Comparison of the Efficacies of Methyl Methacrylate, Bioactive Ceramic and Bioactive Glass on the Prevention of Cranioplasty Infections: An In Vitro Laboratory Study

Kranioplasti Enfeksiyonlarının Önlenmesinde Metil Metakrilat, Biyoaktif Seramik ve Biyoaktif Camın Etkinliklerinin Karşılaştırılması: Laboratuvar Çalışması

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Geliş Tarihi/*Received:* 24.03.2014
Kabul Tarihi/*Accepted:* 18.06.2014

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ABSTRACT Objective: Infection is an important complication of cranioplasty and usually treated with systemic antibiotics and removal of cranioplasty material. The aim of this study was to analyze the antimicrobial activities of three cranioplasty materials and to compare their efficacies on microorganism cultures. **Material and Methods:** Methyl methacrylate, bioactive ceramic and bioactive glass were used in this study. In the first step, small pieces of the materials were cut and incubated. Then, they were washed and placed in agar medium. Finally, the number of colonies was counted. In the second step, the pieces were placed on agar plates containing *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Candida albicans*. The plates were incubated, and then, the inhibition zone for each material was measured. **Results:** No inhibition zone was observed in three plates for any microorganism. The number of colonies was lowest in the plate with methyl methacrylate and highest in the plate with bioactive glass for pseudomonas aeroginosa. The number of colonies was lowest in the plate with bioactive ceramic and highest in the plate with methyl methacrylate for *Staphylococcus aureus*. The number of colonies was lowest in the plate with methyl methacrylate and highest in the plate with bioactive glass for *Staphylococcus epidermidis*. The number of colonies was lowest in the plate with bioactive ceramic and highest in the plate with bioactive glass for candida albicans. **Conclusion:** None of these materials have significant antimicrobial effect. However, colonization was more prominent in bioactive glass. Methyl methacrylate and bioactive ceramic allowed less colonization in agar.

Key Words: 13-93 bioactive glass; bacterial infections; ceramics; craniotomy

ÖZET Amaç: Enfeksiyon, kranioplastinin önemli bir komplikasyonudur, genellikle antibiyoterapi ve kranioplasti materyalinin çıkarılması ile tedavi edilir. Bu çalışmanın amacı, üç kranioplasti materyalinin antimikrobiyal aktivitesini analiz etmek ve mikroorganizma kültürleri üzerindeki etkinliklerini karşılaştırmaktır. **Gereç ve Yöntemler:** Bu çalışmada metil metakrilat, biyoaktif seramik ve biyoaktif cam kullanıldı. İlk aşamada, materyallerden küçük parçalar kesildi ve inkübe edildi. Sonra, bu parçalar yıkandı ve medium agara yerleştirildi. En son olarak, koloni sayıları tespit edildi. İkinci aşamada, bu parçalar, *Staphylococcus aerius, Staphylococcus epidermidis, Pseudomonas aeronigosa* ve *Candida albicans* içeren kaplara yerleştirildi. Sonra kaplar inkübe edildi ve her materyalin inhibisyon alanı ölçüldü. **Bulgular:** Her üç kapta, hiçbir mikroorganizma için inhibisyon alanı izlenmedi. *Pseudomonas aeronigosa* içeren kaplarda koloni sayısı, metil metakrilatta en düşük ve biyoaktif camda en yüksekti. *Staphylococcus aerius* içeren kaplarda koloni sayısı, biyoaktif seramikte en düşük ve metil metakrilatta en yüksekti. *Staphylococcus epidermidis* içeren kaplarda koloni sayısı, metil metakrilatta en düşük ve biyoaktif camda en yüksekti. *Candida albicans* içeren kaplarda koloni sayısı, biyoaktif seramikte en düşük ve biyoaktif camda en yüksekti. **Sonuç:** Bu materyallerinin hiçbirinin kayda değer antimikrobiyal etkisi olmasa da kolonizasyon, biyoaktif camda daha belirgindir. Metil metakrilat ve biyoaktif seramikte, daha az kolonizasyon görüldü.

