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Chlorhexidine loaded MMT as dental composite filler: release evaluation

Luiza Campos[1], Letícia Boaro[2], Tamiris Santos[1], Vinícius Santos[1], Gustavo Varca[1], Duclerc Parra[1]

[1] Nuclear and Energy Research Institute, IPEN-CNEN/SP, São Paulo, SP, Brazil.
e-mail: luizamello@usp.br; 1tamiris1@gmail.com; vinicius.jusan@gmail.com; varca@usp.br; parduclerc@yahoo.com.br.
[2] Department of Biomaterials, School of Dentistry, University of Santo Amaro, São Paulo, SP, Brazil.
e-mail: leticiacidreiraboaro@gmail.com

ABSTRACT

Polymeric restorative materials are widely used, however polymerization shrinkage still stands as a major drawback as it allows microleakage, secondary caries, postoperative sensitivity and cusp deflection. A polymeric material with antimicrobial activity could be very useful in many dental procedures including adhesive cementation, sealants, dental adhesives and direct restorative material. The aim of this study was to develop nanocomposites for dental purposes composed by chlorhexidine loaded montmorillonite (Cloisite 30B) nanoparticles in a BisGMA / TEGDMA organic matrix. The specimens of experimental composites were synthesized with 10 % (w/w) of diacetate chlorhexidine incorporated in a MMT, as filler. The chlorhexidine release at 37 ^{o}C was evaluated *in vitro* in physiological solution at neutral pH for 366 hours. Drug content was estimated by UV spectroscopy at λ=255 nm. Significant drug release initiated after 96 hours and maintained throughout the experiment. In conclusion, the composite was capable of promoting chlorhexidine release over time, and such results in addition to the lack of significant weight loss ($\leq$ 1.43 %) and the very low swelling ($\leq$ 2.22 %) properties, revealed the strong potential of the developed composite for further dental and other biomaterial applications.

Keywords: Dental Composites, MMT, Nanoparticles, Chlorhexidine, Drug Release.

1. INTRODUCTION

Aesthetic dental composites are versatile materials of continuous growth following their introduction on the dental market about five decades ago [1]. Considering their use as direct technique, the composite resin corresponds to one of the main materials applied for dental restorations [2-3]. Improvements made in the polymer matrix and the filler particles have increased significantly the properties of these composites [4].

One of the most important advances of recent years in this field is the application of nanotechnology to composites. In practical terms, the addition of nanoparticles as fillers on dental composites was shown to improve wear resistance, gloss retention [5], elastic modulus [6], flexural strength, diametral tensile strength [7-8], as well as to reduce polymerization shrinkage [9-10-11].

The addition of clay mineral nanoparticle MMT in polymer matrices has been extensively studied, but its use initially attracted the attention of the plastics industry in general due to improvements in optical, thermal and mechanical properties [12]. Polymer / clay nanocomposite plays a key role in the automotive, packaging and aerospace industry considering their important features such as excellent mechanical and thermal properties, and also the possibility to provide a barrier to gases [13-14-15].

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Clay lamellar structure is responsible for their capacity to absorb different kinds of molecules and this absorption process occurs due to the higher surface area, if compared to usual fillers, and its volumetric expansion when the molecules, such as solvents, monomers or polymers penetrate in-between the lamellae [11]. According to some authors [10-16-17] this expansion could minimize polymerization shrinkage and residual stress of these dental composites.

The possibility of MMT nanoparticles incorporation in composites for dental use is also due to their ability to reduce the polymerization shrinkage and residual stress [10-16-18-19], improved lifetime performance, abrasion resistance and resistance to solvent absorption [20].

The ability of the MMT nanoparticle to absorb molecules justifies the used in the pharmaceutical industry as a vehicle for drug controlled release [21-22-23-24]. MMT nanoparticles showed no antibacterial effect, however when added to materials with antimicrobial properties, it may lead to materials such activity [25]. Recent studies showed that the MMT load with chlorhexidine resulted in the release of the drug at a slow kinetics [24]. However, there are no studies on the release of chlorhexidine and its antimicrobial activity when CHX loaded MMT is associated with a conventional dental restorative polymeric matrix such as BisGMA / TEGDMA.

Thus, the aim of this study was to develop nanocomposites for dental purposes composed by chlorhexidine loaded montmorillonite (Cloisite 30B) nanoparticles in a BisGMA / TEGDMA conventional organic matrix.

