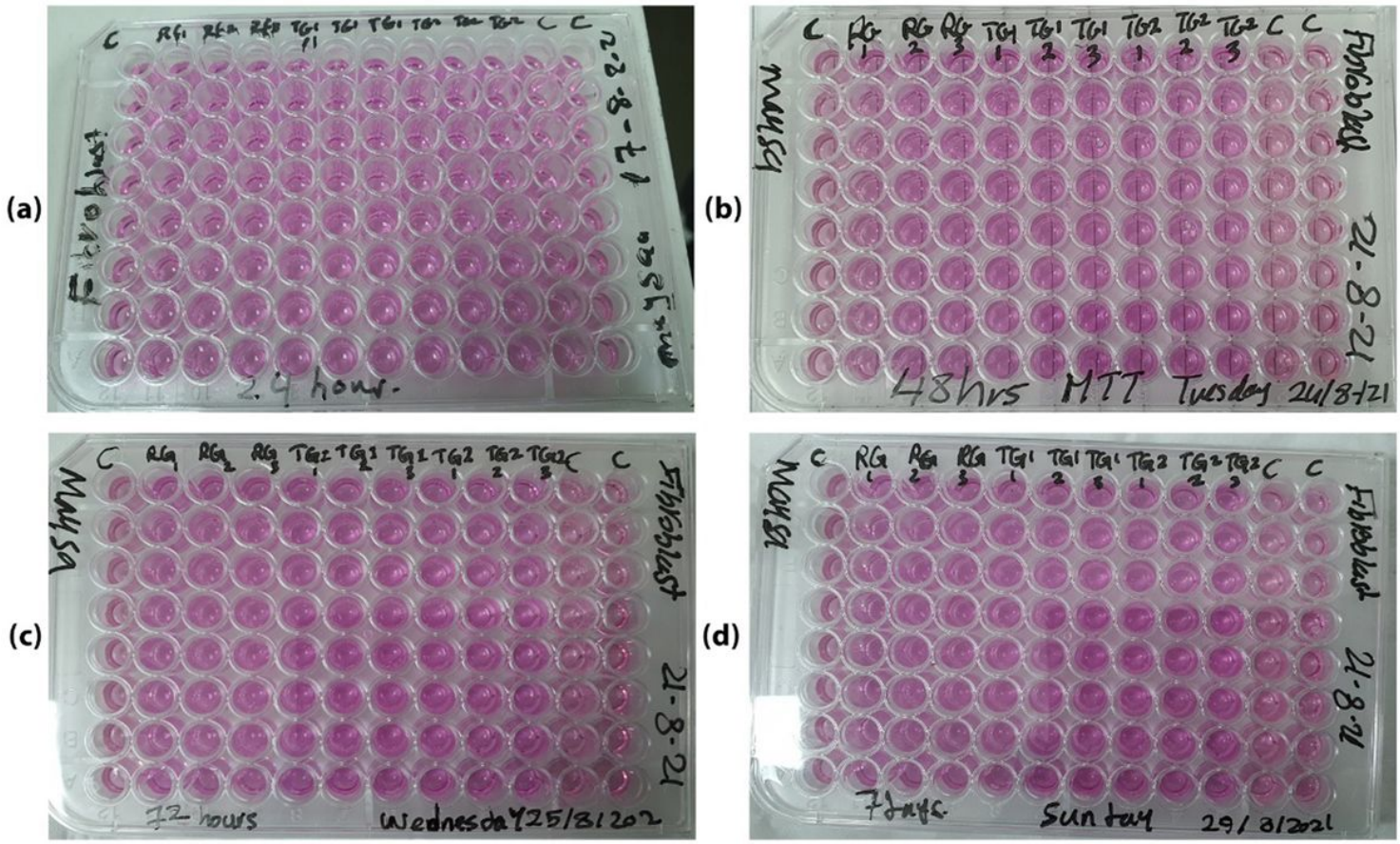

Figure 7

Collection of growth complete media or conditioned media through the time intervals from Heat polymerized acrylic resin (RG), NextDent Base (TG1) and Dental LT Clear (TG2) were transferred to the cells and incubated for (a) 24 hours (b) 48 hours (c) 72 hours (d) 7days.

