Physicochemical, Mechanical, and Biological Properties of Bone Cements Prepared with Functionalized Methacrylates

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ABSTRACT: Bone cements prepared with methyl methacrylate (MMA) as a base monomer and either methacrylic acid (MAA) or diethyl amino ethyl methacrylate (DEAEMA) as comonomers were characterized in terms of curing behavior, mechanical properties, and their *in vitro* biocompatibility.

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The curing time and setting temperature were found to be composition dependent while the residual monomer was not greatly affected by the presence of either acidic or alkaline comonomers in the bone cements. For samples with MAA comonomer, a faster curing time and higher setting temperature were observed when compared to the cement with DEAEMA comonomer.

In terms of mechanical properties, the highest compressive strength was exhibited by formulations containing MAA, while the highest impact strength was shown by the formulations prepared with DEAEMA. There were no differences observed between the two formulations for tensile, shear, and bending strength values. Similarly, fatigue crack propagation studies did not reveal differences with the addition of either DEAEMA or MAA.

No differences were observed in the initial number of attached primary rat femur osteoblasts on the different bone cements and positive controls. However, after 48 h there was a reduced proliferation in the cells grown on bone cements containing MAA.

KEY WORDS: bone cements, methacrylic acid, diethyl amino ethyl methacrylate, mechanical properties, biocompatibility, osteoblasts.

INTRODUCTION

Acrylic bone cements are the most frequently used materials for the fixation of a total joint prosthesis. These polymeric materials are commonly prepared by mixing a solid part comprising of a prepolymerized poly(methyl methacrylate) (PMMA), benzoyl peroxide, and radiopacifiers with a liquid part made of methyl methacrylate (MMA) containing *N*,*N*-dimethyl-*p*-toluidine, and chlorophyll [1]. Since their conception, research into bone cements has aimed at improving among other factors their handling, mechanical properties, and biocompatibility, and bone cements have been modified by changing either the solid or the liquid part. Modifications of the solid component have included the incorporation of various ceramics such as hydroxyapatite, calcium phosphates, and Bioglass® as well as the addition of antibiotics [2,3], whereas in the liquid part, comonomers, cross-linking agents, and new activators have been added [4–6].

In the introduction of new bone cement formulations factors such as curing, mechanical, and biological properties are critical. For these materials, the requirements of either ISO 5833 or ASTM F451 standards must be fulfilled [7,8]. However, these standards are limited as they only cover their curing behavior and some mechanical properties in compression and bending, and a complete mechanical characterization is not generally pursued in the bone cement standards. It is now recognized that a combination of shear, tension, and compression

stresses are found during *in vivo* loading of the artificial joint [9] and therefore these studies should be justified.

Also, bone cement standards do not cover biocompatibility studies but cellular behavior and cell toxicity can be assessed with the use of *in vitro* or *in vivo* methods. *In vitro* methods are generally used for screening purposes as they allow the detection of cellular behavior in a fast, effective, and reproducible fashion. When using cell cultures (either transformed cell lines or primary cultures) the factors that control cell adhesion, proliferation, expression of phenotypic characteristics and, in general, their cytotoxicity must be assessed. The *in vitro* evaluation of bone cements has been extensively covered [10–15].

This study reports on the development and characterization of bone cements prepared with methyl methacrylate (MMA) as the base monomer and either methacrylic acid (MAA) or diethyl amino ethyl methacrylate (DEAEMA) as comonomers. These were characterized in terms of curing behavior, mechanical properties, and their *in vitro* biocompatibility. The mechanical properties were assessed in tension, compression, bending, shear, impact, and fatigue crack propagation. Their *in vitro* biocompatibility was investigated using primary osteoblasts obtained from rat femur.

MATERIALS AND METHODS

Materials

Methyl methacrylate (MMA), methacrylic acid (MAA), diethyl amino ethyl methacrylate (DEAEMA) and *N*,*N*-dimethyl-*p*-toluidine (DMPT) were purchased from Aldrich and used without further purification. Benzoyl peroxide (BPO) was obtained from Merck and used as received. A fast curing–transparent acrylic, Nictone™ (a copolymer of MMA : EMA (90 : 10), average diameter = 60.6 μm, Mn = 305,150, and T_g = 92.5°C) from Manufacturer Dental Continental was used as the solid component of the formulations.

