Acrylic Cement
Advantages or Disadvantages in Orthopaedic Surgery

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Acrylic cement was introduced into practice by Sir John Charnley 40 years ago. The advantages of this cement are the good and durable fixation of the hip and knee prostheses ensuring the distribution of the forces from the implant to the bone. It is also used in vertebroplasties, osteoporosis and bone substitutes. One of the major problems associated with the use of bone cements is the decrease of the mechanical properties in time due to the incompatibility between the organic matrix and the inorganic phase. In this respect we propose the replacement of the inorganic radio-opacifying agent with an iodine-based polymer that is compatible with poly(methylmethacrylate). The new iodine-based polymer proved to have good chemical stability, low toxicity against cells and excellent radio-opacity, so it could be a potential candidate for the manufacture of the orthopaedic cements.

Keywords: cement, PMMA, radio-opacity, cells

Acrylic cement was introduced in medical practice by Sir John Charnley 40 years ago and it is used for hip or knee prostheses. Commercial form, presently used is represented by a methyl ester of methacrylic acid (methyl methacrylate) which may undergo in the presence of benzoyl peroxide (BPO) and dimethyl-p-toluidine (DMPT) an isothermal polymerization reaction. As a result, the poly(methyl methacrylate) can consolidate in 10 minutes the prosthetic components with the host bone.

Nowadays, the number of cemented implants is equal to the number of non-cemented cements and the rate of survival of the first type is about 90% at 15 years. The main role of the cement is to stabilize the prosthesis and to ensure the distribution of the mechanical forces from the implant to the bone [1, 2].

The acrylic cement is also used in vertebroplasty. In this procedure, the cement is directly introduced in the fractured vertebral body under radiological or tomographical control; it is used for osteoporosis to consolidate the spine. A special case is the kyphoplasty where a special balloon is introduced in the vertebral body and then it is filled with acrylic cement.

Another important application of the acrylic cement is to substitute bone defects as a result of major resections (especially fatigue) occurs; become over time places where the mechanical failure (especially fatigue) occurs;

- the dissolution of the inorganic phase
- the filler may leach out of the material.

To overcome this problem, the inorganic fillers are desired to be replaced with radio-opaque polymers that contain covalently bound heavy elements [7-10]. Thus, new polymeric biomaterials, which combine the X-ray visibility with biocompatibility and better mechanical properties, are projected to be developed. These polymers are produced by introduction of a radio-opacifying element into the monomeric building block. It can be expected that, in this way, X-ray visibility is introduced in polymers, without any negative influence on their physico-chemical or mechanical properties, and biocompatibility [9-13]. Moreover, the polymerization or copolymerization of such monomers would produce X-ray radio-opaque materials without the disadvantage of two-phase system.

The cement has the ability to activate the macrophages stimulating in this way the cytokines production (TNF-alpha, IL-1-beta, IL-6), which are substances responsible for massive destruction of the adjacent bone by resorption processes [2].

Another important factor that leads to de-cementation of the prostheses is the cement's porosity due to the mixing of 2 different samples (solid-liquid) and the formation of air bubbles during mixing. A high or a low degree of porosity determines the point-like fixation of the implant, which determines shearing phenomena at the cement-implant interface. Some problems were solved by mixing the components of the cement in vacuum [4].

The simplest manner to induce radio-opacity to polymeric biomaterials is to use an additive that consists of a relatively heavy element, such as barium, zirconium, bismuth, lead, platinum or gold. Nowadays the additive is introduced as a salt (BaSO4), as an oxide (ZrO2), or as powdered metallic particles (Au, Pb). Unfortunately, this practice leads to 2 important undesirable effects due to the fact that fillers (inorganic compounds) are incompatible with the polymeric (organic) matrix. The undesirable effects [5-9] are:

- the phase-boundaries between filler and polymer may become over time places where the mechanical failure (especially fatigue) occurs;
- the filler may leach out of the material.

