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RESEARCH PAPER

Combination of Hyaluronic Acid Hydrogel Scaffold and PLGA Microspheres for Supporting Survival of Neural Stem Cells

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ABSTRACT

Purpose To develop a biomaterial composite for promoting proliferation and migration of neural stem cells (NSCs), as well as angiogenesis on the materials, to rescue central nervous system (CNS) injuries.

Methods A delivery system was constructed based on cross-linked hyaluronic acid (HA) hydrogels, containing embedded BDNF and VEGF-loaded poly(lactic-co-glycolic acid) (PLGA) microspheres for controlled delivery and support for NSCs in the CNS. The surface morphologies were evaluated by SEM and AFM, mechanical property was investigated by rheological tests, and release kinetics were performed by ELISA. Bioactivity of released BDNF and VEGF was assessed by neuron and endothelial cell culture, respectively. Compatibility with NSCs was studied by immunofluorescent staining.

Results Release kinetics showed the delivery of BDNF and VEGF from PLGA microspheres and HA hydrogel composite were sustainable and stable, releasing ~20–30% within 150 h. The bioactivities preserved well to promote survival and growth of the cells. Evaluation of structure and mechanical properties showed the hydrogel composite possessed an elastic scaffold structure. Biocompatibility assay showed NSCs adhered and proliferated well on the hydrogel.

Conclusions Our created HA hydrogel/PLGA microsphere systems have a good potential for controlled delivery of varied biofactors and supporting NSCs for brain repair and implantation.

KEY WORDS central nervous system · controlled release · growth factor · hyaluronic acid hydrogel · PLGA microsphere

ABBREVIATIONS

BDNF	brain-derived neurotrophic factor
CNS	central nervous system
ECM	extracellular matrix
HA	Hyaluronic acid
NSC	neural stem cell
PLGA	poly(lactic-co-glycolic acid)
VEGF	vascular endothelial growth factor

INTRODUCTION

Central nervous system (CNS) injuries cause many nerve cells death and tissue defects, which result in permanent disability. Unfortunately, there have been no effective therapies.

Many studies have shown neural stem cells (NSCs) have an intrinsic ability to regenerate in the treatment of many neurological diseases. But the effect of NSC grafting is often limited by low viability and undesired differentiation (1,2). In addition, the glial scar formation and deficiency of neurotrophic factors and growth factors following the CNS injuries often result in failure of neural regeneration (3,4).

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Therefore, it is critical to overcome these barriers and create suitable microenvironments for neural repair.

Recently, biomaterials used for neural tissue engineering have emerged as a promising treatment for brain or spinal cord injury. Biological scaffolds have been shown to sustain the stem cells for transplantation and neural regrowth *in vivo* (5,6). Furthermore, some results even show some functional improvement (7,8). However, even these advances are still far from successful repair, which depends on good microenvironment to a large extent. A lot of studies have shown that the micro-environment, named "niche," which is composed of cells, blood vessels, growth factors and the extracellular matrix (ECM), plays an important role in regulating neurogenesis, survival and regeneration of NSCs (9,10).

In this research, a complex HA hydrogel/PLGA microsphere system was developed to sustain NSCs and simulate niche, which will create a permissive microenvironment for angiogenesis and neural regeneration. Our prior research has demonstrated that modified HA hydrogels have instinct advantages for neural repair by preventing glial scar formation, inducing endogenous neurons migration in the gel (11–13). Following these promising results, we synthesized HA hydrogel scaffolds to mimic the structure of ECM and mimic the environment of the niche by adding PLGA microspheres in the scaffold for releasing brain-derived neurotrophic factors (BDNF) and vascular endothelial growth factor (VEGF). Thus, multiple attempts are integrated through the hydrogel. It maybe very useful for NSC support in transplantation and rebuilding the micro-environment CNS repair.

MATERIALS AND METHODS

Materials

HA sodium salt (Mw 2.6–2.7 kDa) was purchased from Shandong Fureda Biochem (Shandong, China). The ethyl N,N-dimethylaminopropyl carbodiimide (EDC), adipic dihydrazide (ADH), poly(lactic-co-glycolic acid) (PLGA), dichloromethane, polyvinyl alcohol (PVA), sodium periodate and mouse anti-nestin antibody were obtained from Sigma (St Louis, MO). The recombinant human brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) were purchased from Invitrogen (Paisley, UK). The human VEGF and BDNF ELISA kit were purchased from R&D (Minneapolis, US); DMEM, DMEM/F12, Neurobasal medium, horse serum and fetal bovine serum (FBS) were from Gibco (Paisley, UK); Cell Counting Kit-8 was from Dojindo (Japan).

Synthesis of Bioactive Factors-Loaded PLGA Microspheres

Microspheres of PLGA were prepared using water-in-oil-in-water (w/o/w) emulsion technique to encapsulate BDNF and VEGF. Briefly, 250 mg of PLGA was dissolved in 5 ml dichloromethane; 5 μg BDNF or 10 μg VEGF was dissolved in 1 ml BSA (0.1% wt) and then added into the PLGA solution. The mixture was emulsified and added slowly to PVA (1% w/v) solution, followed by ultrasonic mixing (60 W, 90 s) to form a cloudy white emulsion. Then the emulsion was added into PVA (0.3% w/v) and stirred in an uncovered beaker for several hours to remove dichloromethane. The final emulsion was centrifuged at 4000 rpm for 15 min. Finally, the deposits were resuspended in a minimal amount of deionized water and freeze-dried overnight to obtain PLGA micropsheres.

