Non-biodegradable polymer particles for drug delivery: A new technology for “bio-active” restorative materials

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To develop dental restorative materials with “bio-active” functions, addition of the capability to release active agents is an effective approach. However, such functionality needs to be attained without compromising the basic properties of the restorative materials. We have developed novel non-biodegradable polymer particles for drug delivery, aimed for application in dental resins. The particles are made using 2-hydroxyethyl methacrylate (HEMA) and a cross-linking monomer trimethylolpropane trimethacrylate (TMPT), with a hydrophilic nature to adsorb proteins or water-soluble antimicrobials. The polyHEMA/TMPT particles work as a reservoir to release fibroblast growth factor-2 (FGF-2) or cetylpyridinium chloride (CPC) in an effective manner. Application of the polyHEMA/TMPT particles loaded with FGF-2 to adhesives, or those loaded with CPC to resin-based endodontic sealers or denture bases/crowns is a promising approach to increase the success of the treatments by conferring “bio-active” properties to these materials to induce tissue regeneration or to inhibit bacterial infection.

Keywords: Restorative materials, Resins, Drug delivery, Bio-active, Polymer

INTRODUCTION

The improvement of the basic properties of dental restorative materials, such as their mechanical, physical, aesthetic, and bonding properties, has been dramatic, and the current materials on the market show excellent clinical performance. Therefore, a target of their advancement is being shifted to exhibition of “bio-active” functions to prevent primary/secondary diseases or promote healing of surrounding tissue. Several properties can be proposed as useful “bio-active” functions for restorative materials. One major approach involves addition of “bio-protective” effects such as control of bacterial infection^{1,2}, strengthening of the tooth substrate^{3,4}, prevention of demineralization^{2,5}, or protection of interfacial deterioration⁶⁻⁸. The other approach involves conferring “bio-promoting” effects such as remineralizing effects^{9,10}, control of inflammation, or promotion of tissue regeneration¹¹.

The most common way to make materials “bio-active” is the addition of the capability to release specific agents. However, the incorporation of active agents directly into dental restorative materials has several drawbacks. First, control of the release kinetics is difficult. To attain clinical effectiveness, it is important to have appropriate release of the agent to suit the environment in which the materials are used. Secondly, as the release of the agent occurs, the materials become porous and their mechanical properties decrease over

time. Thirdly, in the case of resinous materials, the incorporation of additives may compromise the curing of resins and hamper their integrity for permanent restoration. These are critical problems since dental restorative materials used to artificially recover lost tissue need to have high mechanical properties, physical and chemical stability, or to strongly bond to adherend.

The application of drug delivery systems is considered to be useful to overcome the problems mentioned above. In particular, for resin-based restorative materials, the use of a polymer-based carrier containing the active agents is effective. In the past, a variety of polymers, such as cellulose¹², gelatin¹³, polysaccharide¹⁴, polyethylene glycol¹⁵, or poly(2-hydroxyethyl methacrylate) (polyHEMA)¹⁶⁻¹⁹, have been reported as carriers for drug delivery. These polymers form biocompatible hydrogels with excellent water absorbability to facilitate the uptake of therapeutic agents. However, most of them are targeted for regenerative therapy and degrade to release the agent in human bodies. Accordingly, they are not applicable for dental materials used for permanent restoration.

In need of non-biodegradable carrier which is stable even after the active agents leach out (Fig. 1), we newly developed non-biodegradable particles for the delivery of water-soluble agents^{20,21}. These particles are made using methacrylate polymers and are compatible with any type of resin used for restorative treatments. In the present paper, the effectiveness of this novel polymer particle as a carrier of growth factors and antimicrobials is summarized. The possible application

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Fig. 1 Schematic diagram of biodegradable and non-biodegradable polymer particles for drug delivery.

of the polymer particles for various restorative materials is also addressed.

