DENTAL MATERIALS 26 (2010) 51–58

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Real-time xCELLigence impedance analysis of the cytotoxicity of dental composite components on human gingival fibroblasts

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ARTICLE INFO

Article history:
Received 8 April 2009
Accepted 22 August 2009

Keywords:
HEMA
TEGDMA
BisGMA
UDMA
Human gingival fibroblasts
Cytotoxicity
xCELLigence system

ABSTRACT

Objectives. Aim of this study was by continuous monitoring to assay the proliferative capacity of human gingival fibroblasts (HGFs), to investigate cytotoxicity of the most common monomers/comonomers in dental resin composites: bisphenol-A-glycidylmethacrylate (BisGMA), hydroxyethylenemethacrylate (HEMA), triethyleneglycoldimethacrylate (TEGDMA), and urethanedimethacrylate (UDMA) in HGFs during 24 h exposure using the xCELLigence system.

Methods. xCELLigence cell index (CI) impedance measurements were performed according to the instructions of the supplier. HGFs were resuspended in medium and subsequently adjusted to 400,000, 200,000, 100,000, and 50,000 cells/mL. After seeding 100 μL of the cell suspensions into the wells of the E-plate 96, HGFs were monitored every 15 min for a period of up to 18 h by the xCELLigence system.

Results. Half maximum effect concentrations (EC_{50}) were determined based on the dose–response curves derived by xCELLigence measurements. Following real-time analysis, significantly increased EC_{50} values of HGFs exposed for 24 h to the following substances were obtained: HEMA[a], TEGDMA[b], UDMA[c]. The EC_{50} values (mean [mmol/L] ± S.E.M.; $n = 5$) were: HEMA 11.20 ± 0.3, TEGDMA[a] 3.61 ± 0.2, UDMA[a,b] 0.20 ± 0.1, and BisGMA[a,b,c] 0.08 ± 0.1. These results are similar to the EC_{50} values previously observed with the XTT end-point assay.

Significance. Our data suggests that the xCELLigence live cell analysis system offers dynamic *live cell* monitoring and combines high data acquisition rates with ease of handling. Therefore, the xCELLigence system can be used as a rapid monitoring tool for cellular viability and be applied in toxicity testing of xenobiotics using *in vitro* cell cultures.

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0109-5641/$ – see front matter
doi:10.1016/j.dental.2009.08.007

1. Introduction

Amalgam has been replaced in increasing rates by dental resin composites that are tooth-colored materials most commonly used to restore dental damage in the permanent dentition [1]. Dental resin composites consist of an organic resin matrix with embedded organic particles. Besides direct filling materials, resins are also used as bonding resins, e.g., dentin adhesives and cements and as luting agents for crowns, inlays and orthodontic brackets [2]. The common components of both resin and bonding components are the monomers/comonomers: bisphenol-A-glycidylmethacrylate (Bis-GMA), hydroxyethylene methacrylate (HEMA), triethyleneglycoldimethacrylate (TEGDMA), and urethanedimethacrylate (UDMA). Previous studies have described that unpolymerized monomers/comonomers can be released from resin composites into the oral cavity [3–5] during implantation and even after polymerization [6,7]. Leaching compounds can, after dilution by the saliva, enter the intestine [8,9] where, after uptake and metabolization they can form toxic and radical intermediates [10–12].

HEMA and TEGDMA are the main comonomers released from resin-based materials [13,14]. In previous animal experiments the uptake, distribution, metabolism, and excretion of HEMA and TEGDMA were investigated [10]. *In vitro* studies revealed mutagenic, teratogenic, genotoxic and estrogenic effects of composite components [15–17]. Numerous cytotoxic responses to dental composite resins and their components have been described [18–22,10]. It has been demonstrated that UDMA and TEGDMA were more cytotoxic than HEMA to human gingival fibroblasts (HGFs) [23,24]. A significant increase in relative toxicity of the monomers/comonomers was found in the XTT-test in the following order: BisGMA > UDMA > TEGDMA > HEMA [25].

