ORIGINAL ARTICLE

Endodontics

Preliminary study of light-cured hydrogel for endodontic drug delivery vehicle

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Keywords
drug delivery, hydrogel, pulp capping.

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Received 23 April 2013; accepted 22 October 2013.

doi: 10.1111/jicd.12118

Abstract

Aim: Direct pulp capping is the treatment of an exposed vital pulp with a dental material to facilitate the formation of reparative dentin and maintenance of vital pulp. A bioengineered drug delivery vehicle has the potential to increase the success rate of pulp capping. The aim of this study was to develop an injectable and light-curing drug delivery vehicle for endodontic treatment including direct pulp capping.

Methods: Polyethylene glycol-maleate-citrate (PEGMC) hydrogel was synthesized as a drug delivery vehicle that is composed of PEGMC (45% w/v), acrylic acid (AA) (5% w/v), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) (0.1% w/v), and deionized water. The association between prehydrogel-solution volume and visible light-curing was examined. The cytotoxicity of the hydrogel was tested using L929 cells in a cell culture system. Ca^{2+} release from the hydrogel was determined using calcium hydroxide as the incorporated medicine.

Results: The results showed that the light-curing time for hydrogel is comparable to composite resin. The hydrogel had cell toxicity similar to adhesive systems. Moreover, controlled Ca^{2+} release was obtained from the calcium hydroxide incorporated hydrogel.

Conclusions: The data suggest that hydrogel should be explored further as a promising drug delivery vehicle for vital pulp therapy and regenerative endodontics.

Introduction

Direct pulp capping is a treatment for exposed vital pulp using dental materials such as calcium hydroxide,[1,2] bonded composite resins,[3] or mineral trioxide aggregate.[4] Pulp capping has been shown to facilitate both the formation of reparative dentin from odontoblasts[5–7] and the maintenance of vital pulp.[8,9] Nevertheless, the 5-year success rates for pulp capping caries-exposed permanent teeth with a mature apex are very low (33–50%),[10–12] due to the lack of methods to simultaneously improve the inflamed pulp condition.[13,14] Steroids,[15–17] osteogenic protein-1,[18] and transforming growth factor-beta[19,20] have been studied as anti-inflammatory agents towards treating inflamed pulp using drug delivery vehicles. However, insufficient degradation of the drug delivery vehicle has been noted in animal studies.[19,20]

Thus, a clinically relevant vehicle that possesses realistic handling and drug delivery properties is needed in direct pulp capping and regenerative endodontic treatment. A vehicle having the above-mentioned properties can be found in bioengineered hydrogel, which is a colloidal gel

suspended in a water medium. Biodegradable hydrogel may be a promising controlled drug delivery system. Specifically, polyethylene glycol-maleate-citrate (PEGMC) hydrogel is a synthetic polyester drug delivery vehicle that is elastomeric, biocompatible, biodegradable, and has light-curing cross-linking properties.[21]

In this study we investigated PEGMC hydrogel as an injectable drug delivery vehicle for regenerative endodontic treatment, including direct pulp capping. The aims of this study were to examine: (a) the association between PEGMC prehydrogel-solution volume and visible light-curing time; (b) the cytotoxicity of the hydrogel drug delivery vehicle and its components in a cell culture system *in vitro*; and (c) the Ca^{2+} release from the hydrogel drug delivery vehicle using calcium hydroxide as a model drug.

Materials and methods

Synthesis of PEGMC

The PEGMC was synthesized by using the three main components as described elsewhere: (a) polyethylene glycol (PEG) (MW 200–1000 Da); (b) maleic acid (MA); and (c) citric acid (CA).[21] Briefly, the components were stirred in a three-necked round-bottom flask (250 mL) fitted with an inlet/outlet adapter for 20 min at 160°C under nitrogen gas flow. This was followed by 4 h stirring at 130°C. The reaction was carefully observed until the desired viscosity was achieved, which was confirmed by the twitching (disturbance in stirring motion) of the stirring bar. Synthesized PEGMC was then purified for 3 days by dialysis, followed by freeze-drying. The ratio of MA and CA in the initial composition was 6:4, and the overall ratio of the acids over the alcohol was 1:1. The material properties, including structure, mechanics, swelling ability, sol-gel contents, and degradation rate, were studied previously.[22–24]

Preparation of PEGMC hydrogel solution

The prehydrogel-solution was prepared by mixing a solution of PEGMC (45% w/v), acrylic acid (AA) cross-linker (5% w/v), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) photoinitiator (0.1% w/v), and deionized (DI) water. Under a visible light-curing system (Model-106; Dentsply Caulk, Milford, DE, USA) the prehydrogel-solution polymerizes into a poly-PEGMC (PPEGMC) gel.

