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Research Article / Araştırma Makalesi

Evaluation of the biocompatibility of various self-adhesive resin cements on subcutaneous connective tissue: An *in vivo* study

Subkutanöz konnektif dokuda çeşitli self adeziv rezin simanların biyouyumluluklarının değerlendirilmesi: *In vivo* çalışma

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ABSTRACT

Background: The purpose of the study was to examine the subcutaneous connective tissue reaction to three self-adhesive resin cements.

Methods: Sixty-three polyethylene tubes were filled with three different self-adhesive resin cements (Group Z [Zirconite], Group P [Panavia SA], and Group H [High Q Bond]). Twenty-one polyethylene tubes remained empty and were used as controls (Group C). All tubes were implanted into the subcutaneous tissue of the 21 rats. Specimens from each cement type were obtained at 7, 30, and 90 days. Quantitative assessments of inflammatory cells were performed in 5 different areas of each specimen.

Results: All animals survive during the follow-up periods except two rats. The mean values of inflammatory cells at 90 days were 14 (±1.8), 15.1 (±3.5), 96 (±12.1), 16 (±2.1) in Groups C, H, P, and Z, respectively. The thicknesses of the fibrous capsule decreased significantly with time in all groups except Group P.

Conclusion: Biocompatibility of self-adhesive resin cements was material-dependent. High Q Bond and Zirconite exhibited satisfactory biocompatibility; however, Panavia demonstrated a moderate inflammatory response at 90 days.

Keywords: biocompatible materials; inflammation; resin cements.

ÖZ

Amaç: Bu calışmanın amacı üç farklı self adeziv rezin simana karşı gelişen subkutanöz doku reaksiyonunu incelemektir.

Gereç ve Yöntemler: 63 adet polietilen tüp içine üç farklı self adeziv rezin siman yerleştirildi. ((Grup Z [Zirconite], Grup P [Panavia SA], and Grup H [High Q Bond]). 21 polietilen tüp boş bırakıldı ve kontrol grubu olarak kullanıldı (Grup C). Bütün tüpler 21 adet farenin subkutanöz dokusuna yerleştirildi. Her siman tipinden subkutan olarak yerleştirilmiş siman örnekleri, 7, 30, 90. günlerde çıkartıldı. İnflamatuar hücrelerinin sayısal değerlendirmesi her örneğin 5 farklı alanından yapıldı.

Bulgular: 2 fare dışında tüm hayvanlar takip periyodları boyunca canlı kaldı. 90. günde Grup C, H, P ve Z de enflamatuar hücrelerin ortalama değerleri sırasıyla 14 (±1.8), 15.1 (±3.5), 96 (±12.1), 16 (±2.1) şeklindedir. Panavya dışında tüm gruplarda fibröz kapsül kalınlığı zaman içinde anlamlı derecede azalmıştır.

Sonuç: Self adesiv rezin simanların biyouyumluluğu materyale bağlı olarak farklılık göstermektedir. 90. Günde High Q Bond ve Zirconite yeterli biyouyumluluk değeri gösterirken Panavya siman da orta derecede inflamatuar cevap gözlemlenmiştir.

Anahtar Kelimeler: Biyouyumlu materyaller; enflamasyon; rezin simanlar

Introduction

Recently, self-adhesive resin cements have been used for several types of indirect restorations making them popular among clinicians. They were developed to combine the advantages and obviate the limitations of traditional and resin-based cements. Furthermore, these cements can bond to an untreated tooth surface, thus cementation can be achieved in a single step.[1] They provide higher strength, lower solubility, and ease of use compared to the other cement types.[2]

