Materials Science and Engineering C 62 (2016) 274–282

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Comparison of a quasi-dynamic and a static extraction method for the cytotoxic evaluation of acrylic bone cements

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ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form 5 January 2016
Accepted 20 January 2016
Available online 21 January 2016

Keywords:
Bone cement
PMMA
Cytotoxicity
In vitro
Extraction conditions
Cell culture

ABSTRACT

In this study, two different extraction approaches were compared in order to evaluate the cytotoxicity of 7 different acrylic bone cements, mainly developed for spinal applications, to osteoblastic cells. Firstly, a static extraction was carried out continuously over 24 h, a method widely used in literature. Secondly, a quasi-dynamic extraction method that allowed the investigation of time-dependent cytotoxic effects of curing acrylic bone cements to cells was introduced. In both cases the extraction of the cements was started at a very early stage of the polymerization process to simulate the conditions during clinical application. Data obtained by the quasi-dynamic extraction method suggest that the cytotoxicity of the setting materials mainly originates from the release of toxic components during the first hour of the polymerization reaction. It was also shown that a static extraction over 24 h generally represents this initial stage of the curing process. Furthermore, compared to the static extraction, time-dependent cytotoxicity profiles could be detected using the quasi-dynamic extraction method. Specifically, a modification of commercial Osteopal®V with castor oil as a plasticizer as well as a customized cement formulation showed clear differences in cytotoxic behavior compared to the other materials during the setting process. In addition, it was observed that unreacted monomer released from the castor oil modified cement was not the main component affecting the toxicity of the material extracts. The quasi-dynamic extraction method is a useful tool to get deeper insight into the cytotoxic potential of curing acrylic bone cements under relevant biological conditions, allowing systematic optimization of materials under development.

1. Introduction

Acrylic bone cements based on poly(methyl methacrylate) (PMMA) are widely used for joint prosthesis fixation as well as injectable augmentation materials in spinal applications such as percutaneous vertebroplasty and balloon-kyphoplasty [1–4]. Despite their widespread use it is well known that bone cements based on PMMA can possess an inherent cytotoxicity although the fully cured cement is considered bioinert. After implantation, acrylic bone cements have been reported to trigger tissue necrosis, fibrosis and impaired bone remodeling in the vicinity of the implant [5,6]. Such adverse effects are believed to be caused mainly by the exothermic setting reaction and the release of cytotoxic components present in the cement. In this context, in vitro studies have shown that extracts from acrylic bone cements may impair the viability and proliferation of different cell types, including fibroblasts [7], osteoblasts [8–10], promyelocytes [9], and lymphocytes [11]. Especially unreacted monomer, methyl methacrylate (MMA), is generally considered as the most cytotoxic cement constituent. This is because it is present in significantly higher amounts than any other liquid constituent [12] and it has also been described as genotoxic [11]. Additionally, N,N-dimethyl-*p*-toluidine (DMPT), used as an activator of the polymerization reaction, is known to have a dose dependent cytotoxic effect on cells, and to reversibly inhibit the cell replication cycle [7,8]. Other cement components, benzoyl peroxide (BPO) and the radiopacifier barium sulfate ($BaSO_4$), have also been identified as cytotoxic and can induce the production of inflammatory cytokines in osteoblastic cells [7,13]. Moreover, free radicals, which are reactive species present during polymerization, are also described to be cytotoxic to osteoblastic cells [14].

The cytotoxicity of injectable acrylic bone cements is generally assessed using material extracts according to the ISO-10993 standard [15]. The standard defines that for in situ curing materials the extraction should be started from the point in the curing process at which the material is used in the final application. In general, acrylic bone cements are applied within the first 10 min after mixing the liquid and powder precursors, depending on cement brand and room temperature. This time limitation is caused by the fact that the viscosity of the material increases after mixing as the polymerization process advances, which restricts the maximum application time especially in minimal invasive procedures such as vertebroplasty. As a result, the cement comes into contact with the bone tissue and the body fluids in a liquid or doughy

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http://dx.doi.org/10.1016/j.msec.2016.01.048
0928-4931/

state. However, in the majority of investigations related to the cytotoxicity of acrylic bone cements, extracts from nearly fully polymerized or fully polymerized cements have been used [7–9,13]. This approach does not replicate the clinical situation and somewhat misleading conclusions may be drawn regarding material biocompatibility. Furthermore, the extraction is normally carried out continuously for at least 24 h. In contrast, the release of cytotoxic components from the cement is time-dependent. For example, the release of residual MMA from the curing cement has been proven to be a diffusion driven process characterized by a classical burst release followed by a plateau phase [12,16,17]. Results of these studies also suggest that the majority of leachable monomer (up to 3% of total content) is transferred to the aqueous phase during the first hour of polymerization. Furthermore, the release of DMPT from the cement matrix is almost constant during the first 24 h and decreases afterwards [8]. Therefore, a proper cytotoxic evaluation of acrylic bone cements should also consider possible time-dependent effects during the curing process.

