

Cytotoxicity(MTT) evaluation of dental instruments made of polymers

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치과용 폴리머 기구의 세포독성(MTT) 평가

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Abstract In order to assess the cell toxicity of 10 instruments made of polymers, the MTT assay which utilizes the L-929 cell was selected. Specimens were eluted at a temperature of 37°C for 24 hours at a rate of 4g per 20mL, RPMI 1640, and then was positively and negatively contrasted with a control test solution, in accordance with the Notification No. 2020-12 Protocols of Medical Apparatus Biological Safety from the Ministry of Drug and Food Safety. As a result of 24 hours of incubation in 37°C, 5% CO₂ Incubator and assessment using an ELISA reader, the results of Intraoral camera indicated a cellular viability of more than 70% at a 50% eluate. But, the Plastic impression tray, 3D printing tweezer, Impression disposable syringe, Dental floss holder, Hand implant scaler, Surgical retractor, Oral scanner tip, Dental mirror, and the Water pick tip all reported a cellular viability of more than 70% at a 100% eluate, which indicates that do not exhibit cytotoxicity, thus allowing it to be used in contact with the mucous membrane of the oral cavity.

Key Words : Dental polymer, MTT assay, Cytotoxicity test, Plastic instruments, Dental materials

요약 최근 치과에서 사용되는 10종의 폴리머 기구에 대한 세포독성을 평가하기 위하여 L-929 세포를 이용한 MTT 시험을 시행하였다. 검체를 4g 당 20mL의 비율로 제조한 37°C의 RPMI 1640 용액에서 24시간 동안 용출한 후 식품의약품안전처 고시 제2020-12호 의리기기 생물학적 안전에 관한 공통기준규격에 따라 검체 용출액과 공시험액, 음성 및 양성대조를 사용하여, 37°C, 5% CO₂ Incubator에서 24시간 배양하여 ELISA reader로 판정한 결과, Intraoral camera는 용출물 농도 약 50%에서 약 70% 이상의 세포 생존율을 나타냈으나 Plastic impression tray, 3D printing tweezer, Impression disposable syringe, Dental floss holder, Hand implant scaler, Surgical retractor, Oral scanner tip, Dental mirror, Water pick tip은 모두 용출물 농도 100%에서 70% 이상의 세포 생존율로 세포독성을 나타내지 않아 구강점막에 직접 접촉하여 사용이 가능한 기구로 평가되었다.

주제어 : 치과용 폴리머, MTT 시험, 세포독성시험, 플라스틱 기구, 치과재료

*This research was supported by Kyungdong University Research Fund, 2021. and by the Korea Medical Device Development Fund grant funded by the Korea government (the Ministry of Health & Welfare)(Project Number: 2014X65) in 2021.

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Received June 27, 2021

Accepted August 20, 2021

Revised July 19, 2021

Published August 28, 2021

1. Introduction

Instruments that are used in dental clinics are required to be assessed their safety and effectiveness by tests for in vitro cytotoxicity, similar to instruments used in medical clinics.

Especially, dental biomaterials are required to undergo the first category of test, or tests in the cellular level, among biological characteristic tests. Dental instruments are usually composed of metal, but instruments that are directly used for treatments are composed of metal and polymers. Polymers, different to metals, have a greater level of plasticity, thus allowing them to be capable of mass production, greater variety to create a wide range of apparatus, and could be easily disinfected and sterilized so that they could be used for small clinical instruments.

Also, as polymer technology has been rapidly advanced, polymers seem to be utilized for handles, tips, and disposable instruments nowadays. Currently, the following polymer apparatuses are used in dental clinics: hand instruments, Cavitron, oral camera, oral scanner, light gun, impression tray, syringe, oral hygiene device, endodontic accessories, polishing kit, tooth preparation bur and implants, ect. In addition, materials and instruments that are actively used in dental clinics are required to be assessed their biological safety as the materials can be introduced to the body, since they are in direct contact with the mucous membrane of the oral cavity. While dental instruments made of metal are standardized throughout the world, which reports that they are very non-toxic in terms of cell toxicity[1], dental polymer instruments do not have a standardized criterion, which suggests that dental polymer instrument could have a standardized criterion to assess their safety in the foreseeable future. Polymers are also assessed their biological safety based on the amount and type of plasticizer, antioxidant, polymer stabilizers, polisher, pigments, filler, and

additives(such as conditioners).