2. MATERIALS AND METHODS

The following materials were applied in this research: **BisGMA:** (Bisphenol A Bis(2-hydroxy-3-methacryloxypropyl)Ether), Esstech, Essington, Pennsylvania, USA; **TEGDMA:** (Triethyleneglycol Dimethacrylate), Esstech, Essington, Pennsylvania, USA; **Camphorquinone:** (camphorquinone, 97%), Sigma-Aldrich Chemie, GmBH, Steinheim, Germany; **DMAEMA:** (2-(Dimethylamino)ethyl methacrylate), Sigma-Aldrich Chemie, GmBH, Steinheim, Germany; **MMT Cloisite® 30B**, BYK Additives INC. Texas, USA. **Chlorhexidine Diacetate Salt Hydrate,** Sigma-Aldrich Chemie, GmBH, Steinheim, Germany.

2.1 Chlorhexidine Loaded MMT

MMT nanoparticle was inserted into an aqueous solution of diacetate chlorhexidine monohydrate, in a weight ratio equivalent to 10% (w/w). The mixture was performed under constant agitation for 3 hours at temperature of 80 °C. After the emulsion process, the compound was lyophilized using an Enterprise II (Terroni, Brazil) lyophilize device.

2.2 Experimental Composite Formulation

Experimental composites were prepared with polymeric matrix based BisGMA / TEGDMA in equal weight proportions (1:1). As filler, were added chlorhexidine loaded MMT nanoparticles (10% wt). The photo initiator camphorquinone and tertiary amine corresponded to 2% (w/w) of all tested formulations. The entire manipulation process was performed under yellow light. Initially the organic matrix was manipulated with the assistance of an analytical balance (Ohaus-Adventure, Shanghai, China). Filler particles were incorporated in the polymeric matrix with the assistance of a mechanical mixer (DAC 150 Speed mixer, Flacktek, Landrum, SC, USA) for 3 minutes at 3500 rpm. The composites were stored in dark sealed vials and maintained under refrigerated conditions until the moment of use.

2.3 CHX Release

The specimens were designed on a silicon matrix with dimensions of 5mm diameter and 1mm thickness, between two glass supports. Photo polymerization was performed us-

ing a dose of approximately 18 J/cm2. The specimens (n=5) were previously weighted and immersed in vials containing 5 mL of saline (0.9 %) phosphate buffer (pH 7, 50 mM) and submitted to incubation on a shaker under temperature of 37 ºC and 80 rpm. The samples were collected as a function of time and Chlorhexidine quantification was performed by UV spectrophotometry analysis at a wavelength of 255 nm [26-27] on a i3x Spectramax (Molecular Devices, USA) microplate reader.

The specimens were weighted after the release experiment and compared to the initial mass of the sample before the experiment. Swelling index was calculated based on Eq. (1):

% Swelling = ((Initial weight/ Final weight *100) – 100**)** **(1)**

After the release experiment, the specimens were stored and oven-dried until constant weight (about 48 hours) at 50 ºC. The specimens were then weighted and the weight loss was determined using Eq. (2):

% Weight loss = (100 – (Initial weight / final weight *100)**)** **(2)**

3 RESULTS AND DISCUSSION

CHX release from the composite was monitored by UV spectrophotometry over time ranging from 0 to 366 hours and revealed an initial release profile up to 4 hours, reaching approximately 0.033 g/ L^{-1} of CHX. The release profile is described in figure 1.

After 24 hours a constant release was registered and maintained throughout the experiment. After 96 hours of release a total CHX release of 0.0985 g/ L^{-1} was achieved. The system reached equilibrium between 168 and 216 hours of the experiment with a CHX total of 0.121 g/ L^{-1}. A slight increase was observed from 264 up to 366, reaching a total CHX value of 0.141 g/ L^{-1}.

The 10% (w/w) MTT based composite incorporated with CHX presented total swelling of about 2.22% (± 0.31) while the weight loss was estimated around 1.43% (±0.36). All measurements were performed at the end of the release experiment.

