Estrogenic activity of monomers and initiators used in resin based dental composites

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Introduction

Due to the possible negative effects of elementary mercury on human health and the environment, the World Health Organization (WHO) has suggested to phase-out using amalgam as a dental restorative[1]. As an alternative, resin based dental materials are often used. Even though these dental composites have already been routinely used for several decades, their biocompatibility has not yet been fully characterized. The resin matrix of dental composites is mainly based on a mixture of dimethacrylates. Currently, the most used dimethacrylates are Bisphenol A glycidyl methacrylate (BisGMA), ethoxylated BisGMA (BisEMA), triethyleneglycol dimethacrylate (TEGDMA) and urethane dimethacrylates (UDMA)[2]. Other than the monomers, the resin matrix also contains an initiator system to start the polymerization process, as well as stabilizers. Besides the resin, the composite further contains inorganic filler and a coupling agent, usually an organo-silane, that bonds the reinforcing filler to the resin matrix[3].

A problem with using these dental composites is that the polymerization process is not complete, with polymerization rates ranging from 50-70%, leaving unreacted monomers that can be released into the mouth[4]. Some of these monomers, such as BisGMA, BisEMA and the less frequently used Bisphenol A Dimethacrylate (BisDMA), are derived from Bisphenol A[5]. Since BPA is used in the manufacturing process, trace amounts of BPA may be present in the final composite[6].

Bisphenol A is one of the highest volume chemicals produced worldwide, with more than 2 million tons produced each year[7]. It's used in number of different applications, such as epoxy resins, polycarbonate plastics, toys, drinking containers, medical equipment and thermal receipts[8]. BPA is also a well-known endocrine disruptor[9]. Due to the possible adverse health effects related to the use of BPA, there has been an increasing need for alternatives. Nowadays, bisphenol analogues such as Bisphenol S, Bisphenol F, Bisphenol C, Bisphenol B etc. are often used as a replacement for BPA[10]. Since these compounds are structural analogues to BPA, their interactions with the estrogen receptor might be similar. BPF is interesting in particular, since BPF epoxy resins are used in several consumer products, including in dental sealants[11].

The aim of this study was to determine the estrogenic activity of 9 monomers (BisGMA, TEGDMA, BisEMA (3), BisEMA (6), BisEMA (10), BisDMA, UDMA, BADGE and TCD-DI-HEA) and 3 initiators (CQ, EDMAB, HEMA), as well as BPA and BPF using the Estrogen Responsive Elements Chemical Activated Luciferase Gene Expression (ERE-CALUX) bioassay.

Materials and methods

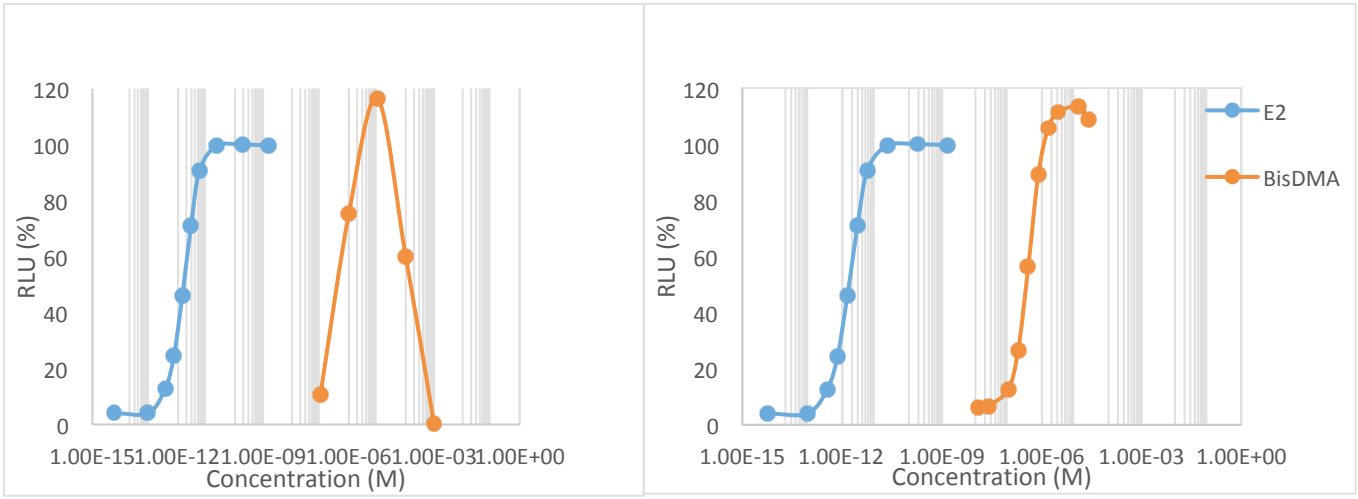
To start, a range finding experiment was performed for all the compounds to see if there was any estrogenic activity present, and in which concentration range. A 5 or 10-point consecutive dilution sequence was made (depending on the potency), starting from a stock solution of 0.1M, with a dilution factor of 10. This provides a broad concentration range, with treatment concentrations ranging from 10^{-3}M to 10^{-13}M. The process was applied to all compounds, except for BPA since the active concentration range is already known. Afterwards, for the compounds that show estrogenic activity, a full dose response curve was made using a 10-point dilution sequence with a dilution factor 2. All dilutions were made using dimethyl sulfoxide (DMSO) as a solvent and carrier agent.