Biocompatibility is the efficiency of the materials to perform with a good host response when a specific implantation was achieved.[3] Thus, in the development of any dental biomaterial, biocompatibility must be required in addition to strength, esthetics, and clinical manipulation. The substances release from the material before and/or after setting can cause adverse reactions on a clinical or subclinical level that may be toxic or allergenic. In the development of any dental biomaterial, biocompatibility must be considered in addition to strength, esthetics, and clinical manipulation.[4,5] Some of the *in vitro* studies reported that self-adhesive resin cements presented satisfactory outcomes when compared to the multi-step resin cements. However, only a limited number of studies researching the biological effects of these cements are available in the literature.[2,6] Zirconite cement was developed for the cementation of zirconia restoration as we know that the cementation of zirconite restoration are still a problem because of their crystalline structure and there was no study about the biological property of this cement. Self adhesive cements are composed of monomer, filler, and initiator. Most of them are dual cure which overcomes the difficulty of light facilitated polymerization of the material in most clinical situations.[7] When the polymerization completed sufficiently, the clinical performance and physical properties of the composite materials were maximizing so adequate polymerization is needed.[8,9] Insufficient polymerization may lead to a low degree of conversion and unreacted monomers being released from dental resin. This can cause adverse biological reactions with surrounding tissues.[7] Those cements contain acidic monomers thus when compared to resin cements, they have a more complex polymerization process. In previous studies, it was reported that the released monomers such as triethylene glycol dimethacrylate (TEGDMA) and urethane dimethacrylate (UDMA), induced cytotoxicity.[6,10] Resin-based dental cements are commonly used in implant-related prosthetic restorations but residual cement has been found to play a vital role in the development of peri-implantitis.[11] It was reported that one of the unfailing methods of evaluating biocompatibility is the subcutaneous connective tissue implantation test in animals [12-15] as inflammatory reactions are characteristic features of all connective tissues.[16]

Although there are a few studies that evaluated the cytotoxicity of self-adhesive cements in the literature [6,17], there is no study providing

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any information about the biological reactions of these cements when implanted in subcutaneous rat tissues. Therefore, the purpose of this study was to examine and compare the histopathological reactions of three different self-adhesive resin cements. The null hypothesis was that self-adhesive dental cements could not present good biocompatibility.

Materials and Methods

This study was approved by the Research Ethics Committee of Gazi University (Protocol number: G.U. ET-14.082). Three different self-adhesive dual-cure resin cements were tested in this study (**Table 1**). Twenty-one sterilized clear polyethylene tubes (2mm in diameter and 10 mm in length) were used for each cement group. Polyethylene tubes were filled with three different self-adhesive resin cements (Group Z [Zirconite], Group P [Panavia SA], and Group H [High Q Bond]) with automix syringes and mixing tips and polymerized (light cure device, Osaka, China) by strictly adhering to the manufacturers' instructions. Twenty-one polyethylene tubes remained empty and were used as controls (Group C).

Table 1. Cements used in the study.

Materials	Cement type	Monomer	Radio-opacity	Manufacturer
Group Z (Zirconite)	Self-adhesive (dual cure)	TEGDMA, 4-META, UDMA	250 % Al	BJM LAB, Yehuda, Israel
Group P (Panavia SA)	Self-adhesive (dual cure)	BISGMA, MDP, TEGDMA, HEMA	150 % Al	Kuraray, Osaka, Japan
Group H (High Q Bond SE)	Self-adhesive (dual cure)	UDMA, TEGDMA, 4-META	250 % Al	BJM LAB, Yehuda, Israel

Specimens from 7 animals for each cement type were obtained 7, 30, and 90 days after the surgical procedure. At the end of each experimental period the animals were euthanized under overdose anesthetic medication, shaved, and the tissues containing the tubes were retrieved and fixed in a 10% formalin solution (Merck, Darmstadt, Germany) for 24-72 hours. After all specimens were placed in the paraffin blocks, they were sectioned in 5µm thicknesses. They were stained with hematoxylin and eosin then evaluated under a light microscope (Leica DM4000-B, Leica microsystems, Wetzlar, Germany). Quantitative data of inflammatory cells (lymphocytes, plasma cells, macrophages, neutrophil leukocytes and giant cells) was performed by Leica DC-500 camera (Leica microsystems, Wetzlar, Germany) in 5 different areas of each specimen at x400 magnification and image analyzer software system (Leica QWin Plus, Leica Corp, Wetzlar, Germany) was used. The mean values of the inflammatory cells were determined and the inflammatory reactions were classified by using the following criteria: [12,18]

0 (no reaction) = None or presence of fewer than 5 cells,

1 (mild reaction) = < 25 cells,

2 (moderate reaction) = 25-125 cells,

3 (severe reaction) = > 125 cells.

