DISTINCT RELEASES OF GROWTH FACTORS FROM THREE DIMENSIONAL FIBROUS SCAFFOLDS COMBINED WITH HYDROGEL FOR DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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ABSTRACT

To mimic the actual microenvironment, independent release control of some growth factors is required in tissue engineering field. Here, we fabricated multifunctional nanofiber-based three dimensional (3D) cell scaffolds which have the capability of release of growth factors independently. From the osteogenesis differentiation of hMSC cultured on the fabricated scaffolds, the capacity as a tissue engineering scaffold was demonstrated successfully. We expect that this independently controlled release system has a great potential for tissue engineering, biochip and other biomedical fields.

KEYWORDS

Controlled dual release, 3D scaffold & culture, Micropatterned fibrous scaffold, Stem cell differentiation.

INTRODUCTION

Manufacturing scaffolds that can provide favorable environments to cells is one of the most important parts in tissue engineering. Among the various factors in designing proper scaffolds, the mechano-structural factors, such as topology and stiffness, should be considered first because most cells exist in a fibrous extracellular matrix in native tissue [1]. Another important factor is biochemical environment. Generally, many biochemical cues such as growth factors are provided simultaneously from the extracellular milieu and affect the cells, thus the release control of each cue make the artifact more similar with real environment. Many researchers have studied functional scaffolds capable of controlled release of growth factors [2]. However, most of those scaffolds contained only one growth factor. Even if multiple growth factors are incorporated into scaffold, only few studies reported independently-controlled release of different growth factors [3, 4]. In the present study we therefore report on a three dimensional fibrous scaffold which is able to release growth factors independently.

EXPERIMENTS & RESULTS

In this study, we fabricated three dimensional (3D) fibrous scaffolds suitable for cell culture that have capability of independently-controlled release of different growth factors. Here, we demonstrated efficient differentiation of human mesenchymal stem cell (hMSC) to osteoblast using basic fibroblast growth factor (bFGF) and bone morphogenetic protein 2 (BMP-2).

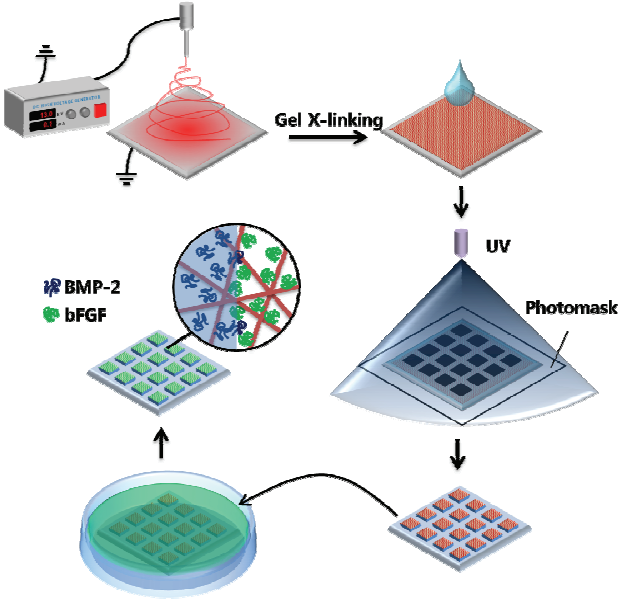

Figure 1. Schematic diagram of preparing nanofiber scaffolds containing growth factors.

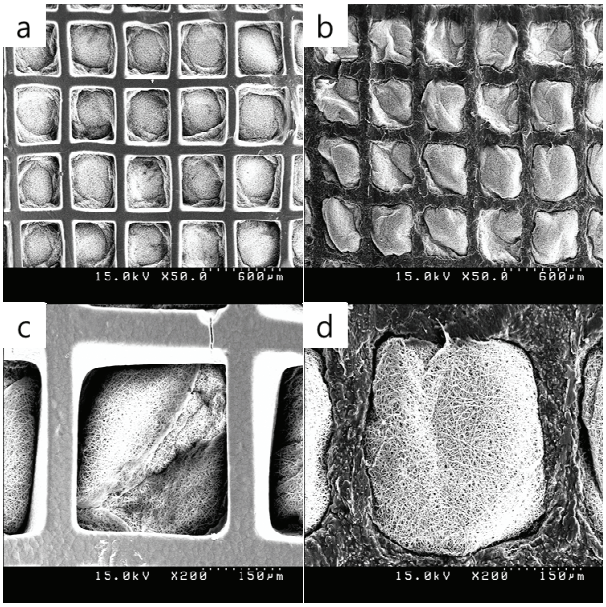

Figure 2. SEM images of fabricated scaffold. (a and c) Upper sides. (b and d) Lower sides.

First, we fabricated PCL/gelatin nanofiber scaffolds incorporated with Poly (ethylene glycol) (PEG) hydrogel microstructures for the controlled release of growth factors individually (Fig. 1). The electrospinning technique, a very easy and versatile method for creating nanofibers, was used for making PCL/gelatin blended nanofiber mat, and then the fiber mat was crosslinked to prevent dissolution of gelatin in aqueous solution. When the hydrogel

microstructures were fabricated via photolithography, BMP-2 was incorporated in hydrogel precursor solution. In case of bFGF, it was electrostatically adsorbed onto the nanofiber domain which has negative charge derived from gelatin. The SEM images show the distinguished domains of unpatterned nanofiber and patterned hydrogel, and the patterning of scaffold was successful from upper to lower sides without disarray (Fig. 2).

