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Review

Evaluation of Biocompatibility Properties of Dental Materials: xCELLigence® System

Dental Materyallerin Biyouyumluluk Özelliklerinin Değerlendirilmesi: xCELLigence® Sistemi

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ABSTRACT

The toxic and biological impacts of dental materials play a pivotal role in their clinical application within dentistry. The assessment of these materials typically commences with *in vitro* tests upon initial development, progressing to *in vivo* animal experiments and clinical trials. *In vitro* cell culture tests afford the examination of tissue responses at the cellular level, allowing the observation of physiological activities. Moreover, these tests offer a cost-effective and time-efficient alternative to animal experiments, rendering them easily applicable and replicable. Recently, real-time cell analysis systems, such as the xCELLigence® system, have emerged as a promising substitute for traditional testing methods, potentially surpassing them in the biocompatibility evaluation of dental materials. The xCELLigence® system facilitates the concurrent observation and analysis of cells within their authentic environment, obviating the need for cell staining or marking. This review seeks to underscore the advantageous features of the xCELLigence® system, which serves to mitigate the drawbacks associated with conventional *in vitro* biocompatibility evaluation methods.

Keywords: Animals; Biocompatibility; Clinical trials; Cytotoxicity tests; Dentistry; Materials testing

ÖZET

Diş hekimliğinde dental materyallerin toksik ve biyolojik etkileri klinik kullanımda büyük bir öneme sahiptir. Dental materyaller yeni geliştirildiğinde canlı dokulardaki etkisi, etik ve yasal yükümlülükler nedeniyle öncelikle *in vitro* testler sonrasında *in vivo* hayvan deneyleri ve klinik deneyler ile değerlendirilmektedir. İn vitro hücre kültürü testleri ile dokuların hücre düzeyinde yanıtları incelenebilmekte ve fizyolojik aktiviteleri taklit edilebilmektedir. Ayrıca hücre kültürü testlerinin hayvan deneylerine göre maliyeti daha düşüktür. Daha kısa süre almakta, kolaylıkla uygulanabilmekte ve tekrar edilebilmektedir. Ancak gelişen ve değişen teknolojiyle birlikte geleneksel test yöntemlerine bir alternatif olan gerçek zamanlı hücre analiz sistemleri (xCELLigence® sistemi), dental materyallerin biyouyumluluk değerlendirmelerinde tercih edilebilir. Bu sistem ile hücrelerin boyanmasına veya işaretlenmesine gerek duyulmadan, hücreleri kendi gerçek ortamlarında gerçeğe eş zamanlı olarak gözlemlemek ve analiz etmek mümkündür. Bu derleme geleneksel *in vitro* biyouyumluluk değerlendirme yöntemlerinin dezavantajlarını elimine eden xCELLigence® sisteminin üstün özelliklerini vurgulamayı amaçlamaktadır.

Anahtar Kelimeler: Biyouyumluluk; Diş hekimliği; Hayvanlar; Klinik denemeler; Materyal testi; Sitotoksisite testleri

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INTRODUCTION

Biocompatibility means that a material does not cause tissue reactions such as allergy, local or systemic toxicity, carcinogenic and mutagenic effects when in contact with tissues.[1] Biocompatibility may vary depending on the type of material, its function, the area where it is applied, the monomers in its structure and the effect of these monomers on cells.[2,3]

Negative tissue reactions to non-biocompatible materials are interpreted as toxic effects. As a result of these reactions, the prevention of synthesis of various macromolecules and the significant deterioration in cell structure and functions are called cytotoxicity.[2]

Before the clinical use of a new material in dentistry, the mutagenic, carcinogenic and teratogenic effects of these materials must be examined through comprehensive tests and their biocompatibility must be evaluated.[2,4] If a material is not biocompatible, having superior physical properties is meaningless.[5]

The International Organization for Standardization (ISO) documents #7405 and #10993 provide guidance on how to perform biocompatibility tests at certain standards. According to these documents, tests should be applied in three stages to evaluate the biocompatibility of medical materials and devices used in dentistry. These test methods are respectively:

1- *In vitro* tests (Phase 1)
2- *In vivo* animal experiments (Phase 2)
3- Usage tests (Phase 3).[6–8]

Biocompatibility tests on materials begin with *in vitro* tests using cell cultures. These tests can be applied more easily. In the next stage, animal tests, which are costlier and take longer, are applied. When positive results are obtained in these tests, more detailed research can be conducted with usage tests.[2]