In addition to available commercial formulations, PMMA-based cements are sometimes modified in the clinics or in an experimental setting for different reasons. Increasing the radiopacifier content of PMMA formulations designed for joint prostheses fixation is a common practice in the clinics to enable the use of the cement in spinal applications [18,19]. Furthermore, several studies focused on modifying commercially available products to decrease the stiffness of the cement [20–24], which has been hypothesized to affect the number of complications associated with vertebroplasty [25–27]. However, most of these modifications interfere with the polymerization reaction, which can favor the leakage of cement components. In turn, this affects the cytotoxicity and makes it necessary to critically investigate the biocompatibility of the resulting materials.

The aim of the present study was to establish a quasi-dynamic extraction method to test the cytotoxicity of commercial as well as investigational acrylic bone cements designed for spinal applications. Based on the issues discussed above, this method intends to more closely emulate the in vivo situation during clinical application. Therefore, the extraction was started at an early stage of polymerization, representing a worst-case scenario under clinical conditions and simultaneously enabling cement curing in a biological environment. Moreover, the extraction media were withdrawn and replaced at different time intervals to assess the time-dependent cytotoxic effects of the materials due to the leakage of cement constituents or by-products from the curing cement matrix. Furthermore, by replacing the extraction media a dilution effect is simulated, which is also a natural event after application in the body. For comparison, an extraction was carried out under static conditions, i.e. continuously over 24 h. Saos-2 human osteoblast-like cells were used to evaluate the extracts' cytotoxicity.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma-Aldrich unless otherwise stated. Linoleic acid and castor oil were sterile filtered using a 0.2 μm syringe filter. $BaSO_4$ and PMMA (mean particle size = 95 μm) powders were sterilized under ultraviolet light for 1 h prior to use.

The chemical compositions of the different bone cements are depicted in Table 1. Three commercially available (unmodified) and four investigational (modified) cements were investigated. One cement for prosthesis fixation, Simplex®P (Stryker Corporation), and two bone cements for vertebroplasty, Vertecem V+ (Synthes GmbH) and Osteopal®V (Heraeus Medical GmbH), were used as unmodified formulations according to the instructions of the manufacturer. Furthermore, the following cement modifications were included in the study:

A modified Simplex®P cement was produced by adding $BaSO_4$ (BS) up to 30 wt.% of the cement powder phase. Increasing the amount of radiopacifier in acrylic bone cements that are intended for prosthesis fixation is a common practice in the clinical setting in order to use them for vertebroplasty, as previously mentioned [18,19].

In addition, Osteopal®V bone cement was modified with either linoleic acid (LA) or castor oil (CO). For both additives it has been shown that the natural oils can act as plasticizer lowering the Young's modulus of the resulting materials [23,24,28]. The cements were obtained by replacing appropriate volumes of the liquid phase by linoleic acid (final concentration 0.75 wt.%) or by adding castor oil to the liquid phase (final concentration 17.8 wt.%). Prior to mixing the cement components, the solutions were thoroughly vortexed to dissolve the additives.

Finally, a laboratory customized cement was prepared. This cement was made to replicate as close as possible the composition of Vertebroplastic® using commercially available, individual components, since such formulations are sometimes found in experimental studies on acrylic bone cements [29].

2.2. Cement preparation and extraction conditions

All steps of the cement preparation and the liquid handling were carried out under a laminar flow hood to assure sterile conditions. In general, the extraction was carried out according to the recommendations of the ISO-10993 standard [15] with some amendments described as follows.

Each cement was produced by adding the liquid phase to the powder phase in a 50 mL centrifuge tube made of polypropylene (PP, Fisher Scientific). The respective powder-to-liquid ratios are

Table 1
Chemical compositions of the used acrylic bone cements (based on the information provided by the manufacturers) and their respective powder-to-liquid ratio (P/L). All components of the customized cement were obtained from Sigma-Aldrich. n/a: declared substance but unknown concentration.

	Simplex®P	Simplex®P + BS	Osteopal®V	Osteopal®V + LA	Osteopal®V + CO	Vertecem V+	Customized cement
Powder (% w/w)							
Poly(methyl methacrylate)	15	11.0	–	–	–	–	69
Poly(methyl acrylate-co-methyl methacrylate)	–	–	54.6	54.6	54.6	44.6	–
Poly(methyl methacrylate-co-styrene)	73.7	51.0	–	–	–	–	–
Barium sulfate	10	37	–	–	–	–	30
Zirconium dioxide	–	–	45	45	45	40	–
Hydroxyapatite	–	–	–	–	–	15	–
Benzoyl peroxide	1.3	1.0	0.4	0.4	0.4	0.4	1
Chlorophyll VIII	–	–	n/a	n/a	n/a	–	–
Liquid (% v/v)							
Methyl methacrylate	97.5	97.5	97.9	94.8	59.10	99.3	98
N,N-dimethyl-p-toluidine	2.5	2.5	2.1	2.1	1.65	0.6	2
Hydroquinone (ppm)	5–9	5–9	n/a	n/a	n/a	60	–
Linoleic acid (LA)	–	–	–	3.1	–	–	–
Castor oil (CO)	–	–	–	–	39.25	–	–
Powder-to-liquid ratio (g/mL)	2	2	2.6	2.6	2.1	2.2	1.7