Preparation of HA Hydrogel/PLGA Microspheres Composites

The PLGA microspheres were embedded in the HA hydrogel, forming a delivery system. HA was dissolved into deionized water to form a 10 mg/ml solution, then BDNF- and VEGF-loaded microspheres of the same weight were added with weight ratio of 5:1 of HA: microsphere. Then ADH (6 × HA weight) was added with thorough stirring. After adjusting the pH of the mixed solution to 3.5–4.75 by adding 0.1NHCl, EDC was added with thorough stirring. The reaction solution was allowed to gel at room temperature overnight. The hydrogel was washed with deionized water five times and then immersed in deionized water overnight to remove any residue.

Characterization of Microspheres and Hydrogel/Microspheres Composites

The microstructure of the lyophilized PLGA microspheres and hydrogel composites was examined by scanning electron microscopy (SEM). The samples were mounted on stubs using colloidal graphite and sputter-coated with a thin layer (approximately 20 nm) of gold. The surface and bulk morphologies were examined with a JSM-6460LV SEM (JEOL, Tokyo, Japan) operating at 10 kV accelerating voltage.

The size and additional higher resolution surface profile of the microspheres were acquired by atomic force microscopy (AFM). The contact mode AFM imaging was carried out in air at room temperature with a large range (100×100 μm) scanner, and the force constant of cantilevers was 0.01–0.1 N/m.

Rheological properties of two disk-shaped hydrogel composites (15 mm diameter, 1.3 mm thick) at swelling equilibrium was measured using a Physica MCR 300

rheometer (Anton Paar, Graz, Austria), operated in a constant stress mode. The hydrogels without microspheres served as control. A detailed description of the method was given previously (11). A parallel-plate geometry type with a variable gap was selected for the test. The samples were kept in 37°C water during the measurements to mimic the body temperature. The tests were carried out by dynamic method at small amplitudes of deformation (1%). The storage (G′) and loss moduli (G″) were examined by small-amplitude oscillatory shear measurements at intervals varying from 0.9 to 1.3 mm and frequencies ranging from 1 to 100 rad/s.

In Vitro Release Kinetics by ELISA

To determine loading, 10 mg microshperes were disrupted with trichloromethane, followed by repeated extraction with 1 ml water. The amount of released BDNF or VEGF content was quantified by ELISA assay, and then the loading and encapsulation efficiencies of microspheres were obtained by comparing with initial protein loading and weight of the PLGA microsphere. The theoretical loading of the hydrogel was obtained by measuring the ratio of microspheres in the gel (1/6,w/w). Then the release kinetics of VEGF and BDNF from the PLGA microspheres and HA hydrogel composite were detected, respectively. Ten mg of VEGF- or BDNF-loaded microspheres were incubated in 1 ml 0.01 M PBS (pH = 7.4) at 25°C, and 100 mg of hydrogels were in 3 ml PBS. The supernatants of 100 μl were harvested at time points from 4 h to 150 h, and the samples were replenished with PBS of the same volume. The amounts of released VEGF and BDNF were quantified using ELISA kits. All the studies were conducted in triplicate with three different independent samples.

Bioactivity Assay of the Released Factors

To test whether the bioactivity was maintained throughout the encapsulation time, the supernatants of released VEGF or BDNF at the last time point, 150 h, were obtained as described above. They were added respectively into the culture medium for neuron and endothelial cell at the concentration of 10 ng/ml according to the ELISA analysis. The culture media were sterilized by filtration through a 0.22 μm membrane filter before use. The bioactivities were examined by cell survival and proliferation.

Released BDNF Bioactivity Assay

The bioactivity of released BDNF was examined by protection against glutamate-induced cytotoxicity on primary cultured rat hippocampal neurons. Briefly, single cells were dissociated and cultured in Neurobasal medium at a density of 10^6 cells/ml on two poly-L-lysine-coated 24-well plates. A 24-h pre-incubation with culture medium containing 10 ng/ml BDNF supernatant for cells of 12 wells was performed; meanwhile, for another two 12 wells, the culture medium not containing the supernatant served as negative control, and controlled BDNF of the same concentration as positive control. Then all cells were subjected to 125 mM glutamate in culture medium for 12 h at 37°C. After stimulation with glutamate, the cell viability was then measured at 24 h and 48 h later by staining with the fluorescent dyes Hoechst 33342 (0.5 mg/ml) and propidium iodide (PI,2.9 mg/ml) in PBS for 15 min at 37°C. The cells were gently washed 3 times with PBS, fixed in 4% paraformaldehyde for 15 min at room temperature and then examined under a Leica fluorescence microscope. For each sample, all the cells of 10 microscopic fields, living or dead, were photographed and counted for the survival rate assay by Leica QWin image analysis system.

Released VEGF Bioactivity Assay

The bioactivity of released VEGF was measured by cell proliferation assay. The human umbilical artery endothelial cells were seeded in 96-well plates with a concentration of 6,000 cells per well in DMEM/F12, 10% serum-medium. After cell adherence, the medium of 48 wells was replaced with medium containing the supernatant of VEGF loaded microspheres (10 ng/ml), and 48 wells were used as negative controls, and another 48 wells adding 10 ng/ml VEGF directly as positive control. The cells were cultured for 72 h, and the proliferation was assessed at 24, 48 and 72 h by CCK-8 assay.

