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Effectiveness of non-biodegradable poly(2-hydroxyethyl methacrylate)-based hydrogel particles as a fibroblast growth factor-2 releasing carrier

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ABSTRACT

Objectives. Dental resin-based restorative materials are used in a variety of dental treatment modalities such as root-end filling, perforation sealing, and adhesion of fractured roots. However, the prognosis after such treatments is not necessarily favorable because they fail to promote healing of the surrounding alveolar tissue. In the present study, non-biodegradable poly-2-hydroxyethyl methacrylate (polyHEMA)-based hydrogel particles were fabricated as a carrier vehicle for drug delivery that is applied to dental resins.

Methods. The loading and release characteristics of bovine serum albumin (BSA) and fibroblast growth factor-2 (FGF-2) from the polyHEMA-based hydrogel particles were evaluated over time in culture. The hydrogel particles were immersed into an aqueous FITC-labeled BSA solution and were observed using confocal laser scanning microscopy (CLSM). To determine the activity of the FGF-2 released from the particles, the proliferation of osteoblast-like cells cultured with eluates collected from the particles for up to 14 days was determined.

Results. CLSM revealed that BSA was adsorbed to the surface of the hydrogel particles. A sustained release of BSA and FGF-2 from the particles was detected for up to 14 days. The eluates from the FGF-2-loaded particles increased the proliferation of the osteoblast-like cells, suggesting that the activity of FGF-2 was maintained for at least 2 weeks within the particles.

Significance. These polyHEMA-based non-degradable hydrogel particles may be useful tools that can be applied to dental restorative materials to achieve sustained delivery of drugs that promote tissue regeneration.

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1. Introduction

The basic properties of many dental resins, including their mechanical, aesthetic, and bonding properties, have been dramatically improved based on materials research performed during the last several decades. Recently, further advancements in dental resins were achieved, and antibacterial adhesives were put into clinical use [1]. Because of these advances in performance, the clinical use of dental resins is expanding. The application of adhesive resins to various treatments such as the restoration of fractured roots [1–4], root-end fillings [5,6], and perforation sealing [7] has generated the most interest. However, conventional adhesive resins are unable to promote healing of the surrounding tissues and a favorable prognosis cannot be expected in cases with severe damage to the surrounding tissue [2]. Moreover, unfavorable results after the treatment of fractured roots, such as occasional induction of ankyloses, using resin-based materials have been reported because of their incompatibility with connective tissue [8].

To improve the biocompatibility of dental resins to connective tissue and further expand their use for new dental and general medical treatment modalities, it may be beneficial to create bio-active dental resins. One possible approach is to create resins that can release growth factors that promote tissue regeneration. However, it is generally difficult to generate an effective release of proteins simply by adding them to the resin. Additionally, various organic components of the resins, as well as their polymerizing reactions, may adversely influence the activities of the proteins. We believe that combining a non-biodegradable hydrogel as the carrier for growth factors with a resin-based material is an effective method for avoiding these problems.

Recently, we developed a novel non-biodegradable hydrogel consisting of 2-hydroxyethyl methacrylate (HEMA) and the cross-linking monomer trimethylolpropane trimethacrylate (TMPT) and reported that these methacrylate-based hydrogels were provided the sustained release of cetylpyridinium chloride [9]. We hypothesized that this hydrogel could also be beneficial for tissue regeneration by releasing growth factors over time. The purpose of the present study was to determine the loading and release profiles of fibroblast growth factor-2 by the non-biodegradable polyHEMA-based hydrogel particles *in vitro*, as well as the effects of released FGF-2 on the proliferation of osteoblast-like cells.

2. Materials and methods

2.1. *Fabrication of the polyHEMA-based hydrogel particles*

To fabricate the non-biodegradable hydrogel, HEMA (Tokyo Chemical Industry Co., Tokyo, Japan) and TMPT (Shin-Nakamura Chemical Co., Wakayama, Japan) (Fig. 1) were mixed at a wt.% of 90:10 with 0.5% benzoyl peroxide. The monomer mixture underwent polymerization at 120 °C for 2 h followed by post polymerization for 16 h at −0.1 MPa. Next, the polymers were pulverized using a planetary ball mill into 550 ± 200-μm diameter particles. The particles were then

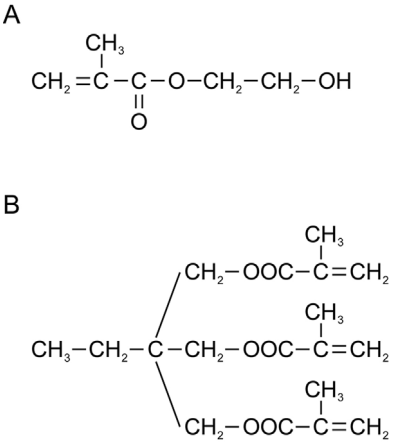

Fig. 1 – The monomers used to fabricate the hydrogel. (A) 2-hydroxyethyl methacrylate (HEMA) and (B) trimethylolpropane trimethacrylate (TMPT).

washed by immersion in distilled water for 48 h and dried at 60 °C for 24 h. To confirm that no residual uncured monomers remained in the specimens, the eluates obtained by immersion of 30 mg of particles in 100 μL of distilled water at 37 °C for 24 h were analyzed by high performance liquid chromatography (HPLC; Prominence, SHIMADZU, Kyoto, Japan).

The water adsorption property of the hydrogel particles was determined essentially as previously described [10], with minor modifications. One gram of the hydrogel particles was immersed in 25 mL of distilled water and stored at 25 °C for 4 days. Based on the weight before (W_a) and after storage (W_b), the water absorption ratio and water content were calculated as follows:

$$\text{Water absorption ratio}\,(\%) = \frac{W_b - W_a}{W_a} \times 100$$

$$\text{Water content}\,(\%) = \frac{W_b - W_a}{W_b} \times 100$$

These experiments were repeated three times.

2.2. *Cytocompatibility of the polyHEMA-based hydrogel particles*

Mouse calvaria-derived osteoblast-like cells (MC3T3-E1; RIKEN Cell Bank, Tsukuba Science City, Japan) were maintained in α-minimum essential medium (α-MEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen) in a humidified 5% CO_2 incubator at 37 °C.

MC3T3-E1 cells were seeded at 2×10^4 cells/well onto 30 mg of hydrogel particles that had been placed in the wells of 48-well tissue culture plates and cultured in a humidified 5% CO_2 incubator at 37 °C. After 3 days, the specimens were rinsed with phosphate-buffered saline (PBS), fixed with 0.1 M half-strength Karnovsky solution, and dehydrated in a series of ethanol concentrations up to 100%. The samples were then lyophilized using t-butyl alcohol and sputter-coated with platinum. The attachment of the cells to the particles and

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