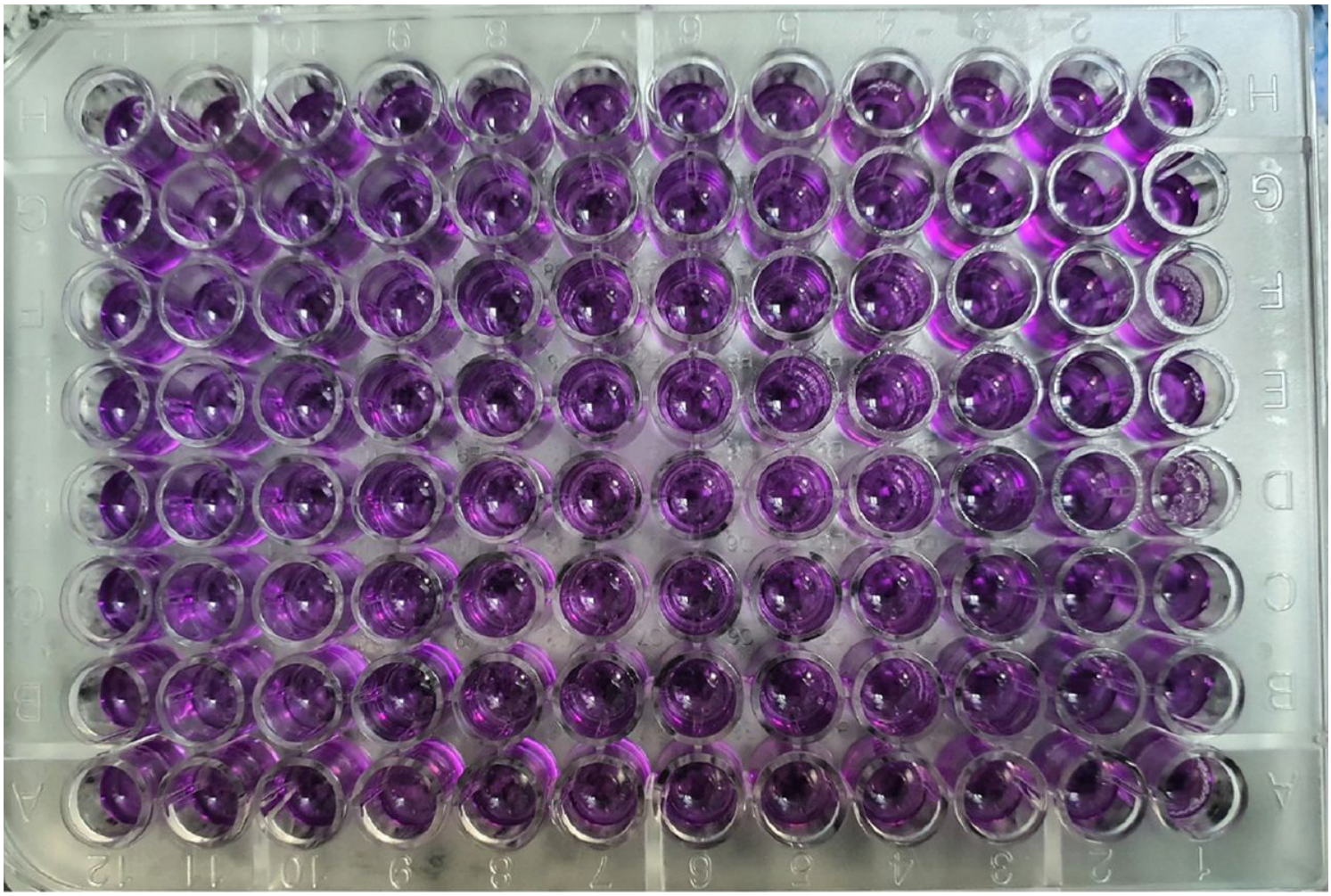

Figure 8

Cell viability of HGFs were measured by an MTT test.