Methods

Bone Cement Preparation

Experimental bone cements were prepared with MMA as the base monomer and either MAA or DEAEMA as comonomer. The acidic comonomer, MAA, was incorporated at 0.3 molar fraction while the alkaline comonomer, DEAEMA, was added at 0.08 molar fraction.

These formulations were chosen on the basis of their good static mechanical properties [16]. DMPT was added to the monomer mixture at 2.5% by volume fraction while BPO was added to the polymer at 1% by weight. The polymer to liquid ratio used was 2:1 and both components were hand mixed.

Determination of Curing Properties

Determination of Maximum Temperature and Setting Time

Maximum temperature during polymerization (T_{max}), setting temperature (T_{set}), and setting time (t_{set}) were determined according to ISO 5833 (Annex C) in a water bath at 20°C. The change in temperature with time was recorded immediately after the mixing of powder and liquid. The average of at least three measurements was used.

Determination of Residual Monomer

Residual monomer content was calculated by ^{1}H NMR on a Varian Gemini 200 (0.05 g in 0.6 mL). The monomer was quantified on bone cement plates ($30 \times 10 \times 1\,mm^3$) after 7 days of preparation. Samples containing DEAEMA and those without comonomer were dissolved in $CDCl_3$ while those containing MAA were dissolved in THF-d_8. The areas for the $CH_2=C-$ at δ 5.6 and 6.1 ppm and at 3.5 ppm for OCH_3 group of the MMA monomer were used to determine the percentage of monomer present in the total sample.

Determination of Glass Transition Temperature

The glass transition temperature (T_g) of bone cements was determined by means of a Perkin-Elmer DMA-7 (Perkin-Elmer Instruments) in tension. Bone cements machined to $20 \times 3 \times 0.1\,mm^3$ strips were deformed under a static force of 60 mN and with a dynamic force of 40 mN at a frequency of 1 Hz. Experiments were conducted from −50 to 150°C at a heating rate of 2°C/min and under nitrogen flow. T_g was determined from the peak of the Tan δ against temperature curve and the average of two measurements was used.

Determination of Mechanical Properties

Tensile strength (σ_T), compressive strength (σ_C), and bending strength (σ_B) values were measured as reported previously [16]. Shear strength (τ) was determined according to ASTM D5379 standard at a crosshead speed of 1 mm/min using a Iosipescu shear test fixture supplied by Wyoming Test Fixtures Inc. The special coupon was a rectangular flat strip

($76 \times 19 \times 2\,mm^3$) with symmetric centrally located v-notches (45°) with a 12-mm separation. For each type of deformation, at least five specimens were tested on an Instron 1125 after storing them at 25°C for 1 week. Impact strength (σ_I) was determined using notched (45°) rectangular beams according to ASTM D256 standard (Izod specimens). Beam dimensions were $63.5 \times 12.7 \times 6.35\,mm^3$ with a 10.16 ± 0.05 mm distance from the notch end and the edge of the beam. Samples were tested with an impact pendulum Resil 25 CEAST 6545 with a 0.5 J hammer and an impact speed of 3.46 m/s.

Fatigue analysis was carried out where the fatigue crack propagation rate was evaluated using the compact tension specimens ($B = 6$ mm, $W = 23$ mm, $a = 11.35$ mm, $\phi = 6$ mm, $H = 1.22$ W, $P = 0.55$ W, and $D = 1.25$ W). Tension–tension experiments with a constant load ratio ($R = P_{min}/P_{max} = 1/3$) were conducted at 2 Hz under load control. A mean load of 50 N for either MMA alone or MAA formulations and 60 N for DEAEMA containing bone cements was used. For each test, the number of cycles (N) and the length of the crack (a) were recorded. The crack length was measured by following the crack tip by means of a video camera CCD 4005R and video measuring system (VMS) with a resolution of 1 μm. From a plot of N versus a, the rate of crack propagation in one cycle (da/dN) was estimated from the slope. The amplitude stress intensity factor in mode I ($\Delta K_I = K_{IMax} - K_{IMin}$) was calculated by using Equations (1) and (2):