The ERE-CALUX bioassay was used to determine the estrogenic activity of the compounds. This is a bioanalytical tool that uses a human breast cancer cell line, which is stably transfected with an ER responsive luciferase reporter gene (VM7Luc4E2). 17β-Estradiol (E2) was used as a reference standard.

The cells were maintained in α-MEM (alpha Minimum Essential Medium), supplemented with 1% pen-strep, 2% L-glutamine and 10% FBS (Fetal Bovine Serum). The cells are incubated at 37°C, 5% CO_2 and 80% humidity. At least 48 hours before dosing, the cells were transferred to DMEM (Dulbecco's Modification of Eagle's Medium), supplemented with 4.5% charcoal stripped FBS, 2% L-glutamine, 1% pen-strep and 1% sodium pyruvate. For harvesting, phenol-red free trypsin was added to collect the cells, which were counted and the cell suspension was diluted to 200 000 cells/mL. When seeding 200µL in every well of the 96-well plate, the final amount will be 40 000 cells/well. After incubating for 24h, the medium was removed and each well was dosed with 190µL DMEM, containing 1% DMSO with the reference standard (E2) or the compound of interest, which are both dosed in triplicate. After incubating the 96-well plate for 19-22 hours, lysis was added and measurements were performed using a luminometer (Glomax, Promega, USA), which automatically adds luciferine to each well. Data analysis was performed in Excel.

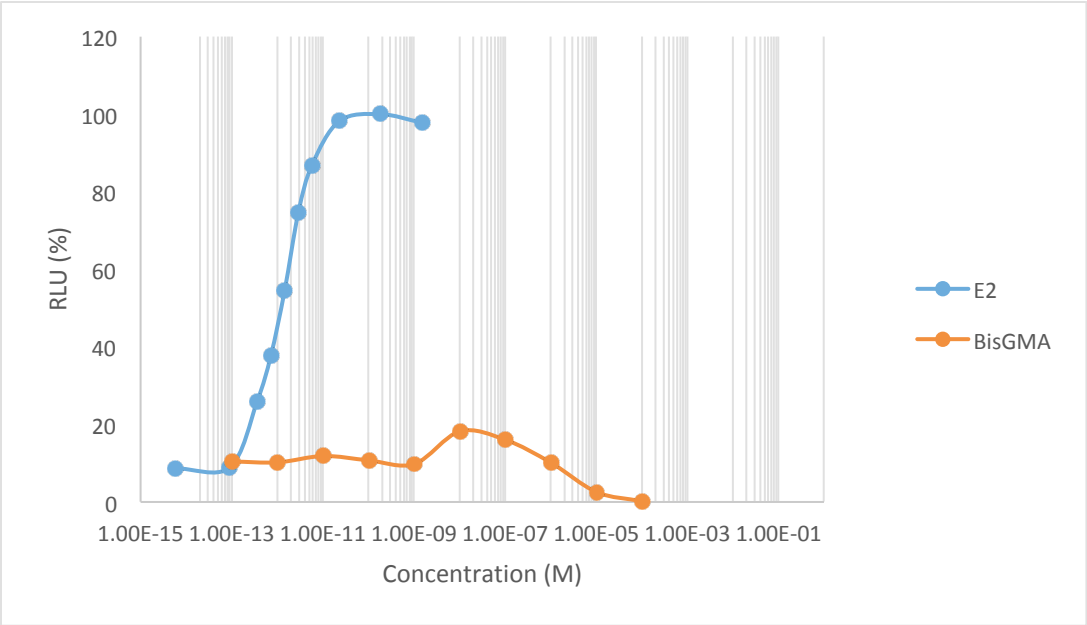
Results and discussion

For the analyzed monomers, only BisDMA showed a clear agonistic estrogenic activity, as can be seen in graph 1. For BisGMA, there was a small agonistic estrogenic activity present, with a response equal to or lower than 20% of the E2 maximum. At the highest concentrations, cell death occurred which is demonstrated in graph 2 (response dips below the background value).