Fibrous capsule thicknesses from 10 different areas of each specimen at x200 magnification was also measured with a camera (Leica DC-500, Wetzlar, Germany). For statistical analysis, Kruskal-Wallis and Wilcoxon signed rank tests were used. Statistical significance was set at $P < 0.05$.

Results

All animals survived during the follow-up periods of 7 and 30 days but 2 rats died before the specimen collection on day 90. No post-operative complications were observed and no infection was seen at the surgical sites. **Table 2** shows the mean and the standard deviation values regarding inflammatory cells. The statistical comparisons of the inflammation scores among the experimental groups are given in **Table 3**. The fibrous capsule thickness values of the materials in each time period are shown in **Table 4**. A clear fibrous capsule was seen in all groups after day 7. At 90 day period, the thicknesses of the fibrous capsule significantly decreased in all groups except Group P.

Table 2. The mean values (±SD) of inflammatory cells on days 7, 30, and 90.

Materials	Day 7	Day 30	Day 90
	Mean (±SD)	Mean (±SD)	Mean (±SD)
Group C	164.4 (±5.2) A a	118 (±11.9) A b	14 (±1.8) A c
Group H	170.4 (±6.7) B a	75.8 (±4.5) B b	15.1 (±3.5) A c
Group P	172.7 (±4.3) B a	124.3 (±10.7) A b	96 (±12.1) B c
Group Z	165.3 (±5.6) A a	116.8 (±6.3) A b	16 (±2.1) A c

SD: Standard deviation
Same uppercase letters vertically indicate that mean values were not significantly different among the resin cement groups in the same time period (P >0.05).
Same lowercase letters horizontally indicate that mean values were not significantly different among the time period days in the same resin cement group (P >0.05).

Table 3. Statistical comparisons of tissue reaction among the materials in each time period.

Materials		n	Median	Min	Max	Mean rank
Day 7	Group C	7	3.00 A	3.00	3.00	16.50
	Group H	7	3.00 A	3.00	3.00	16.50
	Group P	7	3.00 A	3.00	3.00	16.50
	Group Z	7	3.00 A	3.00	3.00	16.50
Day 30	Group C	7	2.00 B	2.00	2.00	15.00
	Group H	7	2.00 B	2.00	2.00	15.00
	Group P	7	2.00 C	2.00	3.00	21.00
	Group Z	7	2.00 B	2.00	2.00	15.00
Day 90	Group C	5	1.00 D	1.00	1.00	9.50
	Group H	5	1.00 D	1.00	1.00	9.50
	Group P	5	2.00 E	2.00	2.00	21.50
	Group Z	5	1.00 D	1.00	1.00	9.50

Same uppercase letters vertically indicate that median values were not significantly different among the resin cement groups in the same time period (P >0.05).

Table 4. Statistical comparisons of fibrous capsule thickness values (µm).

Groups	Day 7	Day 30	Day 90
	Mean (±SD)	Mean (±SD)	Mean (±SD)
Group C	141.42 (±29.9) A a	109.69 (±7.9) A a	79.66 (±3.4) A b
Group H	184.99 (±26.8) BD a	102.81 (±18.5) AD b	50.29 (±8.5) B c
Group P	171.79 (±41.6) BC a	231.57 (±27.7) B a	145.91(±92.5) C a
Group Z	212.82 (±26.3) D a	217.71 (±41.7) C a	72.66 (±13.9) AD b

SD: Standard deviation
Same uppercase letters vertically indicate that mean values were not significantly different among the resin cement groups in the same time period (P >0.05).
Same lowercase letters horizontally indicate that mean values were not significantly different among the time period days in the same resin cement group (P >0.05).

Day 7

On day 7, all test and control groups showed severe inflammatory reactions. Lymphocyte-rich inflammatory cell infiltration was seen around the tubes and at their open-ends for all test and control groups (**Figure1**). Plasma cells and macrophages were mixed. Necrosis and foreign-body giant cells were not observed. The number of inflammatory cells was significantly higher in the Group P than the other groups. Fibrous capsule thicknesses were significantly thicker in all test groups than the control groups.