$$\Delta K_I = f(a/W)\frac{\Delta P}{B \cdot W^{1/2}} \tag{1}$$

$$f(a/W) = \frac{(2 + a/W)}{(1 - a/W)^{3/2}} \cdot \left[0.866 + 4.64 \cdot \left(\frac{a}{W}\right) - 13.32 \cdot \left(\frac{a}{W}\right)^2 + 14.72 \cdot \left(\frac{a}{W}\right)^3 - 5.6 \cdot \left(\frac{a}{W}\right)^4\right] \tag{2}$$

Constants for the Paris law, m the rate of increase of crack velocity during propagation and A the length/cycle, were obtained by plotting (da/dN) against ΔK_I on a logarithmic scale where m and A were the slope and intercept, respectively.

Porosity Determinations

Density measurements were used to estimate porosity, which is the ratio of observed to theoretical densities. The density was determined by Archimedes' Principle using a density kit attached to an Ohaus

Voyager V12130 balance. Water at 20°C was used as the standard of known density. Theoretical densities were calculated following the rule of mixtures, $\rho_{PMMA} = 1.2\,g/cm^3$, $\rho_{PDEAEMA} = 1.047\,g/cm^3$, and $\rho_{PMAA} = 1.293\,g/cm^3$.

Determination of *in vitro* Biocompatibility

Cell Adhesion and Proliferation

Bone cement discs of 10 mm diameter and 0.1 mm thickness were sterilized by dipping in absolute ethanol for 15 min and placed at the bottom of 24-well culture plates with Dubelcos Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum. The discs were seeded with primary osteoblasts (OB), obtained from the bone marrow of neonatal rat femur at a density of 5×10^3 cells/well and left in the culture for 24 and 48 h. After this period, samples were washed with Phosphate Buffer Saline (PBS) to remove nonadherent cells. The number of cells attached to the surface was determined by the MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay where 20 μL of MTT test solution was added to each well, incubated for 1 h and plates were read at 490 nm using a SLT Spectra I Spectrophotometer. Tissue culture plastic (TCPS) was used as a positive control and polyvinyl chloride (PVC) was used as a negative control.

Cell Morphology

Osteoblasts attached to TCPS after 4 h and those attached to bone cements, TCPS, and PVC after 48 h were fixed with 2.5% glutaraldehyde in PBS at 4°C for 30 min. Each sample was washed twice with cold PBS (30 min) and postfixed with 1% osmium tetroxide in Sabatini buffer (pH 7.4) containing sucrose (260 mOsm/L). After 1 h, samples were rinsed twice with Sabatini buffer and gradually dehydrated using a graded series of acetone washes (30, 50, 70, 90, 95, 100%). Samples were critically point-dried (Balzers CPD-020) and coated with gold/palladium (~20 nm), for SEM observation using a S-500 Hitachi microscope.

Statistical Analysis

For mechanical properties, the average and standard deviations of at least five repetitions were used. Statistical analysis was performed using one-way ANOVA where $p < 0.05$ was considered to be significant. For the biological properties study, the average and standard deviations of three repetitions were used. Statistical analysis was performed using the Student's *t*-test where $p < 0.05$ was considered to be significant.

RESULTS

Effect of Bone Cement Composition on the Curing Properties

The setting properties of the analyzed bone cements are summarized in Table 1. A high temperature (72.5°C) and a short setting time (2.6 min) were observed when MAA was present in the formulation. In contrast, a significantly lower temperature (37.5°C) and a longer setting time (9.1 min) were obtained when using DEAEMA in the formulation. Despite the differences in the chemical nature of these bone cements, the amount of residual monomer was similar in formulations with MAA or DEAEMA, which were higher than that of the formulation containing MMA alone. The addition of MAA to MMA rendered bone cements with a T_g of 120°C while the addition of DEAEMA resulted in a T_g of 83.9°C.