Anahtar Kelimeler: 13-93 bioaktif cam; bakteri enfeksiyonları; seramikler; kraniotomi

Turkiye Klinikleri J Neur 2014;9(3):93-9

Cranioplasty is performed after craniectomy for cosmetic or functional reasons in order to protect the brain.[1,-8] The most common indications of cranioplasty are decompressive craniectomy secondary to trauma, bone flap infarct or tumor invasion of the skull.[1,2,9,10] There are many options for cranioplasty materials.[5,11,12] Autograft or synthetic materials such as methyl methacrylate, porous polyethylene, titanium, bioceramic or bioactive glasses are currently in use for cranioplasty.[1,2,4-7,12-14] Methyl methacrylate is the most commonly used acrylic material used today among other synthetic materials, due to its properties such as easy administration, good tissue compatibility, low price, competency for regeneration, high tolerance by the soft tissue.[1,3,5,9,15,16] The most common disadvantage of methyl methacrylate is the high rate of infection.[9] Bioactive glasses are one of the synthetic biomaterials.[15,17] These glasses are comprised of 53% SiO_2, 23% Na_2O, 20% CaO and 4% P_2O_5 and it is suggested that these glasses have an antimicrobial effects.[15,18] Recently, bioactive ceramics are also in use on the closure of bone defects in the human body and also on the skull. These ceramics contains mainly hydroxyapatite (HA) which is one of the materials used for cranioplasty and is one of the components of the bone is a bioceramic material showing similarities to the bone tissue.[15,19-23] The addition of Mg, Na, Sr, Si and Zn to this material, improves the mechanical properties of HA and increases the antibacterial efficacy of bioactive ceramics.[13,21]

The most significant complications of cranioplasty are infection in the late period and foreign body reaction secondary to cranioplasty material.[3,11,15] Although infection is monitored 3-10 months following cranioplasty, it is shown that the infection may develop although rarely, even 20 years after cranioplasty.[3] The development of infection regarding the implant may be due to many reasons including material contamination directly or based on adjacent tissues, blood-bone contamination or by superficial infection invasion. Most of the infections develop due to direct contamination.[24-26] The infection rate after cranioplasty varies between about 1% and 13.5% in the clinical series.[3,6] Ninety percent of the infections related all the implants are caused by gram-positive *Staphylococcus aureus (S. aureus)*, whereas *Enterococcus faecalis (E. faecalis)* which is a clinically expressive type of gram positive bacteria and gram-negative *Pseudomonas aeruginosa (P. aeruginosa)* are pathogenous microorganisms responsible for infections in relation with implants.[27] Teterycz et al. showed that 66% of the patients were contaminated by methicilline resistant *S. aureus* (MRSA), 79% methicilline sensitive *S. aureus* (MSSA) and 75% Coagulase-negative *Staphylococcus.*[28]

The aim of this study was to investigate and compare the 3 synthetic cranioplasty materials (methyl metacrylate, bioactive glass, bioactive ceramic) for their antimicrobial efficacy on 4 microorganism culture plates.

MATERIAL AND METHODS

This study has been carried out in the microbiology laboratory of our institution. After the approval of our institutional ethics committee, this in vitro experimental study was constituted in accordance with 3 main factors: Materials, Microorganisms and the Test model.

Material: Three synthetic cranioplasty materials were used for this study; First material was a bioactive ceramic (Silisium-strontium hydroxyapatatite-Si-Sr HA, Middle East Technical University, Turkey), second material was methyl methacrylate cranioplastic kit (Codman&Shurtlef Inc, USA), and third material was bioactive glass (Bonalive®, Vivodix, Finland).

Microorganisms: In this study, the most common 4 microorganisms related to cranioplasty such as *P. aeruginosa, S. aureus, S. epidermidis, Candida albicans* were used.