DESIGNING NON-BIODEGRADABLE POLYMER PARTICLES FOR DRUG DELIVERY

Non-biodegradable methacrylate-based polymer particles
Among the several methacrylate monomers used for dental restorative materials, HEMA is highly hydrophilic^{22,23}) and a candidate to fabricate particles to carry water-soluble agents. However, a homo-polymer made of HEMA is not completely non-biodegradable¹⁹). In addition, HEMA-polymer easily aggregates and is difficult to grind into particles. Therefore, we designed a new polymer by combining HEMA and a cross-linking monomer trimethylolpropane trimethacrylate (TMPT, Fig. 2). The HEMA/TMPT binary resin system forms a cross-linked structure and provides a solid polymer that can be pulverized through milling.

We fabricated five types of polymer, by mixing HEMA and TMPT at different weight ratios (Table 1). The mixture of HEMA and TMPT was polymerized using 0.5% benzoyl peroxide by heating at 120°C for 2 h and then under diminished pressure (−0.1 MPa) for 16 h. The polymerized resin was ground into particles with an average diameter of 500–550 μm using a mill, and washed through immersion in distilled water for 48 h. Figure 3 shows the 90% HEMA/10% TMPT polymer particles prepared. The particles are colorless, transparent, and have irregular shapes.

Characterization of the polyHEMA/TMPT particles

The water absorbability, hydrophobicity, and surface electric charge of the five polymer particles fabricated are summarized in Table 1. The average water absorption ratio ranged from 5.5–21.6%. An increase in the HEMA content clearly resulted in greater water absorbability,

Fig. 2 The monomers used to fabricate the non-biodegradable polymers. (A) 2-hydroxyethyl methacrylate (HEMA). (B) trimethylolpropane trimethacrylate (TMPT).

indicating that the particles show water absorbability based on the hydrophilic nature of HEMA.

Interestingly, when the *H*-index^{24,25}) of each polymer was measured, the polymers with higher ratios of HEMA showed greater hydrophobicity (Table 1). While the polymers with a greater HEMA content can contain more water because of the hydrophilic hydroxyl group, the alkyl chains of the polymerized HEMA in the network possibly act to provide a hydrophobic characteristic.

HEMA possesses an anionic character, which is present in its polymeric form *via* the polar hydroxyl

Table 1 Water absorbability, hydrophobicity, and surface electric charge of polyHEMA/TMPT particles

Abbreviation	HEMA/TMPT (wt%)	Water absorbability (Water absorption ratio, %)	Hydrophobicity (<i>H</i> -index)	Surface electric charge (Zeta potential, mV)
H10	10/90	5.5±2.8	1.00±0.03	−27.9±1.2
H30	30/70	12.7±4.5	0.99±0.02	−31.0±0.1
H50	50/50	13.3±5.2	1.09±0.04	−25.2±0.2
H70	70/30	19.7±1.6	1.17±0.04	−26.1±0.8
H90	90/10	21.6±2.5	1.30±0.07	−25.8±1.8

Water absorbability was determined by measuring the weight of the hydrogel particles immersed in water for 4 days at 25°C. Water absorption ratio (%) = $(W_b - W_a)/W_a \times 100$, where W_b and W_a are the weight of polymer particles before and after storage in water, respectively. To assess hydrophobicity, 100 mg (W_a) of the hydrogel particle was immersed in 1 mL of distilled water or aqueous 70 vol% isopropanol for 36 h, and the swollen weight (W_s) was measured. The equilibrium mass swelling ratio (q) in solvent (170: 70 vol% isopropanol, H₂O: distilled water) was calculated as: $q_s = W_s/W_a$, and the *H*-index was determined as: $H = q_{170}/q_{H_2O}$.

Fig. 3 PolyHEMA/TMPT particles (H90).

groups^{26,27}. The zeta potential of each polymer particle was measured, and all five polymers presented a negative surface charge but their charges were not significantly different (Table 1).

Cytocompatibility of polyHEMA/TMPT particles

To assess the toxicity of the polyHEMA/TMPT particles to cells, mouse calvaria-derived osteoblast-like cells (MC3T3-E1 cells) were cultured with the particles. Through observations using a scanning electron microscope, the cells were found to spread well on the surface of the particles and exhibited spindle or polygonal shapes after a 3-day culture period. A Live/Dead staining examination revealed that the cells below and around the polymer particles were all alive. The polyHEMA/TMPT particles fabricated have high polymerization rates and no release of unpolymerized HEMA or TMPT from the particles after the washing

procedure was detected.

