Growth Factor-Carrying Hybrid Hydrogel/Nanogel Composite for the Treatment of Urethral Incontinence

K. M. Park¹, J. Y. Son¹, J. H. Choi¹, I. G. Kim², J. Y. Lee², and K. D. Park¹

¹Dept. of Molecular Science and Technology, Ajou University, Suwon 443-749; ²Dept. of Urology, Seoul St. Mary’s Hospital, Seoul 137-701, Republic of Korea
kdp@ajou.ac.kr

ABSTRACT SUMMARY
We present a hybrid hydrogel/nanogel composite that can serve as an injectable and bioactive bulking material for the treatment of urethral incontinence. The hybrid composite was prepared via an enzymatic reaction in the presence of horseradish peroxidase and H₂O₂. We found that the bFGF-carrying hybrid composites could stimulate the regeneration of the smooth muscle tissue surrounding the urethral wall and promote the recovery of the biological function of the urethral muscle when injected in vivo.

INTRODUCTION
Urethral incontinence is caused by sneezing or coughing and affects a patient’s quality of life. Over the past decade, a myriad of therapeutic methods, including surgical repair, drug therapy, and implantation of bulking materials, have been explored to enhance urethral resistance by external abnormal pressure. Specifically, injectable materials (i.e., particulates and hydrogels), based on a minimally invasive technique, have attracted substantial interest as bulking matrices to narrow the urethral lumen. However, they have several limitations such as untimely reabsorption and particle migration after injection, resulted in poor regeneration of the urethral muscle that regulates the exit of urine.

We previously reported horseradish peroxidase (HRP)-catalyzed in situ forming gelatin-polyethylene glycol-tyramine (GPT) hydrogels as an injectable material. Self-assembled heparin-Pluronic (HP) nanogels were also developed as a multi-functional vehicle for a long-term delivery of bFGF. In this study, we investigated a hybrid hydrogel-nanogel composite composed of GPT and HP that may serve as an injectable and bioactive bulking material platform for the treatment of urethral incontinence.

Figure 1. Regeneration strategy of Urethral incontinence and fabrication of hybrid hydrogel/nanogel composites.

EXPERIMENTAL METHODS
GPT and HP conjugates were synthesized as previously reported. bFGF-loaded HP nanogels (HP/bFGF) were prepared using a direct dissolution method. To prepare a hybrid hydrogel/nanogel composite, 3 wt% GPT solution including 0.025 mg/mL HRP and 1 mg/mL HP/bFGF was mixed with the same volume of 3 wt% GPT solution including 0.0125% H₂O₂. The mixture was gently shaken at 37 °C. All solutions used were dissolved in 0.01 M phosphate buffered saline (PBS) at pH 7.4. For in vitro bFGF release test, the hybrid composites were incubated in 1 mL of 0.01 M PBS including 1% BSA. At predetermined time intervals, the release media were withdrawn and replaced with fresh PBS. The ELISA analysis
was used to quantify the released amounts of bFGF.

To evaluate the urethral muscle regenerating-ability of hybrid composites, the urinary incontinent rats were prepared by denervation of the sciatic nerve. One week after the denervation, the sterilized hybrid composites were injected into the periurethral submucosa, with microscopic guidance to minimize surrounding tissue damage. After 4 weeks of implantation, the physical urethral sphincter function was evaluated by a leaking pressure point (LPP) assay. After the leaking pressure point (LPP) measurement, the specimens were vertically cut into 4 µm slices and subjected to immunohistochemistry for α-smooth muscle actin (SMA). We also performed a contractility test to confirm the physical function and regeneration of the nerve surrounding the urethral tissue.

RESULTS AND DISCUSSION

After bFGF loading into HP nanogels, the loading efficiency and amount were measured to be 90.8 ± 1.0% and 199.9 ± 2.2 ng/mg of HP polymer, respectively. We observed round-shaped HP nanogels and a similar size distribution in each sample. A significant difference was found in bFGF release behavior between the GPT hydrogel encapsulating free bFGF and the GPT/HP/bFGF composite. After 22% initial burst, the GPT/HP/bFGF composite exhibited a sustained bFGF release for 28 days.

The GPT/HP/bFGF hybrid composite showed a significantly higher density of SM surrounding the urethral wall, compared to the other groups, demonstrating that the GPT/HP hybrid hydrogel promoted SM regeneration via sustained release of bFGF. The GPT/HP/bFGF showed a significantly higher LPP level (48.7 ± 3.3 cm H$_2$O) compared with the denervation group (30.2 ± 2.5 cm H$_2$O) and GPT (37.0 ± 2.9 cm H$_2$O) but not the GPT/bFGF (42.3 ± 6.9 cm H$_2$O). It was also observed that the GPT/HP/bFGF showed significantly increased muscle contraction (4.5 ± 0.7 g tension/g tissue) compared with the other defect groups.

CONCLUSION

The hybrid hydrogels were formed via an enzyme-mediated crosslinking reaction, with encapsulation of bFGF-loaded HP nanogels. We demonstrated that incorporation of a heparin-based nanogels into the hydrogel allowed a sustained release of bFGF. The bFGF-carrying hybrid composites could stimulate the regeneration of the smooth muscle tissue surrounding the urethral wall and promoted the recovery of the biological function of the urethral muscle when injected in vivo.

REFERENCES


ACKNOWLEDGMENTS

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Ministry of Science, ICT & Future Planning (NRF-2012R1A2A2A06046885).