and the primary rat osteoblast cells are then seeded onto these patterns. The cell experiments suggest that the osteoblast cells preferentially attach onto the protein areas and display different cell morphologies and spreading behavior on different protein patterns. The protein pattern shapes can significantly influence the cell adhesion, distribution, spreading, alignment and cell orientation and so on. It is possible to control the cell morphology and even cell function by carefully designing the pattern shapes and sizes. Compared with the control samples (isotropic FN coating on PS surface), the osteoblast cells on FN patterns display better growth and spreading behavior. The spreading behavior of cells can obviously affect the cell function such as protein expression. The cells with better spreading exhibit enhanced collagen I and osteocalcin expression. Therefore, the present study suggests that ECM patterns can be used to modify the tissue-engineered scaffold surfaces for construction of bone implants to control the living osteoblast cell behavior, especially controls of cell adhesion, alignment and protein expression are needed.

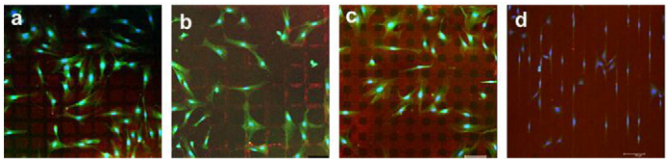

Fig. 1. Osteoblast cells adhered on different FN patterns. The cytoskeletons were stained by Alexa Fluor 488-Phalloidin (Green). The cell nuclei were stained using DAPI (blue). The protein pattern (red) and the cell morphology were observed and recorded simultaneously by confocal laser scanning microscopy. The scale bar indicates 100 μm.

Keywords: protein patterns, tissue engineering, osteoblast cell, bone

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Biodegradable microparticles for regulating differentiation of neural stem/progenitor cells for treatment of Parkinson's disease

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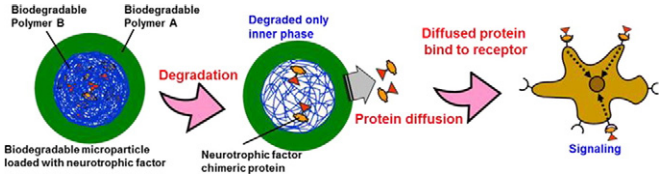
Transplantation of neural stem/progenitor cells (NSPCs) has been noticed as a powerful method for treatment of Parkinson's disease. However, cell-based therapy for treatment of Parkinson's disease has two big problems; one is the improvement of graft survival, and the other is the strict regulation of neuronal differentiation. In our research, biodegradable microparticles (MPs) were designed to strictly regulate the differentiation of transplanted NSPCs in the brain tissue.

These MPs are constructed with PLGA as an outer phase, atelocollagen as an inner phase, and glial cell line-derived neurotrophic factor (GDNF) fused with collagen-binding peptide (CBP), that is GDNF-CBP chimeric protein, immobilized to collagen of the inner phase [1]. The novel character of the designed MP is that the release of neurotrophic factor encapsulated into the MP is time-dependently regulated by using bio-degradation of collagen with a specific protease (Scheme 1).

GDNF-CBP, a fusion protein was synthesized using *Escherichia coli* and was incorporated in the microparticles utilizing the ability of CBP to bind to collagen. Microparticles were prepared using the W–O–W emulsion technique and when analyzed using microscopy and SEM showed an average size of 27.68 ± 3.70 μm. GDNF-CBP encapsulated in MPs showed hardly any release in PBS while an immediate start of release was observed in collagenase solution. Bioactivity of GDNF-CBP released from MPs was analyzed with differentiation of neural progenitor cells (NPCs) into neuronal cells. NPCs cultured for 10 days with MPs in the medium containing collagenase effectively differentiated into MAP2-positive neurons, which is the mature neuron marker indicating that the released GDNF-CBP has bioactivity. Consequently, it was shown that our designed MPs have the ability for strictly regulating the differentiation of transplanted NSPCs.



Scheme 1. Mechanism of strict regulation of cells using the designed microparticles.

Keywords: Cell transplantation, chimeric protein, protein delivery, regulation of protein release

Acknowledgments

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Characterization and fabrication of multi-layer films based on polysaccharide and cellulose

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Biodegradable polysaccharides as drug carriers have many advantages, including good biocompatibility, nontoxicity, and adjustable controlled release properties [1–3].

This paper describes the characterization and fabrication of multi-layer films based on carboxymethyl chitosan (CMC)–alginate–CMC/cellulose fabricated using a layer-by-layer assembly process (A). Cellulose was dissolved in an NaOH/urea aqueous solution precooled at −7 °C. Then a membrane based on a blend of CMC and cellulose was regenerated in a coagulation bath. The multi-layer films were fabricated using a layer-by-layer assembly process by alternatively