Biomimetic hydrogels with dual function peptides for modulating angiogenesis and neurogenesis

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ABSTRACT SUMMARY

Angiogenesis and neurogenesis is two major factors for tissue regeneration. It has been known that neuropeptides such as substance P also involved in the angiogenesis. We evaluated the angiogenic activity of well known neuropeptides, secretoneurin, neuropeptide Y, and substance P. Neuropeptides are immobilized in the hyaluronic acid based hydrogels. Stem cells in the hydrogels showed the differential angiogenic and neurogenic activities depending on the neuropeptides. We could confirm that these neuropeptides has angiogenic as well as neurogenic activity. This novel hydrogel can be used for regeneration of complex tissues, which requires angiogenesis as well as neurogenesis.

INTRODUCTION

Neuropeptides including secretoneurin (SN), substance P (SP) and neuropeptide Y (NPY) are synthesized in nerve cells and stored in large dense core vesicles in nerve endings and biological effects on target cells via specific receptors.

Recently, it has been found that neuropeptides such as secretoneurin (SN), substance P(SP), and neuropeptide Y(NPY) have involved in angiogenesis as well as neurogenesis.

SN, a 33-amino acid neuropeptide derived from secretogranin II(chromogranin C,CHGC) induces angiogenesis and postnatal vasculogenesis1. NPY, a 36-amino acid peptide, is a mediator of neurogenic angiogenesis during development 2. SP is a member of tachykinin family neuropeptides which are small molecules(11-amino acid). SP mediates pain perception and regulates wound healing, inflammation, tumor cell proliferation, and angiogenesis. Especially, SP induces angiogenesis through an enhanced recruitment of angiogenic cells.3

For complex tissue regeneration, angiogenesis and neurogenesis is the important factors. In this study, we compare the neurogenic activity as well as angiogenic potentials of neuropeptides by immobilizing in the hydrogels. We evaluated the behavior and differentiation mesenchymal stem cells in vitro.

EXPERIMENTAL METHODS

Acrylation and gel preparation was achieved by following the previous study4.

Mesenchymal stem cells were cultured in a T-75 cell culture plate, and adherent cells were isolated by trypsin. The resulting CB-MSC suspension was mixed with hydrogels with peptides (5x10^5 cells/50µl gel). The following four experimental groups were prepared: (A) HA-ac+ MMP-s 80% +RGD20% +CB-MSC; (B) HA-ac+ MMP-s 80% + SP 80%+CB-MSC; (C) HA-ac + MPP-s 80% +NYP 20%; (D) HA-ac + MPP-s 80% +SP 20%. The cell-containing hydrogels were cultured in complete EGM-2 medium(Lonza) for 21 days(5%, CO2,37°C).

Cellular morphology was observed at day 7 and 21. Gene expression of cells in each group was measured using real time PCR.

RESULTS AND DISCUSSION

Figure 1. synthetic scheme of hyaluronic acid-based hydrogel immobilized with Neuropeptide. and photography of HA base hydrogel. (MW; 800kDa, 3wt%)
Hydrogel was prepared by incubating reaction mixtures at 37°C. Michael type addition reaction allowed the immobilization of cysteine containing neuropeptides on the hydrogel. Stem cells was also entrapped in the hydrogel during hydrogel preparation (figure 1).

Cellular morphology was changed in the hydrogels with neuropeptides compared to the RGD peptide immobilized hydrogels. Stem cells in the SN, NYP and SP showed the cell aggregation and extensive tubular structure formation. Especially, stem cells in neuropeptide Y showed the extensive cell aggregation among samples (figure 2).

Figure 2. Evaluation of cell morphology of CB-MSCs in HA-based hydrogel. CB-MSCs cultured for 7 days and 21 days in HA based hydrogel. 5X10^5 CB-MSCs per construct. HA + MMPs 80% + RGD 20%, 7 days (A) and 12 days (E). HA + MMPs 80% + SN 20%, 7 days (B) and 21 days (F), HA + MMPs 80% + NYP 20%, 7 days (C) and (G), HA + MMPs 80% + SP 20%, 7 days (D) and (H).

We analyzed the gene expression of stem cells in the dual function neuropeptide immobilized hydrogels. Figure 3 showed the gene expression of angiogenic factors such as vWF, CD31, and CD105. This result shows that hydrogels with SP showed the highest gene expression among samples. Interestingly, neurogenic marker genes such as Pax 6, and nestin showed the higher level only in the SP immobilized group. Sox 2 showed the lower expression compared to RGD immobilized hydrogels. Based on the results, it is concluded that substance P is the promising dual function peptides for angiogenesis and neurogenesis.

Figure 3. Gene expression of hMSCs in various HA-based hydrogel by real-time RT-PCR at 21 days. (A) HA+MMPs 80% + RGD 20% (B) HA+MMPs 80% + SN 20% (C) HA+MMPs 80% + NYP 20% (D) HA+MMPs 80% + SP 20%.

In the future, we will apply this peptides in myocardial infarction model for in vivo evaluation.

CONCLUSION
We evaluated and compared the angiogenic as well as neurogenic activity on the hydrogels immobilized with neuropeptides. Even though all three neuropeptides have known to angiogenic potential, only substance P showed the dual functions in our system. Dual function peptide based hydrogels could be used for the complex tissue regeneration where angiogenesis and neurogenesis is required in the defect area.

REFERENCES