EFFECTIVENESS AS CARRIERS OF GROWTH FACTORS

It is well known that growth factors are important molecules to promote tissue regeneration, and a lot of research to utilize growth factors for regenerative therapy has been conducted in the field of dentistry. A promising concept involves combining growth factors with restorative materials to make them “bio-promoting”. However, since growth factors are proteins, simply adding them to restorative materials is not recommended because of the following reasons; i) dental restorative materials consist of various components and some ingredients may impair the action of growth factors; ii) proteins are large molecules and may hamper setting or curing of the materials. In addition, the release of large molecules can deteriorate the materials as a function of time; iii) proteins are sensitive to thermal conditions. Especially in the case of resin-based materials, the heat generated by polymerization may denature proteins. To exclude these problems and obtain release in an effective manner, we attempted to use polyHEMA/TMPT particles as a carrier vehicle for growth factors²¹.

Loading and release of model protein

To investigate the feasibility of polyHEMA/TMPT particles as a protein reservoir, the uptake of protein by 90% HEMA/10% TMPT-polymer particles was first investigated using bovine serum albumin (BSA) as a model protein. When the polymer particles were immersed in FITC-labeled BSA solution at 500 μg/mL for 24 h and observed using confocal laser scanning microscopy (CLSM), adsorption of BSA onto the particle surface was clearly observed (Fig. 4). Such BSA-loaded polymer particles released BSA into distilled water at least up to 14 days. The release pattern demonstrated an initial burst and a gradual decrease thereafter,

indicating that BSA was mostly adsorbed to the outer surface layer of the particles.

Loading and release of fibroblast growth factor-2 (FGF-2)

FGF-2 is widely used to accelerate wound healing^{28,29}. FGF-2 promotes the proliferation of various types of cells such as vascular endothelial cells, chondrocytes, osteoblasts, and mesenchymal stem cells^{30–33}. It also stimulates angiogenesis and tissue development^{28,34}. Based on the outstanding clinical outcome of FGF-2 for periodontal regeneration³⁵, Regroth® (Kaken Pharmaceutical, Tokyo, Japan), of which the main active ingredient is recombinant human FGF-2, was launched on the market recently, as the world's first pharmaceutical for periodontal regeneration. Therefore, we chose FGF-2 as the growth factor to be loaded into the polyHEMA/TMPT particles.

To load FGF-2, the polymer particles were immersed in FGF-2 solution (Fiblast®, Kaken Pharmaceutical) at 500 µg/mL and incubated for 24 h. FGF-2 uptake by the polymer particles was achieved using this procedure, at a

16.3% adsorption ratio. When the FGF-2-loaded particles were immersed in distilled water, they showed a similar release profile as that of the BSA-loaded particles. After an initial discharge of the large amount during the first 12 h, a continuous release of a small amount of FGF-2 was observed for up to 14 days. However, the initial burst release was greater for FGF-2 compared with that of BSA. This is probably because of the difference in the molecular size of BSA and FGF-2. The size of FGF-2 and BSA are 17 and 67 kDa, respectively, and the average diameter is 3–4 nm for FGF-2²⁹ and 7–9 nm for BSA³⁶. For the release of proteins based on the simple mechanism of water diffusion, smaller sized molecules appear to be easily desorbed from the polyHEMA/TMPT particles.

Effects of FGF-2-loaded polymer particles on osteoblasts

To examine if the activity of FGF-2 is maintained after being loaded into the particles, samples of eluates from FGF-2-loaded polymer particles into culture medium (alpha-MEM) were collected periodically and MC3T3-E1 cells were cultured in the presence of those eluates. Cell proliferation was significantly promoted by the eluates (Fig. 5) at all collection periods up to 14 days. The FGF-2 released into the medium from the polymer particles was confirmed as maintaining its activity.

