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Simple method for increasing drug elution from polymethylmethacrylate bone cement

Polimetilmetakrilat kemik çimentosundan ilaç elüsyonunu artırmak için basit bir yöntem

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ABSTRACT

Objectives: This study aims to investigate the effects of the use of citric acid and sodium bicarbonate mixture as an effervescent in polymethylmethacrylate bone cement in terms of better drug elution.

Patients and methods: Multiple cylinder cement blocks each containing 10 g of glucose and different amounts of effervescent (E), with different numbers of pieces (P), surface areas, volumes, and porosities were prepared. Physical properties of all blocks were calculated. Blocks were placed in jars containing saline solutions and the released glucose concentrations were measured at predefined intervals. Correlations between elution rate and physical properties were defined.

Results: Elution rates were significantly higher in samples with effervescent. At the end of 45th day, E^0P_1 released mean 21% of its glucose content. This value was 38%, 61%, 82% and 88% for E^0P_2, E^0P_4, E^2P_1 and E^4P_1, respectively. Strong correlations were detected between water absorption ratio, surface areas, porosity and glucose elution rates ($r=0.942$, $p<0.01$; $r=0.894$, $p<0.05$; $r=0.918$, $p<0.05$).

Conclusion: Using sodium bicarbonate and citric acid as effervescent in bone cement provides satisfactory porosity development for better antibiotic elution. This method may be useful when a monolithic spacer and better local antibiotic elution are required.

Keywords: Antibiotic, bone cement; cement spacer; elution; polymethylmethacrylate.

ÖZ

Amaç: Bu çalışmada sitrik asit ve sodyum bikarbonat karışımının polimetilmetakrilat kemik çimentosunda efervesan olarak kullanımının daha iyi ilaç elüsyonu açısından etkileri araştırıldı.

Hastalar ve yöntemler: İçeriğinde 10'ar gram glikoz ve farklı miktarlarda efervesan (E) olan; parça sayıları (P), yüzey alanları, hacimleri ve poroziteleri farklı silindir çimento bloklar hazırlandı. Tüm blokların fiziksel özellikleri hesaplandı. Bloklar serum fizyolojik içeren kavanozlara konuldu ve sıvı içerisine salınan glikoz konsantrasyonları önceden belirlenen aralıklarda ölçüldü. Elüsyon hızı ve fiziksel özellikler arasındaki ilişkiler tanımlandı.

Bulgular: Efervesanlı numunelerden elüsyon oranları anlamlı derecede daha yüksekti. Kırk beşinci günün sonunda E^0P_1 glikoz içeriğinin ortalama %21'ini saldı. Bu değer E^0P_2, E^0P_4, E^2P_1 ve E^4P_1 için sırasıyla %38, %61, %82 ve %88 idi. Su absorbsiyon oranları, yüzey alanları, porozite ve glikoz elüsyonu oranları arasında güçlü korelasyonlar saptandı ($r=0,942$, $p<0,01$; $r=0,894$, $p<0,05$; $r=0,918$, $p<0,05$).

Sonuç: Sodyum bikarbonat ve sitrik asidin efervesan olarak kemik çimentosu içinde kullanılması, daha iyi antibiyotik elüsyonu için tatmin edici porozite oluşumunu sağlamaktadır. Yekpare bir spacer ve daha iyi lokal antibiyotik elüsyonu gereken durumlarda bu yöntem işe yarayabilir.

Anahtar sözcükler: Antibiyotik, kemik çimentosu; çimento spacer; elüsyon; polimetilmetakrilat.

