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An enzyme-responsive nanoplatform for preventing collagen degradation in dental tissues

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Keywords

Peptide Hydrolases, Matrix Metalloproteinases, Matrix Metalloproteinase Inhibitors, Dental Caries Susceptibility, Periodontal Diseases, Micelles

Abstract

The collagen degradation mediated by host proteolytic enzymes, such as the metalloproteinases (MMPs), is strongly associated to the development of secondary caries at the restorative material-dental tissues interface and periodontal disease. While some potent MMP inhibitors have been identified, their incorporation in the dental biomaterials remains as a challenge due to chemical incompatibility, poor diffusion, and limited substantivity. Therefore, this study is aimed at developing an innovative MMP-responsive nanocarrier to serve as a delivery vehicle for enzyme inhibitors and promote their sustainable and on-demand release.

The newly designed nanocarrier is based on a micellar nanoparticle composed of a block-copolymer with MMP-9 recognizable/cleavable sequences. For the block-copolymer synthesis, the compounds (N-Benzyl)-5-norbornene-exo-2,3-dicarboximide and 1-{[(2S)-bicyclo[2.2.1]hept-5-en-2-ylcarbonyl]oxy}-2,5-pyrrolidinedione were synthesized.The polymerization was catalyzed by a modified Grubbs catalyst. The final block-copolymer was obtained via precipitation in cold methanol and centrifugation, and characterized by NMR spectroscopy.

The copolymer (50 mg/mL) was dissolved in an anhydrous mixture of dimethylformamide and dimethyl sulfoxide containing peptides with the amino acid sequence GPLGLAGGWGERDGS and N,N-Diisopropylethylamine (1:4:16 copolymer:peptide:DIPEA). After stirring for 27 hr, the solution was precipitated in cold methanol and centrifuged—the peptide-copolymer was characterized by NMR spectroscopy.

To form micelles, the block copolymer-peptide compound was first dissolved in DMSO and distilled water was added until reaching the critical micelle concentration (30% v/v aqueous). After 2 days of stirring, water was added until reaching 50% v/v aqueous. The final solution was transferred to dialysis tubing and placed in water. The buffer was exchanged 3x per day for 2 days, then the procedure was repeated once with Dulbecco's phosphate-buffered saline solution.

The micelles were characterized by transmission electron microscopy after being concentrated. The responsivity to MMP-9 was tested by incubating the micelles in buffer for 24 hr at 37 °C, and further analyzed by TEM.