Therefore, even for finished products, dental instruments must be conducted a safety test as medical instruments are obliged to follow certain requirements to be approved[2]. This, study aims to quantitatively assess the cell toxicity of 10 polymer instruments that are actively utilized in dental clinics. In order to do so, this study adopted the protocols from Notification No. 2020-12 Protocols of Medical Apparatus Biological Safety from the Ministry of Drug and Food Safety and ISO 10993-5:2009 Biological evaluation of medical devices Part 5 : Tests for in vitro cytotoxicity'[3,4] to assess the biological safety by the cytotoxicity of MTT assay. This study ultimately aims to be used as a database of worldwide standardization of polymers for dental clinics.

2. Materials and Methods

2.1 Study Materials and Specimen

2.1.1 Specimen

1) A(Intraoral camera)



Fig. 1. Intra oral camera

2) B(Plastic impression tray)

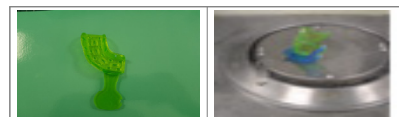


Fig. 2. Plastic impression tray

3) C(3D printing tweezer)

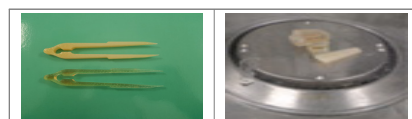


Fig. 3. 3D printing tweezer

4) D(Impression disposable syringe)

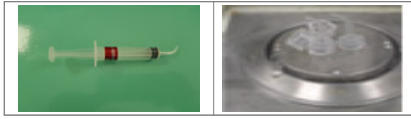


Fig. 4. Impression disposable syringe

5) E(Dental floss holder)

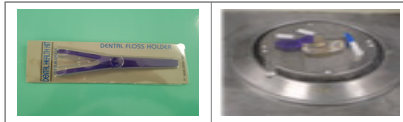


Fig. 5. Dental floss holder

6) F(Hand implant scaler)



Fig. 6. Hand implant scaler

7) G(Surgical retractor)

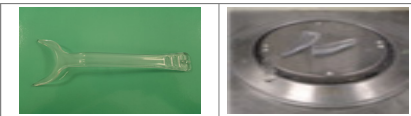


Fig. 7. Surgical retractor

8) H(Oral scanner tip)

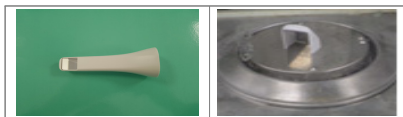


Fig. 8. Oral scanner tip

9) I(Dental mirror)

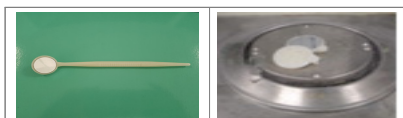


Fig. 9. Dental mirror

10) J(Water pick tip)



Fig. 10. Water pick tip

2.2 Preparation of Experimental Materials

2.2.1 Elution Solvent

10% FBS(Fetal Bovine Serum, Welgene, Korea) added to RPMI(RPMI 1640 Medium, Welgene, Korea)

2.2.2 Preparation of Specimens

Specimens were cut into appropriate pieces and sizes before being utilized.

2.2.3 Elution of specimens

- Specimen A: 1.94g of specimen was eluted by injecting 9.7mL of Elution Solvent (4g/20mL)
- Specimen B: 1.94g of specimen was eluted by injecting 9.7mL of Elution Solvent (4g/20mL)
- Specimen C: 3.0g of specimen was eluted by injecting 15.0mL of Elution Solvent (4g/20mL)
- Specimen D: 2.2g of specimen was eluted by injecting 11.0mL of Elution Solvent (4g/20mL)
- Specimen E: 2.9g of specimen was eluted by injecting 14.5mL of Elution Solvent (4g/20mL)
- Specimen F: 1.93g of specimen was eluted by injecting 9.65mL of Elution Solvent (4g/20mL)
- Specimen G: 2.2g of specimen was eluted by injecting 11.0mL of Elution Solvent (4g/20mL)
- Specimen H: 3.6g of specimen was eluted by injecting 18.0mL of Elution Solvent (4g/20mL)
- Specimen I: 3.0g of specimen was eluted by injecting 15.0mL of Elution Solvent (4g/20mL)
- Specimen J: 2.0g of specimen was eluted by injecting 10.0mL of Elution Solvent (4g/20mL)