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Antibiotic-loaded polymethylmethacrylate (PMMA) cements - e.g. as a knee spacer - help us to get higher intra-wound antibiotic concentrations without systemic toxicity.[1,2] Various methods were described to increase the elution of antibiotics from the cement spacers. Increasing the surface area is a solution as in septopal beads.[1] Agitation increases the rate of dissolution like stirring the sugar into your coffee or cyclic loading to a cement spacer.[3] These findings simply depend on dissolution rate principles. Agitation, surface area, temperature and the chemical properties of the medium like pH and common ions are the determinants of dissolution rate.[4] *In vivo*, temperature and chemical properties of the body fluids cannot be modified but surface area or agitation can be. Porosity defined as pores or voids exist within the solid. Permeability refers to the ability of a solid to allow the passage of fluids through itself. These two qualities are closely related. Porosity and therefore permeability of PMMA are very low. Increasing the porosity would also increase the elution rate. For clinical use, the method for doing that must be easy, safe and cost-effective. Cimatti and Engel described such a method for increasing the porosity of PMMA cement.[5] They proposed to use citric acid and sodium bicarbonate mixture as an effervescent in PMMA cement. Therefore, in this study, we aimed to investigate the effects of the use of citric acid and sodium bicarbonate mixture as an effervescent in PMMA bone cement in terms of better drug elution.

PATIENTS AND METHODS

We set up an experiment at Medical Faculty of Erzincan University, Mengücek Gazi Training and Researh Hospital between April 2016 and June 2016 using:

- Standard viscosity PMMA cement (Cement Oliga 1, Modena, Italy)
- Effervescent (one-to-one mixture of sodium bicarbonate and citric acid powder)
- Glucose powder
- Cylindrical molds (4.5 cm in diameter)
- One liter of saline filled jars

Each cement had 40 g of powder component (PMMA, barium sulfate, benzoyl peroxide) and liquid monomer (methyl methacrylate, benzenamine). Multiple cylindrical cement blocks were prepared inside the molds using either none or different amounts of effervescent but all containing 10 grams of glucose. Two of the blocks without effervescent were divided into two and four equal pieces to test surface area theory. One control sample was prepared without glucose or effervescent.

Acronyms were used to tag the samples. E: effervescent in grams, P: number of pieces, C: control (Table I). Remnants of the preparation were collected, ground and dissolved in hot saline solutions. glucose was tested and wasted part was calculated for each specimen. Each specimen was soaked into the separate 1 L of saline filled jars in a fixed position and kept in the incubator at 37 °C. We took a pair of 1 mL samples from each sample just after soaking and a gentle stirring at t zero and at first, second, sixth, 12th and 24th hours, and second, fourth, sixth, eighth, 10th,

TABLE I

Properties of specimens and glucose concentrations

Sample	Control	E^0P_1	E^0P_2	E^0P_4	E^4P_1	E^2P_1
Dimensions (mm)	45x30	45x33	2 pieces	4 pieces	45x77	45x65
Expansion (%)	-	10	10	10	157	117
Weight (g)	51.4	60.7	57.5	52.5	63.3	62.5
Bulk volume (cm^3)	47.7	52.5	50.2	45.6	123	102.5
Void volume (cm^3)	-	4.8	-	-	75.3	54.8
ϕ (porosity) (%)	-	9.1	9.1	9.1	61.2	53.5
Density (g/cm^3)	1.08	1.15	1.15	1.15	0.51	0.61
Surface (cm^2)	74.2	78.5	108	168	140	123
Effective glucose in 12 h	6.6	6.6	6.6	6.6	6.7	6.6
Effective glucose (g)	0	9.8	9.6	9.4	9.1	9.6
Final weight (g)						
Wet	51.6	62.8	60.3	53.9	89	70.8
Dried	51.4	56.3	52.2	47.2	45.4	49
Water absorption (%)	0.3	11.5	15.5	14.1	104.1	44.5
Entrapped (%)	0	61.2	35.4	23.4	6.6	5.2