NSC Culture in Hydrogel/Microspheres Composites

The NSCs were cultured as described previously (14). The cells were isolated from E13.5 Sprague–Dawley rat embryos forebrain and cultured as neurospheres in serum-free DMEM/F12 growth medium containing 20 ng/ml EGF, bFGF and 2 mM B27 Supplement. Following 7 days of primary culture, neurospheres were dissociated into a single cell suspension and seeded in 24 hydrogel composites ($1 \times 1 \times 0.3$ cm^3), and the other 24 HA hydrogels of the same size without PLGA microspheres were used as control. All the gels were sterilized by ethylene oxide before use. One hundred μl cell suspension was injected into each hydrogel at density of 1×10^6 cells/ml using a syringe, then cultured in 24-well plates for 5 days at 37°C with the same media as above. The adherence and distribution of cells in 6 gels were visualized randomly by SEM and immunofluorescent staining of nestin (1:200), and the images were obtained using SEM and laser scanning confocal microscope (Leica Microsystems LAS AF-TCS SP5). The proliferation of cells on the rest gels were analyzed by CCK-8 array.

Statistics

All data were presented as mean ± standard deviation. The bioactivity of the released factors was analyzed by one-way ANOVA and Student's *t*-test for statistical comparisons between pairs of samples, where the "*" and "#" indicate significant difference between the sample and control ($p<0.05$).

RESULTS

Morphology of Microspheres

The SEM image showed the PLGA microspheres were about 1~5 μm in diameter (Fig. 1a), with shell structure providing enough space for encapsulated factors (Fig. 1b) and irregular small pores on the surface (Fig. 1c).

The 3-D reconstruction of PLGA microspheres by AFM images showed that the microspheres-encapsulated biofactors were about 2 μm in diameter and 0.5 μm in visual height (Fig. 2a, b).

Characterization of Hydrogel/Microspheres Composites

The morphology study by SEM indicated the structure of HA hydrogel with or without PLGA microspheres was similar to that we described before (11) and showed no significant difference except for the surface (Fig. 3a, d). The surface of hydrogel/microspheres composite was relatively rough, while that of the HA hydrogel alone was smooth (Fig. 3b, c, e, f).

Rheological test was used to measure mechanical properties of the swollen gel when subjected to compression and decompression cycles. The HA hydrogel synthesized in our former studies has shown suitable rheological properties for CNS tissue engineering (11). Similarly, in this study, the HA hydrogel composites still showed a typical and well-crosslinked gel behavior, as shown in Fig. 4, the storage modulus (G′) >> the loss modulus (G″), and G″/G′<0.1, as a type of soft gel. The elastic properties (G′) are constant over the range of frequencies from 1 to 100 Hz. The G′ and G″ of the composite increased a little compared to the HA hydrogel alone, so the elastic property of hydrogel was enhanced by microspheres.

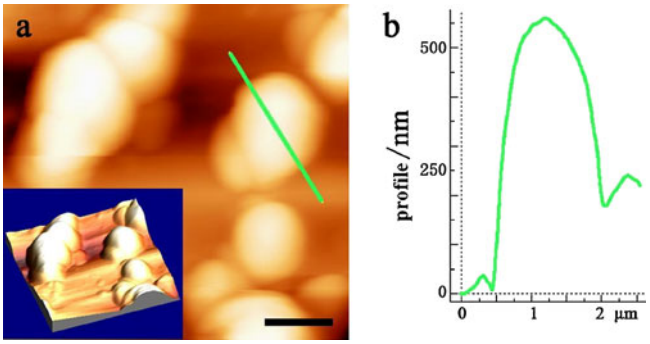

Fig. 2 AFM image of biofactors-loaded PLGA microspheres. (**a**) The three-dimensional surface of PLGA microspheres, about 2 μm in diameter. (**b**) The profiles of microspheres through the line. (Bar = 2 μm).

In Vitro Release Kinetics of PLGA Microspheres and HA Hydrogel/Microsphere Composites

The contents of BDNF and VEGF proteins were 10.4 and 34 ng/ml by ELISA, so the loading of BDNF and VEGF in microspheres was 0.10 and 0.34 μg/mg microspheres, respectively, corresponding to 52% and 84% loading efficiency. When embedded with microspheres, the loading efficiencies of HA gel composites were 17.4 and 56.6 ng/mg, respectively.

Within the period of 6 days, the BDNF and VEGF were released from the microspheres constantly and cumulatively, without initial burst, probably due to the thorough washing process since the initial burst was believed to come from the proteins on the surface. It provided a slow and linear release profile during the test period. The concentration of BDNF and VEGF respectively reached a high value of 24 ng/ml and 95 ng/ml on the 6th day, releasing about 20–30% of the loading proteins (Fig. 5a,b). Given that the degradation of PLGA was insignificant during this period, the factors were released mostly by diffusion from areas near the surface.

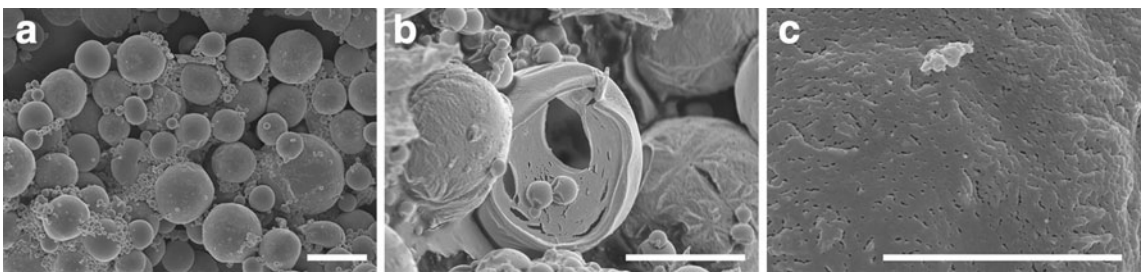

Fig. 1 SEM images of biofactors-loaded PLGA microspheres. (**a**) The profile of PLGA microspheres, about 1~5 μm in diameter. (**b**) The core-shell structure of the microsphere for the delivery of biofactors. (**c**) The pores on the surface of microsphere. (Bar = 2 μm).