Test model: The antimicrobial efficacies of 3 cranioplasty materials were evaluated by counting the number of colonies and measuring the inhibition zones in the agar plates containing 4 microorganisms.

RESULTS

BIOACTIVE CERAMIC (SI-SR-HA)

No inhibition zones were monitored for the 4 microorganisms tested on the bioactive ceramic. It was observed that there is a significant bacteria growth for each microorganism, especially there was an extensive number of *P. aeruginosa* when the number of colonies was evaluated. The number of colonies for *P. aeruginosa* was measured as 1640 CFU/ml (Table 1).

METHYL METHACRYLATE

No inhibition zones were detected for the 4 microorganisms tested in methyl methacrylate. When the number of colonies was evaluated it was observed that there was a significant bacteria growth for each organism and especially there was an extensive number of *P. aeruginosa.* The number of *S. aureus* was measured as +4000 CFU/ml (Table 1).

BIOACTIVE GLASS

No inhibition zones were detected for the 4 microorganisms tested in bioactive ceramic. When the number of colonies was evaluated it was observed that there was a significant bacteria growth for each organism and especially there was an extensive number of *Pseudomonas aeruginosas*. The number of *P. aeruginosa* was measured as +4000 CFU/ml (Table 1).

DISCUSSION

Our study showed that 3 materials which are in use in cranioplasty operations have no significant antimicrobial effect. We showed that the colonization is more prominent in bioactive glass. Methyl methacrylate and bioactive ceramic allow less colonization in agar plates. Antimicrobial effects of bioactive ceramic may be related Strontium elements and HA molecule.

Infection is an important complication of cranioplasty operations, especially when the defects are closed with synthetic materials. The infection rate of cranioplasty is ranged between 1% and 13.5% in the published series.[3,6] Yadla et al. performed a systematic review of 18 studies related to cranioplasty infections and they found that infection rate varies between 0% and 21.4% and the mean infection rate is 7.9%.[10]

Lee et al. at the Seoul National University Bundang Hospital, where the surgical results of 140 patients, the infection rates were determined as 7.86% and the most frequent microorganisms growing in culture were MRSA (9 patients) and Klebsiella (2 patients).[6] Likewise, Teterycz et al. showed that 66% of the patients were contaminated by MRSA, 79% MSSA and 75% Coagulase-negative staphylococcus.[28] Similarly, Jaberi et al. performed a study on 70 patients with cranial defects, indicated that the infection developed on the postoperative days 15-507 and the most isolated microorganisms in the cultures were 67% *S. aureus.*[5] Whereas the retrospective cohort study of Im et al. carried out to determine the relationship between the time of cranioplasty with the graft used and the infection at the surgical zone on 131 patients the infection rate was detected as 10.8% (14 patients) and the microorganisms with the highest growth rate were MRSA, methicilline resistant coagulase-negative *Staphylococci, S. aureus, P. aeruginosa, Enterobacter aerogenes, Staphylococcus chromogenes* and *Candida guilliermondi.*[4] In our study, we used 4 microorganisms which are mostly detected in cranioplasty infections and mostly col-

TABLE 1: Evaluation of 3 materials regarding the number of colonies and the inhibition zones.

	Bioceramic		Methyl Methacrylate		Bioactive glass	
	Mean colony number CFU/ml	Measured inhibition zone mm	Mean colony number CFU/ml	Measured inhibition zone Mm	Mean colony number CFU/ml	Measured inhibition zone mm
Pseudomonas aeruginosa	1640 CFU/ml	0	1200 CFU/ml	0	+4000 CFU/ml	0
Staphylococcus aureus	820 CFU/ml	0	+4000 CFU/ml	0	2000 CFU/ml	0
Staphylococcus epidermidis	120 CFU/ml	0	20 CFU/ml	0	1100 CFU/ml	0
Candida albicans	20 CFU/ml	0	80 CFU/ml	0	840 CFU/ml	0

