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Eugenol derivatives immobilized in auto-polymerizing formulations as an approach to avoid inhibition interferences and improve biofunctionality in dental and orthopedic cements

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Abstract

Auto-polymerizing formulations based on poly(ethyl methacrylate) (PEMA) and eugenol derivatives are reported for dental and orthopedic applications. Spherical beads of PEMA were used as the pre-polymer powder and mixed with combinations of ethyl methacrylate and eugenyl methacrylate (EgMA) or ethoxyeugenyl methacrylate (EEgMA). A range of concentrations from 10 to 30 wt.% of EgMA or EEgMA were used to impart bioactivity properties to the cements. Increasing concentrations of the eugenol derivatives decreased the maximum polymerization temperatures from 69 to 37 °C without altering the working or setting time. At concentrations of 10 and 15 wt.% of EgMA or EEgMA a noticeable increase in the compressive (8%), flexural (40%) and tensile (24%) strengths were recorded in comparison to the control cements containing PEMA/ethyl methacrylate only. In addition to the improvement in mechanical properties the cements yielded a slightly crosslinked network due to the participation of the allylic group present in the eugenol derivatives, the presence of which has an intrinsically bactericidal effect against *Escherichia coli* and *Streptococcus mutans* strains, as reported in a previous study, thus enhancing the properties of the cements.

Keywords: Poly(ethyl methacrylate); Auto-polymerizing formulations; Eugenol derivatives; Dental cements; Orthopedic cements

1. Introduction

Auto-polymerizing cements based on methacrylates are widely used as synthetic biomaterials in dentistry [1], and craniofacial and orthopedic surgery [2]. The most commonly employed materials used clinically consist of poly (methyl methacrylate) (PMMA) microparticles (beads) with methyl methacrylate (MMA) as the polymerizable monomer, which reacts via a redox reaction based on benzoyl peroxide (BPO) as initiator and a tertiary aromatic amine (*N*,*N*-dimethyl-4-toluidine), as the activator under physiological conditions [3,4].

This traditional composition has been employed over the last 40 years without significant changes despite some disadvantages derived from its components and way of application, such as the high exotherm of the polymerization reaction which can evoke cellular necrosis in the surrounding tissues or the prosthesis loosening in the longer term. The toxicity of the aromatic tertiary amine (*N*,*N*-dimethyl-4-toluidine) [5] is of concern and other chemically stable compounds such as phenolates and free radicals produced during the curing reaction are likely to produce adverse cytotoxic effects during several weeks post polymerization, inducing a local inflammatory response in the surrounding tissues [6–8].

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Self-polymerizing systems based on poly(ethyl methacrylate/*n*-butyl methacrylate) with better handling, lower modulus and higher ductility in comparison to the conventional PMMA-based cements have been reported for use as orthopedic bone cements [9–11]. Other successful modifications include the incorporation of crosslinking agents that have resulted in improved mechanical properties due to the formation of three-dimensional networks via crosslinking [12–15]. In general terms, it can be said that crosslinking points are able to restrain excessive movements, maintaining the properties of the network and enhancing its mechanical resistance without altering the handling properties or curing kinetics.

On the other hand, it is current practice to use zinc-oxide-eugenol auto-polymerizing cements in dentistry in combination with other widespread restorative materials based on dimethacrylates and other root canal sealers. One particular problem associated with the use of such combination is the incompatibility of both systems as a consequence of the presence of free eugenol, which acts as a free radical scavenger inhibiting the cure of dental composite resins [16]. However, the analgesic and anti-inflammatory activities [17], antimicrobial and anti-aggregating function [18], antipyretic activity [19], anti-anaphylactic properties preventing mast cell degranulation [20] and the capacity to prevent lipidic peroxidation [21] of eugenol makes it an attractive component in the cements for both dental and orthopedic applications. Thus, combining the properties of the eugenol-based restorative materials and auto-polymerizing acrylic resins in the same system is an interesting concept which has not been exploited so far due to the inhibitory effects of the eugenol molecule.

