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Comparison of the Bond Strengths of Glass Ionomer Bone Cement Combined with Various Pretreatments

Çeşitli Ön İşlemlerle Birleştirilmiş Cam İyonomer Kemik Çimentosunun Bağ Kuvvetlerinin Karşılaştırılması

Onur ERGÜN[a], Bilge HAKAN YÜCE[a], Eda ÇAKMAK[b], Sinem PEHLİVAN[c], Gülçin Şefiye AŞKIN[d]

[a]Clinic of Otorhinolaryngology, Head and Neck Surgery, Başkent University Hospital, Ankara, Türkiye
[b]Department of Audiology, Başkent University Faculty of Health Sciences, Ankara, Türkiye
[c]Başkent University, Experimental Animal Breeding and Research Center, Ankara, Türkiye
[d]Department of Mechanical Engineering, Hacettepe University Institute of Science, Ankara, Türkiye

ABSTRACT Objective: Our aim in this study is to determine if a pretreatment could make bone cement connection stronger and reduce the failure rate. **Material and Methods:** Twenty-four 6-9 months old female rats were divided into 2 groups as "day 0" and "day 90". Four different applications were made to the 2x2 cm^2 surgical area. Freshly prepared glass ionomer bone cement (GIBC) was applied by removing the periosteum with bipolar in the first application, by keeping the perichondrium intact in the second application, by removing the periosteum with trichloroacetic acid (TCA) solution in the third application, and by removing the periosteum mechanically in the last application. After these rats were sacrificed on day 0 and day 90, mechanical tests were made to the bone cements and shear strength values were calculated by dividing the maximum measured forces by the apparent contact areas. **Results:** Although shear forces were higher on day 90 than on day 0 ($p=0.007$), subgroup analyses revealed that the differences were significant in only the subperiosteum group ($p=0.003$). According to the pairwise comparisons, subperiosteal bone cement application yielded significantly higher shear forces on day 90 compared to the others ($p<0.003$) while the supraperiosteum, TCA, and electrocoagulation groups were similar to each other. **Conclusion:** GIBC reached its highest shear strength with time after a subperiosteal application. We do not recommend any of the pre-treatments tried in this study over a supra periosteal application before a bone cement ossiculoplasty since no increase in shear strength could be obtained.

Keywords: Bone cements; stapes surgery; trichloroacetic acid; periosteum

ÖZET Amaç: Bu çalışmadaki amacımız, hangi kemik çimentosu uygulamasının bağlantıyı daha güçlü hâle getirebileceği ve dislokasyon oranının daha düşük olacağıdır. **Gereç ve Yöntemler:** Yirmi dört adet 6-9 aylık dişi rat "0. gün" ve "90. gün" olmak üzere 2 gruba ayrıldı. 2x2 cm^2'lik cerrahi alana 4 farklı uygulama yapıldı. Cam iyonomer kemik çimentosu 1. uygulamada bipolar ile periost kaldırılarak, 2. uygulamada periost kaldırılmayarak, 3. uygulamada ise trikloroasetik asit [trichloroacetic acid (TCA)] solüsyonu ile periost kaldırılarak ve son uygulamada periost mekanik olarak kaldırılarak uygulandı. Bu dişi ratlara sakrifiye edildikten sonra mekanik testler uygulandı. Dayanıklık güçleri ölçülen maksimum kuvvetin temas alanına bölünmesiyle ölçüldü. **Bulgular:** Kesme kuvvetleri 90. günde 0. güne göre daha yüksek olmasına rağmen ($p=0,007$), alt grup analizlerinde farklılıkların sadece subperiosteum grubunda anlamlı olduğu görüldü ($p=0,003$). İkili karşılaştırmalara bakıldığında, subperiosteal kemik çimentosu uygulaması 90. günde diğerlerine göre anlamlı olarak daha yüksek kesme kuvvetleri verirken ($p<0,003$), supraperiosteum, TCA ve elektrokoagülasyon grupları birbirine benzerdi. **Sonuç:** Cam iyonomerin en yüksek kesme kuvvetine subperiosteum grubunda ulaşıldı. Bu çalışmada, denenen diğer yöntemler anlamlı bulunmadığı için bu diğer yöntemlerin uygulanmasını önermiyoruz.