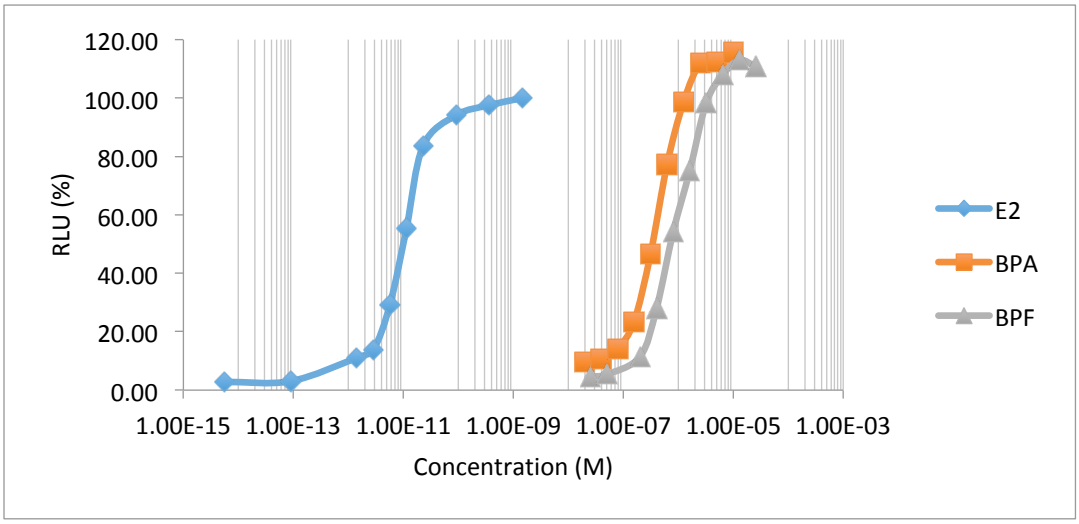
Graph 1: On the left, the results of the range finding experiment for Bisphenol A dimethacrylate (BisDMA). On the right, ERE-CALUX full dose-response curve for BisDMA. The concentration is depicted in M, the response in Relative Light Units (RLU), which are scaled to the maximum of the E2 standard curve (100%).

All three BisEMA compounds, TEGDMA, UDMA, BADGE, and TCD-DI-HEA showed no agonistic estrogenic effects. The lack of estrogenic activity for both TEGDMA and UDMA is to be expected, since both compounds are not derived from BPA and show no structural relationship to BPA. BisEMA, BADGE and TCD-DI-HEA are all large compounds with a high molecular weight, which could explain their inability to bind to the estrogen receptor. BisDMA on the other hand is rather small, with a very high structural relationship to BPA, which could explain their similar behavior. This indicates that the size of the monomers used has a big influence on their estrogenic activity, which needs to be taken into account when synthesizing new composites. The 3 initiators that were analyzed, CQ, EDMAB and HEMA, also showed no agonistic estrogenic activity.



Graph 2: ERE-CALUX dose response curve for Bisphenol A glycidyl methacrylate (BisGMA). The concentration is depicted in M, response in Relative Light Units (RLU), scaled to the maximum of the E2 standard curve (100%).

The use of BPA analogues is becoming more popular due to the possible health effects related to BPA. BPF is such an analogue that is used in the synthesis of epoxy resins which are used in dental sealants. As can be seen from graph 3, BPF is a clear estrogenic agonist, which shows a similar behavior to BPA. This shows that caution needs to be taken when switching to BPA analogues, since most show structural relationships to BPA and they can have similar effects.



Graph 3: ERE-CALUX dose response curves for Bisphenol A (BPA) and Bishphenol F (BPF). The concentration is depicted in M, the response in Relative Light Units (RLU), scaled to the maximum of the E_2 standard curve (100%).

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