Association between prehydrogel-solution volume and visible light-curing time

To examine the association between prehydrogel-solution volume and visible light-curing (450–495 nm) time in an *in vitro* dental setting, magnetic microstir bars were placed in 96-well plates (effective area 0.32 cm^2/well) containing the hydrogel solution on a magnetic stir plate (Figures 1a and 1b), and a visible light-curing was used for polymerization (Figure 1c). Gelation time (in sec) was defined as the point when the stir bar ceased stirring, indicating formation of a hard gel after polymerization (Figure 1d). Various volumes of the prehydrogel solution (50, 100, 150, and 200 μL) were used for photopolymerization; the experiment was repeated 10 times per group to measure the gelation time.

In vitro cytotoxicity of the hydrogel drug delivery vehicle and its components

Various concentrations of the polymerized hydrogel and its components were prepared in the cell culture medium as in a previously reported dose-dependent cytotoxicity study.[25] L929 cells (American Type Culture Collection, Manassas, VA, USA) were cultured in a complete medium consisting of Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The cells were incubated in a humid environment at 37°C and 5% CO_2. Upon 80–90% confluency, the cells were seeded in either 96-well plates at 5000 cells/well or 48-well plates at 10 000 cells/well. Cells in the 96-well plates were subjected to 200 μL of PEGMC at 0–1.41% w/v, AA at 0–5% w/v, AAPH at 0–4% w/v and exposed to light (450 nm) for 0–90 sec. For the cured PPEGMC gel toxicity, gels prepared from prehydrogel solution in a 96-well plate, were exposed to the cells in the 48-well plates. The cells exposed only to the medium served as controls in all groups. After 6 h of incubation, the cell survival was evaluated by [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS, CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay; Promega Corp., Madison, WI, USA) assays. The linear regression was fitted to investigate the association between the gelation time and the solution volume. The data were analyzed by an ANOVA and if there was significance among the groups, pairwise comparisons were conducted using Student's *t*-test with Bonferroni corrections accounting for multiple comparisons.

Ca^{2+} release from the hydrogel drug delivery vehicle

Ca^{2+} release of $Ca(OH)_2$-incorporated hydrogel solution (experimental group) were compared with $Ca(OH)_2$ (Fisher, Pittsburgh, PA, USA) as a control. An amount of 500 mg/mL of the materials were incubated at 37°C in a Float-A-Lyzer membrane (500–1000 Da; Spectrum Laboratories, Rancho Dominguez, CA, USA). At predetermined time points (1–96 h), 1 mL dialysate containing released