Anahtar Kelimeler: Kemik çimentosu; stapes cerrahisi; trikoloroasetik asit; periosteum

The term "bone cement" may confuse since it is used for many different chemical formulations for bonding or space-filling. Polymethylmethacrylate (PMMA) is a polymer commonly used for implant fixation in orthopedy.[1] It acts as a filler and a bonding material. The addition of inorganic glass particles to a polymer matrix makes glass ionomer bone cement (GIBC). Both PMMA and GIBC have exothermic polymerization phases that covalent bonds form once 2 components of the formulation are mixed. GIBC has higher biocompatibility, lower exothermic potential, and therefore heat-induced tissue necrosis risk. Hydroxyapatite (HA) is also sometimes referred to as bone cement. HA solidifies with precipitation instead of a chemical reaction, making a mechanical-only adhesion.[1] Although HA has excellent biocompatibility, its lack of polymerization means it is a pure filler. In the otology contest, GIBC is the preferred

Correspondence: Bilge HAKAN YÜCE
Clinic of Otorhinolaryngology, Head and Neck Surgery, Başkent University Hospital, Ankara, Türkiye
E-mail: bhakanyuce@gmail.com

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bone cement type and will be referred to as “bone cement” for the rest of this paper.

GIBC, which can be prepared, shaped, and used according to the case’s specific needs, has widespread use in otological surgeries. It is a cost-effective alternative with a similar success rate to partial ossicular replacement prostheses.[2] It can be used not only for bridging small gaps between the ossicles but also for increasingly complex ossiculoplasties.[3] Since the hearing outcome is dependent on an intact fixation, the bone cement must retain its bonding strength in the long term.

The long-term durability of bone cement ossiculoplasty, which is often applied on an intact periosteum, unlike dental or orthopedic surgeries, remains a question mark. Ráth et al. found that the long-term bone cement separation frequency in a rabbit model was significantly higher when it was applied over the intact periosteum than the application after denudation.[4] But mechanical stripping of the periosteum from mobile and fragile ossicles may not always be safe or possible.

Electrocoagulation was proposed to improve bone cement results as an alternative to mechanical denudation of the ossicles. But a controlled comparison was not included to support that argument.[5] Trichloroacetic acid (TCA) solution has been used for chemical coagulation and de-epithelization of perforation margins for more than a century.[6] The use of 50% and near-saturated TCA solutions was reported with successful results.[6,7]

This study was designed to determine whether the short and long-term mechanical strength of GIBC could be increased with a pre-treatment in a rat model. As far as we know, this is the first study in the otorhinolaryngology literature that incorporates mechanical tests to determine and compare the long-term durability of bone cement applications.

MATERIAL AND METHODS

This study was approved by Başkent University Animal Experiments Local Ethical Committee (date: May 16, 2022, no: 22/17). All animals have received humane care in compliance with the Guide for the Care and Use of Laboratory Animals and indicating approval by the institutional ethical review board. Twenty-four 6-9 months old female rats were divided into 2 groups as “day 0” and “day 90”. The operations were performed under general anesthesia by the same surgeon.

SURGICAL TECHNIQUE

The surgical area was cleaned with an iodine solution, then local anesthetic with 1% lidocaine, and 1:100000 adrenaline was injected. A 2 cm incision was made with a number 15 scalpel and subcutaneous dissection was performed down to the periosteum. The 2x2 cm^2 surgical area which corresponded to the frontal and parietal bones around the bregma was kept exposed with retention sutures. The periosteum was mechanically removed in the anterior left, bipolar electrocauterization (ITKA GSD Basic 250 electrosurgical unit, Finland) of the periosteum was done at 5% power (3.75watt) in the anterior right, chemically cauterization was performed with a drop of 70% trichloroacetic acid solution in the posterior right. Freshly prepared GIBC (3M ESPE Ketac Cem Easy Mix Glass Ionomer Cement, USA) was applied to 4 spots making sure that the surgical field is dry (Figure 1). Waited for 10 minutes for the bone cement samples to be fixed. Then the incision was sutured with 4.0 Vicryl (Ethicon, USA).

After the surgical procedure, the day 0 group was sacrificed. The upper caps of the scalps were carefully dissected and resected, taking care not to touch the bone cement samples and crack the bone. The “day 90” group recovered from anesthesia and followed up for 90 days before sacrification. The bone samples were placed in soaked surgical sponges, put in an ice box, and immediately transferred to the mechanical engineering laboratory for testing.