Application of FGF-2-loaded polymer particles to restorative materials

The most common “bio-promoting” material currently used in restorative dentistry is mineral trioxide aggregate (MTA). MTA is a type of calcium silicate cement and is used for various treatments such as direct pulp capping, root-end filling, root canal filling, perforation repair, and apexogenesis^{37,38}. However, the application of dental adhesive resins is also expanding to include various treatments such as restoration of fractured roots^{39–42}, root-end fillings⁴³, or perforation sealing⁴⁴. Among a plethora of dental adhesive resins, a 4-methacryloxyethyl trimellitate anhydride (4-META)/methyl methacrylate (MMA)-based resin is

Fig. 4 Loading of BSA into polyHEMA/TMPT particles (CLSM image using FITC-labeled BSA).

Fig. 5 Promotion of proliferation of MC3T3-E1 cells by the eluates from FGF-2-loaded polymer particles.

Phase contrast microscopy image of MC3T3-E1 cells cultured in the presence of the eluates from (A) the control polymer particles without FGF-2 loading and (B) the particles loaded with FGF-2.

highly biocompatible, and exhibits excellent bonding and sealing capabilities in wet conditions⁴⁵. Our study demonstrated that osteoblastic cells could proliferate well on cured 4-META/MMA-based resins and the differentiation of pluripotent mesenchymal precursor cells was not hampered^{46,47}. Considering these advantages, the commercial 4-META/MMA-based adhesive resin sold as Super Bond C&B® (SB; Sun Medical, Moriyama, Japan) is clinically used to treat fractured roots. However, conventional adhesive resins including SB have no ability to promote tissue healing and a favorable prognosis cannot be expected for cases with severe damage of surrounding tissue⁴⁰. In some cases, incomplete healing has been reported for the bonding of fractured roots with adhesives because of insufficient regeneration of periodontal tissue⁴⁸. To increase the success rate of treatments using adhesive materials, it is beneficial to provide the ability to release FGF-2.

It has already been demonstrated that the heat generated by the polymerization of SB is not in the range to influence proteins⁴⁹. The temperature inside the adhesive during polymerization does not reach 41°C, which is enough low to maintain the structure and function of FGF-2. Unpolymerized 4-MET or MMA released from the cured SB had no negative influence on the ability of FGF-2 to promote the proliferation of osteoblast-like cells⁴⁹. We have confirmed that FGF-2 released from SB combined with FGF-2-loaded polyHEMA/TMPT particles could promote osteoblastic proliferation. The effectiveness of an experimental adhesive containing FGF-2-loaded particles, in terms of tissue regeneration *in vivo*, is currently under investigation.

EFFECTIVENESS AS CARRIERS OF ANTIMICROBIALS

Many dental diseases are caused by the oral microbiome, and restorative materials with antimicrobial effects could be useful for preventing such infectious diseases. A conventional approach to provide dental restorative materials with infection control abilities is to incorporate water-soluble antimicrobials and enable their release in a wet environment⁵⁰. However, the duration of the antimicrobial effects produced using this method is limited to a short period and continuous delivery of the agents is not possible. Aiming at achievement of reconstructive materials which exhibit long-lasting antimicrobial effects, we incorporated non-biodegradable polyHEMA/TMPT particles into resin-based materials as reservoirs for water-soluble antimicrobials. Since synthetic antimicrobials are more stable compared with proteins, it is possible to load them into polyHEMA/TMPT particles using two different methods. One method is similar to that used for growth factors; *i.e.* immersion of the particles into an aqueous solution of the antimicrobial to facilitate uptake. The other method is to add antimicrobials into the HEMA/TMPT monomer mixture and cure it to

produce polymers.

Loading of cetylpyridinium chloride using the immersion method

Cetylpyridinium chloride (CPC), a quaternary ammonium compound, is highly soluble in water, and is frequently incorporated in oral rinses or tooth pastes. CPC has a strong antimicrobial activity against oral bacteria and fungi^{51–55}, and was chosen as the antimicrobial to be loaded into the polymer particles.

To load CPC using the immersion method, the polyHEMA/TMPT particles were placed into 30 µL of CPC aqueous solution at 500 µg/mL (total amount of CPC=15 µg) and stored for 24 h. The particles were washed in water to remove loosely bound CPC. The polymer particles with HEMA at 50% or more (H50, H70, H90) demonstrated greater adsorption of CPC than the particles with HEMA at 30% or less (H10 and H30). Especially, H70 and H90 demonstrated adsorption at more than 97% (Fig. 6).