onized microorganisms were *S. aureus* and *P. aeruginosa.*

The entrance of bacteria such as *S. aureus* and *S. epidermidis* to the surface of the implant takes place either before or during the surgery.[22] The infection may develop via many different ways for example such as directly or by material contamination based on adjacent tissues, blood-bone contamination or by superficial infection invasion. A great majority of these take place due to direct contamination.[26,29] Therefore, the main strategy for inhibition of the development of infection is to provide sustained sterilization in every step of implantation, to minimize contamination during the operation and administration of preoperative antibiotics.[12] To treat a severe infection, the implant should be removed, surgical debridement should be carried out and a long-term wide spectrum antibiotic therapy should be administrated.[20] On the other hand, addition of antibiotics to the cranioplasty material or bathing the material with an antibiotics solution during the operation are suggested to decrease the rate of infection.[1,30] The use of tobramycin, gentamycin or vancomycin to decrease the postoperative infection, is also suggested in the literature.[12] Shapiro conducted a study on 65 patients using methyl methacrylate for cranial and spinal defects.[31] Shapiro suggested irrigation with antibiotics and systemic antibiotics administration during the operation and the addition of antibiotics into the cranioplasty cement to decrease the infection rate. Recent studies showed that the antibiotics or synthetic bone grafts which release ions from the material could help inhibit the infection related to implant and thus the antibiotics could be coated on the surface of the implant.[27] Since the antibiotics have a fragile nature, there is a concern that the antibiotics may decompose during the sterilization of the implant. Another concern is the development of antibiotic resistant microorganisms due to use of such materials.[20,32] Because of these concerns, recent studies have focused on the antimicrobial efficacy of the implants which indicate that the ideal antibiotic releasing material is a material that releases a drug substance within the first couple weeks.[20,32] We did not add any drug substance or antimicrobial agent on the cranioplasty materials during the study. We cultured them in a media with microorganisms and evaluated their efficacy against microorganisms with their purest phases.

The infection is developed on the implantation zone of cranioplasty material due to the bacterial adhesion and colonization that cause a layer of biofilm whereas 45% are based on nosocomial infections.[22,27] The bacteria have many abilities such as attaching to the surface of the implant, matrix synthesis which consist of a great majority of extracellular polysaccharides, to form a layer of biofilm that includes the cells and to avoid the effects of an antibiotics therapy.[22] The extracellular matrix bacteria, developed by bacteria later become a protective layer.[26] Generally the pathogens grow in the microcolonies attached to the microfilm layer, infect the surface of the implant under the bone and inhibit the growth of new bone tissues. The colonized implant may rapidly cause an infection. The best treatment of colonization is to remove the implant and administration of systemic antibiotics. The prevention of bacterial adhesion on the implant is the important step to hamper infection related to bacteria and to provide the tissue-implant interference.[22] When the bacterial adhesion takes place before the tissue regeneration, the defense mechanisms cannot inhibit the surface colonization and a biofilm layer is formed. Thus, the inhibition of bacterial adhesion is important to prevent infection related to implants.[27] We found that all 3 cranioplasty materials had bacterial colonization. Most colonization was observed on bioactive glass material. However, the two other materials also had the colonization of microorganisms.

Antibacterial efficacy of bioactive ceramics develop due to mechanisms such as the degeneration of electron transport chain, inhibition of DNA replication, division of DNA, formation of reactive oxygen and the inhibition of oxygen.[22] It is thought that the strontium release in the bioceramic being used and its effect on local pH is also related to the antibacterial efficacy.[33] The antibacterial activity of the HA which is within the structure of the bioactive ceramic is related not only with the low rate ion release but also with the free radicals released