In Vitro Tests

In vitro tests are tests performed outside a living organism. In these tests, flasks, test tubes, mammalian cells in cell culture, tissues, organelles, some enzyme types or bacteria can be used. And the response created by the cells as a result of direct/indirect contact with the material to be examined is evaluated.[9] This biocompatibility evaluation is made by measuring the viability rate, metabolic functions, development rate or other functions of the cells.[10] Tests performed to evaluate the general toxicity of materials such as cytotoxicity, carcinogenic effect tests, systemic toxicity, inhalation and hemolysis are first level *in vitro* tests.[5]

The advantages of *in vitro* tests are that they are able to examine a specific function of cell metabolism, are performed quickly and economically, give quantitative and comparable findings, are easily standardized and reproducible, and have a wider range of use compared to animal experiments and usage tests. In addition, there are disadvantages such as using a single type of cell for each experiment, culture cells differing from host cells, and the absence of the inflammatory / immune system and circulatory system that would protect from adverse effects.[9,10]

With *in vitro* cytotoxicity tests, potential reactions that may be caused by a material in body tissues can be imitated and observed in the laboratory environment.[1]

The most commonly used *in vitro* tests for cytotoxicity evaluations of dental materials are cell cultures.[12]

Cell Culture

Cell cultures, cell organelles and organ cultures are biological systems used in cytotoxicity tests. The most commonly used of these is cell culture.[13] The main purpose of cell culture applications is the survival of cells taken by mechanical and enzymatic disruption under *in vitro* conditions, spontaneous migration from living tissues, and their reproduction by feeding in environments that imitative the body's unique physiological state and body temperature.[2,13–15]

The structure of the cell, its physiological properties, repair and reproduction mechanisms, and pathological changes occurring in the cell can be examined with cell cultures. The effects of materials or drugs on cells can be detected, and structural and chromosomal disorders that may occur as a result of possible mutagenic effects can be observed.[14]

In cell culture research, two types of cell lines are used: primary cells and continuous cells.[2,4,14,15] Primary cells are obtained by taking them directly from

a living tissue or organ and culturing them for more than 24 hours. These cells reflect the physiological state of the tissue. In addition, they show the same characteristics as the original tissue cell in terms of genotype and phenotype.[4,13] Subcultures are formed as a result of primary cell cultures being moved from one culture medium to another after the initial passaging process. By performing this process quickly, continuous cell lines are formed.[13]

The use of continuous cell lines in cell culture experiments to evaluate the biocompatibility of materials is reported to be a more accurate approach in terms of standardization. Because primary cell lines have limited reproductive ability and can quickly lose their functions similar to the tissue from which they are taken. The number of proliferation cycles of continuous cell lines that undergo transformation is not limited, and their metabolic and genetic stability is better. In addition, these cells have higher cloning efficiency, growth rate and tumorigenicity. Persistent cell lines can be easily propagated.[11,12,16,17]

In most studies investigating the cytotoxic effects of materials used in dental applications, rat fibroblasts (L929 and 3T3) or human epithelial cells (HeLa) are used as continuous cell lines. Additionally, human and animal pulp cells, human THP-1 monocytes, and immortalized rat odontoblast cells can also be used.[4,14,16] Due to the homogeneous morphology of these cells and their reproductive characteristics, it becomes easier to detect *in vitro* cytotoxicity.[1,12]

Advantages of cell cultures:

• Environmental conditions can be standardized.
• It is low cost.
• Useful in evaluating short-term interactions.
• Standard measurements can be made by directly observing the effects on the cell.
• It is replicable and results can be obtained faster.
• The temperature, pH, osmotic pressure, humidity, oxygen and carbon dioxide amount of the medium can be controlled.[11,17]

Disadvantages of cell cultures:

• The complex effects of chemical substances cannot be examined.
• It cannot provide sufficient information on its own.
• Preparation of cultures and microscopic examination requires experience and expertise.
• A sterile laboratory environment free of bacterial and chemical contamination is required.
• Cells of the desired purity cannot always be obtained.
• It takes time to produce sufficient number of cells.
• Freezing cells for a long time causes biochemical and genetic changes. This may affect the results of the experiment.
• As time passes, the proliferation abilities of the cells decrease.
• Since the experiment can be performed with a single cell type, information about the effect of the material on different cell types cannot be obtained with just one experiment.[11,17]