Effect of Bone Cement Composition on the Mechanical Properties

Table 2 summarizes the mechanical properties of the analyzed bone cements. Those prepared with MAA as comonomer exhibited significantly higher ($p < 0.05$) compressive strength values. However, the addition of either DEAEMA or MAA to MMA significantly reduced ($p < 0.05$) their tensile strength values. DEAEMA monomer incorporation resulted in a significantly higher ($p < 0.05$) impact strength while having no significant effect on the bending and shear strength values. Table 2 also shows that there is no statistically significant differences ($p < 0.05$) in the fatigue crack propagation rate within these bone cements. Generally, the mechanical properties exhibited by these bone cements correspond to materials of low porosity. Porosity is in the range of 1.0–2.3% being higher in formulations containing DEAEMA.

Table 1. Curing behavior of bone cements prepared with functionalized methacrylates.

Bone Cement	Residual Monomer Content (%)	T_g (°C)	T_{max} (°C)	T_{set} (°C)	t_{set} (min)
MMA	1.1	95.2	60.5 ± 3.8	40.2 ± 1.9	6.7 ± 0.2
MAA	3.5	120.0	72.5 ± 0.1	46.2 ± 0.1	2.6 ± 0.1
DEAEMA	3.8	83.9	37.7 ± 1.8	28.8 ± 0.9	9.1 ± 0.1

Table 2. Mechanical properties of bone cements prepared with functionalized methacrylates.

Bone Cement	Porosity (%)	σ_T (MPa)	σ_C (MPa)	σ_B (MPa)	τ (MPa)	σ_I (kJ/m^2)	ΔK_I (MPa m$^{0.5}$)	m	A (mm/cycle)
MMA	2.3	44.3 ± 4.7	104.6 ± 6.1	35.4 ± 3.8	23.8 ± 5.0	1.6 ± 0.1	0.893	7.14 ± 1.85	0.0068 ± 0.005
MAA	0.9	33.0 ± 3.6	131.8 ± 4.8	42.9 ± 7.7	24.9 ± 4.5	1.4 ± 0.2	0.9946	11.15 ± 3.15	0.0055 ± 0.0009
DEAEMA	1.2	33.3 ± 4.4	86.3 ± 2.8	35.6 ± 7.8	22.8 ± 1.9	1.8 ± 0.3	1.0015	7.07 ± 1.15	0.0003 ± 0.0009

Effect of Bone Cement Composition on Cell Adhesion and Proliferation

Figure 1(a) and (b) show the morphology exhibited by osteoblasts attached to TCPS at different incubation periods. The OB (1–2 μm) were round and oval after 4 h, while flat cells were observed after 48 h on these positive controls. Similar flat looking osteoblasts were observed on different bone cements after 48 h (Figure 1(c)–(e)). In contrast, OB