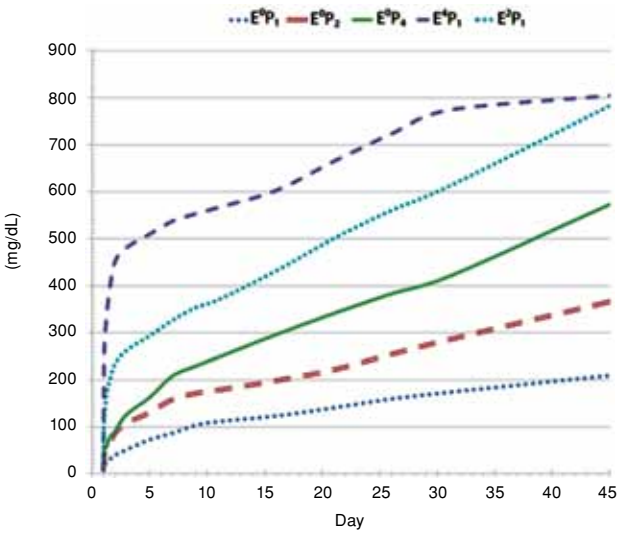

Figure 1. Elution time graph. Slopes of lines give information about rates.

15th, 20th, 25th, 30th and 45th days and tested them for glucose levels.

At the end of the experiment, we rinsed all the specimens, and weighed them when wet and dried to find out the water absorption ratio. We grounded and mixed each in hot 500 mL saline solutions and calculated the entrapped glucose amount. Each type of cement blocks was prepared separately and immersed in the saline solution with radiopaque dye and computed tomography (CT) scan was obtained at first, fifth and 15th days to visualize the gradual liquid permeation into the cement blocks.

Volume and surface area of the cement blocks were calculated using the relevant cylinder formulas: volume= πr^2 h, surface area= 2 πr^2 +2 πr h

Porosities were represented in percent terms using the formula:

$$\phi^{(\text{porosity})} = V_v^{(\text{void volume})} / V_t^{(\text{total volume})}$$

Beckman Coulter AU2700Plus (Beckman Coulter, CA, USA) was used for glucose level measurements. Siemens Somatom Emotion (Siemens AG, Munich, Germany) was used for CT scans. The study protocol was approved by the Erzincan University Medical Faculty, Mengücek Gazi Training and Research Hospital Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA) software. ANOVA and Bonferroni test used to compare the elution rates. Spearman correlation test used to find out the correlations between elution rate, porosity, water absorption and surface area.

RESULTS

The final products were all cylinder cements of 4.5 cm diameter with various heights and weights (Table I). We calculated the physical properties, porosity and the effective glucose ingredient of each cylinder block. Glucose levels in the saline solutions were recorded at predefined intervals (Figure 1). Elution rates for different intervals were calculated (Table II).

Analysis of variance test showed significant differences between the mean glucose elution rates ($p=0.03$). Then, Bonferroni test was used to determine which means differed. There were significant differences between the elution rates of E^0P_1 and E^4P_1 ($p<0.05$), and E^0P_1 and E^2P_1 ($p<0.05$). There was no difference between the elution rates of E^0P_1, E^0P_2 and E^0P_4. There was also no difference between the elution rates of E^4P_1, E^2P_1, E^0P_2 and E^0P_4.

Compared to the control, E^0P_1 was 3 mm taller (Figure 2), which meant that 10 g glucose occupied 4.8 cm^3 in the cement block.

Using the given formula, porosity of the E^0P_1 was calculated: $\phi = V_v/V_t = 4.8/52.5 = 9.1\%$

Water absorption ratio of the control specimen was as negligible as 0.3%. However, other specimens showed significant water absorption ratio (Table I).

TABLE II

Elution characteristics of specimens

	E^0P_1	E^0P_2	E^0P_4	E^4P_1	E^2P_1
Elution (mg/day)	45	66	110	108	127
12th hour elution (%)	3	6	8	42	20
1st to 30th day elution (%)	17	29	51	38	57
45th day elution (%)	21	38	61	82	88

Figure 2. Control, E^0P_1 and E^4P_1 specimens.

Spearman correlation test showed high correlation between water absorption and elution ratio ($r=0.942$, $p<0.01$); surface area and elution rates ($r=0.894$, $p<0.05$); porosity and elution rates ($r=0.918$, $p<0.05$), and porosity and water absorption ratio ($r=0.922$, $p<0.05$). Computed tomography scan showed gradual liquid permeation into the cement block E^2P_1 (Figure 3).

