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Development of a novel dental resin cement incorporating FGF-2-loaded polymer particles with the ability to promote tissue regeneration

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ABSTRACT

Objective. Aiming to achieve bioactive dental resins that promote healing of surrounding tissues, we developed novel poly(2-hydroxyethyl methacrylate/trimethylolpropane trimethacrylate) (polyHEMA/TMPT) particles. These particles have been reported to be useful as a non-biodegradable carrier for fibroblast growth factor-2 (FGF-2) *in vitro*. The aim of this study was to evaluate the ability of an adhesive resin incorporating FGF-2-loaded polymer particles to promote tissue regeneration *in vitro* and *in vivo*.

Methods. Experimental adhesive resins were prepared by incorporating FGF-2-loaded polyHEMA/TMPT particles into a 4-META/MMA-based adhesive resin, and the release profiles of FGF-2 were evaluated. The proliferation of osteoblast-like cells in the eluate from cured experimental resin was assessed. When the experimental resin was implanted into rat calvaria defects, bone regeneration was evaluated by microcomputed tomography and histological observations.

Results. Sustained release of FGF-2 from the experimental resin was observed for 14 days. Eluate from the cured experimental resin significantly promoted the proliferation of osteoblast-like cells. Significantly greater bone regeneration was observed using the experimental resin compared with the control resin without FGF-2.

Significance. 4-META/MMA-based adhesive resin incorporating FGF-2-loaded polymer particles is useful to promote tissue regeneration, suggesting that its application would be beneficial for root-end filling or the repair of fractured roots in cases with severely damaged periodontal tissue.

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1. Introduction

Dental adhesives are widely used for dental restoration. Recently, they have been used for many other applications, such as root-end filling [1–3], perforation sealing [4], and repair of fractured roots [5–8], owing to the improvement of their physical and sealing properties. However, conventional adhesive resins have no ability to promote tissue regeneration, and a favorable prognosis cannot be expected in cases with severely damaged periodontal tissue. To increase the success rate of treatments using adhesive resins in periodontal tissue, it would be beneficial to provide the ability to promote tissue regeneration.

Growth factors are important molecules that promote tissue regeneration, which are used for regenerative therapy in the field of dentistry [9–11]. Therefore, combining growth factors with adhesive resins is a promising approach to provide them with the ability to regenerate tissues by the release of active agents [12]. However, simply adding growth factors to adhesive resins is not recommended. Adhesive resins consist of various components that may impair the action of growth factors. In addition, because growth factors are large protein molecules, they may hamper setting or curing of adhesives. To circumvent these problems, the use a non-biodegradable vehicle as a carrier for delivery of growth factors is beneficial to provide adhesive resins with the ability to regenerate tissues. Recently, we have developed non-biodegradable polymer particles for delivery of antimicrobials or growth factors for application in dental resins [12,13]. Because the particle consisting a hydrophilic monomer 2-hydroxyethyl methacrylate (HEMA) and cross-linking monomer trimethylolpropane trimethacrylate (TMPT) is a non-biodegradable carrier, it is stable even after drugs leach out. Our *in vitro* study reported that polyHEMA/TMPT particles are cytocompatible and effectively act as a reservoir to release fibroblast growth factor-2 (FGF-2) [14]. When FGF-2 was loaded into such particles, sustained release of FGF-2 was maintained for up to 14 days. In addition, FGF-2 released from the particles promoted the proliferation of osteoblast-like cells. Based on these previous findings, it is expected that FGF-2-loaded polyHEMA/TMPT particles incorporated into adhesive resins will demonstrate sustained release of FGF-2 and promote tissue regeneration.