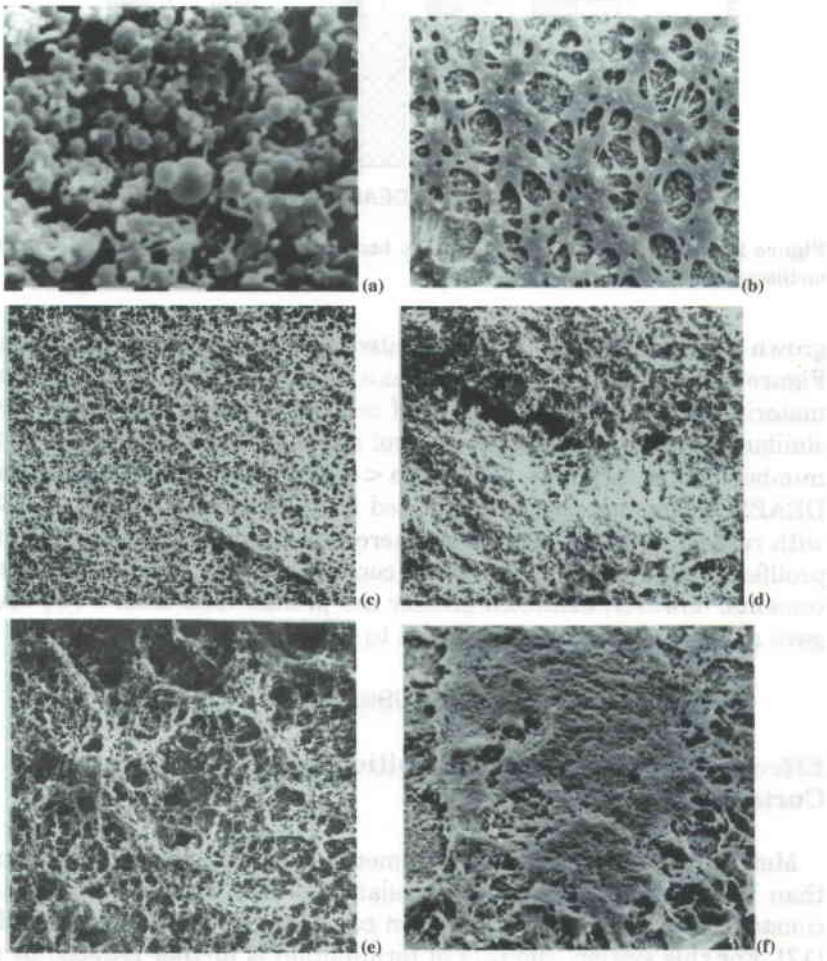

Figure 1. SEM micrographs of osteoblasts grown on: TCPS after 4 h (a); TCPS (b); MMA (c); MAA (d); DEAEMA (e); and PVC (f) after 48 h. Micrographs taken at 250×.

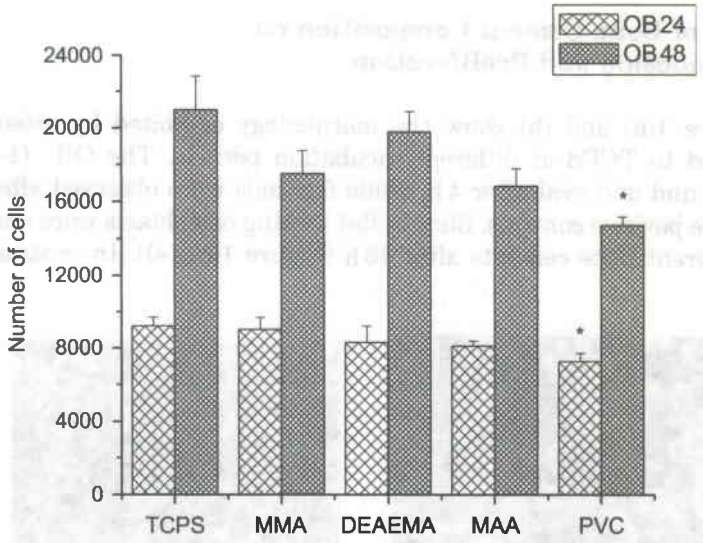

Figure 2. Number of OB cells attached to bone cements prepared with functionalized methacrylates.

grown on PVC (Figure 1(f)) exhibited characteristics of cell damage. Figure 2 shows the number of cells as a function of time for the analyzed materials. After 24 h the number of cells on the different cements was similar to that on the positive control and significantly higher than the number on the negative control ($p < 0.05$). After 48 h, the MMA and DEAEMA cements samples exhibited good osteoblast growth (80–85% with respect to TCPS). However, there was a reduction observed in the proliferation of cells on the MAA cement samples. All of these bone cements, however, exhibited greater cell proliferation than PVC, which gave a 55–60% reduction compared to TCPS.

DISCUSSION

Effect of Bone Cement Composition on the Curing Properties

Methacrylic acid-incorporated cements exhibited faster curing times than MMA cements. This can be related to MAA's higher propagation constant (K_p) and lower termination constant (K_t) than those for MMA [17]. For this system, the rate of termination is further reduced by the presence of the prepolymerized polymer beads (Nictone^{TM}) by increasing the viscosity of the medium, which implies that the K_p/K_t ratio is

further increased. To our knowledge, the rate constant for DEAEMA have not been determined, but following the aforementioned reasoning we can expect a lower propagation rate constant for DEAEMA and explain their corresponding longer curing times. As a consequence of the vitrification process, a low amount of monomer remained unreacted although this amount is within the values exhibited by commercial formulations.