The time the material is in contact is an important factor for the tests applied. ISO 10993 defines contact periods of less than 24 hours as limited contact, 24 hours to 30 days as extended contact, and contacts longer than 30 days as continuous contact.[6,8] The toxicity of the applied material may vary depending on the density of the material components and the interaction process with the tissue.[18,19] Necrosis, apoptosis and autophagy develop in cells that are exposed to a cytotoxic material for a period of time. As a result of these biological events, cells may lose their viability or proliferation ability.[20]

In evaluating the cytotoxicity of dental materials, the physical structure of the applied material and its contact with the cell culture are important. This contact can occur directly, indirectly or through the extract of the biomaterial. In direct contact tests, cells and culture medium are in direct contact. In indirect contact tests, there is a permeable barrier between the cells and the test materials.[12,21] ISO has determined some criteria so that tests can be carried out according to certain standards. According to ISO 10993-5 criteria, *in vitro* cytotoxicity test methods that can be applied to dental materials can be listed as follows:[2,6,8]

1. a) Direct cell culture test
   i. Direct contact test
   ii. Extract test
   b) Barrier test method
2. Agar diffusion test
3. Filter diffusion test
4. Dentin barrier test

Direct cell culture test: Dental materials or compo-

nents are applied to cells in culture for a short time (less than 24 hours) in the direct contact test. In the test performed with this method, the material is in physical contact with the cells or culture medium. Direct contact of materials and cells, without any barrier between them, is essential. Water-soluble materials can dissolve in the medium and provide successful material-cell contact. For water-insoluble materials, direct contact can be achieved using different methods. Placing the test sample as close to the cells as possible, applying it on the cells, placing it on the bottom of the cell culture container, applying the cell suspension on the sample, or culturing the cells by placing them directly on the samples are some of these methods.[13,21,22]

In the extract test, cytotoxicity evaluation is made by contacting the dissolved components of the material kept in a liquid solvent with the cells. Serum-containing medium, serum-free medium, physiological salt solution or one of other suitable solvents can be used as the solvent extraction liquid. The samples are added to the test tubes and the selected extraction liquid is added to them, then the test tube is left under the recommended environmental conditions so that the sample material can dissolve and release. At the end of this period, the extracts obtained are replaced with the medium in the prepared cell cultures and the cytotoxic effects resulting from the experiment are reported.[2,6]

In order to accurately determine the toxic effect of the material, the extraction liquid must imitate the clinical use conditions of the material and this environment must not affect the chemical structure of the material. The concentration of the material in the extract depends on factors such as the volume of the extraction liquid, temperature, time, surface area of the material, pH, solubility, diffusion rate and osmolarity of the material. 37±2ºC for not less than 24 hours, 50±2ºC for 72±2 hours, 70±2ºC for 24±2 hours or 121±2ºC for 1±0.2 hours are the extraction environments recommended by ISO.[2,6,7]

Barrier test method: Dentin in the oral environment acts as a barrier between the pulp and the material applied to the cavity. For this reason, tests in which cells come into direct contact with the material are not sufficient to imitate the clinical situation. In the barrier test method, various substances that resemble dentin and allow the diffusion of the applied material components are used as barriers.[6]

Agar diffusion test: The agar diffusion test is the longest-used barrier test method in toxicity experiments. It is a simple and inexpensive method. In this test method, cells stained with neutral dye are covered with agar and the sample material is placed on the agar. Then, the toxicity of the components of the diffusing test materials is examined. Cytotoxicity is evaluated according to the amount of accumulation of the dye in lysosomes at the end of the 24-hour incubation period, depending on the permeability of the cell membrane.[6,8,17]

Filter diffusion test: In the filter diffusion test, a cellulose acetate filter is placed between the incubated cells and the material. In order for the cytotoxic effect on cells to be observed, the material must diffuse through the filter with pores of 0.45 µm and reach the target cells. Damages occurring in the cells are determined by examining the staining intensity with a spectrophotometer or by measuring the decolorization area after staining with neutral red dye.[6,8,16,17,23]

Dentin barrier test: Dentin barrier tests are a complementary development to cytotoxicity tests and are considered a testing method that can resemble *in vivo* conditions. In this method, the diffusion ability of the monomers of the test material is measured. Sterilized dentin discs obtained from bovine or human dentin are used as the dentin barrier, and cells are placed on one side of the barrier and material is placed on the other side.[6,17,24,25]

Animal Experiments

Animal experiments are performed on experimental animals by imitating the clinical use of dental materials.[5] Mammals such as rats, rabbits and pigs are generally preferred in these biocompatibility tests. The difference between these tests and *in vitro* cytotoxicity tests is that *in vivo* systems such as metabolic transformation and detoxification can be examined.[26]