DISCUSSION

Antibiotic-loaded PMMA cements are widely used to achieve higher intra-wound antibiotic concentrations during primary or revision arthroplasties, while antibiotic prophylaxis is an important issue.[6,7] These are composite materials, which mainly consist of highly hydrophobic, water stable polymer and highly soluble drugs. Soluble part of this composite material creates the porosity by itself. Not the weight but the volume of the ingredients in a composite material determines the porosity. Effervescent added into this material increases the porosity remarkably by creating interconnected macro-pores, which also helps permeability. Water needs to reach deep inside the cement and solve the drug to act. This process requires networked cracks and voids to work, or the drug might be entrapped.

Cimatti et al.[5] proposed a method for creating "interconnected macro-pores" inside the cement. They proposed that this type of cement may be used to fill irregularly shaped bone defects by expansion and those macro-pores may act as a scaffold for tissue ingrowth. They also showed that the strength of aforementioned cement was comparable with cancellous bone.[5]

In this study, we demonstrated the efficacy of this novel porosity inducing method for better drug elution. We explained the elution of drugs through PMMA spacers by using porosity, permeability, and dissolution rate principles and criticized the literature for neglecting and not reporting on such important data in relevant studies.

Vancomycin, gentamicin, tobramycin and cefazolin, commonly used in bone cements, are considered highly soluble in water as well as glucose.[8] Thus, we used glucose as the surrogate for antibiotics in our study to reduce the cost.

In our experiment, we first experimented with the method proposed by Cimatti et al.[5] and noted that the final products porosity may be greater than required for this purpose. Thus, we decided to reduce the amount of effervescent and created E^2P_1 batch for the test.

The main variant of our experiment was the volumetric expansion, which is directly related to porosity. In our experiment, E^0P_1 with 10 g of glucose was 4.8 cm^3 larger than the control sample. Therefore, 10 g of glucose powder created 9.1% porosity in E^0P_1, E^0P_2, and E^0P_4. With effervescent, we increased this

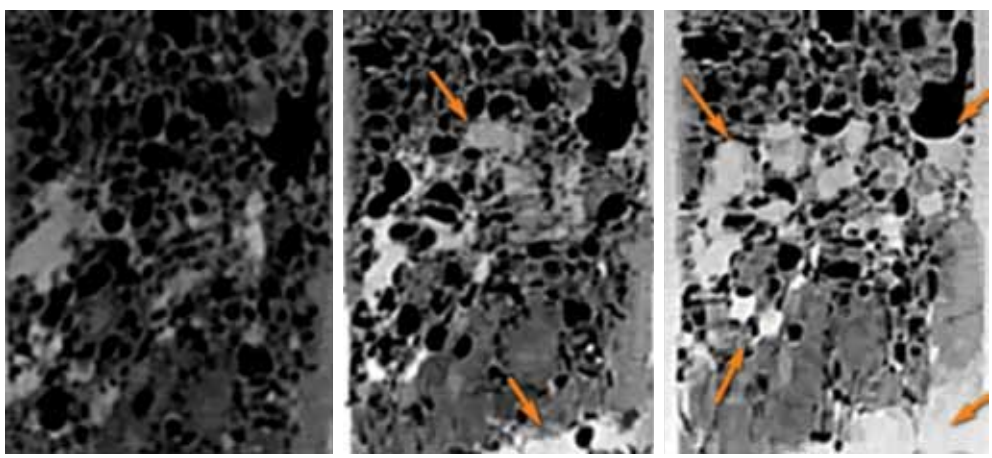

Figure 3. From left to right first, fifth and 15th day radiopaque liquid invasion into E^2P_1 cement block. Arrows show gradual liquid penetration into pores.