Commercial bone cements exhibit longer curing times than the bone cements developed here but have similar maximum temperatures [1]. Although high temperatures are enough to cause bone necrosis it is generally accepted that the temperatures measured *in vitro* do not correspond with the actual values *in vivo*. This is because the actual temperature depends on the cement mantle thickness and heat dissipation by the surrounding fluids. The temperatures registered during this study were for cylindrical specimens ($6 \times 68\,mm^2$) prepared in accordance with ISO 5833 and it is expected that in the final application the maximum temperature reached would be lower.

Effect of Bone Cement Composition on the Mechanical Properties

The incorporation of either acidic or alkaline comonomers to standard bone cement formulations were shown to have a significant effect on some of the mechanical properties. By adding MAA, higher compressive strength values were observed. On the other hand, the addition of DEAEMA improved their impact properties.

The contrasting properties exhibited by MAA and DEAEMA containing formulation can be related to their glass transition temperature and porosity. Although cross-linking reactions have been suggested for systems containing tertiary amine [18], such an effect was not observed here with DEAEMA-based bone cements as the material dissolved in chloroform. The higher impact strength values exhibited by the DEAEMA containing formulations could be due to the long lateral chain of this comonomer.

Residual monomer has also been suggested to have an effect on the mechanical behavior of bone cements as they tend to plasticize the polymeric matrix. However, in all the analyzed cement formulations, the amount of residual monomer was not enough to plasticize the cement as their T_g were not severely reduced. The small T_g reduction observed in formulations prepared only with MMA can be attributed to the presence of minor amounts of ethyl methacrylate in the base polymer, Nictone™.

All the analyzed formulations fulfilled the minimum compressive strength (MCS = 70 MPa) requirements for bone cement applications, however, DEAEMA containing formulations exhibited a significantly lower ($p < 0.05$) compressive strength value than that prepared with MAA (Table 2). In terms of tensile strength values, neither ISO 5833 or F451 standards give the minimum requirements, therefore the lowest previously reported tensile strength values (MTS = 30 MPa) were used as the criteria for bone cements applications [9,19]. The cements analyzed here fulfilled this requirement and no significant differences ($p < 0.05$) were observed between those containing either MAA or DEAEMA. In contrast, the minimum bending strength (MBS = 50 MPa) was not satisfied by any of the formulations analyzed here and there were no significant differences ($p < 0.05$) between the acidic or alkaline bone cements. Currently, there are no minimum shear strength requirements for bone cements but results obtained here compared well with the value of 21.78 MPa, reported for PMMA by Funk and Litsky [20], and no significant differences were between the analyzed formulations.

There are a few reports on the impact properties of bone cements, however, these cannot be compared directly as they were measured according to DIN 53435 standards and using unnotched specimens [1]. This study demonstrates that a significantly higher impact strength ($p < 0.05$) is exhibited by low T_g bone cements, i.e. DEAEMA containing formulations, which can result in a greater difficulty to remove the cement mantle.

The fatigue crack propagation properties of bone cements were determined since these materials can fail well below their quasi-static strengths once they are subjected to cyclic loading. Table 2 shows that bone cements prepared with DEAEMA exhibited the highest ΔK_I in spite of using a greater average load and load average amplitude. Therefore, this formulation exhibited a lower rate of crack propagation. Although a large variation in the fatigue data was observed, an attempt was made to obtain m and A (Table 2). Samples containing MAA exhibited the highest value of m suggesting a higher rate of crack velocity during propagation. These values are in agreement with those found in literature, which range from 6.5 to 11.8 [21]. However, statistical analysis of these results revealed that there were no significant differences ($p < 0.05$) for m, A, and ΔK_I values among the different formulations suggesting that the addition of either functionalized monomer did not alter the fatigue properties.