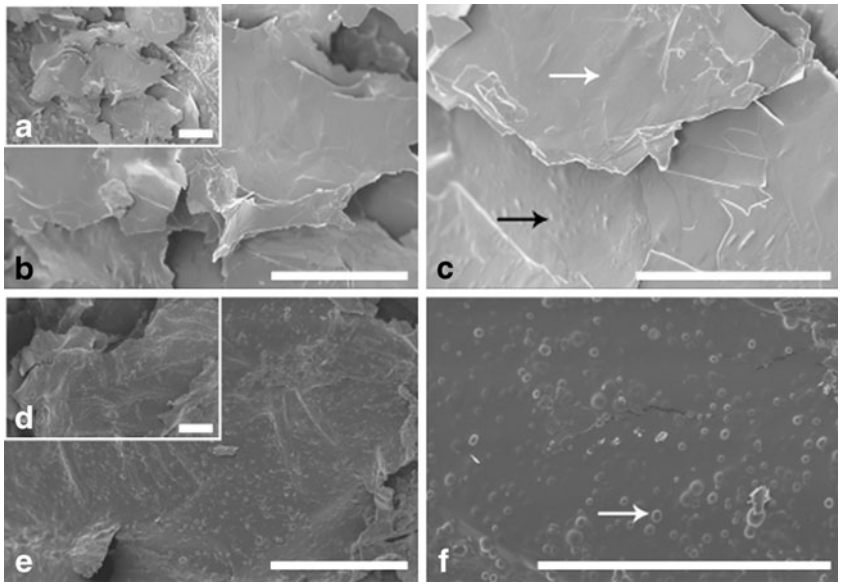

Fig. 3 SEM images of HA hydrogels with/without PLGA microspheres. (**a–c**) The profile of HA hydrogel without PLGA microspheres as control. The surface of the scaffold is smooth. (**d–f**) HA hydrogel/PLGA microspheres composites. There are many small particles on the surface of the scaffold. (Bar = 200 μm)

The release of VEGF and BDNF from the hydrogel embedded with microspheres revealed an initial "burst" followed by a stable release phase, as shown in Fig. 5c,d. This release profile may be attributed to the diffusion through the hydrogel matrix, since the released factors remained in the hydrogel during the cross-link processing. The releasing of the two factors was about 12% and 13% of total loading. Apparently, the presence of the surrounding HA hydrogel has a delayed or deposited effect on the release profile of the biofactors, or a portion of factors might be lost during the crosslink.

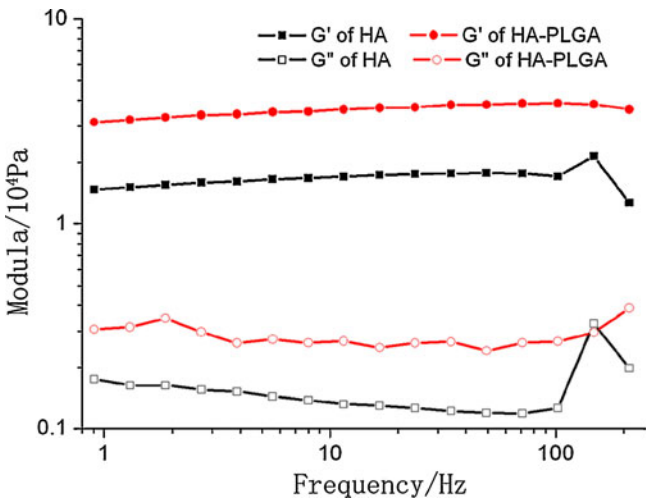

Fig. 4 The Rheological properties of the hydrogels by dynamic modulus. For both gels, the constant storage modulus G′>> loss modulus G″ over the range of frequencies, presented a typical soft gel. The moduli of G′ and G″ of hydrogel/microsphere composites (HA-PLGA, *red*) increased a little during the frequency from 1 to 100 Hz compared to HA hydrogel (HA, *black*).

Bioactivity of the Released Factors

Protective Effect of Released BDNF on Neurons

The protective effect of released BDNF from PLGA microspheres on glutamate-induced neurotoxicity was examined by rat hippocampus neurons. As shown in control of Fig. 6a and b, the cell viability was significantly reduced by exposure to glutamate for 24 h, which resulted in large-scale apoptosis (red nuclei). In contrast, the neurotoxicity was greatly prevented by the application of released supernatant, in which apoptotic cells were significantly reduced at 48 h, similar to that of BDNF control. So the bioactivity of the released BDNF was kept well enough until the test time and was helpful in increasing the survival rate of neurons subjected to the neurotoxicity.

Proliferation Effect of Released VEGF on Endothelial Cells

The supernatant of released VEGF form PLGA microspheres was added into the culture medium of endothelial cells for 3 days. The CCK-8 cell proliferation assay showed that the released VEGF significantly promoted the growth of cells compared to control group, especially 48 h later, and was similar to the effect of positive VEGF control (Fig. 7). The bioactivity of the released VEGF was preserved well enough.

