HA hydrogel modified with Nogo-66 receptor antibody inducing the neural regeneration in vitro

Cui FZ[a], Wei YT[a], Tian WM[a], Xu QY[b]
a. Department of Material Science and Engineering, Tsinghua University, Beijing 100084, PR China
b. Beijing Institute for Neuroscience, Capital University of Medical Sciences, Beijing 100054, PR China

Statement of Purpose:

CNS (central nervous system) injury often leads to functional loss. Functional recovery is restricted mainly due to the limited regeneration and plasticity of injured axons. Different approaches have used to enhance axonal regeneration. However to the best of our knowledge, the effect of the antibody to neurite growth inhibitors is not clear sofar. In this paper, the antibody modified HA hydorgel has been observed the effect of the antibody for axonal regeneration.

Methods:

1. The fragment (YDNNLQALPDNTERD) of receptor protein sequence was synthesized by solid phase, linked with keyhole limpet haemocyanin (KTH) then. The conjugation with Freund's adjuvant was immunized to rabbits several times in 3 months. Finally the titer of antibodies was 1:100,000 by ELISA detection.
2. The preparation method of HA-ADH hydrogel has been reported previously [1]. The antibody containing aldehyde group was prepared by oxidation of saccharide moieties in the Fc part of the antibody by the reaction with sodium periodate [2]. Then the hydrogel was soaked in the solution of oxidized antibodies in 5 ml volume of PBS and left overnight under rotary mixing at 4-8^{o}C [3].
3. Stability and rate of release of antibodies from hydrogel were investigated after incubation of polymer samples in 3 ml sodium acetate buffers at 37.8^{o}C. The IgG concentration was measured by UV spectrophotometer 3100 (Biochrom England). The concentration (μg/ml) of the antibody can be calculated using the empirical formula developed by Waddell as below: Antibody (μg/ml) = 144*(Abs_{215}-Abs_{225}).
4. Dorsal root ganglia (DRG) were processed to co-culture with the modified hydrogel which was close to the ganglia in order to observe a possible interaction between them [4]. In this co-culture system, the gels were located in the center and the ganglia scattered around it peripherally. Dishes were incubated for 72 h. The growth of neurites of DRG was observed under reverse phase microscope and digital images were acquired.

Results/Discussion:

1. The hydrogel with covalently attached antibody was incubated individually in buffer with different pH 5, 6 and 7.4, respectively. As shown in Fig.1, most of antibody (60–80%) was released from the hydrogel within 50 h. However, in pH 7.4 buffer solution antibody was released much slower and the duration time last at least 500 h. The release behavior is different from physically absorption, which would release most of antibodies with a few hours. But the antibody, directly incorporated into a biodegradable polymer scaffold, is released by a controlled mechanism that is regulated by chemically bond control the release of the protein.
2. In the co-culture system, DRG were planted and cultured beside the modified hydrogels. The distinct dissimilarities between side A and side B of ganglia were detected. At 72 h, many long neurites were sent up toward the gels on side A. However, the amount and length of neuritis on side B were visibly fewer than that on side A. Similar phenomena did not exist in the group of unmodified hydrogels. It informs that an anti-NgR delivery system has been processed successfully, and this system can gradually release antibody in certain behavior in the culture condition. Furthermore, the deduction that NgR antagonist might promote recovery and growth of injured neural cell was confirmed in vitro. Lastly the concentration gradient caused by continuous release of Nogo-66 receptor antibody may be the important reason of inducing axonal regeneration.

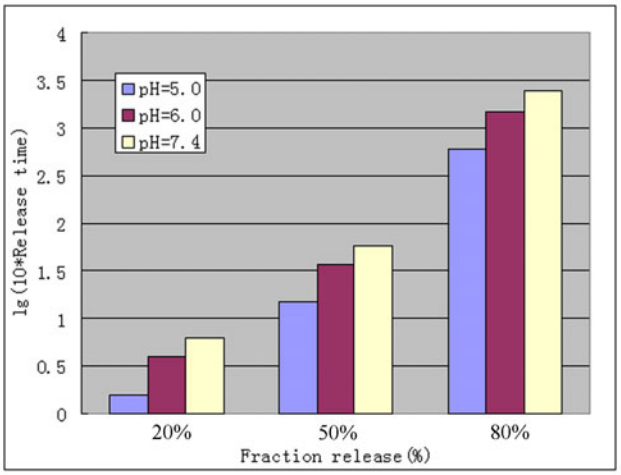

Figure 1. The releasing behavior in vitro of (a) IgG covalently attached to HA in different pH value.

Conclusions:

In this study we synthesized the hydrogel with antibody drug through the pH-sensitive hydrazone linkage. The linkage enabled controlled release of antibody from the hydrogel depended on pH change. The antibody released from the hydrogel can induce neurite outgrowth in vitro. The results presented here might lead to a potential therapeutic approach for CNS injury in the future.

References:

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