In the earlier studies several methods and techniques were used to investigate the cytotoxicity of dental resin materials, e.g., lactate dehydrogenase (LDH) assay [2], 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzol-disulfonate (WST-1) assay [26], sodium 3′[1-phenyl-aminocarbonyl]-3,4-tetrazolium bis-[4-methoxy-6-nitro] benzene sulfonic acid hydrate assay [25], 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) assay [17], bromodeoxiuridine (BrdU) assay [2], and fluorescence microscopy [25]. All these methods, which are designed for the analysis of cell proliferation, viability and cytotoxicity, are single end-point qualitative measures of cell fitness. The established assays are labor intensive and comprise a number of manipulation steps that potentially can induce variation of the end-points. In addition there is a great tendency for compound interference because of the optics-based detection methods for most assays, such as absorbance, luminescence or fluorescence, which are vulnerable to distortions. In this sense there is an important requirement for the competency of quantitative monitoring cell biological parameters in real-time in *in vitro* cell culture. Hence, an automated assay that combines high reproducibility with respect to *in vitro* cell proliferation and viability with easy manipulation is much appreciated.

Recently, Roche Applied Science and ACEA Biosciences conjointly launched the Real-Time Cell Analyzer Single Plate (RTCA SP®) system under the xCELLigence™ name, which follows the predecessor impedance-based Real-Time Cell Electronic Sensing (RT-CES®) system. The RT-CES system has been previously described in detail [27–29].

Real-time and continuous monitoring allows label-free assessment of cell proliferation, viability and cytotoxicity, revealing the physiological state of the cells and at the same time saves expensive reagents used in conventional cell analysis. In the xCELLigence system, the kinetic control of cellular status during entire experiment runs reveals continuous information about cell growth, morphological changes and cell death. Furthermore, the xCELLigence system allows for the calculation of time-dependent physiological EC_{50} values, which can be more informative than single EC_{50} end-points of classical toxicity testing.

In our present experiments, we conducted experiments with the new xCELLigence system that investigated the cytotoxicity of the dental composite compounds: BisGMA, HEMA, TEGDMA and UDMA on HGFs by real-time and continuous monitoring of the cell growth, proliferation and viability.

2. Materials and methods

2.1. Chemicals

The monomers/comonomers triethyleneglycoldimethacrylate (TEGDMA; CAS-No. 109-16-0), bisphenol-A-glycidylmethacrylate (BisGMA; CAS-No. 1565-94-2), hydroxyethylenemethacrylate (HEMA; CAS-No. 868-77-9), and urethanedimethacrylate (UDMA; CAS-No. 72869-86-4) were obtained from Evonik Röhm (Essen, Germany).

HEMA and TEGDMA were directly dissolved in medium. BisGMA and UDMA were dissolved in dimethyl sulfoxide (DMSO, 99% purity, Merck, Darmstadt, Germany) and diluted with medium (final DMSO concentration: 0.20%). Control experiments contained DMSO (0.20%) in medium only.

2.2. Cell culture

The human gingival fibroblast (HGF) cultures used in this study were produced by Provitro on the base of human tissues and obtained from Cell-Lining, Berlin, Germany, Cat-No.: 1210412. The HGFs (passage 9) were grown on 175 cm^2 cell culture flasks to approximately 75–85% confluence and maintained in an incubator with 5% CO_2 atmosphere at 100% humidity and 37 °C. Quantum 333 medium supplemented with L-glutamine and 1% antibiotic/antimycotic solution (10,000 Units/mL penicillin, 25 mg/mL streptomycin sulfate, 25 μg/mL amphotericin B) was used in the experiments. After reaching confluence the cells were washed with Dulbecco's phosphate buffered saline (PBS), detached from the flasks by a brief treatment with trypsin/EDTA. Quantum 333, antibiotic–antibiotic solution, PBS and trypsin/EDTA were purchased from PAA Laboratories GmbH, Cölbe, Germany.