Supporting of Neural Stem Cells with HA Hydrogel/Microspheres Composite

To investigate the biological potential of this hydrogel scaffold as a NSC delivery system, the NSCs were seeded in

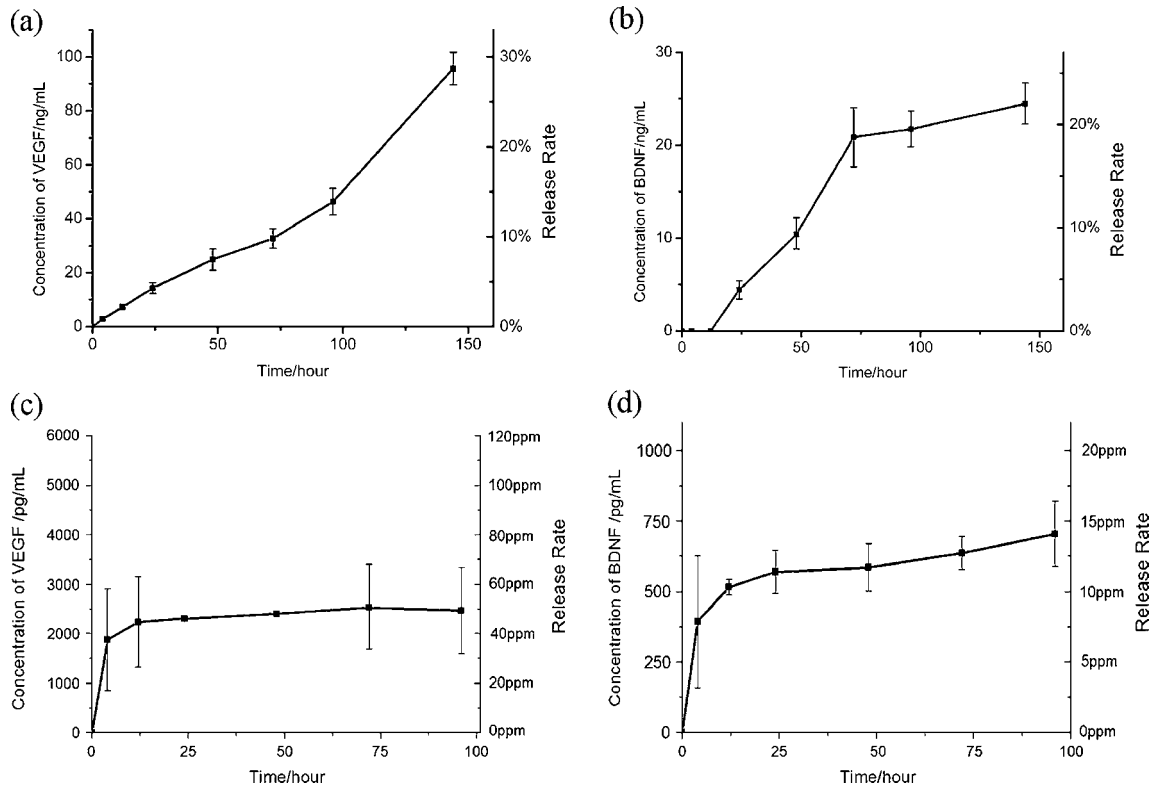

Fig. 5 *In vitro* release of VEGF and BDNF from PLGA microspheres and HA hydrogel/PLGA microsphere composites by ELISA. (**a**) VEGF and (**b**) BDNF from microspheres showed constant and cumulative releasing profile. The concentration of VEGF and BDNF reached 95 and 24 ng/ml, respectively, releasing about 20~30% of loaded drugs on the sixth day. (**c**) VEGF and (**d**) BDNF from HA hydrogel/PLGA microsphere composites showed an initial burst, followed by a stable release phase. The concentration of VEGF and BDNF reached to 2500 and 700 pg/ml at 100 h, releasing about 12–13% of total loading, respectively.

scaffolds for a 5-day culture. The fluorescent staining of nestin, the marker of NSC, showed the cells adhered onto the scaffold, dispersed widely through the entire hydrogel along the wall of the scaffold (Fig. 8a, an overall view), extended long neurite and formed an extensive network (Fig. 8b,c). Furthermore, the proliferation by CCK-8 array showed the NSCs grew faster in the hydrogel with encapsulated growth factors than in HA hydrogel control (Fig. 8 d). The hydrogel-containing encapsulated factors showed good biocompatibility with NSCs and provided better environment and support for cell growth.

DISCUSSION

The reconstruction of damaged CNS has been a daunting task because of the complicated changes of tissue defect, cell loss, and micro-environment destruction following injuries (3,4). Strategies of NSC transplantation and biofactor delivery are the most promising approaches for brain repair. But the transplanted cells encounter poor survival without suitable microenvironment. Therefore, to construct a good environment is crucial for successful neural repair and neural tissue engineering. Although the biofactors can improve this situation, their usages are limited greatly by fast inactivation and poor ability to cross the blood–brain barrier into the brain (15,16).

In this study, an HA hydrogel scaffold embedded with biofactor-encapsulated PLGA microspheres was constructed as a multiple delivery system for CNS regeneration. The properties of the composite were assayed by morphology, mechanical property and bioactivity tests. The results show the HA hydrogel composite has excellent cytocompatibility with NSCs. Since HA is the main component of extracellular matrix, it follows that scaffold of HA is analogous to the tissue (17) to provide good mechanical support for cell adherence, proliferation or migration. As shown in our former data, the HA hydrogel can support the seeded cells *in vitro* and the endogenous migrated cells *in vivo* as well (11,18). In this study, the PLGA