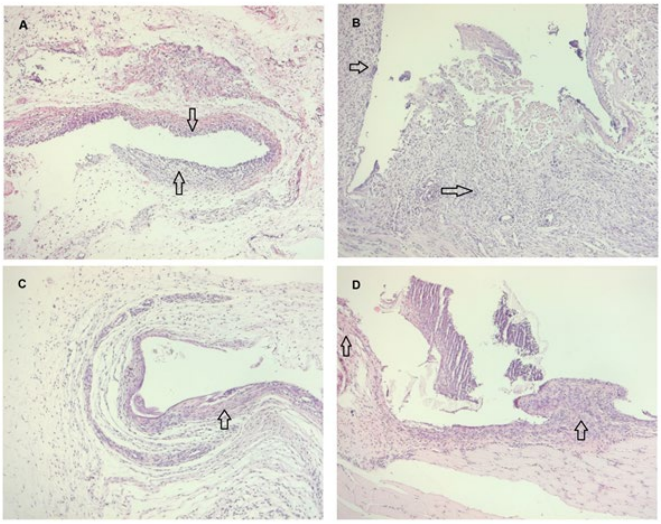

Figure 1. Inflammatory cell infiltration at the tube circumference and the end of tube on day 7 A) Control B) High Q Bond C) Panavia SA D) Zirconite

Day 30

According to the histolojic evaluation, the amount of inflammation decreased in number from day 7 to day 30, and a moderate inflammatory reaction was seen in all test and control groups (**Figure 2**). Necrosis and foreign-body giant cells were not observed. It was seen that the decrease was more evident in Group H. The thickness of the fibrous capsule reduced on day 30 in all groups. The mean thickness values of fibrous capsule were 109.69 µm, 102.81 µm, 231.57 µm, and 217.71 µm in Groups C, H, P and Z, respectively.

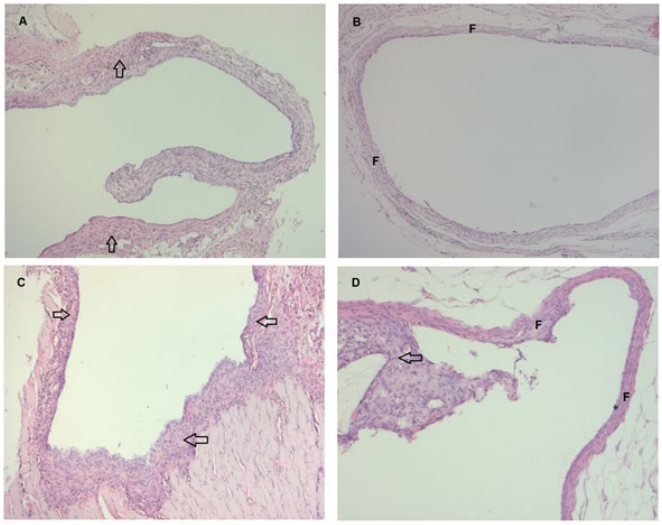

Figure 2. A) Inflammatory cells around the tube and connective tissue proliferation into the tube on day 30 in Control group.

B) Fibrous capsule-like connective tissue formation around the tube and at the end of tube on day 30 in High Q Bond group.

C) Inflammation of the end of tube and around the tube on day 30 in Panavia SA group.

D) Fibrous capsule-like connective tissue formation around the tube and at the end of tube on the day 30 in Zirconite group.

Day 90

On day 90, it was seen that in all groups the number of inflammatory cells were significantly decreased. Mild inflammatory reactions were noted in Groups Z and H. Although inflammatory cells decreased from day 30 to day 90, a moderate reaction was recorded in Group P. Necrosis and foreign-body giant cells were not observed. Cell-poor fibrous band formation was seen around the tubes and their open-ends in Groups Z and H. Group P showed fibrous band formation and scant inflamation around the tubes' open ends where the particles embed the capsule into the surrounding connective tissue (**Figure 3**). The fibrous capsule thicknesses significantly decreased in all groups except Group P.