MECHANICAL TESTING

Mechanical tests were conducted with a 3-axis force sensor testing equipment (Kistler model 9317B, Kistler Corporation, USA) and a motorized vertical translation stage (MLJ150/M, Thorlabs, USA). Skull samples were firmly attached to 3D-printed single-use base plates with hot-melt adhesive. Plates were attached vertically to the testing equipment. A custom-made metal testing tip was designed and produced to have enough clearance and apply pressure to a single bone cement sample without touching the

FIGURE 1: An example of bone cement application surgery.

others or the bone surface. A camera system and a monitor were set up to display the real-time magnified view of the tip. 0.5 mm/min speed was chosen to apply the force in a controlled manner. The testing equipment was connected to a computer through a NI DAQ USB-6003, multifunction I/O device (National Instruments, USA) and a dynamic signal recorder (SIRIUSi 6xACC,2xACC+, DEWESoft) to display and record the values with the DEWESoft software (DEWESoft, USA). The maximum forces, measured just before detachments, were determined. The apparent contact area dimensions of the detached bone cement samples were measured in 2 perpendicular axes with a 150 mm digital caliper (TorQ, China) (Figure 2). Shear strength values were calculated by dividing the maximum measured forces by the apparent contact areas.

STATISTICAL ANALYSIS

Statistical analyses were performed with SPSS version 25.0 (IBM, USA). The Kruskal-Wallis test was used for the comparison of bone cement shear strength on day 0 and day 90, respectively. The Wilcoxon sign rank test was used to evaluate the difference in bone cement shear force between 0 and 90 days. The sphericity assumption was evaluated by Mauchly's W test. Then, the repeated measures two-way analysis of variance was performed to evaluate the bone cement shear force between groups depending on time. Least significant difference test was used to determine the difference between groups. The level of statistical significance was set off $p<0.05$ in all data.

RESULTS

One animal died on the 83rd day of an unknown cause. No infection, tissue reaction, or granulation was noted on day 90. One supraperiosteal GIBC specimen was too small to be tested accurately, therefore excluded. While no detachment was observed in the subperiosteal application, 2 detachments were observed in the supraperiosteum and electrocoagulation groups, and 3 in the TCA group. Detached GIBC droplets were occasionally displaced several millimeters over intact periost. Those were included in the calculation with a value of "0 N/mm^2". Regardless of the application method, all intact bone cement droplets were covered by a healthy periost on day 90. The results were listed in Table 1.

Although shear forces were higher on day 90 than on day 0 ($p=0.007$), sub-group analyses revealed that the differences were significant in only the subperiosteum group ($p=0.003$) (Table 2). Once the detached samples were removed from the statistical analysis then all groups had significantly higher 90th-day results ($p=0.017$-0.044) (Figure 3). There was no difference between the groups on day 0 ($p=0.172$). According to the pairwise comparisons, subperiosteal bone cement application yielded significantly higher shear forces on day 90 compared to the others

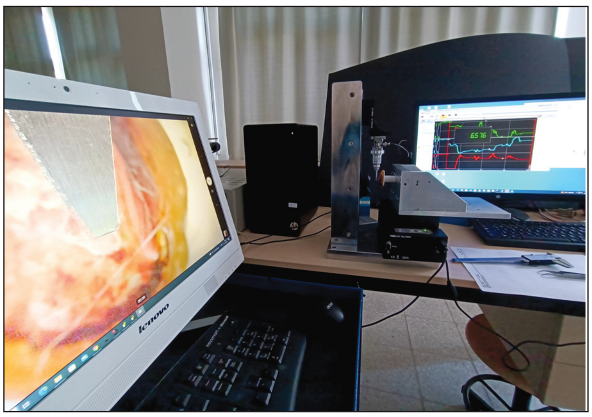

FIGURE 2: The setup of the mechanical test laboratory is seen. The real-time video feed of the metal tip with a large magnification is seen on the left screen. The mechanical test device, the computer that displays and records the data, and the digital caliper are also seen.

TABLE 1: Shear strength of each sample is given. Shear strength is calculated by dividing the measured shear force by the apparent contact area (N/mm^2). Please note that the detached bone cement samples are those with a value of "0".

	Subperiosteum		Supraperiosteum		TCA		Electrocoagulation	
	Day 0	Day 90	Day 0	Day 90	Day 0	Day 90	Day 0	Day 90
1	0.5	1.2	0.3	0.9	0.4	0.7	0.6	0.9
2	0.8	1	0.6	0.5	0.7	0.8	0.4	1.1
3	0.6	1.3	0.5	0.6	0.4	0	0.3	0.4
4	0.4	0.8	0.3	0	0.3	0.6	0.9	0.4
5	0.4	0.7	0.5	0	0.2	0.9	0.4	0.6
6	0.4	0.9	0.2	-	0.1	0	0.3	0.9
7	0.6	1.2	0.3	0.8	0.2	0.9	0.4	0.9
8	0.5	1.9	0.3	0.9	0.6	0.6	0.8	1
9	0.4	1.2	0.4	1.3	0.4	1	0.3	1.2
10	0.7	1.2	0.4	0.5	0.6	0	0.5	0
11	0.5	2.5	0.6	1.2	0.2	0.8	0.2	0
12	0.9		0.4		0.6		0.2	

TCA: Trichloroacetic acid.