Further studies are required to understand the mechanical behavior of these bone cements such as the use of the stress versus number of cycles (S–N curves) or the determination of their fracture toughness (K_{IC}).

Effect of Bone Cement Composition on Cell Adhesion and Proliferation

Cell adhesion to polymeric substrates is controlled by factors such as wettability, charge, and topography [22]. In this study, it is observed that the effect of chemical composition on cell adhesion is dependent on the incubation period. Osteoblast adhesion is similar after 24 h and there are no significant differences between the number of cells attached on the different bone cements (see Figure 2). After 48 h bone cements prepared only with MMA and those prepared with DEAEMA exhibited an increased cell number whereas, bone cements prepared with MAA exhibited a marked reduction in cell numbers. However, even in this case, the effect was not as severe as compared to the negative control, PVC.

A potential source of cytotoxicity in bone cements is the amount of residual monomer and other low-molecular weight compounds such as the tertiary amine used as activator [23]. Considering that the amount of DMPT is the same in all formulations, and that the amount of residual monomer is also similar for bone cements containing either MAA or DEAEMA (see Table 1), a more plausible explanation for the poor biocompatibility of MAA bone cements is the formation of its homopolymer. The formed poly(methacrylic acid) may diffuse from the cement due to its water solubility and reduce the pH of the culture medium. Although, it has been stated that methacrylates bearing longer alkyl chains are more cytotoxic as the membrane lipids are solubilized by the monomer [24], DEAEMA bone cements exhibited lower cytotoxicity. This may be due to its presence in low concentration and its cationic character (pKa of the polymer approximately 8), which would in turn allow for a better interaction with a negatively charged cell membrane.

The results of this study showed that some of the curing, mechanical, and biological properties can benefit from the addition of either acidic or alkaline methacrylate comonomers to MMA-based bone cements. Additional advantages could be expected after conditioning in simulated body fluid as a result of their more hydrophilic character as they can render low modulus bone cement that would yield or flow before fracture and result in better stress distribution. Similarly, the leaching of soluble homopolymers can expose bioactive fillers if incorporated in these formulations. Another advantage that could be considered is related to the ability of DEAEMA, a tertiary amine, to act as an activator in a similar manner to DMPT. DMPT may also induce the deprotonation of MAA leading to the formation of free radicals capable of initiating polymerization of acrylic monomers as it has shown in other systems [25,26].

CONCLUSIONS

The physical, mechanical, and biological properties of the bone cements developed in this study showed comparable behavior with MMA-based formulations. By adding MAA, the compressive properties were improved although their setting properties and biocompatibility were compromised. In contrast, the addition of DEAEMA improved the impact properties without altering their biocompatibility.

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REFERENCES

1. Kühn, K.D. (2000). Bone Cements: Up-to-date Comparison of Physical and Chemical Properties of Commercial Materials, pp. 36–135, Springer, Berlin.
2. Harper, E.J. (1998). Bioactive Bone Cements, In: *Proc. Instn Mech. Engrs*, 212H, pp. 113–120.
3. Saito, M., Maruoka, A., Muri, T., Sugano, N. and Hino, K. (1994). Experimental Studies on a New Bioactive Bone Cement: Hydroxyapatite Composite Resin, *Biomaterials*, **15**: 156–160.
4. Deb, S., Vázquez, B. and Bonfield, W. (1997). Effect of Crosslinking Agents on Acrylic Bone Cements based on Poly(methyl methacrylate), *J. Biomed. Mater. Res.*, **37**: 465–473.
5. Tanzi, M.C., Sket, I., Gatti, A.M. and Monari, E. (1991). Physical Characterization of Acrylic Bone Cement Cured with New Accelerator Systems, *Clin. Mater.*, **8**: 131–136.
6. Liso, P.A., Vázquez, B., Rebuelta, M., Hernaez, A.L., Rotger, R. and San Román, J. (1997). Analysis of the Leaching and Toxicity of New Amine Activators for the Curing of Acrylic Bone Cements and Composites, *Biomaterials*, **18**: 15–20.
7. ISO 5833: 1992 (E). Implants for Surgery – Acrylic Resin Cements.
8. ASTM F451-86 (1986). Standard Specification for Acrylic Bone Cement, In: *Annual Book of ASTM Standards, Medical Devices*, American Society for Testing Materials, Philadelphia.
9. Lewis, G. (1997). Properties of Acrylic Bone Cement: State of the Art Review, *J. Biomed. Mater. Res. (Appl. Biomater.)*, **38**: 155–182.
10. Deb, S., Di Silvio, L., Vázquez, B. and San Román, J. (1999). Water Absorption Characteristics and Cytotoxicity and Biological Evaluation