In previous works, our research group has synthesized two eugenyl methacrylate derivatives, eugenyl methacrylate (EgMA) and ethoxyeugenyl methacrylate (EEgMA), which were successfully copolymerized with alkyl methacrylates [22,23]. Rheological analysis of these systems revealed a pseudo-solid-like behaviour with relatively high values of average molecular weights between crosslinks ($\sim 10^4$ Da), confirming the proposed network structures of branched systems with a slightly crosslinked degree, in which a certain amount of the eugenyl moieties remained pendant from the main chain [24]. It was also demonstrated by different agar-test methods that these new materials possessed intrinsically bactericidal properties, showing clear halos of inhibition, up to 7 mm, against different strains such as *Escherichia coli* and *Streptococcus mutans*, and consequently indicating activity against microorganisms commonly found in both dental and osseous cavities [25]. In addition, the immobilization of eugenol is advantageous as it avoids the migration of this molecule to the surrounding tissues and improves its hydrolytic stability.

Thus, the incorporation of eugenyl methacrylate derivatives (MEg) in auto-polymerizing formulations based on methacrylates via copolymerization reaction yielding slightly crosslinked polymer networks is an attractive alternative to impart biological properties and improved mechanical resistance to the resultant cements without the customary inhibitory effects of the inclusion of native eugenol. The objective of this study was to examine the effect of EgMA and EEgMA in poly(ethyl methacrylate)/ethyl methacrylate-based self-curing cements. Furthermore 4,4′-bis(dimethylamino)diphenyl carbinol (BZN) was employed as an activator in the cement formulations due to its established lower cytotoxicity [5].

2. Materials and methods

2.1. Materials

Eugenyl methacrylate (EgMA) and ethoxyeugenyl methacrylate (EEgMA) monomers were synthesized in our laboratories as reported previously [22]. Ethyl methacrylate (EMA) and methyl methacrylate (MMA) stabilized with 100 ppm monomethylether of hydroquinone were supplied by Acros (Spain) and used as received without further purification. Benzoyl peroxide (BPO) supplied by Merck (Germany) was used after fractional crystallization from ethanol. 4,4′-bis(dimethylamino)diphenyl carbinol (BZN) (Fluka, Spain), poly(vinyl alcohol) (PVA) powder (99%-hydrolysed, $M_w = 90{,}000$) (Aldrich, Spain) used as surfactant, poly(methyl methacrylate) (PMMA) beads (Degussa, Spain) and poly(ethyl methacrylate) (PEMA) beads (Aldrich, Spain), were used as received.

2.2. Synthesis of PEMA beads

PEMA beads were prepared using suspension polymerization [26]. An aqueous solution containing 3% (w/v) of PVA was added as a suspension agent to a three necked flask fitted with a nitrogen inlet, mechanical stirrer and a condenser, which was heated to 70 °C, stirred at 600 rpm and flushed with nitrogen for 10 min prior to the addition of 200 ml of EMA monomer in which the initiator, BPO (2% w/w with respect to monomer) was dissolved. Polymerization was carried out at 70 °C over 3 h followed by another 2 h at 80 °C to complete the reaction. The microparticles obtained were filtered, thoroughly washed with water and dried at 60 °C until constant weight. The chemical composition of microparticles was analysed by proton NMR spectroscopy. ^{1}H NMR spectra were recorded at 25 °C in $CDCl_3$ using tetramethylsilane (TMS) as internal standard on a Varian XLR-300 spectrometer. The morphology of the microparticles was analysed by scanning electron microscopy (SEM) using a Philips XL 30 ESEM apparatus at an accelerating voltage of 15 keV. The samples were sputter-coated with gold before examination.

The particle size distribution was determined by laser scattering using a Coulter LS320 (Beckman). Approximately 10 mg of the sample was thoroughly dispersed in 2 ml of water:ethanol 1:1 and an average of three readings were recorded for each sample. Number and weight average molecular weights were determined by size exclusion

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