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**Materials and method**

**Polymer synthesis**

MMA (Merck) was used after a preliminary purification by distillation in vacuum (63°C and 200 mmHg). The iodine-containing monomer namely 2-(2'-iodobenzoyle)-ethylmethacrylate (IBEM) was prepared in pure form. 2-hydroxyethyl methacrylate (HEMA) (Fluka) was distilled under reduced pressure (45°C, 2.1·10⁻³ bar) and stored at -20°C. Ethylene glycol dimethacrylate (EGDMA) (Aldrich) was used as such without any further purification. Benzoyl peroxide (BPO) was employed from Merck and purified through recrystallization from methanol at 40°C. Toluene (S.C. Reagent) was distilled (110°C, 1 bar pressure) and stored at +8°C. Dichloromethane and triethylamine were purchased from Aldrich and used without purification. Tetrahydrofuran (THF, Merck) was purified by distillation in the following manner: first, it was distilled on cuprous chloride (CuCl), then the medium fraction was deposited on potassium hydroxide pellet (KOH) overnight and finally it was rectified over metallic sodium (66.8°C).

**Synthesis of IBEM (fig. 1)**

A solution of 2-iodobenzoyle chloride (10g, 37.5 mmoles) in 50mL of anhydrous dichloromethane was added dropwise (60 min) at -5°C to a magnetically stirred solution of 2-hydroxyethyl methacrylate (5.85g, 45 mmoles) and triethylamine (7.5g, 75 mmoles) in 150mL of dichloromethane. The stirring was continued for 1 hour at room temperature.

The yellow mixture was then cooled down at -5°C and 200mL of water were added carefully. The organic phase was separated and washed with 150mL of NaHCO₃ saturated solution and 150mL of saturated brine solution. The organic phase was dried over MgSO₄, filtered and stored at +8°C. Dichloromethane and triethylamine were transferred to Photoshop CS (Adobe Inc software) and the mean grey level of each polymer sample was determined and ranged from 0 to 255. The X-ray absorbance was determined as A= 255-grey level and ranged from 0 (no X-ray absorption) to 255 (100% absorption), expressed in arbitrary unit control.

**Preparation of the copolymers MMA-IBEM**

Copolymers were prepared in solution for primary characterization and in bulk for preparing pellets and cylinders that can be used for in vitro experiments. Copolymerisation in solution of MMA-TIBOM is done for 2 different compositions MMA-IBEM (90:10 and 80:20 molar ratio). The reaction was carried out in the presence of benzoyl peroxide (8·10⁻³ mole/L) as initiator and toluene as solvent. The copolymerization was performed at 75°C and under nitrogen atmosphere. The copolymers obtained were precipitated in ethyl alcohol, dried at 40°C and purified by repeated washing with ethyl alcohol.

**For bulk copolymerization**, the monomers, initiator ([BPO]=10⁻² moles/moles monomers) and the cross-linking agent EGDMA (3% molar with respect to monomers mixture) were mixed together by vortexing at 30 Hz, then poured into polyethylene moulds (20 mm in diameter and 30 mm in height). Polymerizations were carried out in inert atmosphere, at 75°C for 5 h, followed by post-polymerization at 110°C, for 3 h. The obtained pellets were immersed in ethanol for 2 days to extract the residual monomers.

For toxicity assays, cells were harvested by splitting with trypsin and seeded in 24-multi-well plated (φ = 15 mm), at the concentration of 1·10⁵ cells/mL (1mL/well). The cells were maintained in culture in RPMI medium (containing FCS) during 2 days. After 2 days of culture, medium was removed, cells were washed 3 times in phosphate buffer solution (PBS 1x) (to remove dead cells and debris), fresh medium was added and pellets of polymer were introduced in each well (one pellet per well). Cells were incubated over night in contact with pellets.

**Measurement of X-ray radio-opacity of the copolymers**

The X-ray opacity of the samples was measured on 2D sections of cylinders examined with an X-ray microtomograph (Elias Hospital, Bucharest, University of Angers, France). MMA-IBEM copolymers were fixed on a brass stub with plasticine and a piece of human trabecular bone was used as control. 2D sections were obtained after reconstruction from the projection images obtained in the cone beam mode. On each 2D section coded on 8 bits, the different polymer cylinders were evidenced in cross section. Five sections (separated by 200 µm) were transferred to Photoshop CS (Adobe Inc software) and the mean grey level of each polymer sample was determined and ranged from 0 to 255. The X-ray absorbance was determined as A= 255-grey level and ranged from 0 (no X-ray absorption) to 255 (100% absorption), expressed in arbitrary unit control.