2.2.4 Elution temperature : 37°C, 24 hours

2.2.5 Elution apparatus : Shaking incubator

2.2.6 Examination of eluant : clear

2.3 Preparation of control

2.3.1 Negative control : Alumina(Samhwa ceramic, Lot No. 20150717)

- 1) Elution solvent: 10% FBS(Fetal Bovine Serum, Welgene, Korea) added into RPMI growth medium (RPMI 1640 Medium, Welgene, Korea)
- 2) Preparation of negative control: Alumina 1.56g was eluted by injecting 7.8mL of elution solvent (4g/20mL)
- 3) Elution apparatus: Shaking incubator
- 4) Examination of eluant: clear

2.3.2 Positive control

Phenol diluent(SIGMA-ALDRICH, Lot No. BCBP4612V)

2.3.3 Blank solution

RPMI 1640(10% FBS + 1% Penicillin-streptomycin

2.4 Experimental Apparatus

- 1) Sterile air hood (Clean bench)
- 2) CO₂ incubator
- 3) Centrifuge
- 4) Phase-contrast microscope
- 5) Constant temperature water bath
- 6) Elisa reader
- 7) Shaking incubator
- 8) Electric balance

2.5 Suppliers

- 1) Cell lines
American Type Culture Collection CCL1(L-929, KCLB No. 10001).
- 2) Suppliers
Name of supplier: Korea Cell Bank
Address: Seoul, Jongro Gu, Daehak Ro 101, Korea Cell Bank:
- 3) Culture Medium
RPMI growth medium (RPMI 1640 Medium, Welgene, Korea) with 1% Penicillin-Streptomycin (Welgene, Korea) and 10% FBS(Fetal Bovine

Serum, Welgene, Korea.

2.6 Experimental procedures

- 1) L-929 cell suspension was injected into the 96 well plate, 100μL each with a concentration of 1×10^4 cells/well, and then incubated for 24 hours with a condition of 37°C, 5% CO₂ incubator.
- 2) The incubation status of the one-layer cell(more than 80% of the incubation area) and the shape of the cells were examined using a microscope.
- 3) The culture medium was removed, followed by injection of Blank, Negative control, Positive control(1.0%, 0.5%, 0.25%, 0.125% dilution), experimental material(100%, 50%, 25%, 12.5%, 6.25% dilution) 100μL each, and an incubation of 24 hours with a condition of 37°C, 5% CO₂ incubator.
- 4) The culture medium of Blank, Negative control, Positive control(1.0%, 0.5%, 0.25%, 0.125% dilution) and Experimental materials(100%, 50%, 25%, 12.5%, 6.25% dilution) was removed, followed by a two-hour reaction of 50μL of the MTT solution. The MTT solution was prepared in each well with a condition of 37°C and 5% CO₂ incubator.
- 5) After removal of the MTT solution, 100μL of isopropanol was injected into each well and were allowed for reaction.
- 6) Using the ELISA reader, the absorbance was measured at 570nm.(Reference wavelength 650nm).

2.7 Evaluation of cytotoxicity

The measured absorbance value was input to the following formula to calculate a qualitative cellular viability.

$$\text{Cellular Viability (\%)} = \frac{OD_{570 \text{ Experimental Group}}}{OD_{570 \text{ Blank}}} \times 100$$

In case the cellular viability decreases less than 70% of the blank solution, this study concluded that it indicates potential cytotoxicity. When the experimental material was tested with a concentration of 50%, the cellular viability is supposed to be equal or higher than that of 100%.

3. Results

3.1 Intraoral camera

At an eluate concentration of 50%, the materials that constitute the Intraoral camera was reported to have a cellular viability of more than 70%. Fig. 11 shows the results of the Intraoral camera.

3.2 Plastic impression tray

At an eluate concentration of 100%, the materials that constitute the Plastic impression tray was reported to have a cellular viability of more than 70%. Fig. 12 shows the results of the Plastic impression tray.

3.3 3D printing tweezer

At an eluate concentration of 100%, the materials that constitute the 3D printing tweezer was reported to have a cellular viability of more than 70%. Fig. 13 shows the results of the 3D printing tweezer.