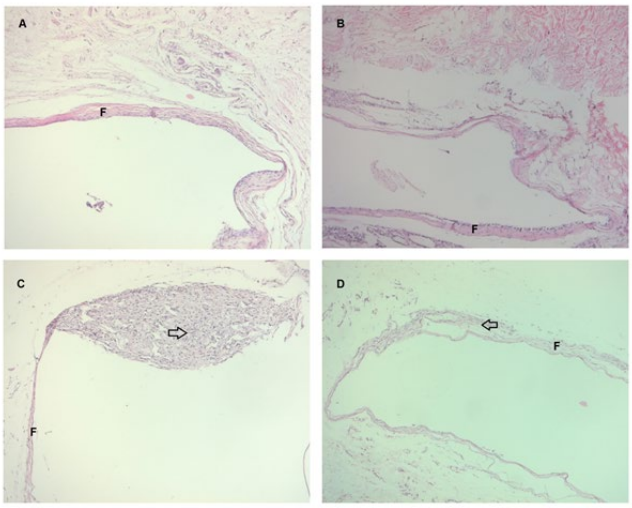

Figure 3. A) Cell-poor fibrous band formation in and around the end of tube on day 90 in Control group.

b) Fibrous band formation around the tube on day 90 in High Q Bond group.

c) Formation of a fibrous band around the tube at the end of tube on day 90 in Panavia SA group and scant inflammation around the particles embedded in the connective tissue at the end of tube.

d) Mild inflammation and fibrous band formation at the end of tube and around the tube on day 90 in Zirconite group.

(Arrows are showing inflammatory cell infiltration areas and F: fibrous capsule-like tissue formation, h.e. x100).

Discussion

Self-adhesive cements offer a promising new approach to indirect restorative procedures and implant-related prosthetic restorations. Self-adhesive cements tested in the present study are commonly used cements in prosthetic restorations. Zirconite cement was manufactured for the cementation of zirconia restorations and Paniavia cement was also recommended for the zirconia restorations. Because of the glass free structure zirconia is an un-etchable material thus it has limited adhesive luting potential. It has been reported that the residual cement has a significant effect in the development of peri-implantitis but very little information regarding the biological response of these cements exists in the literature.[6,7]

Cell culture and subcutaneous implantation tests were the recommended tests to evaluate the biocompatibility of dental materials.[13-15] Cell culture and genotoxicity tests provide information about the *in vitro* cytotoxicity of the materials.[6,19] Subcutaneous surgical placement of a testing material with polyethylene tube has been recommended for testing the biocompatibility and tissue reaction *in vivo*. Not only the ideal properties (chemical, physical, or mechanical) but also the biological response is important so the material should not start an inflammatory or foreign body reaction, and it should be non-carcinogenic.[12,13] In the present study, the null hypothesis that self-adhesive resin cements could not present good biocompatibility was partially accepted Panavia SA showed a moderate tissue reaction at the 90-day time period. However High Q Bond and Zirconite cements exhibited good biocompatibility.

Correlative studies between cell culture and in vivo testing are

available in the literature, and generally they reported a poor correlation between the two methods.[20,21] Self-adhesive resin cements are dual-cured materials and several factors can affect their biocompatibility. Some in vitro studies indicated that these cements had cytotoxic effects in cell culture. This is mainly caused by insufficiently polymerized cement that releases unconverted monomers.[22] Dalpino et al., [23] reported that the exposure of odontoblastic cells to different self-adhesive cements induced injury to odontoblastic cells. A significant reduction in the percentage of viable cells was also reported. Moreover, they mentioned that the results of their study might be due to the composition of materials used and the release of cytotoxic substances from the materials. They noted that the choice of polymerization protocols in most of the cements they tested affected the cytotoxicity. A study by De Souza *et al.*,[24] reported that ensuring a high degree of conversion was crucial to obtain the best chemical, physical, and biocompatibility properties of resin cements. Sun *et al.*, [25] evaluated the cytotoxicity of self-adhesive cements with or without light irradiation on human periodontal ligament fibroblasts. They found that the extract solution of these cements used in their study was cytotoxic to the human periodontal ligament fibroblasts, which could inhibit cell growth and induce cell apoptosis/necrosis. It was reported in some previous studies that resin cements significantly reduced the cell viability and the composition of the material might have caused different cytotoxic effects.[23,25]

