Chitosan-coated nanofibrous sheet including human growth factor for treating oral mucositis

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ABSTRACT SUMMARY
Many cancer patients suffer from oral mucositis caused by chemotherapy and radiotherapy, however, there is a lack of drug-carriers to delivery for treating the mucositis. Therefore, in this study, a chitosan-coated nanofibrous sheet composed of Eudragit L100 and human growth hormone [CEL/hGH NS] as an anionic model drug was prepared to enhance oral mucou-wound healing by electrospinning. The CEL/hGF NS showed a sustain release profile of hGH from the sheet and the released hGH promoted a proliferation rate of human dermal fibroblasts. Also, when an oral muco-ulcer was dressed with the CEL/hGH NS in vivo, the wound was fully recovered compared to non-treatment after 7 days of treating. Therefore, the CEL/hGH NS showed a potential of a new drug carrier for treating mucositis.

INTRODUCTION
Oral mucositis occurs on mucous membrane of patient’s mouth and throat during chemotherapy and radiotherapy to treat cancer. 48% of cancer patients suffer from the mucositis, however, there is a lack of drug-carriers to delivery for treating the mucositis.1 Eudragit® have been developed to control a targeted release area of oral mediation without toxicity by Röhm GmbH (Germany).2 Among of the Eudragit® series, anionic Eudragit® L 100 [EL] composed of methacrylic acid and methyl methacrylate (1:1) dissolves above pH 6.0. Chitosan has been used as a coating material for a drug carrier to control a dissolution rate of the carrier and a release rate of the drug.3 Also, their positive charges could possibly have an intrinsic bio-adhesiveness property owing to developing molecular attraction forces by electrostatic interaction with negative charged mucosal surface.4,5 In this study, a nanofibrous sheet composed of EL and hGH as an anionic model drug was prepared to enhance oral mucou-ulcer healing by electrospinning. Subsequently, the sheet was physically surface-modified with chitosan in order to control a dissolving rate of EL in the sheet and release of hGH (Figure 1.). Then, CEL/hGH NS was confirmed healing efficacy of acid-induced oral mucositis.

EXPERIMENTAL METHODS
In order to prepare CEL/hGH NS, 12wt% EL solution including hGH (120μg/ml) in a mixture of N,N-dimethylacetamide and ethanol (5:1) was electrospun with following parameters; 1ml/h of a flow rate, 20kV of an electric potential, and 12cm of a horizontal distance from the end of needle to a collector. Each EL/hGH NS was immersed with 0, 0.5, and 1.0wt% of chitosan solution in 1.0% acetic acid for 6h, then, the chitosan-coated EL/hGH NS was washed with distilled water. After completely air-drying, for investigating release profile of hGH from CEL/hGH NS, 2mg CEL/hGH NS was incubated with 5 ml PBS (pH 7.4) at 37°C for 90min. Then an amount of released hGH was quantitative-
ely determined by ELISA. Human dermal fibroblasts (HDFs) (1x10^4 cells/well) with 1mg of CEL/hGH NS were incubated without growth supplements for 3 days. The proliferation of HDFs was determined by MTT assay. In order to confirm recovery efficacy on acid-induced oral muco-ulcers of a beagle, ulcers were covered with various CEL/hGH NSs (1x1cm^2). After 3 days of ulcer dressing with CEL/hGH NSs, ulcers were applied identical CEL/hGH NSs for secondary dressing. After 4 days of the second dressing, the regenerated muco-membranes were dissected for H&E staining.

RESULTS AND DISCUSSION

Loaded hGH was controlled a release behavior from CEL NS as increasing the amount of chitosan on a surface of CEL NS (Figure 2). While the loaded hGH released 33.15% from EL NS after 10 min of the release procedure, 0.5% and 1.0% CEL NS showed sustained release of 27.06% and 22.39% of the loaded hGH, respectively, after 180 min. Also, a release rate (v = 0.37) of hGH from 1.0% CEL NS was slower than that (v = 0.89) from 0.5% CEL NS at 10 min.

As shown in Figure 3, while EL/hGH NS showed lower cell proliferation compared to TCPS for 3 days, 0.5% and 1.0% CEL/hGH NSs showed 1.7- and 1.5-fold increase of cell proliferation, respectively, compared to TCPS at day 3 because hGH sustainedly released from the CEL/hGHs NS for 3 days. Interestingly, 0.5% CEL NS showed approx. 1.2-fold higher of the cell proliferation than 1.0% CEL NS because hGH dose-dependently stimulates cell proliferation. Hence, the cell proliferation was efficiently enhanced by a larger amount of released hGH from 0.5% CEL NS compared to that from 1.0% CEL NS (Fig. 2).

As shown in Figure 4, an ulcer completely closed which was no treatment (Fig. 4a) or dressed with 0.5% CEL/hGH NS (Fig. 4c). However, the oral mucous membrane dressed with 0.5% CEL/hGH NS showed a thick regenerated epidermis whereas the mucous membrane with no treatment showed an extremely thin regenerated epidermis. Because released hGH from the 0.5% CEL NS would promote a rate of cell proliferation in epidermis and dermis, and hGH stimulates synthesis of insulin-like growth factor (IGF) which is a mitogen. However, an ulcer still opened which was covered with EL/hGH NS (Fig. 4b) or 1.0% CEL/hGH NS (Fig. 4d).

CONCLUSION

EL NS containing hGH was successfully fabricated by electrospinning. hGH was slowly released from the CEL/hGH NS owing to surface-coated chitosan layer and the released hGH promoted a proliferation of HDFs. Oral muco-ulcer dressed with the 0.5% CEL/hGH NS was fully recovered compared to CEL NS and no treatment for 7 days. Therefore, the CEL/hGH NS showed a potential of a new drug carrier for treating mucositis.

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