($p<0.003$) while the supraperiosteum, TCA, and electrocoagulation groups were similar to each other ($p=0.358$-0.827) (Figure 3).

DISCUSSION

Attempting to remove the periosteum from the ossicles so that the GIBC can be used the way it is used in dentistry is dangerous and nearly impossible. Rough manipulation of the ossicles may disrupt the fragile connections between them, and cause perilymph fistula or inner ear trauma. To avoid acoustic trauma, an ossicle directly or indirectly in contact with the inner ear should also not be touched with a diamond burr. Also, even if some of the periosteum could be removed, the bleeding of the mucosa could become a bigger problem. Due to these limitations and knowing that bone cement adhesion already feels strong enough, GIBC is usually applied on an intact mucosal lining in otology.

Ráth et al. used a rabbit model to examine the long-term histological interactions between bone-GIBC and periosteum.[4] Their findings revealed that as the periosteum under the bone cement disappeared, the growth of the peripheral mucosal lining, which took place between the 14-60th days, was key to determining the long-term stability. If the re-growing mucosa and periosteum covered the bone cement, instead of wedging between the surface of the bone and the GIBC, a strong long-term integration with no visible foreign object reaction was obtained.[4] Our findings supported their hypothesis. All mobile GIBC droplets were over the periosteum and displaced to some extent, while intact ones were beneath.

TABLE 2: Descriptive statistics and p-values of the groups. Please note that the detached bone cement samples are included in the statistical analysis of this table. There was no difference between the day 0 results. Subperiosteal samples had no detachments by the 90th day and only they had significantly higher results.

		Day 0		Day 90		Day 0	Day 90	Day 0-90
	n	$\bar{X}$	SD	$\bar{X}$	SD	p^{KW}	p^{KW}	p^{W}
Subperiosteum	11	0.527	0.135	1.264	0.518	0.172	0.007*	0.003*
Supraperiosteum	10	0.420	0.123	0.670	0.442			0.101
TCA	11	0.373	0.195	0.573	0.388			0.152
Electrocoagulation	11	0.464	0.220	0.673	0.422			0.153

*$p<0.05$; SD: Standard deviation; TCA: Trichloroacetic acid; KW: Kruskal-Wallis test; W: Wilcoxon signed rank test.

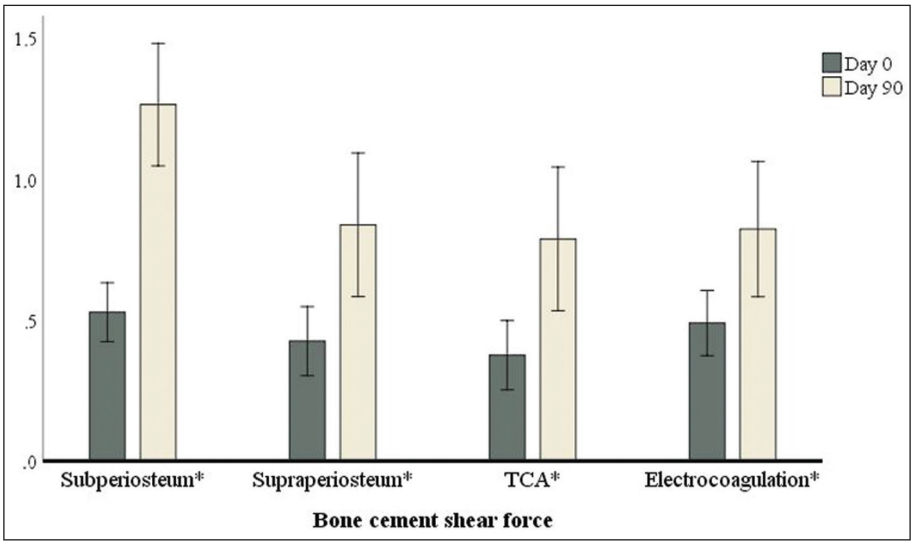

FIGURE 3: Bone cement shear strengths calculated on days 0 and 90 in different groups are shown with bar graphs.
All groups had significantly increased results at the 90th day. Please note that only the values of the intact bone cement samples by day 90 were represented in this graph. Two detachments in the supraperiosteum and electrocoagulation groups, and 3 in the TCA group were excluded, *p<0.05; TCA: Trichloroacetic acid.