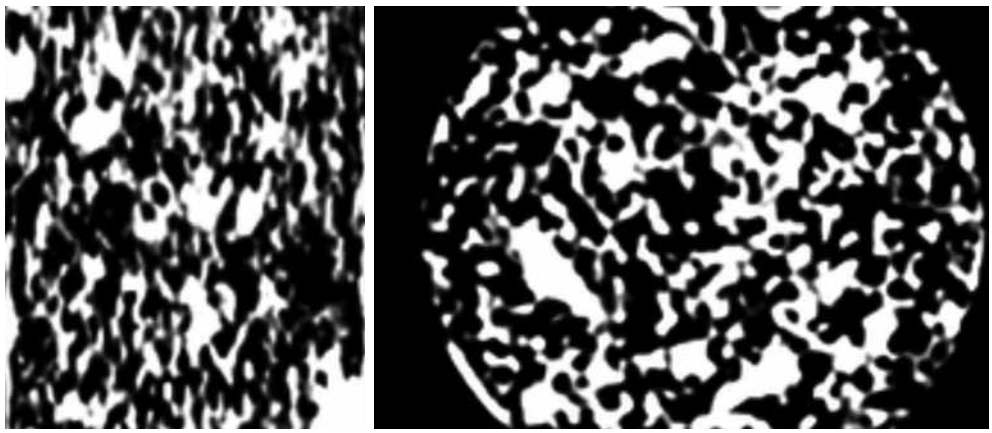

Figure 4. Computed tomography scan of the specimen E^4P_1, coronal and transverse views.

porosity remarkably in E^2P_1 and E^4P_1 without using much solid compound. Without effervescent, E^0P_1 with 9.1% porosity showed consistent elution in our setup (Figure 1). However, only 20% of the ingredient was eluted in 45 days with that (Table II). That would be a waste for a six-week planned antibiotic-loaded joint spacer treatment. To increase the rate of elution, dividing the cement into smaller pieces to increase the surface area also worked well as stated before. E^0P_2 and E^0P_4 specimens showed proportional elution rates with their surface area (Table II) (Figure 1). However, sometimes, the surgeon needs a monolithic spacer, which cannot be divided into smaller pieces. This method is useless in such scenarios.

In our study, E^2P_1 and E^4P_1 were both prepared with effervescent (Cimatti's recipe). Calculated porosities were 53.5% and 61.2% (Cimatti's 62.7%); while calculated densities were 0.6 and 0.5 g/cm^3 respectively, which were both more than Cimatti's 0.4 g/cm^3. Therefore, we believe that our samples' mechanical properties were also better than their samples, which were measured and were close to bovine bone.[5] It is not known how much compressive strength a knee spacer must have. Although we did not measure this, it was hard to break our sample even with a large chisel or hammer.

The effervescent reaction creates carbon dioxide bubbles. They are trapped inside the cement and they create porosity. Initiating the reaction requires water, which should be added when the cement has a sticky dough consistency. The chemical reaction was fast in that stage and rapid mixing with an appropriate beater is necessary for maintaining homogeneity. The same amount of cement occupied significantly larger volume in specimens with effervescent (Figure 2). The surfaces of the specimens were smooth. Macro-pores created by effervescent could be seen in CT scans (Figures 4, 5).

With Cimatti's recipe, E^4P_1 had more than 150% expansion and porosity of 61.2%. With such massive porosity, elution became faster than needed. E^2P_1 with 117% expansion and 53.5% porosity would be better for a 45-day spacer job.

For effervescent reaction, substances should be in aqueous form. However, polymer is highly hydrophobic. Water tends to stay outside the cement and while doing this, it dissolves glucose and brings it out to the surface of the blocks. Thus, the amount of water should be minimized to maintain volumetric homogeneity.