Animal tests, which are second level tests, are local toxicity tests such as sensitization, subcutaneous implantation, intraosseous implantation and oral mucous membrane irritation tests.[5] The effect of experimental materials placed subcutaneously, intramuscularly or intrabony on experimental animals

is evaluated microscopically and macroscopically at different implantation periods (1 week to several months). After the short implantation period (1-2 weeks), the level of inflammation around the implanted material is first determined. In the later stages of the period, a connective tissue capsule can be observed. Thanks to the mucous membrane irritation test, the inflammation caused by the test material in the mucosa or eroded skin can be examined. Buhler test and maximization test are used to detect allergic effects.[4]

Usage Tests

Usage tests are third level tests, they are performed by applying dental treatment to experimental animals or humans.[5] Tests performed on humans are called 'clinical trials'. These clinical trials set the gold standard for usage tests.[9]

Usage tests are quite complex and costly. When long-term effects are investigated, study periods as long as months or years may be required. For clinical trials conducted in humans, there must be approval from government agencies and informed consent from the patient. There are many legal responsibilities in these tests.[9]

Cytotoxicity Evaluation Methods

In the evaluation of cytotoxicity test methods applied according to ISO 7405 and 10993-5 conditions, parameters such as cell count, cell membrane damage, staining and metabolic changes are examined. The methods determined by ISO to ensure standardization are as follows:[2,6,17]

1- Viability assessment tests
2- Life evaluation tests
3- Proliferation evaluation tests
4- Metabolism evaluation tests

Viability Assessment Tests: With viability assessment tests, the proportion of cells that can survive in cell culture as a result of the short-term toxic effect of the experimental material is calculated. In these tests, evaluation is made by staining cells with impaired and intact cell membrane integrity.[2,16,27]

Life Evaluation Tests: Viability evaluation tests evaluate the colony-forming ability of cells in a low-density uniform cell suspension.[2] With these tests, the long-term effects of toxic reactions caused by the material applied to the cells on cell viability are examined. These tests are short-term tests. Although they are useful, easily applied and rapid tests, they do not provide sufficient information to determine the long-term toxic effects of the material because they only show dead cells during the test. However, cells exposed to toxic effects may need several hours, days or longer to show the consequences of toxicity. For this reason, long-term tests are used instead of short-term tests as life evaluation tests.[2,22,27]

Proliferation Evaluation Tests: Proliferation evaluation tests are one of the oldest and most widely used methods in which the effects of various components of the experimental material on cell proliferation are examined. By counting the cells in the cell culture after a few days, the effect of the components of the material on cell proliferation is determined. Cell counting at a specific time during the test period does not give a clear result. Therefore, it is necessary to obtain a growth curve in the early stages of testing.[2,17,22] A moment in the growth curve should be chosen when the control cells are in the log phase (reproductive phase) or mid-log phase. When a significant effect is detected, the obtained improvement curve should be supported by a second improvement curve or other evaluation methods should be applied.[2]

Metabolism Evaluation Tests: Metabolism evaluation tests have been developed as alternative test methods because the number of samples is large, the preparation phase of life evaluation tests takes a long time, and test analyzes are time-consuming and laborious. It is not possible to directly evaluate the life of cells with metabolism evaluation tests, but thanks to these tests, ongoing metabolic activity can be detected by determining the increase in the number of cells, DNA or protein synthesis. Through metabolism tests and protein content tests, the metabolic capacities of cells are measured to understand the damage that will occur in the long term.[2]

In metabolism evaluation tests, which are cheap and quick methods, the viability of cells is determined with the help of a spectrophotometer with a microplate reader. Lactate dehydrogenase (LDH) test, alamar blue test and colorimetric MTT test are included in this group.[2,22]

xCELLigence® System

Real-time cell analysis system (RTCA, xCELLigence®) is a system that provides information about cell characterization. Cell proliferation and cytotoxicity can be determined through this system. The xCELLigence® system consists of a cell-based microelectronic cell sensor array that measures the connection or non-connection of cells to electrodes using electrical impedance technology. Electronic impedance is measured with sensors and changes in the electrodes are detected. Cell index is used to measure changes in electrical impedance. Electrode impedance is affected by cell viability, number and morphology. The data determined based on the increase or decrease in the cell index is evaluated and finalized by the software.[28,29]