The wetting characteristics of polymer particles loaded into a solution of FGF-2, as prepared previously, have less operability and might undermine the physical properties of the base resin. It is hypothesized that lyophilization treatment, which is a dehydration process to make particles more convenient for handling while preserving a protein, may overcome these drawbacks. The purpose of this study was to assess the influence of lyophilization treatment of FGF-2-loaded polymer particles on the release profile of FGF-2 and the proliferation of osteoblast-like cells *in vitro*. Furthermore, experimental adhesive resins were prepared by incorporating freeze-dried FGF-2-loaded polyHEMA/TMPT particles into a commercial 4-[2-(methacryloyloxy)ethoxycarbonyl]phthalic anhydride (4-META)/methyl methacrylate (MMA)-based adhesive resin, and their capacity for tissue regeneration was evaluated *in vitro* and *in vivo*.

2. Materials and methods

2.1. Preparation of polyHEMA/TMPT particles

Procedures to prepare the polymer particles have been described previously [14]. Briefly, a mixture of HEMA (Tokyo Chemical Industry Inc., Tokyo, Japan) and TMPT (Shin-Nakamura Chemical Inc., Wakayama, Japan) at a weight ratio of 90/10 was polymerized at 120 °C for 2 h, followed by post-polymerization for 16 h at −0.1 MPa. These particles were pulverized into an average diameter of 591.2 ± 297.1 μm. Subsequently, the obtained polymer particles were immersed in distilled water for 9 days to remove unpolymerized monomers, dried at 60 °C for 24 h, and then subjected to the following experiments.

2.2. Evaluation of FGF-2 release from freeze-dried polymer particles

Polymer particles (30 mg) were immersed in a 30 μL solution of FGF-2 (Fiblast, Kaken Pharmaceutical Inc., Tokyo, Japan) at 500 μg/mL and stored at room temperature for 24 h. FGF-2-loaded polymer particles were irrigated with distilled water, excess water was removed, and the particles were stored at −80 °C overnight. Frozen particles were dried in a vacuum dryer (JFD-310, JEOL Inc., Tokyo, Japan) at −4 °C for 12 h.

To determine the release profile of FGF-2, 30 mg of freeze-dried particles were immersed into 200 μL of distilled water and incubated at 37 °C. After 15 and 30 min, 1, 3, 6, and 12 h, and 1 and 2 days, the water was replaced and the concentrations of FGF-2 in the eluates were measured using the Micro BCA protein assay kit (Thermo Fisher Scientific Inc., Kanagawa, Japan). These experiments were repeated five times.

2.3. Effects of FGF-2 released from the polymer particles on proliferation of osteoblast-like cells

Osteoblast-like cell line MC3T3-E1 was cultured in α-minimum essential medium with L-glutamine and phenol red (α-MEM) (Wako Pure Chemical Industries Inc., Osaka, Japan) supplemented with 10% fetal bovine serum (Equitech-Bio Inc., Kerrville, TX, USA) and 1% penicillin/streptomycin in a humidified 5% CO_2 incubator at 37 °C. FGF-2-loaded polymer particles (30 mg) were immersed into 100 μL of α-MEM at 37 °C for 15 min, and then collected to evaluate the effect of released FGF-2 on cells. MC3T3-E1 cells were seeded in 96-well plates at 2×10^4 cells/well and cultured overnight. Next, the medium was replaced with 10-fold-diluted eluates, and cell culture was continued for another 24 h. The cell number was determined by MTT assays. Briefly, 20 μL of 3-(4,5-dimethylthiazoly1-2)-2,5-diphenyl-tetrazolium bromide solution (Sigma Aldrich Inc., St. Louis, MO, USA) was added to each well, and the plates were incubated for 4 h at 37 °C. After removing the medium, 200 μL of dimethyl sulfoxide (Wako Pure Chemical Industries Inc., Osaka, Japan) was added to each well. The absorbance at 570 nm was measured using a microplate reader (Wallac 1420 ARVO Mx/Light; PerkinElmer Inc., Waltham, MA, USA). Medium collected after 15 min of particle immersion, which had not been loaded with FGF-2, and cells cultured with

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