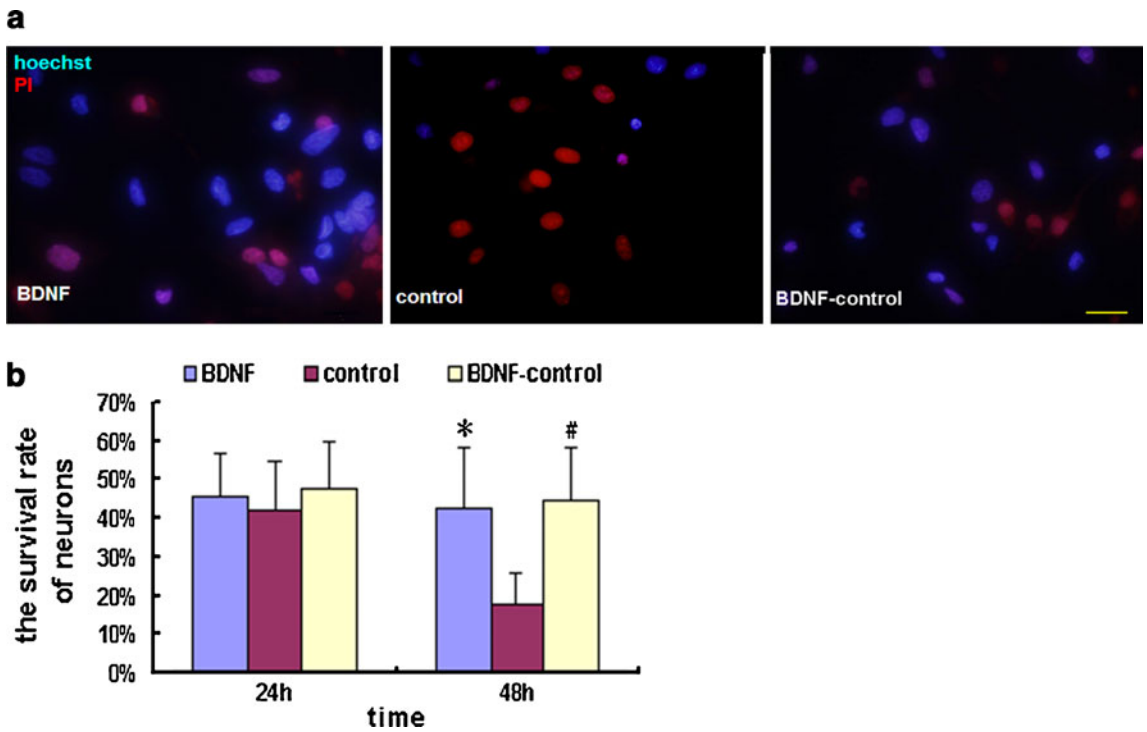

Fig. 6 Results of protective effects of released BDNF on glutamate-induced primary hippocampus neurons cytotoxicity. (**a**) The 12-h pre-incubation of released BDNF reduced the apoptosis of neurons after exposing to glutamate for 48 h compared to the negative controls without BDNF, and similar to positive BDNF control. Hoechst/P.I. staining showed the live cells (*blue* nucleus) of the two pre-incubation groups were more than control, which had more necrotic or apoptotic cells (*red*). (Bar = 20 μm). (**b**) The survival rate of neurons was increased by adding the released BDNF (*) or controlled BDNF (#) into the culture medium compare to the control (*,# $p < 0.05$).

microspheres are successfully embedded in the hydrogel during the gel cross-link procedure for biofactors delivery. The hydrogel composite appears similar in structure to HA hydrogel control with a relative rough surface due to the presence of microspheres. Apparently, the embedding has little effect on *in situ* cross-linkability and whole structure of the gel. This may be because the amount (1/6 of gel) and size (1~2 μm) of PLGA microspheres are insufficient to change the structure of gel. The hydrogel composite shows a typical mechanical property of soft gel, which is thought to be suitable for soft tissue. While the gel appears, there is a little increase in storage modulus when embedded with particles, that is to say, the particles enhance the mechanical property of gel to a small extent. Actually, the rheologic property of hydrogel composite can be controlled by adjusting the dose of cross-linking agent or particles to get a softer or harder hydrogel for better matching to the brain tissue.

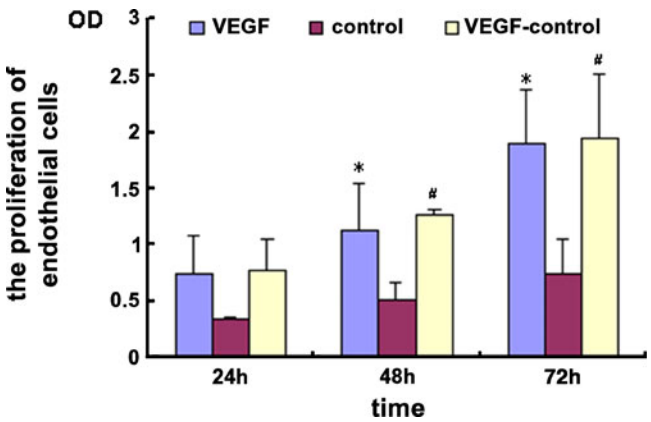

Fig. 7 The effect of released VEGF on human umbilical artery endothelial cells by CCK-8 cell proliferation assay. The endothelial cells cultured with released VEGF grew faster than the control group, especially on 48 and 72 h (*$p < 0.05$). The effect was similar to that of the positive control added VEGF directly. (# $p < 0.05$, *vs.* control).