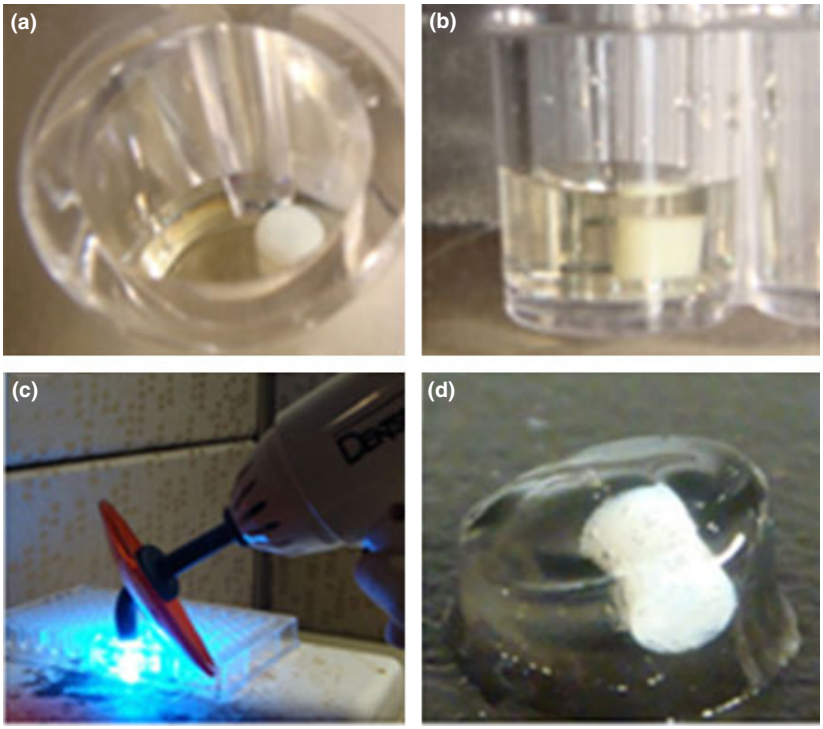

Figure 1. (a and b) The prehydrogel solution with a magnetic microstir bar in 96-well plates, (c) the visible light-curing system, and (d) the formed hard gel.

Ca^{2+} was collected and reconstituted with 1 mL fresh distilled water. The Ca^{2+} content was then quantified using a calcium reagent (Pointe Scientific, Canton, MI, USA) and spectrometer at 550 nm. The percentage of Ca^{2+} release from the experimental and control groups was compared using a generalized linear mixed model analysis. Degradation characteristics of PPEGMC have been studied previously.[21] Data are expressed as mean ± SD.

Clinical simulation of injection in plastic root canal block

The viscosity of the hydrogel solution needs to be examined if it is suitable for maxillary injection because the solution will leak out due to gravity before the light-curing procedure can be conducted. An injection syringe with a needle (BD 0.5 cc tuberculin syringe 27G) was used to examine the handling property in solution before the light-curing polymerization. Root canal plastic blocks were used as if the solution were to be injected into the maxillary area, simulating the clinical scenario.

Results

Association between prehydrogel-solution volume and visible light-curing time

The gelation times were 85.7 ± 10.7, 91.6 ± 10.5, 116.0 ± 11.5, and 147.3 ± 26.7 sec for 50, 100, 150, and 200 μL of solution, respectively. Gel was formed after photo-polymerization. The fitted linear regression was time = 57.85 + 0.42 × volume, with R^2 = 0.66 (Figure 2), which predicted that the gelation time would decrease on reduction in the volume of the solution. The predicted mean values at 25 and 12.5 μL were 68.3 and 63.1 sec, respectively.

In vitro cytotoxicity of the hydrogel drug delivery vehicle and its components

Light exposure onto L929 cells for 30 and 60 sec resulted in cell viability of 92.5 and 79.7% (Figure 3a). When the

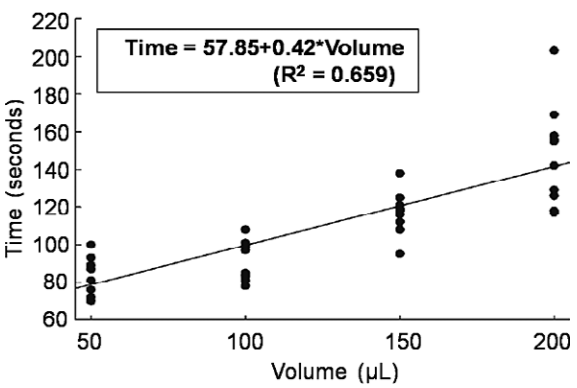

Figure 2. Association between prehydrogel-solution volume and visible light-curing time.