Guttuso [26] and Olsson *et al.*[16] suggested small experimental animals for subcutaneous connective tissue tests to examine the local effects of materials. To our knowledge, there is no information in the literature regarding the *in vivo* biocompatibility of the self-adhesive resin cements especially the cements tested in the current study and there are a few studies about the biocompatibility of conventional resin cements, *in vivo*.[27,28] A study by Shimada et al.[27] evaluated the pulpal responses of the light and self cured resin cements on monkeys' teeth. Authors reported that all self and light cured resin cements had an acceptable biological response. Another study by Bezzon *et al.*[28] evaluated the tissue response of resin luting materials (Rely X Unicem and Multilink) on dogs' teeth and the authors noted that these materials caused no adverse tissue reactions. The materials and test methods used in these studies are different from the present study, so no direct comparison to the present study can be made. In our study, severe inflammatory reactions were seen in all test and control groups at 7 days. Trauma produced during the implantation of the material could be the reason of this.[29] Three different self-adhesive dual-cure cements (Zirconite, Panavia SA and High Q Bond SE) were tested. Although a severe inflammatory reaction was seen in all materials at 7 days, the lowest number of inflammatory cells was found at the Zirconite cement. The severity of inflammatory responses and fibrous capsule thickness both decreased in all groups over time. The number of inflammatory cells decreased at the 30-day time period and a moderate reaction was experienced. When compared to the Zirconite (116.8) and High Q Bond cements (75.8), Panavia SA cement (124.3) exhibited a statistically higher number of inflammatory cells at day 30. The Zirconite and High Q Bond cement showed better tissue response than the Panavia SA cement on day 90. This was indicated by a moderate reaction while the Zirconite and High Q Bond showed a mild reaction. The difference in inflammatory response among these cements could be explained as the chemical composition and quantity of chemotoxic leachables were different in these cements. Migration of high levels of leachables, specifically uncured monomers and additives from the Panavia cement could have resulted in its high inflammation score. In the current study, the amount of the monomer in self adhesive cements was unknown. Kong *et al.*[22] examined the cytotoxicity of three resin-based cements and found that all cements induced slight cytotoxicity. In addition, cytotoxic effects of TEGDMA, UDMA and HEMA were also reported in the other studies.[22,30,31,32] Although these studies are not similar to the present study, some of these monomers were found in the self-adhesive cements tested in our study. Our study has a limitation that 2 of the animals died on day 90 thus the evaluated specimens on 90 days were fewer than the 7 and 30 day periods. Further in vivo and *in vitro* studies are needed to evaluate the leachable components and determine the responsible component for the adverse tissue reactions.

Conclusions

Within the limitations of this study, the following conclusions were drawn;

a) Zirconite and High Q Bond self-adhesive cements showed mild inflammatory reaction and good biocompatibility,
b) The fibrous capsule thicknesses became thinner over time in all tested groups,
c) Although the number of inflammatory cells decreased over time, moderate inflammatory reaction was seen with the Panavia SA cement on day 90.

Değerlendirme / Peer-Review

İki Dış Hakem / Çift Taraflı Körleme

Etik Beyan / Ethical statement

Bu çalışmanın hazırlanma sürecinde bilimsel ve etik ilkelere uyulduğu ve yararlanılan tüm çalışmaların kaynakçada belirtildiği beyan olunur.

It is declared that during the preparation process of this study, scientific and ethical principles were followed and all the studies benefited are stated in the bibliography.

Benzerlik Taraması / Similarity scan

Yapıldı – ithenticate

Etik Bildirim / Ethical statement

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Çıkar Çatışması / Conflict of Interest

Yazarlar çıkar çatışması bildirmemiştir. | The authors have no conflict of interest to declare.

Yazar Katkıları / Author Contributions

Çalışmanın Tasarlanması | Design of Study: BTB(%65),AG(%35)
Veri Toplanması | Data Acquisition: AG(%75), BTB (%25)
Veri Analizi | Data Analysis: EB (75), AG (%25)
Makalenin Yazımı | Writing up: BT(60),AG (%40)
Makale Gönderimi ve Revizyonu | Submission and Revision: BTB(%75), EB(25)

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