It is reasonable to use mechanical test equipments to objectively measure the bond strength of bone cement. This method, which requires a multidisciplinary collaboration between medicine and engineering, has previously been used to compare the strengths of different types of bone cement.[8] Kalcıoğlu et al. used a universal testing machine to evaluate and compare the shear bond strengths (N/mm^2) of different GIBCs and one type of HA cement applied on fixed incuses. Their shear bond strength values with Ketac-Cem (3M ESPE, USA). were almost 15 times higher than our day 0-subperiosteal application results.[8] This might have been because their application was made on perfectly dried cadaveric incuses, which also had burred concave surfaces to enhance mechanical fixation while ours were done in-vivo and on a flat bone surface.

Conducting controlled mechanical tests by applying the GIBC to the ossicles would inevitably cause some difficulties. First of all, it would be necessary to choose a larger animal model, which would not only limit the number of animals that could be used but would also make the relatively long follow-up period of 3 months problematic. Moreover, operating in a small closed surgical field could make it harder to be sure about the GIBC applied under dry conditions. In addition, the curved 3-dimensional shapes of the ossicles would affect the mechanical test results, making them difficult to interpret. Also, measuring the surface area of cement, which directly affects its strength, would be difficult. Considering all these, it was thought that the application on a flat bone surface such as the rat forehead would be the most appropriate in terms of application, testing, and interpretation. Although it can be argued that the results of bone cement applied in an airy environment such as the middle ear may be different from our model, this variable was neglected because this study was all about the effects of various pretreatments and application ways on the adhesion strength of GIBC.

Strengthening of the GIBC in the long term was an unexpected result. The significant increase in the shear strength of the subperiosteum group could be explained by the GIBC-bone integration within 90 days with the ongoing bone turnover. If the detached bone cement samples were not included in the calculation, intact bone cement samples in all groups got significantly stronger until the 90th day. Such an integration correlates with the histological outcomes of Ráth et al. It might also be said that the integration process was faster following the removal of the periosteum and an increase in strength could be expected to continue in other groups over a longer period.[4]

Subperiosteally applied bone cement was the strongest both on days 0 and 90, as expected. The subperiosteal group had been considered as a positive control, establishing the benchmark for the others. In the selection of pretreatments to be tested, the applicable methods and chemicals used in otology that are likely to improve the results were considered. Seventy percent TCA solution used in myringoplasty was chosen to represent chemical methods, and 3.75W bipolar electrocauterization was chosen to represent the mechanical methods as alternatives to mechanical denudation. However, they did not provide any additional benefit over the supraperiosteal application.

TCA is a highly corrosive chemical used for chemical cauterization.[6] If not carefully controlled, contamination of TCA to middle ear structures could lead to uncontrolled mucosal damage and long-term scarring. TCA could also have an ototoxic potential. Considering that bone cement fixation after prosthesis placement is frequently applied in stapes surgeries, an uncontrolled TCA application could even cause catastrophic outcomes by leaking into the inner ear. Therefore, TCA pretreatment should not be used before a bone cement application since no additional benefits were gained.

Electrocoagulation was suggested to be used before bone cement ossiculoplasty applications to achieve a dry surface and have a better bonding.[5] Although this approach sounds logical at first and a dry surface is essential for a strong bond, it did not show any benefit in our study. Even though bipolar electrocoagulation was applied at a very low-power setting, thermal injury to the surrounding tissue and bone could have adversely affected healing, increased inflammation, and hindered any potential benefits. So, electrocoagulation should not be used before a bone cement application.

CONCLUSION

As a result, GIBC reached its highest shear strength with time after a subperiosteal application. If no detachment happened, the strength of the bone cement was higher in the follow-up. We do not recommend any of the pre-treatments tried in this study over a supra periosteal application before a bone cement ossiculoplasty since no increase in shear strength could be obtained.

Source of Finance

This study was approved way Başkent University Ethical Committee for Experimental Research on Animals (Project no: DA22/19) and supported by Başkent University Research Fund.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

***Idea/Concept:** Onur Ergün, Bilge Hakan Yüce; **Design:** Onur Ergün, Bilge Hakan Yüce; **Control/Supervision:** Onur Ergün; **Data Collection and/or Processing:** Sinem Pehlivan, Gülçin Şefiye Aşkın; **Analysis and/or Interpretation:** Onur Ergün, Bilge Hakan Yüce; **Literature Review:** Bilge Hakan Yüce; **Writing the Article:**Onur Ergün, Bilge Hakan Yüce, Eda Çakmak; **Critical Review:** Onur Ergün; **References and Fundings:** Onur Ergün; **Materials:** Onur Ergün.*

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