However, the release profile of CPC was totally different among the five CPC-loaded particles (Fig. 6). It was found that more than 91% of the CPC adsorbed to the H10 and H30 particles was rapidly released within 24 h. On the contrary, only a small amount of adsorbed CPC (<4%) was released from H70 and H90 particles, and most of the CPC remained trapped within the particles. The particles comprising 50% HEMA/50% TMPT (H50) showed the largest amounts of release (Fig. 6). As for the release duration, H50 showed release over 48 h, the longest period of release among the five particles.

To analyze the binding mechanism of CPC to polyHEMA/TMPT, Triton X-100 or NaCl solution was used as an elution medium and the release of CPC was evaluated. Because Triton X-100 hinders hydrophobic interaction^{56,57}, CPC adsorbed to polymers through hydrophobic interaction is desorbed by this surfactant. An NaCl solution is effective to assess the involvement of electrostatic interaction in the binding of CPC to polyHEMA/TMPT since CPC is cationic and shows strong interaction with negatively charged substances²⁷.

Fig. 6 Total amounts of CPC adsorbed to the polymer particles and released into water within 48 h.

The results indicate that almost all the loaded CPC was released into Triton X-100 from all the polymer particles, while the adsorbed CPC was not desorbed by the NaCl solution. These results indicate that CPC binds to the frame of the HEMA/TMPT polymer mainly through hydrophobic interactions.

The loading of CPC and its release from polyHEMA/TMPT can be explained by the state of CPC, polymer mesh sizes, and the hydrophobicity of the polymer as shown in Fig. 7. As a cationic surfactant, CPC forms micelles at concentrations above 322 $\mu\text{g/mL}$ ⁵⁸⁾. Since the polymers were immersed into a 500 $\mu\text{g/mL}$ CPC solution, CPC in the small micellar state was taken up by the polymers. The average size of such CPC micelles is approximately 1 nm⁵⁹⁾. On the other hand, it has been reported that an increase in the HEMA content resulted in a larger polymer network mesh size⁶⁰⁾. For H10, which has the smallest mesh size among the five polymers, a small amount of CPC micelles is adsorbed to the superficial part of the polymer and most of the adsorbed CPC is rapidly released within a short period. For H30, which has a larger polymer mesh size than H10, more micellar CPC is adsorbed to the polymer, and a longer release of CPC is possible than from H10. As for H70 and H90, which have higher water absorbability and larger mesh sizes, a greater amount of CPC is trapped more deeply within the polymer, compared with H10, H30, and H50. However, CPC taken up by these polymers is desorbed less because of strong adsorption through hydrophobic interaction. Among the five polymers fabricated, the polymer particles comprising 50% HEMA/50% TMPT have an optimal mesh size for the penetration of micellar CPC and a binding affinity with CPC, showing the largest amounts of release, for

the longest period.

Loading of CPC using a pre-mixing method

It has been reported that methacrylate resins to which chlorhexidine powder was added before polymerization exhibit release of the agent for 4–5 weeks^{61,62)}. Through the pre-mixing method, the antimicrobial is dispersed homogeneously inside the polymer and water-diffusion based release can be slow and proceed for longer. The CPC-loaded polymer particles, prepared through mixing CPC powder with the 50% HEMA/50% TMPT monomer before polymerization, demonstrated a longer period of CPC release, as expected. The extension of the release period was remarkable compared with immersion-loaded H50 polymers, from which release became non-detectable after 72 h. The polymer pre-mixed with 0.5% CPC demonstrated release of CPC into distilled water for 20 days. For 5% or 10% CPC-pre-mixed polymers, the release of CPC continued over 120 days. In addition to the homogenous dispersion of CPC inside the polymer, CPC dissolved in a monomer mixture of HEMA and TMPT does not form micelles as it does in water. Therefore, it is possible that binding of non-micellar CPC to the HEMA/TMPT polymer by hydrophobic interaction is stronger and therefore long-term release is attained.