The xCELLigence® system consists of four main components: RTCA analyzer, RTCA single-plate station, RTCA computer with integrated software and disposable E-plate 16. This system is used to measure cell viability according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany and ACEA Biosciences, Inc., San Diego, CA, USA). The RTCA single-plate station fits into a standard tissue culture incubator and measurements are transferred to the computer with a software analyzer. E-plate 16 is a disposable plate used to perform cell-based analyzes on the RTCA single plate station device. There are gold cell sensor arrays at the bottom of these plates. With E-plate 16, cells in each well can be monitored, experiments can be performed separately in each well, and their results can be evaluated separately. Each well on E-plate 16 has a bottom diameter of 5.0 mm ± 0.05 mm and a total volume of 243 ± 5 µL. The plate has a low evaporation lid design. Approximately 80% of the ground area of the wells in the plate is covered with circular electrodes designed to be used in ambient conditions between +15 and +40°C, at a maximum relative humidity of 98% without condensation. Physiological changes of the cells are detected by the electronic impedances detected by the sensor electrodes. The voltage applied to the electrodes during measurement is approximately 20 mV. The impedance value measured between the electrodes in each well varies depending on the ion concentration in the well, electron geometry and whether the cells are connected to the electrodes or not. The electrode impedance value increases proportionally with the cell density. In addition, data such as cell index (CI), graph, average value, maximum and minimum values, standard deviation, concentration that produces half the maximum effect (EC50) and half concentration of maximum inhibition (IC50) can be obtained through RTCA software.[30]

With the xCELLigence® system, proliferation and death in cell culture are demonstrated by simultaneously and continuously detecting impedance (resistance shown by cells to electric current). As the amount of cells adhering to the gold electrodes on the bottom surface of the E-plates increases, the resistance to the current increases, and as it decreases, the resistance decreases. Thanks to this system used in cell culture laboratories, studies such as cell characterization, proliferation and cytotoxicity determination, adhesion and receptor-mediated signal transmission can be carried out. Additionally, cell proliferation and death can be recorded continuously and in real time. The recorded data is transferred to the computer screen in graphic form.[31]

The advantages of the xCELLigence® system are that it is less invasive than traditional testing methods, can make easy, simultaneous measurements in a shorter time, and provides more reliable results.[31] With these new systems that offer real-time analysis, cellular data can be received at minimum 15-second intervals. Data obtained from the cells in the wells can be displayed simultaneously on the computer screen, and thus instant changes can be made to the experimental protocol, such as stopping the experiment and adding a new substance. Since data acquisition in the xCELLigence® system is based solely on impedance measurement, the same cells can be reused in another experiment. For example, while a material is being examined in real time for cytotoxicity, when necessary, the experiment can be stopped, cells can be collected from the wells, and its genotoxicity can be evaluated by isolating nucleic acid. In this way, both time and cost can be saved.[30]

CONCLUSION

With the development of the product range in dental materials, the number and diversity of tests evaluating the biocompatibility of materials have also increased. The xCELLigence® system as a cell culture method for evaluating the biocompatibility of dental

materials has emerged as a successful alternative to traditional methods. With the xCELLigence® system, cell proliferation and viability levels can be evaluated and comments can be made on the biocompatibility of dental materials.

Real-time cell analysis systems (xCELLigence® system) enable researchers to perform biocompatibility evaluations of dental materials in a shorter time, at a lower cost, and more comprehensively and accurately. Considering these advantages of the system, the xCELLigence® system can be preferred as a cell culture method.

REFERENCES

1. Hanks C, Wataha J, Sun Z. *In vitro* models of biocompatibility: A review. Dent Mater 1996;12:186–93.

2. Tuncer S, Demirci M. Dental materyallerde biyouyumluluk değerlendirmeleri. Atatürk Üniv Diş Hek Fak Derg 2011;21:141–9.

3. Adabi M, Naghibzadeh M, Zarrinfard MA, Esnaashari SS, Seifalian AM, Faridi-Majidi R, *et al*. Biocompatibility and nanostructured materials: Applications in nanomedicine. Artif Cells, Nanomedicine Biotechnol 2017;45:833–42.

4. Schmalz G, Arenholt-Bindslev D. Biocompatibility of dental materials. Berlin: Springer; 2009.

5. Koçak S, Erten H. Mineral trioksit agregat'ın biyouyumluluğunun değerlendirilmesi. Acta Odontol Turc 2012;29:63–71.

6. Murray PE, Garcia Godoy C, Garcia Godoy F. How is the biocompatibility of dental materials evaluated? Med Oral Patol Cir Bucal 2007;12:E258-66.