3.4 Impression disposable syringe

At an eluate concentration of 100%, the materials that constitute the Impression disposable syringe was reported to have a cellular viability of more than 70%. Fig. 14 shows the results of the Impression disposable syringe.

3.5 Dental floss holder

At an eluate concentration of 100%, the

materials that constitute the Dental floss holder was reported to have a cellular viability of more than 70%. Fig. 15 shows the results of the Dental floss holder.

3.6 Hand implant scaler

At an eluate concentration of 100%, the materials that constitute the Hand implant scaler was reported to have a cellular viability of more than 70%. Fig. 16 shows the results of the Hand implant scaler.

3.7 Surgical retractor

At an eluate concentration of 100%, the materials that constitute the Surgical retractor was reported to have a cellular viability of more than 70%. Fig. 17 shows the results of the Surgical retractor.

3.8 Oral scanner tip

At an eluate concentration of 100%, the materials that constitute the Oral scanner tip was reported to have a cellular viability of more than 70%. Fig. 18 shows the results of the Oral scanner tip.

3.9 Dental mirror

At an eluate concentration of 100%, the materials that constitute the Dental mirror was reported to have a cellular viability of more than 70%. Fig. 19 shows the results of the Dental mirror.

3.10 Water pick tip

At an eluate concentration of 100%, the materials that constitute the Water pick tip was reported to have a cellular viability of more than 70%. Fig. 20 shows the results of the Water pick tip.

Table 1. Absorbance of test sample extraction, blank, negative control and positive control

Classification	Absorbance	Blank	Negative control	Positive control concentration (%)				Extraction concentration (%)				
				1	0.5	0.25	0.125	100	50	25	12.5	6.25
Intraoral camera	Average	1.395	1.194	0.008	0.013	0.018	0.018	0.274	1.047	1.206	1.247	1.345
	Stdev	0.114	0.132	0.001	0.001	0.002	0.002	0.043	0.069	0.152	0.141	0.121
	Viability(%)	100.00	85.58	0.54	0.90	1.32	1.26	19.62	75.06	86.41	89.39	96.39
Plastic impression tray	Average	1.333	1.241	0.008	0.013	0.018	0.018	0.998	1.046	1.088	1.131	1.218
	Stdev	0.103	0.086	0.001	0.001	0.002	0.002	0.094	0.076	0.089	0.018	0.113
	Viability(%)	100.00	93.08	0.57	0.95	1.38	1.32	74.88	78.47	81.64	84.85	91.40
3D printing tweezer	Average	1.601	1.533	0.008	0.013	0.018	0.018	1.323	1.368	1.401	1.431	1.584
	Stdev	0.143	0.137	0.001	0.001	0.002	0.002	0.179	0.275	0.192	0.229	0.197
	Viability(%)	100.00	95.75	0.47	0.79	1.15	1.10	82.64	85.42	87.50	89.37	98.95
Impression disposable syringe	Average	1.344	1.282	0.008	0.013	0.018	0.018	1.239	1.265	1.286	1.310	1.321
	Stdev	0.137	0.145	0.001	0.001	0.002	0.002	0.284	0.090	0.221	0.263	0.154
	Viability(%)	100.00	95.36	0.57	0.94	1.37	1.31	92.17	94.15	95.71	97.47	98.29
Oral hygiene device(dental floss handle)	Average	1.487	1.443	0.008	0.013	0.018	0.018	1.065	1.226	1.268	1.319	1.323
	Stdev	0.100	0.044	0.001	0.001	0.002	0.002	0.079	0.124	0.126	0.102	0.099
	Viability(%)	100.00	97.03	0.51	0.85	1.24	1.18	71.63	82.47	85.27	88.70	88.96
Hand implant scaler	Average	1.405	1.307	0.008	0.013	0.018	0.018	1.073	1.194	1.222	1.255	1.320
	Stdev	0.142	0.134	0.001	0.001	0.002	0.002	0.114	0.192	0.106	0.258	0.147
	Viability(%)	100.00	93.03	0.54	0.90	1.31	1.25	76.36	84.94	86.99	89.33	93.95
Surgical retractor	Average	1.349	1.287	0.008	0.013	0.018	0.018	1.059	1.132	1.149	1.236	1.269
	Stdev	0.128	0.066	0.001	0.001	0.002	0.002	0.096	0.169	0.093	0.120	0.162
	Viability(%)	100.00	95.37	0.56	0.93	1.36	1.30	78.50	83.94	85.14	91.65	94.07
Oral scanner tip	Average	1.320	1.318	0.008	0.013	0.018	0.018	1.143	1.150	1.158	1.164	1.179
	Stdev	0.132	0.089	0.001	0.001	0.002	0.002	0.096	0.056	0.102	0.137	0.090
	Viability(%)	100.00	99.82	0.58	0.95	1.39	1.33	86.59	87.09	87.70	88.17	89.30
Dental mirror	Average	1.338	1.195	0.008	0.013	0.018	0.018	1.121	1.146	1.207	1.235	1.273
	Stdev	0.112	0.141	0.001	0.001	0.002	0.002	0.067	0.108	0.058	0.173	0.208
	Viability(%)	100.00	89.26	0.57	0.94	1.37	1.32	83.76	85.59	90.21	92.29	95.14
Water pick tip	Average	1.440	1.359	0.008	0.013	0.018	0.018	1.122	1.184	1.199	1.266	1.272
	Stdev	0.136	0.066	0.001	0.001	0.002	0.002	0.085	0.140	0.053	0.088	0.061
	Viability(%)	100.00	94.35	0.53	0.87	1.28	1.22	77.89	82.22	83.22	87.90	88.29