Antibacterial activity of CPC-loaded polymer particles

CPC has a wide antibacterial spectrum and is effective in inhibiting various oral bacteria. To confirm that CPC-pre-mixed polymer particles exhibit antibacterial effects by releasing CPC, agar diffusion tests were conducted. The 0.5, 5, and 10% CPC-pre-mixed polymer particles demonstrated inhibition against *Enterococcus faecalis*, *Streptococcus mutans*, *Lactobacillus casei*, or *Candida*

Fig. 7 Schematic diagram of loading of CPC and its release from polyHEMA/TMPT particles.

Table 2 Size of inhibition zones produced by CPC-pre-mixed polymer particles by agar diffusion tests

Bacteria	Particles	0.5% CPC-premixed polymer particles	5% CPC-premixed polymer particles	10% CPC-premixed polymer particles
<i>Streptococcus mutans</i> NCTC10449		1.00±0.14	4.22±0.14	4.98±0.15
<i>Lactobacillus casei</i> ATCC4646		1.37±0.06	3.57±0.37	4.40±0.15
<i>Enterococcus faecalis</i> SS497		0.93±0.07	2.16±0.12	4.89±0.48
<i>Candida albicans</i> SC5314		1.70±0.22	4.36±0.57	5.57±0.23
(Mean±SD (mm))				

The 0.5, 5 or 10% CPC-pre-mixed polymer particles was filled into wells of an agar plate inoculated with each bacterium. After 72 h of incubation at 37°C, production of inhibition zones (mm) was determined.

Fig. 8 Exhibition of long-lasting antibacterial effects produced by an experimental endodontic sealer containing CPC-loaded polymer particles. An experimental sealer containing CPC-pre-mixed polyHEMA/TMPT particles demonstrates inhibition against *E. faecalis* after immersion in water for 14 days (left) or 28 days (right).

albicans. In addition, as the amount of CPC loaded into the polymer was increased, greater inhibition was observed (Table 2).

Sustained release of CPC through recharging

It was found that smaller molecule CPC is easily taken up by the polyHEMA/TMPT particles. Based on such characteristics, recharge of CPC to the particles is possible after CPC is released and depleted. As expected, by exposing 50% HEMA/50% TMPT polymer particles depleted of CPC, to CPC solutions, recovery of CPC release was possible. This recharging process can be repeated and is applicable for both immersion-loaded and pre-mixing-loaded particles. It is also possible to control the recovery level of CPC release by adjusting the concentration of the recharging solution and the exposure time. For the 0.5% CPC-pre-mixed polymer, the release of CPC above the minimum inhibitory concentrations (MIC) values against *Streptococcus mutans*, *Lactobacillus casei*, *Enterococcus faecalis* or *Candida albicans* was maintained by a 5 min exposure to a CPC solution at 500 µg/mL. Therefore, the combination

of pre-mix loading of CPC powder and recharging using a CPC solution is effective to achieve persistent antimicrobial effects with a sustained release of CPC.

Application of CPC-loaded polymer particles to restorative materials

Currently, an attempt to fabricate a novel endodontic sealer containing CPC-pre-mixed polymer particles is under investigation. To provide endodontic filling materials which exhibit long-lasting antibacterial effects after root canal treatment, an approach to apply these particles which facilitate the long-term release of CPC is likely to be beneficial. The experimental sealers were prepared by combining CPC-pre-mixed polymer particles with a commercial HEMA-based endodontic sealer (MetaSEAL Soft®, Sun Medical). This experimental sealer containing CPC-pre-mixed particles exhibited long-lasting antibacterial activity against *E. faecalis* (Fig. 8).

Other applications of this CPC-sustained release system include denture bases or provisional crowns. Resinous materials composed of polymethyl methacrylate

(PMMA) are prone to plaque accumulation, which is responsible for a variety of clinical problems, including denture stomatitis, periodontal disease, and dental caries. Incorporation of CPC-loaded-polyHEMA/TMPT particles with the ability to recharge CPC has the potential to exhibit sustained antibacterial effects to inhibit biofilm formation.

CONCLUSIONS

The non-biodegradable polymer particles which we developed are useful as carriers of growth factors or antimicrobials to be combined with restorative materials. Such technology is beneficial for a big leap of restorative materials to next generation with “bio-active” functions. Their application may be further expanded to other materials to provide preventive effects against oral diseases and the promotion of tissue regeneration.

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