7. International Organization for Standardization. Dentistry - Evaluation of biocompatibility of medical devices used in dentistry. Test methods for dental materials. ISO 7405; 2008.

8. International Organization for Standardization. Dentistry-Biological evaluation of medical devices. Tests for *in vitro* cytotoxicity. ISO 10993-5; 2009.

9. Uzun İ, Bayındır F. Dental materyallerin biyouyumluluk test yöntemleri. Gazi Üniversitesi Diş Hekim Fakültesi Derg 2011;28:115–22.

10. Wataha JC. Principles of biocompability for dental practioners. J Prosthet Dent 2001;86:203–9.

11. Pizzoferrato A, Ciapetti G, Stea S, Cenni E, Arciola C GD. Cell culture methods for testing biocompability. Clin Mater 1994;15:173–90.

12. Schmalz G. Use of cell cultures for toxicity testing of dental materials-advantages and limitations. J Dent 1994;22:6–11.

13. Schmalz G. Concepts in biocompatibility testing of dental restorative materials. Clin Oral Investig 1997;1:154–62.

14. Helgason C, Miller C. Methods in moleculer biology. Third Edit. Tatowa: Humana Press; 2005. p.1–12.

15. Tokur O, Aksoy A. *In Vitro* Sitotoksisite Testleri. Harran Üniv Vet Fak Derg 2017;6:112–8.

16. Powers J, Sakaguchi R. Craig's restorative dental materials. 12th ed. St. Louis: Mosby Elsevier; 2006. p.97–125.

17. Yıldırım ZS, Bakır EP, Bakır Ş, Aydın MS. Diş hekimliğinde biyouyumluluk ve değerlendirme yöntemleri. Selcuk Dent J 2017;4:162–9.

18. Saw TY, Cao T, Yap AUJ, Ng MML. Tooth slice organ culture and established cell line culture models for cytotoxicity assesment of dental materials. Toxicol Vitr 2005;19:145–54.

19. Mallineni SK, Nuvvula S, Matinlinna JP, Yiu CK, King NM. Biocompatibility of various dental materials in comtemporary dentistry: a narrative insight. J Investig Clin Dent 2013;4:9–19.

20. Galluzzi L ve ark. Guidelines for the use and interpretation of assays for monitoring cell death in higher eukaryotes. Cell Death Differ 2009;16:1093–107.

21. Polyzois GL. *In vitro* evaluation of dental materials. Clin Mater 1994;16:21–60.

22. Moharamzadeh K, Brook IM NR. Biocompatibility of Resin-based Dental Materials. Materials 2009;2:514–48.

23. Wennberg A, Hasselgren G TL. A method for toxicity screening of biomaterials using cells cultured on Millipore filters. J Biomed Mater Res 1979;13:109–20.

24. Outhwaite WC, McKenzie DM PD. A versatile split-chamber device for studying dentin permeability. J Dent Res 1974;53:1503.

25. Schmalz G, Hiller K, Nunez L, Stoll J, Weis K. Permeability characteristics of bovine and human dentin under different pretreatment conditions. J Endod 2001;27:23–30.

26. Zorba Y, Yıldız M. Adeziv Restoratif Materyallerde Biyouyumluluk Testleri ve Kriterleri. Atatürk Üniv Diş Hek Fak Derg 2007;2:15–21.

27. Adan A, Kiraz Y, Baran Y. Cell Proliferation and Cytotoxicity Assays. Curr Pharm Biotechnol 2016;17:1213–21.

28. Ke N, Wang X, Xu X AY. The xCELLigence system for real-time and label-free monitoring of cell viability. Methods Mol Biol 2011;740:33–43.

29. Erol S, Öztürk Kurt B, Dinç B, Özdemir S. HEK 293 hücrelerine farklı doz ve sürelerde uygulanan klorpirifos ve tannik asitin hücre canlılığı üzerine etkilerinin araştırılması. Sağlık Bilim İleri Araştırmalar Derg 2023;6:56–63.

30. Atilan Yavuz S, Sürmelioğlu D. Evaluation of Cytotoxicity of Different Universal Bonds Using the Xcelligence System. Cumhur Dent J 2020;23:371–81.

31. xCELLigence RTCA DP Real Time Cell Analyzer – Dual Purpose. https://www.agilent.com/en/product/cell-analysis/real-time-cell-analysis/rtca-analyzers