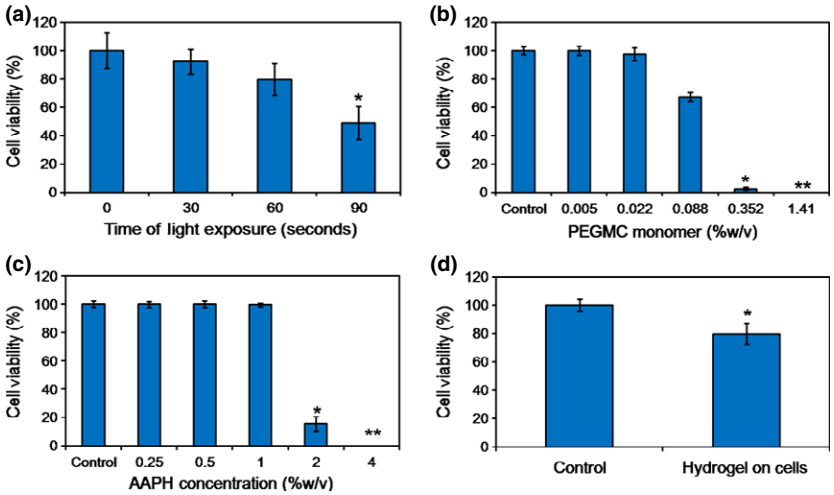

Figure 3. Cytotoxicity studies on L929 cells based on (a) time of light exposure, (b) concentration of PEGMC monomer, (c) concentration of AAPH, and (d) PPEGMC.

cells were exposed to 90 sec of light there was significant decrease in cell viability of 48.9%. For the PEGMC monomer, maximal cell viability of 97.4% was observed with a monomer concentration of 0.088%, while there was a significant drop in viability to 2.6% at 0.35% concentration ($P < 0.05$); there was no cell survival at 1.41% concentration (Figure 3b), and the free form of AA was found to be highly toxic to the cells (results not shown). Cell viability on AAPH remained at 100% up to 1% concentration but dropped to 15.6% at 2% concentration ($P < 0.05$), with no cell survival at 4% concentration (Figure 3c). Lastly, for the hydrogel system, cell viability over 80% was observed after a period of 6 h (Figure 3d).

Ca^{2+} release from the hydrogel drug delivery vehicle

The Ca^{2+} release in the $Ca(OH)_2$-incorporated hydrogel system, compared with free (not incorporated in any material) $Ca(OH)_2$, is summarized in Figure 4. The Ca^{2+} release characteristics display a steady increment from 12% release at the first hour, followed by 82% at the 16-h time point, and finally, 94% release at 96 h.

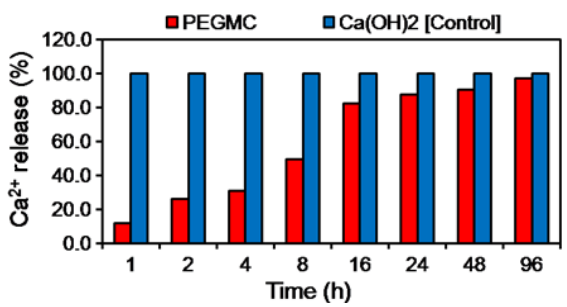

Figure 4. Comparison of percentage Ca^{2+} release between the experimental and control groups. (■) PEGMC and (■) Ca(OH)2 [Control].

Clinical simulation of injection in the plastic root canal block

In a simulated dental setting using an injection syringe with a needle confirmed injection ability and viscosity (Figure 5a). After injection into plastic root canal blocks, any unexpected solution drop or flow was not observed for both mandibular (Figure 5b) and maxillary teeth (Figure 5c). The estimated amount needed for direct pulp capping in a real dental setting was <50 μL.

Discussion

In this study we tested a hydrogel solution for tooth that solidifies upon light curing. Unlike low-viscosity hydrogel vehicles,[26,27] the viscosity of the proposed prehydrogel solution was suitable for both maxillary and mandibular teeth. Low-viscosity materials are not suitable for maxillary injections because the solution will leak out due to gravity before the light-curing procedure can be conducted. As a result of the gelation time test, the predicted light-curing times at 25 and 12.5 μL were 68.3 and 63.1 sec, respectively. The preferred light-curing times for composite resin materials in current dental practice range from 30 to 90 sec; therefore, hydrogel gelation time should fall within that range. Significant cell viability was observed with exposure over this time frame. In general, PEG-based hydrogels typically swell considerably after polymerization in moist environments, therefore, the polymerized hydrogel is expected to be covered by restorative material in order to minimize polymerization contraction.[26,27]