of Bone Cements Formulated with a Novel Activator, *J. Biomed. Mater. Res. (Appl. Biomater.)*, **48**: 719–725.

11. Méndez, J.A., Aguilar, M.R., Abraham, G.A., Vázquez, B., Dalby, M., Di Silvio, L. and San Román, J. (2002). New Acrylic Bone Cements Conjugated to Vitamin E: Curing Parameters, Properties, and Biocompatibility, *J. Biomed. Mater. Res.*, **62**: 299–307.
12. Zambonin, G., Colucci, S., Cantatore, F. and Grano, M. (1998). Response of Human Osteoblasts to Poly(methyl methacrylate) *in vitro*, *Calcif. Tissue Int.*, **62**: 362–365.
13. Ciapetti, G., Granchi, D., Stea, S., Cervellati, M., Pizzoferrato, A. and Toni, A. (2000). *In vitro* Testing of Ten Bone Cements after Different Time Intervals from Polymerization, *J. Biomater. Sci. Polym. Ed.*, **11**: 481–493.
14. Granchi, D., Ciapetti, G., Savarino, L., Cenni, E., Pizzoferrato, A., Baldini, N. and Giunti, A. (2002). Effect of Bone Cement Extracts on the Cell-mediated Immune Response, *Biomaterials*, **23**: 1033–1041.
15. Cenni, E., Granchi, D., Ciapetti, G., Savarino, L. and Corradini, A. (2002). Effect of Four Acrylic Bone Cements on Transforming Growth Factors – Beta1 Expression by Osteoblast-like Cells MG63, *Biomaterials*, **23**: 305–311.
16. Islas-Blancas, M.E., Cervantes-Uc, J.M., Vargas-Coronado, R., Cauich-Rodríguez, J.V., Vera-Graziano, R. and Martínez-Richa, A. (2001). *J. Biomat. Sci.: Polym. Edn*, **12**: 893–910.
17. Koros, R. and O'Driscoll, K.F. (1975). *Polymer Handbook*, John Wiley & Sons, New York, USA, II-45.
18. De Feng, X. (1992). *Makromol. Chem. Macromol. Symp.*, **63**: 1.
19. Kusy, R.P. (1978). *J. Biomed. Mater. Res.*, **12**: 271.
20. Funk, M.J. and Litsky, A.J. (1998). *Biomaterials*, **19**: 1561.
21. Planell, J.A., Vila, M.M., Gil, F.J. and Driessens, F.C.M. (1995). *Acrylic Bone Cements*, In: Wise, D.L., Trantolo, D.J., Altobelli, D.E., Yaszemski, M.J., Gresser, J.D. and Schwartz, E.R. (eds), *Encyclopedic Handbook of Biomaterials and Bioengineering*, Vol. 2, Part B, pp. 879–92, Marcell Dekker, New York.
22. Anselme, K. (2000). *Biomaterials*, **21**: 667.
23. Taningher, M., Pasquini, R. and Bonatti, S. (1993). *Environ. Mol. Mutagen*, **21**: 349.
24. Yoshii, E. (1997). *J. Biomed. Mater. Res.*, **37**: 517.
25. Horrison, J., Jerome, R. and Teyssie, P. (1986). *J. Polym. Sci. Polym. Lett. Ed.*, **24**: 69.
26. Farahani, M., Antonucci, J.M. and Karam, L.R. (1998). *J. Appl. Polym. Sci.*, **67**: 1545.