**Fig. 1. Chemical strudure of 2-(2'-iodobenzoyle)-ethyl methacrylate**

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A series of copolymers MMA-IBEM were synthesized as filaments with increasing concentrations of IBEM (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 molar composition). The reactions were carried out in polyethylene ampoules with BPO as initiator, at 75°C under inert atmosphere, for 8 h. The polymers were extracted with ethanol for 2 days to remove the residual monomers.

**Characterization techniques of the copolymers**

The copolymers were purified by repeated extraction with alcohol in Soxhlet to remove the unreacted monomer and other impurities.

**FTIR characterization of the polymeric biomaterials**

The copolymers were characterized by FTIR to confirm their structure by measuring the absorption spectrum for the identification of the chemical bonds in their molecule.

**Evaluation of the cytotoxicity by in vitro assays**

The macrophage is considered to be an important cell in the initial non-specific host response against biomaterials. Macrophages are responsible of the elimination of foreign bodies in the organism.

**1. Cell culture**

The cells were maintained in Roswell Park Memorial Institute (RPMI) culture medium containing 10% foetal calf serum (FCS) and 100 UI/mL of penicillin and 100mg/mL of streptomycin sulphate. Cells were cultured at 37°C in a humidified incubator with 5%CO₂.

**For cytotoxicity assays, pellets were sterilized in RPMI under UV light during 1 night.**

For toxicity assays, cells were harvested by splitting with trypsin and seeded in 24-multi-well plated (φ = 15 mm), at the concentration of 1·10⁵ cells/mL (1mL/well). The cells were maintained in culture in RPMI medium (containing FCS) during 2 days. After 2 days of culture, medium was removed, cells were washed 3 times in phosphate buffer solution (PBS 1x) (to remove dead cells and debris), fresh medium was added and pellets of polymer were introduced in each well (one pellet per well). Cells were incubated over night in contact with pellets.

**2. Toxicity test**

After one day of contact with pellets, polymer was removed and cells were stained by trypan blue (0.1% in PBS 1x) during 2 min. Trypan blue was removed and photos were taken.
Results and discussions

Characterisation of the copolymers

Comparison of the FTIR spectra of PMMA and the copolymer MMA-IBEM confirmed the presence of specific bands: iodine bonded to the phenyl ring – 993.0 cm⁻¹, C=O band for methacrylate moiety – 1732 cm⁻¹ (spectra not shown).

X-ray analysis

MicroCT analysis is a very important device in evaluation the radio-opacity of bone and different polymeric biomaterials.

In figure 2, copolymers MMA-IBEM with different fractions of IBEM are compared with the X-ray opacity of a piece of human trabecular bone and with commercial cement containing as radio-opacifying agent zirconium dioxide grains.

The X-ray opacity of the copolymer MMA-IBEM increased linearly with IBEM fraction. One can see from the graph that a concentration of 0.2 molar in IBEM within the polymer confer similar radio-opacity than the zirconium particles of the orthopaedic cement.

This copolymer shows an excellent radio-opacity due to the presence of the iodine atom bound to the polymer matrix and therefore, the copolymer MMA-IBEM could successfully replace the barium sulphate or zirconium dioxide from the classical bone cements.

Cytocompatibility assays using in vitro culture

The results from the cytotoxicity tests, as expressed by SEM microphotographs, are presented in figures 3 and 4. The cells were encountered at the surface of the polymer disks after two days. Cells were mainly found in a round shape, but sometimes, they exhibited an elongated shape. Some cells exhibited numerous thin filopodia, allowing the anchorage to the disks. Cells with short extensions are also present that could lead to their death. It is possible to have in that region some ppm of unreacted MMA monomer that could lead to the presence of dead cells.

As a conclusion we may say that the biocompatibility of the copolymer MMA-IBEM is good, so from this point of view the copolymer could be a potential candidate for incorporation into bone cements.

Conclusions

This article offers an important perspective in the obtaining of bone cements with new iodine-based polymers as radio-opacifying agents in the final recipe. The copolymer MMA-IBEM could be a potential candidate for the manufacture of bone cements as it has good chemical stability, low cytotoxicity combined with excellent radio-opacity. In a future article the mechanical properties of the new cement will be presented as compared to the mechanical properties of the commercial barium sulphate or zirconium dioxide cements.

References

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