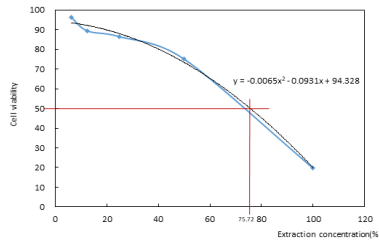


Fig. 11. The graph of cell viability of Intraoral camera depends on extraction concentration.

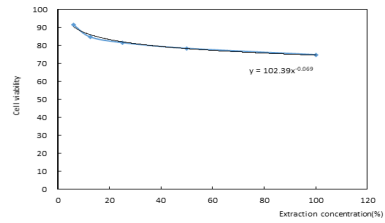


Fig. 12. The graph of cell viability of Plastic impression tray depends on extraction concentration.

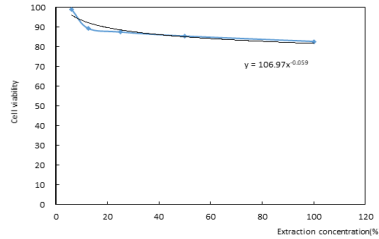


Fig. 13. The graph of cell viability of 3D printing tweezer depends on extraction concentration.

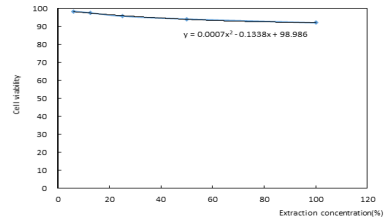


Fig. 14. The graph of cell viability of Impression disposable syringe depends on extraction concentration.

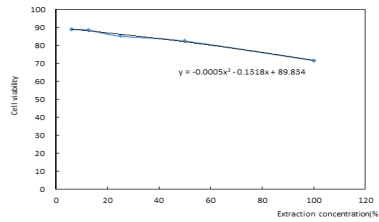


Fig. 15. The graph of cell viability of Dental floss holder depends on extraction concentration.

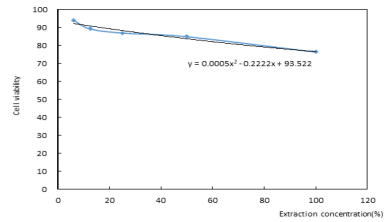


Fig. 16. The graph of cell viability of Hand implant scaler depends on extraction concentration.

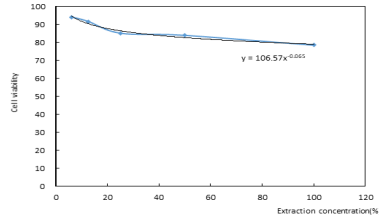


Fig. 17. The graph of cell viability of Surgical retractor depends on extraction concentration.

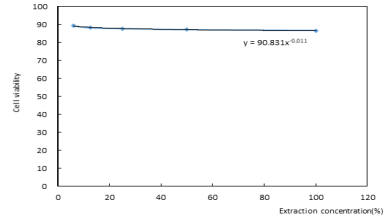


Fig. 18. The graph of cell viability of Oral scanner tip depends on extraction concentration.