In the delivery system, BDNF and VEGF are encapsulated in PLGA microspheres and then embedded in the HA hydrogel for controlled release. Their *in vitro* release shows that the loading efficiency of BDNF and VEGF are about 52% and 84%, respectively, with a 20–30% releasing rate during the test. With respect to the overall delivery system, it shows a stable and sustainable release profile, with an initial rapid burst and a relatively low releasing rate of 12–

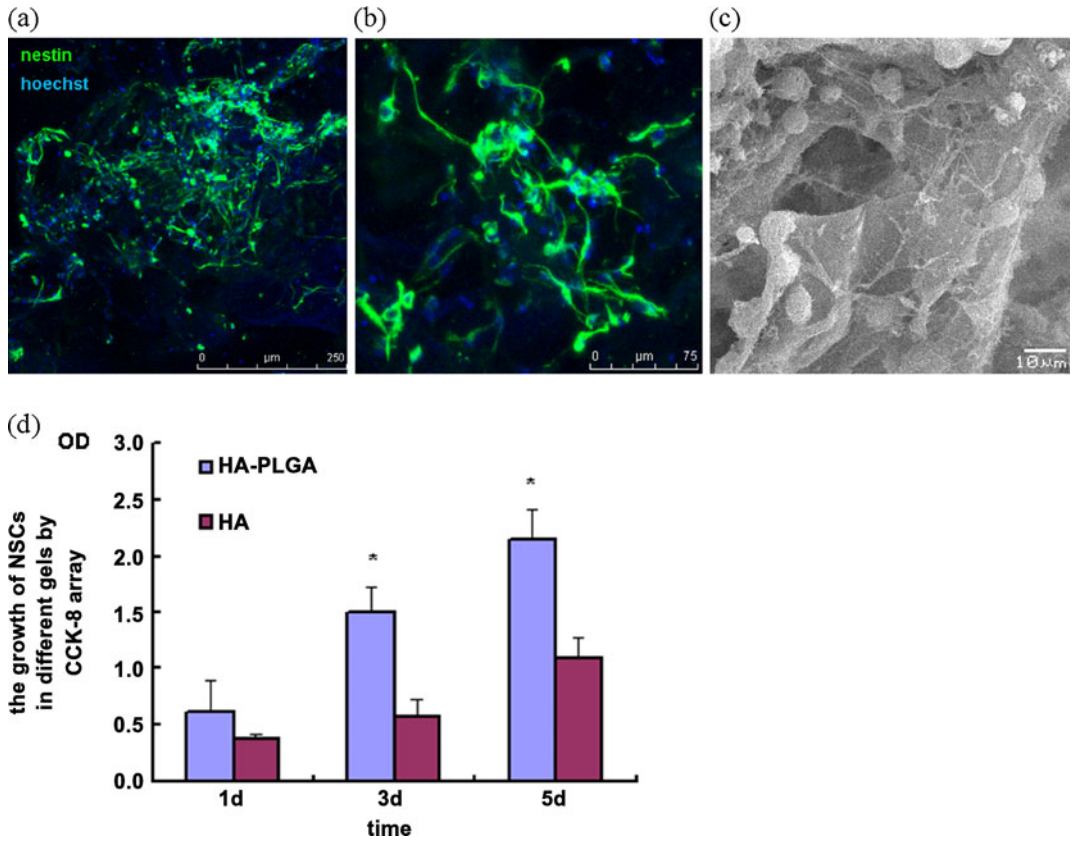

Fig. 8 The growth of NSCs on the HA hydrogel composite for 5 days. (**a**, **b**) The fluorescent staining of nestin showed the NSCs (*green*) adhered on the gel, grew well and spread widely along the wall of the scaffold (**a**, overall view), extending long neurites (**b**, high magnification). (**c**) The hydrogel showed good biocompatibility and support for NSCs by SEM. (**d**) The proliferation array by CCK-8 showed the NSCs grew faster on the hydrogel composite than on the HA hrdrogel control (*$p < 0.05$).

13% compared to the microsphere. Therefore, the surrounding cross-linking gel may delay the release by forming a barrier of matrix through which the proteins have to diffuse out. Additionally, some factors may be lost during the cross-link proceeding. However, it is still an effective delivery vector with a high releasing level.

In addition, the bioactivities of released factors are well-preserved to work on endothelial cells proliferation and neuron protection. It is crucial to the availability of delivery proteins; hence, the hydrogel composite containing BDNF and VEGF shows a significant effect on NSC proliferation by comparison with particle-free gel. The stable release of BDNF and VEGF may greatly improve the microenvironment for NSC (19). It is well known that BDNF and VEGF have notable effects on NSC proliferation *in vitro* and *in vivo* (20); also, they play important protective roles on nervous system repair by supporting neuron survival and nerve fiber outgrowth (21,22). In addition, the VEGF can strongly promote the endothelial cells proliferation and angiogenesis to rebuild the blood supply (23–26). The constant BDNF and VEGF delivery by the hydrogel can mimic and rebuild the "niche," which is essential to NSCs and tissue formation (22). Therefore, as an effective protein delivery device, the HA hydrogel composite may be very useful not only for the transplanted NSCs but also for the endogenous cells.

As our previous studies have shown, the HA hydrogel can also serve as an NSC transporter (18) and promote brain injury repair when implanted *in vivo* (11–13). In this research, the hydrogel composite shows good support and functions for NSCs, promoting the growth of cells. In consideration of its good performance in NSC support and factor delivery, as well as the soft property suitable for central nervous tissue, the HA hydrogel/PLGA microsphere composite shows a great potential to be used *in vivo* for NSCs transplantation and neural repair.

CONCLUSION

The properties of this HA hydrogel/PLGA microsphere composite show that it plays a useful role in supporting neural stem cells and biofactors delivery. It may have a good

potential for controlling the delivery of varied bioactive factors and neural stem cells for brain implantation.

