Low Molecular Weight Methylcellulose-Based Injectable Hydrogel for Protein Delivery

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ABSTRACT SUMMARY
Low Molecular Weight Methylcellulose (LMWMC)-based injectable hydrogel was developed for protein delivery. The molecular weight of methylcellulose was controlled by the addition of cellulase and confirmed by the gel permeation chromatography method. Protein drugs, exenatide and FGF21, were successfully loaded into methylcellulose based hydrogel and in vitro release result showed sustained release profile of loaded protein drugs.

INTRODUCTION
Although various protein based drugs have been produced for the treatment of several diseases, there are several challenges such as stability, enzymatic degradations and efficacy in the body1, 2. To overcome these limitations of protein drugs, various delivery systems have been applied for protein delivery. Delivery systems have shown to increase the stability of protein in vivo by protecting protein from antibody neutralization and proteolysis. In situ forming hydrogel is one of drug delivery systems, which has been applied for various biomedical applications. In situ forming hydrogels can be formed using several mechanisms such as pH, solvent exchange, UV-irradiation, cross-linking and temperature.

We previously reported injectable, thermo-reversible hydrogels for protein drug delivery. Methylcellulose is a hydrophobically modified natural polymer, which forms physical gels in aqueous solution by increasing temperature. For clinical application, molecular weight of methylcellulose should be reduced to form a gel at the body temperature and be eliminated by globular filtration3.

In this study, we reduced the molecular weight of methylcellulose using cellulase hydrolyzing 1, 4-beta-D-glycosideic linkages of methylcellulose. The molecular weight of methylcellulose was confirmed by the gel permeation chromatography and in vitro release profiles were determined with exenatide and FGF21.

EXPERIMENTAL METHODS
LMWMC was prepared using commercially available methylcellulose. The 30U/ml cellulase was added to 4% methylcellulose stock solution and incubated at room temperature with stirring. After 3 days, MC solution was heated for 10 min at 80°C and transferred into cellulose ester dialysis membrane (MWCO 2000 Da). The dialysis bag was immersed in D.W and stirred for 3 days. The final low molecular weight methylcellulose solution was collected and freeze-dried.

The release profiles of exenatide and FGF21 from hydrogel were measured at 37°C. The exenatide and FGF21 were mixed with 25% methylcellulose stock solution. After gel formation, pre-warmed PBS, pH7.4, was filled over the gel and shaken at 150rpm. The released
protein concentration was measured by the D.C protein assay.

RESULTS AND DISCUSSION

The molecular weight of methylcellulose was controlled depending on the cellulase incubation time. Figure 1 represents molecular weights of original methylcellulose and degraded methylcellulose. The cellulase treatment successfully reduced the molecular weight of methylcellulose by hydrolysis of 1, 4-beta-D-glycosidic linkages in methylcellulose. The average molecular weight of degraded methylcellulose was 14kDa.

Figure 1. GPC results of original methylcellulose (a) and low molecular weight methylcellulose (b).

As shown in figure 2, exenatide and FGF21 were released from 20% methylcellulose based hydrogel with biphasic and sustained release manners over a period of 7 days. The methylcellulose forms a unique gel structure by hydrophobic interaction between methyl groups. This gel structure is viscous enough to hold exenatide and FGF21 and slowly release exenatide and FGF21.

Figure 2. In vitro release of exenatide and FGF21 from hydrogel

CONCLUSION

The low molecular weight methylcellulose which is practically applicable in vivo was produced through the cellulase chopping process. The molecular weight of methylcellulose was confirmed using gel permeation chromatography and in vitro release of protein also tested. The gel permeation chromatography result demonstrated a reduction of MC molecular weight. The exenatide and FGF21 were easily and 100% loaded into methylcellulose based hydrogel and slowly released from hydrogel with no initial burst. Low molecular weight MC-based injectable hydrogel provides a strong potential to be used for efficient protein drug delivery.

REFERENCES


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