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Antimicrobial effect and physicochemical properties of an adhesive system containing nanocapsules

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ABSTRACT

Objective. To incorporate indomethacin and triclosan-loaded nanocapsules into primer and adhesive, and evaluate its properties.
Methods. Indomethacin and triclosan were encapsulated by deposition of preformed polymer and subsequently characterized regarding morphology, particle size, drug content and cytotoxicity. Nanocapsules (NCs) were incorporated into primer at 2% and into adhesive at 1, 2, 5, and 10% concentrations. Degree of conversion (DC) and softening in ethanol of the adhesive were evaluated. Drug release and drug diffusion through dentin was quantified by high performance liquid chromatography. Antimicrobial test was performed until 96 h.
Results. Spherical and biocompatible NCs presented mean size of 159 nm. Drugs content was 3 mg indomethacin/g powder and 2 mg triclosan/g powder. Incorporating NCs in adhesive showed no influence in DC ($p = 0.335$). The addition of 2% of NCs showed no influence in softening in ethanol ($p > 0.05$). After 120 h, 93% of indomethacin and 80% of triclosan were released from primer, 20% of indomethacin and 17% of triclosan were released from adhesive with 10% of NCs. Indomethacin showed diffusion through dentin. In 24 h, adhesive containing 2 and 5% of NCs using primer with NCs showed antimicrobial effect. In 96 h, adhesives containing different concentration of NCs promoted antimicrobial effect.
Conclusions. Indomethacin and triclosan-loaded nanocapsules were successfully incorporated into primer and adhesive, promoting controlled drugs release, indomethacin diffusion through dentin and antimicrobial effect without compromising its physicochemical properties.

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Significance. Indomethacin and triclosan-loaded nanocapsules have potential to prevent recurrent caries and to be used in deep cavities controlling pulpar inflammatory process.

1. Introduction

The failure rate of composite resin restorations as a result of recurrent caries was reported between 40 and 70% [1–3]. The increase of bacterial oral colonization around restorations leads to a pH decrease and consequently recurrent desmineralization process [4]. Post-operative sensitivity is another reason of failure with an occurrence rate around 10% [3]. The sensitivity occurs in deep cavities with a pulpal involvement that can lead to an acute or chronic inflammation or necrosis of pulp tissue [5,6].

In order to reduce failures of restorations, antibacterial agents have been added to adhesive systems [7–9]. Overall, agents are effective but for a short time. Further, the increase of concentration of agents can negatively affect the properties of material [7,10–12]. Regarding pulp sensitivity, indirect pulp capping using calcium hydroxide is the most widely applied treatment to prevent progress of pulp inflammation in deep cavities through dentin repair [13]. However, calcium hydroxide as pulp capping resulted no improvement in long-term success rate of restorations [14–16]. Therefore, new alternatives have been developed to improve the prevention of pulp inflammatory progress [17–19]. Nonetheless, there is no study focused on development of adhesive system promoting antimicrobial and anti-inflammatory effects.

An alternative strategy to improve bioavailability and efficacy is drug-loaded nanocapsules [20]. Due to encapsulation, drug release is controlled, effect occurs even in sub-therapeutic doses, and adverse effects are sparse [21,22]. In addition to carrier systems, the selection of drugs is essential. Triclosan presented broad-spectrum of antimicrobial activities through structural perturbations resulting a loss of permeability-barrier functions [23]. Due to its ability to inhibit membrane enzymes and glycolysis of *Streptococcus mutans* in biofilms, triclosan is considered an anti-caries agent [24]. Indomethacin has presented successful anti-inflammatory effect, including in pulp tissue [25], due to inhibition of prostaglandins by reversibly blocking cyclooxygenases [26]. Further, indomethacin has been showed high effective nanoencapsulation and the resultant nanocapsules presented potent anti-inflammatory effect [22].

Therefore, the aim of this study was to incorporate indomethacin and triclosan-loaded nanocapsules into a primer and an adhesive resin and evaluate its properties.

2. Materials and methods

2.1. Preparation of indomethacin and triclosan-loaded NCs

Indomethacin and triclosan-loaded NCs were prepared by the interfacial deposition of preformed polymer in a miniemulsion. The reagents were obtained from Sigma Chemical (St. Louis, USA). An organic phase was composed by polymer (MMA-co-MAA), Eudragit® S100 (0.50 g), indomethacin (0.025 g), triclosan (0.025 g), medium chain triglycerides (0.81 mL), sorbitan monostearate (0.19 g) and acetone (125 mL). An aqueous phase contained polysorbate 80 (0.385 g) and water (250 mL). The organic phase was added through a funnel to aqueous phase under magnetic stirring at 25 °C. Acetone and water excess were eliminated using a rotary evaporator (Rotavapor II, Buchi, Flawi, Switzerland), a B-740 recirculating chiller (Buchi, Flawi, Switzerland) and a U-700 vacuum pump (Buchi, Flawi, Switzerland). The suspension containing NCs was spray dried (B-290, Buchi, Flawi, Switzerland) using hydrophilic fumed silicon dioxide (Aerosil® 200) in amount of 1.5% of the suspension content as an adjuvante to avoid the aggregation on internal wall of equipment. The inlet temperature at the drying chamber was approximately 150 ± 4 °C, and the outlet temperature was 107 ± 4 °C.

2.2. Characterization of indomethacin and triclosan-loaded NCs

The mean size ($d_{4.3}$) of indomethacin and triclosan-loaded NCs were measured by laser diffractometry (Mastersizer 2000, Malvern, Worcestershire, United Kingdom), in wet and dried states for NCs in aqueous suspension and dried NCs respectively. The distribution of the particle size (span) values was calculated by $(d_{0.9} - d_{0.1})/d_{0.5}$, where $d_{0.9}$, $d_{0.5}$, and $d_{0.1}$ were the particle diameters at the 90th, 50th, and 10th percentile of particles. Zeta potential of the suspension was determined using a Zetasizer nano-ZS ZEN 3600 model (Malvern Instruments, Malvern, Worcestershire, United Kingdom). The samples were then diluted with 1 mM NaCl aqueous solution. The measurements were made in triplicate to assure accuracy.

The morphological analysis was realized using a transmission electron microscopy (TEM, JEM 1200 Exll, Jeol, Tokyo, Japan) at 80 kV. An amount of 20 μL of NCs suspension at a dilution of 1:10 was deposited in a Formvar-Carbon support film on a specimen grid and negatively stained with uranyl acetate solution (2% m/v). Dried NCs (0.01 g) were processed using gold-sputter-coating and submitted to scanning electron microscopy (SEM, JSM 6060, Jeol, Tokyo, Japan) at an accelerating voltage of 10 kV.

2.3. Determination of drugs content for dried indomethacin and triclosan-loaded NCs

The dried NCs (20 mg) were dissolved in acetonitrile (10 mL) under 30 min of ultrasound stirring. The solution was filtered using a 0.45-μm (Millipore) filter, and free drugs was measured using high performance liquid chromatography (HPLC, Shimadzu LC 10-A Shimadzu, Kyoto, Japan), a UV/vis detector (λ = 280 nm) and Nova-Pak® C18 3.9 × 150 mm (4 μm) Waters column. A mobile phase (60:40 acetonitrile:water solution