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REFERENCES

1. Yiu G, He Z. Glial inhibition of CNS axon regeneration. Nat Rev Neurosci. 2006;7:617–27.
2. Wang KC, Koprivica V, Kim JA, Sivasankaran R, Guo Y, Neve RL, *et al.* Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature. 2002;417:941–4.
3. Delcroix GJR, Schiller PC, Benoit J-P, Montero-Menei CN. Adult cell therapy for brain neuronal damages and the role of tissue engineering. Biomaterials. 2010;31:2105–20.
4. Chiara P, Laura L, Gian LB, Cristina R, Carolina F, Ferruccio P, *et al.* Arterially perfused neurosphere-derived cells distribute outside the ischemic core in a model of transient focal ischemia and reperfusion *in vitro*. PLoS ONE. 2008;7:1–8.
5. Rutledge G, Ellis B, Yu XL, Si WY, David KCT, Shu GZ, *et al.* Nano neuro knitting: Peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision. Proc Natl Acad Sci. 2006;103(13):5054–9.
6. Hicks AU, Lappalainen RS, Narkilahti S, Suuronen R, Corbett D, Sivenius J, *et al.* Transplantation of human embryonic stem cell-derived neural precursor cells and enriched environment after cortical stroke in rats: cell survival and functional recovery. Eur J Neurosci. 2009;29:562–74.
7. Kazanis I, Lathia J, Moss L, ffrench CC. The neural stem cell microenvironment StemBook, The Stem Cell Research Community; 2008.
8. Jason MB, Hideki K, Shahin R. Instructive role of the vascular niche in promoting tumour growth and tissue repair by angiocrine factors. Nat Rev. 2010;10:138–46.
9. Peter AW, Kevin RA, Fernando J, Shinil KS, Matthew TH, Brijesh SG, *et al.* Advances in progenitor cell therapy using scaffolding constructs for central nervous system injury. Stem Cell Rev. 2009;5:283–300.
10. Lauren L, Kevin EH, David S. Engineering biomaterials for synthetic neural stem cell microenvironments. Chem Rev. 2008;108:1787–96.
11. Hou SP, Xu QY, Tian WM, Cui FZ, Cai Q, Ma J, *et al.* The repair of brain lesion by implantation of hyaluronic acid hydrogels modified with laminin. J Neurosci Meth. 2005;148(1):60–70.
12. Tian WM, Zhang CL, Hou SP, Yu X, Cui FZ, Xu QY, *et al.* Hyaluronic acid hydrogel as Nogo-66 receptor antibody delivery system for the repairing of injured rat brain: *in vitro*. J Control Release. 2005;102(1):13–22.
13. Hou SP, Tian WM, Xu QY, Cui FZ, Zhang J, Lu Q, *et al.* The enhancement of cell adherence and inducement of neurite outgrowth of dorsal root ganglia co-cultured with hyaluronic acid hydrogels modified with Nogo-66 receptor antagonist *in vitro*. Neuroscience. 2006;137(2):519–29.
14. Johe KK, Hazel TG, T Muller, Dugich DMM, McKay RD. Single factors direct the differentiation of stem cells from the fetal and the adult central nervous system. Genes. 1996; 3129–40.
15. Pardridge WM, Kang YS, Buciak JL. Transport of human recombinant brain-derived neurotrophic factor (BDNF) through the rat blood-brain barrier *in vivo* using vector-mediated peptide drug delivery. Pharma Res. 1994;11:738–46.
16. Pardridge WM, Wu D, Sakane T. Combined use of carboxyldirected protein pegylation and vector-mediated blood-brain barrier drug delivery system optimizes brain uptake of brain-derived neurotrophic factor following intravenous administration. Pharma Res. 1998;15:576–82.
17. Amico B, Mark H, Doris D. Hyaluronic acid and hyaluronic acid-binding proteins in brain extracellular matrix. Anat Embryol. 1993;188:419–33.
18. Yong JR, Zi YZ, Fu ZC, Ying W, Jun PZ, Qun YX. Hyaluronic acid/polylysine hydrogel as a transfer system for transplantation of neural stem cells. J Bioact Compat Polym. 2009;24:56–62.
19. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci. 2001;24:677–736.
20. Islam O, Loo TX, Heese K. Brain-derived neurotrophic factor (BDNF) has proliferative effects on neural stem cells through the truncated TRK-B receptor, MAP kinase, AKT, and STAT-3 signaling pathways. Curr Neurovasc Res. 2009;6(1):42–53.
21. Schänzer A, Wachs FP, Wilhelm D, Acker T, Cooper-Kuhn C, Beck H, *et al.* Direct stimulation of adult neural stem cells *in vitro* and neurogenesis *in vivo* by vascular endothelial growth factor. Brain Pathol. 2004;14(3):237–48.
22. Abe K. Therapeutic potential of neurotrophic factors and neural stem cells against Ischemic brain injury. J Cereb Blood Flow Metab. 2000;20(10):1393–408.
23. Qin S, Yue W, Erzsebet K, Gang L, Shu MC, Susan KG, *et al.* Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. Cell Stem Cell. 2008;3:289–300.
24. Tang J, Hua Y, Su J, Zhang P, Zhu X, Wu L, *et al.* Expression of VEGF and neural repair after alprostadil treatment in a rat model of sciatic nerve crush injury. Neurol India. 2009;57:387–94.
25. Taoufik E, Petit E, Divoux D, Tseveleki V, Mengozzi M, Roberts ML. TNF receptor I sensitizes neurons to erythropoietin- and VEGF-mediated neuroprotection after ischemic and excitotoxic injury. Proc Natl Acad Sci. 2008;105:6185–90.
26. Ke C, Yin ZL, William SK. Three-dimensional polymer scaffolds for high throughput cell-based assay systems. Biomaterials. 2008;29(18):2802–12.