Antibiotics added into cement must be water-soluble for elution. Otherwise, desired concentrations cannot be achieved with poorly soluble antibiotics like cefepime or ampicillin.[8-10] Nonetheless, this method may work in the same way even if the molecule

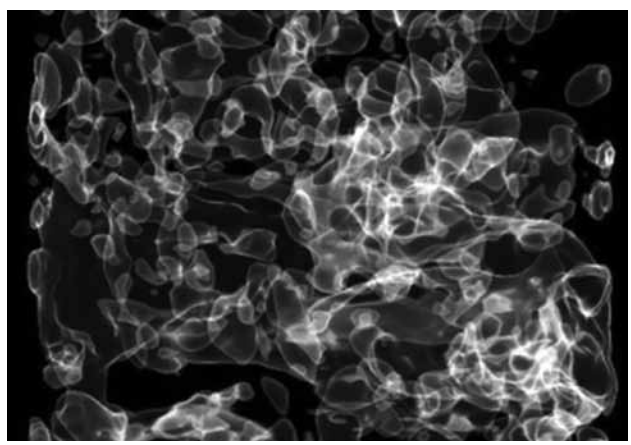

Figure 5. Three-dimension reconstruction of voids inside E^4P_1 specimen.

had high or low solubility. The effect might be more prominent in low solubility drugs. This issue needs to be studied further.

Without the limitation of the medium -in our case PMMA- any highly soluble powdered substances dissolve in their solvent as they meet. However, in our case, time was measured by days. Thus, one of the important rate-determining steps in such a scenario was the permeation of the solvent into the medium, which takes time. To visualize this gradual permeation, we immersed our separately prepared samples in radiopaque dye filled saline solutions and had CT scans at first, fifth and 15th days (Figure 3).

Researchers found that some cement brands are better for antibiotic elution, and some keep their strength better with manually added antibiotics.[1,2,11,12] Type and form of the antibiotic also change the properties of cement differently.[2,3]

As already shown and explained in our study, the main variant in such an experiment is volumetric expansion. Not only the necessary amounts of drugs but also the desired porosities of final products should be reported in such studies for warranting reproducibility. Unfortunately, to our best knowledge, none of the related studies had such an important volume data and thus comparison among studies or validation of our method was impossible.

Only one study in the literature addressed this problem with a dramatic experience with generic and proprietary tobramycin. They observed that 1.2 g of generic tobramycin powder was 3.6 times greater than the proprietary one (12.5 vs. 3.5 mL), which affected the elution rate remarkably.[13]

Porosity is not defined by the weight but is defined by the volume of the porogen. Therefore, studies, which do not mention volume data, lack significant information.

The main limitation of this study was the use of glucose instead of antibiotics. However, both substances are in powdered form and have high solubility.[8,14] Hence, the solution never reaches the saturation point and the rate of dissolution is never limited. Solubility and dissolution rates are two different properties, which should not be confused and are not necessarily related. The factors that affect the rate of dissolution according to Noyes-Whitney equation are the diffusion coefficient, the surface area of the solute, the concentration of the solute in the boundary layer and the height of the boundary layer. Thus, even though the molecules were different, the result would be similar with different drugs with similar properties. Molecular size also should not be a concern in such an experiment because physical crack to molecule size rate is practically infinite for all. Thus, instead of a water-soluble antibiotic, glucose was our choice of solute thanks to easy and cheap measurement of its amount in any liquid.

The use of this method was proposed for different purposes; however, to our knowledge, has not been tested *in vivo* yet. Thus, biocompability of the product may be a concern. However; sodium bicarbonate and citric acid are widely used in medicine and body fluids are able to buffer both in natural ways. Citric acid is also used in calcium phosphate cement to reduce the inflammation and increase the biocompatibility.[15] Another byproduct of this reaction is sodium citrate, which is also already in use for metabolic acidosis. Moreover, pH of our saline solutions did not show significant alteration. It was also reported that the increased porosity in PMMA cement helps *in vivo* biocompatibility.[16,17] Carbon dioxide trapped inside the cement block will be released gradually as the liquid occupies more space inside the pores. We estimate that the amount of gas would be negligible enough for compensation if the effervescent reaction exhausts outside the body. The reaction is quite fast and already negligible when the cement is hardened and cold.

In conclusion, using sodium bicarbonate and citric acid as effervescent in bone cement provides satisfactory porosity development for better antibiotic elution. This method may be useful when a monolithic spacer and better local antibiotic elution are required, promising better drug elution. However, further *in vivo* studies are needed to clarify this.

Declaration of conflicting interests

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