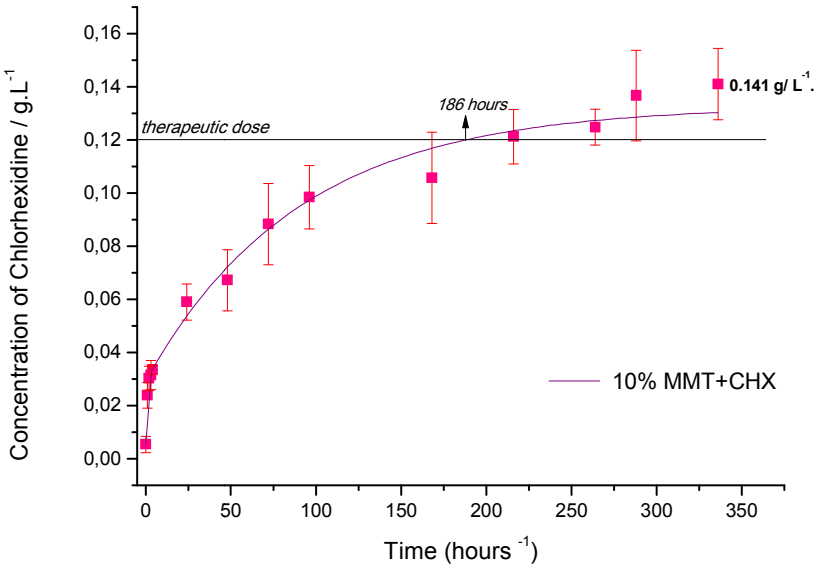

Figure 1: CHX release profile from 10% (w/w) MTT based composite evaluated by UV spectrophotometry (λ= 255 nm).

Mineral clays are inorganic materials currently on the spotlight of the pharmaceutical area due to the physical-chemical properties of such silicates. In a more specific way, the properties that assure the applications of mineral clays in pharmaceutical applications are related but not limited to its absorption properties, large surface area, low toxicity, and the fact that such materials are chemically inert [28].

The MMT property in interlayer organic compounds is particularly important in the preparation of drug delivery systems. The MMT nanoparticle used as a carrier may have an influence on two very important aspects in drug bioavailability, as release and stability. In the literature there are successful examples of drugs intercalated in MMT which kept the pharmacological activities after released from lamellar system [29-30].

To maximize the benefits of chlorhexidine, one can find support in nanotechnology through controlled drug delivery systems, which holds several advantages over conventional systems. Among the advantages, highlights are directed towards increased therapeutic efficacy provided by the gradual and controlled release of the drug from the nanoparticle and the significant reduction in toxicity due to pharmacological features of the advanced release system. Additionally, this system may avoid the instability and decomposition of the drug (premature bio-inactivating), as well as enable deeper tissue penetration due to its small size [27].

The release profile obtained for the composite are in correlation with previous studies, where chlorexidine acetate (CHX) was incorporated into MMT nanoparticles and held a release profile with an initial release observed in the first 24 hours, and maintained up to 72 hours [31]. However, the developed composite was capable of promoting a release profile up to 366 hours, highlighting a better delivery system for long-term release, as well as confirming the novelty embroidered in the system.

The swelling index obtained for the composite, established as 2.22 %, revealed a negligible or very low swelling profile, essential for dental applications. Apart from an application point of view, such value also indicates a very strong binding between the chains of the polymer matrix, which does not allow water molecules to penetrate. Previous studies pointed out swelling values of 3.14 % ± 0.26 up to 5.19 % ± 0.93 after immersion in water as a function of time of a BisGMA/TEGDMA copolymer [32]. According to Santos *et al*., (2002) [33], the water uptake occurred in the polymeric matrix and was affected by the overall structure and the percentage of such components in the system or composite. In terms of compliance to international standards, resins for restauration materials or similar applications water uptake must be inferior than 40 μg/ mm³ and solubility values inferior than 7,5 μg/ mm³ for a seven days period (norm 4049 of ISO). Thus the composite falls within this limit, being identified as adequate for further biomedical or dental applications.

In terms of weight loss, the experimental composite revealed a minimum loss of approximately 1.43 %. According to Cattani-Lorente *et al*., (1999) [34], composite resins may undergo chemical degradations as a result of diffusion properties of molecules and ions of residual monomers. As the resin is immersed in aqueous solution, water absorption takes place between the polymer chains, even though silanes were used in the composite to increase its mechanical properties, some degradation may take place. Whatsoever, the low values obtained for this experimental parameter revealed the strong integrity of the composite structure, and thus corroborating the swelling experiments which also revealed a strong linkage of the polymer chains by the low swelling index registered. Thus, the weight loss values were more likely to be attributed to the CHX release, rather than a degradation of the system itself.

4 CONCLUSION

In conclusion, the composite was capable of promoting chlorhexidine release over time, and such results in addition to the lack of significant weight loss (≤ 1.43 %) and the very low swelling (≤ 2.22 %) properties, revealed the strong potential of the developed composite for further dental and other biomaterial applications.

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