After the injection ability and gelation time were examined, the cytotoxicity of the material was tested on L929 fibroblasts. The toxicity was generally high because we tested all the hydrogel materials directly on cells at very

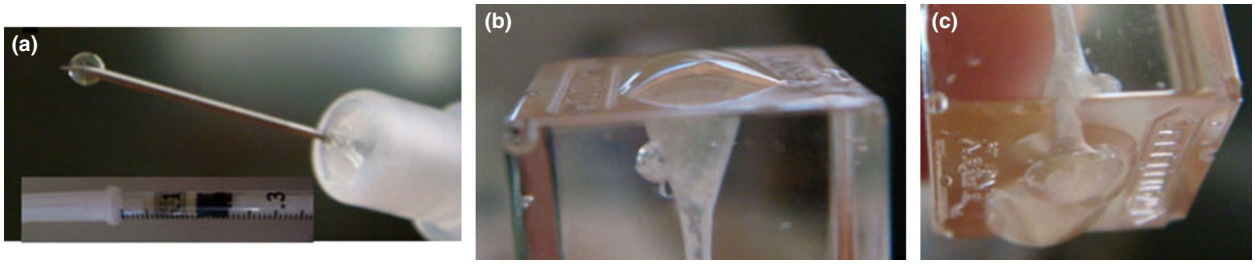

Figure 5. Simulated clinical setting: (a) confirmed injection ability, (b) mandibular teeth, and (c) maxillary teeth. No unexpected solution drop or flow was observed in a simulated dental setting.

high concentrations of material components. However, in the clinical setting, all the materials will be used up in the gelling process when the hydrogel is formed (set). Accordingly, as hydrogels form in real time, there will be a negligible amount of unreacted components, which will not make the system cytotoxic. This was evident from the results of PPEGMC gel toxicity, where cell viability of over 80% was observed, suggesting the hydrogel system can be successfully employed in clinical settings.

The current initiator selection (AAPH) was appropriate for the proposed visible light-curing system, as evidenced by the clinically acceptable curing times and low cytotoxicity observed in the results. When designing new bioengineered materials/hydrogels for dental pulp, an AAPH concentration of 1% or below is recommended. When redesigning a hydrogel vehicle for dental pulp, its cytotoxicity and the individual component amounts within their biocompatibility range should be considered.

The hydrogel also has the advantage that it can be used as a drug delivery system. The disadvantage of traditional root-canal drug delivery systems is that only a small percentage of the drug within the root-canal placement material actually reaches the affected area. The time course of drug delivery by these systems is also an issue. The lack of drug reaching canal tissue in the proper time course limits the current systems therapeutic value.[28–30] However, state-of-the-art bioengineering techniques have recently led to maximizing therapeutic doses in various types of controlled drug delivery systems.[29,31–33] The advantages of these systems include improved targeted efficacy and local delivery, long-term drug release, and reduced overdoses and potential side-effects.[31,33,34] Normally, the approach of simply mixing a drug such as Ca $(OH)_2$ into a hydrogel precursor is expected to cause rapid burst-type release kinetics. In the present study, Ca^{2+} release by the hydrogel showed kinetics of a controlled release and thus suggests that hydrogel may be a superior drug delivery system than those that are currently available. In future studies, other drugs, such as growth factors and anti-inflammatory medicine, will be tested.

The properties of the hydrogels will have a clinical impact on regenerative endodontics in the following ways. First, when the hydrogels are incorporated with an anti-inflammatory drug and injected at the pulpotomy or direct pulp-capping site they can release the drugs, reducing the pulp inflammation. The hydrogels could be designed to have their degradation rates parallel to the reparative dentin-healing rate. Second, the hydrogel could function as a scaffold that could be used in conjunction with dental stem cells for pulp regeneration or to induce calcification to seal the capping site. In summary, the innovative aspects of this study include: (a) developing a hydrogel drug delivery system to dental treatment for direct pulp capping and regenerative endodontics; and (b) developing a new treatment modality to increase the successful rate of vital pulp therapy. A subsequent animal study is the next vital step towards future clinical human trials.

Acknowledgments

This paper was supported in part by NIH UL1T-R0000451.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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