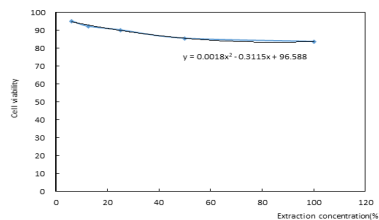


Fig. 19. The graph of cell viability of Dental mirror depends on extraction concentration.

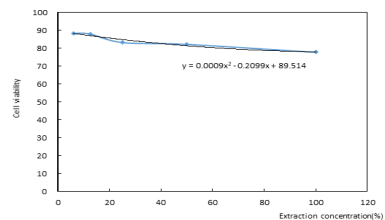


Fig. 20. The graph of cell viability of Water pick tip depends on extraction concentration.

4. Discussion

This study evaluated the biocompatibility in accordance with 'ISO 10993-5:2009 Biological evaluation of medical devices Part 5 : Tests for in vitro cytotoxicity' in order to evaluate the cytotoxicity of dental polymer instruments[4].

Generally, most materials that are in direct contact with the body, not limited to but including plastic food containers, foods and ingredients are required to have their biosafety evaluations by the cytotoxicity test[5,6].

Since dental instruments are in direct contact with the mucous membrane of the oral cavity, they must have a low cytotoxicity and must not be made with toxic and hazardous materials. Most medical devices as well as dental clinical instruments must have a high biosafety rating as they are composed of metal or polymers[7-9].

Also, dental clinical instruments are utilized within the oral cavity, which means that they may be consistently exposed to the saliva. Hazardous materials, potentially included in some of the instruments, may be dissolved to the saliva and get introduced to the body. Moreover, instruments that contain latex may initiate allergic reactions when in contact with the mucous membrane[10].

Thus, they must have their safety tested by toxicity tests[11,12]. These toxicity tests may be done via the following methods: Cell viability assay, MTT assay, LDH leakage assay, Neutral red assay[3].

Therefore, the polymer instruments were tested via the MTT assay method, which are appropriate for cytotoxicity tests. The tests were conducted by examining the cellular viability of cells in vitro, when polymer instruments which are currently utilized in dental clinics were exposed to cells[13, 14].

Although this study acknowledges that dental instruments and materials were already tested their safety and effectiveness by the

manufacturing companies by the MTT assay methods(MTT assay methods have their reliability proven due to its accuracy, accessibility and convenience), polymer instruments may have their chemical and physical properties change due to changes in the concentration, ratio, type of additives, stiffness and crafting methods. In particular, the following additives may change the chemical and physical properties of polymers: high-molecule monomers, additives(compounding agents), polishers, fillers and stabilizers[15].

The cytotoxicity test for Vinyl Polysiloxane elastomer, a type of dental polymer that is used as an impression material for impression taking, was done, as well as cytotoxicity tests for nano particles of latex bands, used for orthodontics[16,17]. Because dental polymers have various types of ingredients, they must undergo a biosafety test before they are actually used in the oral cavity. Previous studies have reported that dental instruments made of metal have a low cytotoxicity[1-3], this study claims that dental polymer must have their cytotoxicity studied constantly as they are sterilized constantly. Dental polymer instruments are particularly in interest as manufacturers tend to increase significantly nowadays as they are easy to manufacture, which may often lead to problems as their composition and cytotoxicity yet to be standardized. This is partially due to the fact that those dental instruments are often disposable goods or used temporarily. Therefore, in par with this study, dental polymer instruments and their composing materials must undergo cytotoxicity tests constantly, as well as studies for domestic and international standardization of dental instruments in general.

This study reports that 10 polymer instruments used in the oral cavity are not cytotoxic.

5. Conclusions

The results above show that the intra oral

camera had a cellular viability of more than 70% at an eluate concentration of 50%. The following materials reported to have a cellular viability of 70% at an eluate concentration of 100%: Plastic impression tray, 3D printing tweezer, Impression disposable syringe, Dental floss holer, Hand implant scaler, Surgical retractor, Oral scanner tip, Dental mirror, and Water pick tip.

Future studies must be conducted and evaluate harmful and toxic compounds, in order to standardize biosafety of dental polymer instruments and evaluate elution tests.

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