from the surface of HA.[22] The interference between HA and the monocytes cause the release of inflammatory cytokines such as TNF-a, IL-6, IL-18. On the other hand, inflammatory cytokines such as IL-10 are also being generated.[34] Recent studies show that the antibacterial efficacy structure of hydroxyapatite on *E. coli, S. aureus, Lactobacillus* has increased with the addition of strontium along with calcium.[19] Whereas the study of Buache et al. indicated that it decreases the generation of strontium TNF-a and IL-6 while having no effect on IL-1β or IL-18.[34] IL-1β and TNF-a are strong activators which stimulate the immune cells. When these are activated, they stimulate the generation of other pro-inflammatory factors such as proteolytic mediators. Although the studies regarding the antibacterial efficacy of strontium are limited with in vitro and in vivo studies, it is said that the cements consisting of strontium prohibit the postoperative complications due to residual or contaminated bacterium.[22,33,35] Ravi et al. found a significantly low amount of E. Coli and S. Aureus in the strontium coated material during their study.[19] Only 10% of the strontium coated material has shown maximum antimicrobial activity. Fielding et al. discussed the fact that the number of live bacteria in the HA and Sr-HA cultures after 24 hours was high, whereas the number of dead bacteria was significantly low.[20] However, it is said that the number of dead bacteria in argentiferous HA and silver-Sr-HA are high, whereas the number of live bacteria was low. In our study, bioactive ceramics with strontium content were used, but no inhibition zone has been monitored in their surroundings. Nevertheless, the least colonization of S. Aureus and Candida albicans were especially in bioactive ceramics. The bioactive ceramics containing strontium had higher efficacy against these two organism. It was also shown that the bioactive glass has properties such as stimulating the bone it is in interaction with along with other antibacterial properties.[36-38]

The antibacterial efficacy of the bioactive glass is effected by the chemical compounds of the glass along with the dissolution circumstances in the media.[39] The bond between the bone and the glass is formed following a number of chemical reactions.[18,36-39] For the bioactive glasses to bond rapidly, they need to consist of SiO_2 with a ratio of 45% to 52%.[40] The chemical reaction on bioactive glasses is activated following interaction with body fluids. Then a rapid transition from the bioactive glass and H+ and H_3O ion transition from the extracellular fluid takes place.[40] The chemical reaction develops due to the negative surface potential of the bioactive glasses. The negative surface formed, makes up double layer electricity and attracts Ca and Na ions. Additionally a Si rich layer is formed on the negative surface potential. This mechanism, generally cause an adhesion between the negative load bacteria and negative loaded surface.[37] The HA layer is stabilized over this layer. Then this layer forms a chemical bond with the bone.[18,36-39] Afterwards ions such as the sodium, calcium, phosphate and salts of silicic acid are freed and thus causes and increase of pH and osmotic pressure in the media.[37,38,41] The osmotic effect which forms with the dissolution of glass, is used to explain the antibacterial efficacy of the bioactive glass.[18,36,39] On the other hand it is indicated that the high concentration of Ca along with the alkaline ions freed from the bioactive glass may be effective also and that this may cause the degeneration of the bacteria membrane potential.[41] Stoor et al. showed that no infection was detected in the patients where bioactive glass (BonAlive®) was used and no increase in the number of microorganisms adhering to the surface of bioactive glass throughout the long period of incubation.[37] Thus it has been indicated that the no colonization or biofilm layer has been monitored on the surface. The study of Stoor et al. was carried out on patients whereas our study was carried out on specific microorganism cultures.[38] Our study showed the growth of microorganisms on the bioactive glass material and this growth was more when compared to the other synthetic materials. The same bioactive glass (BonAlive®) was used in our study. However our results have shown that the bioactive glass does not carry any antimicrobial properties unlike previous studies.

CONCLUSION

Methyl methacrylate, bioactive glass and bioactive ceramics have no significant antimicrobial effect on the operation site. Although less colonization was observed on the bioactive ceramics, none of them create an inhibition zone for these microorganisms. The best way to prevent the cranioplasty infection is clear operation site in association with good antimicrobial treatment.

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