As mentioned before, the crosslinked gelatin has negative charge, so the bFGF which has weak positive charge can be attached on the nanofibers. We demonstrated the electric interaction between bFGF and crosslinked gelatin, and the release profiles were measured using fluorescence dye conjugated bFGF (Fig. 3). During the 12 hours of loading time, the bFGF was considerably attached on the crosslinked PCL/gelatin fiber, and there were few or no fluorescence signals on the other fibers. It would be explained that the physical adsorption on the PCL fiber was much weaker than electrostatic interaction between bFGF and gelatin, and the gelatin of uncrosslinked PCL/gelatin fiber would had washed out in aqueous solution. The intensities of crosslinked PCL/gelatin fiber were steadily decreased until 4 days and maintained the intensity level, so the result represent that bFGF attached on the crosslinked PCL/gelatin fiber was released gradually for 4 days.

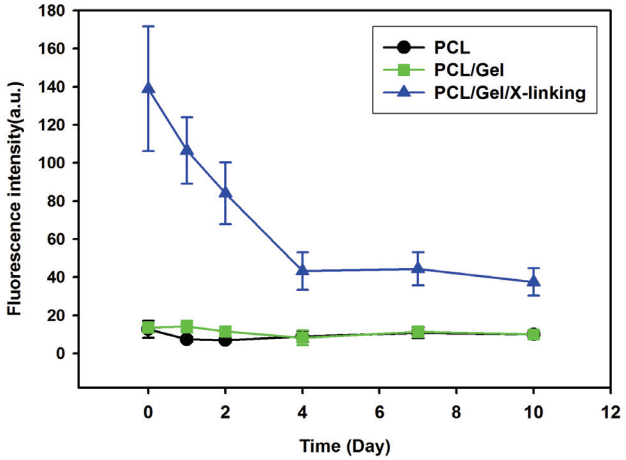

Figure 3. Fluorescence intensities of dye conjugated bFGFs attached on the fibers.

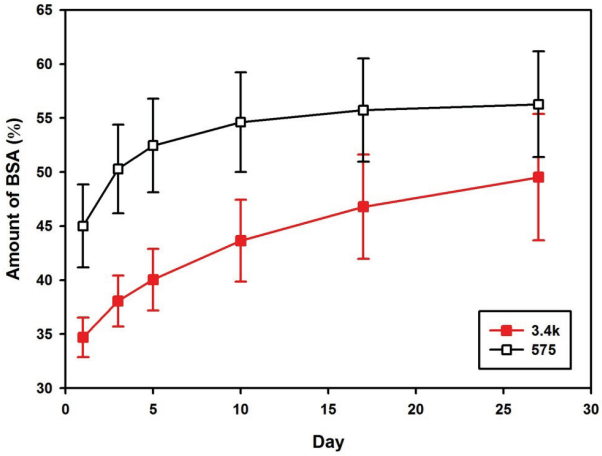

Figure 4. BSA releases from hydrogels of different molecular weights (3400 and 575 g/mol).

Furthermore, the release rate of BMP-2 was also controllable from the change of molecular weight (MW) of PEG which has various ranges, so the release term can be adjusted from the property of PEG hydrogel. The release profiles were determined using bovine serum albumin (BSA) which is the model protein of the BMP-2 from two different MW of PEG (3400 and 575 g/mol) (Fig. 4). From the result, although the initial release from 575 was higher than 3400, sustained release was maintained for a longer period in case of 3400. To be precise, the releases from 575 and 3400 hydrogel were sustained for about 10 days and a month, respectively. Thus, we used 3400 PEG hydrogel to stimuli hMSC using BMP-2 for more three weeks. In short, we can expect that the release profile of fabricated scaffold would consist of bFGF and BMP-2 release for 4 days at the beginning and following BMP-2 without bFGF release over 3 weeks.

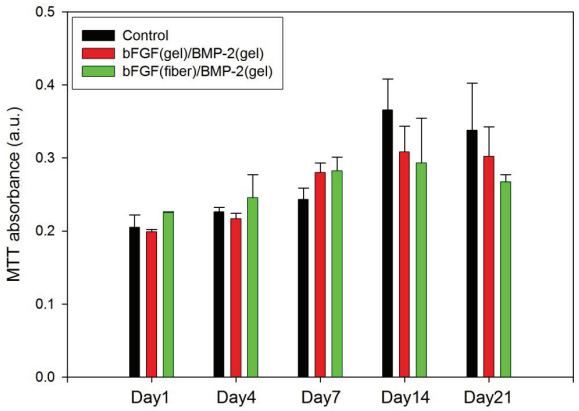

Figure 5. MTT assay of hMSC. Black; no growth factors, Red; simultaneous release of bFGF and BMP-2, Green; sequential release of bFGF and BMP-2.

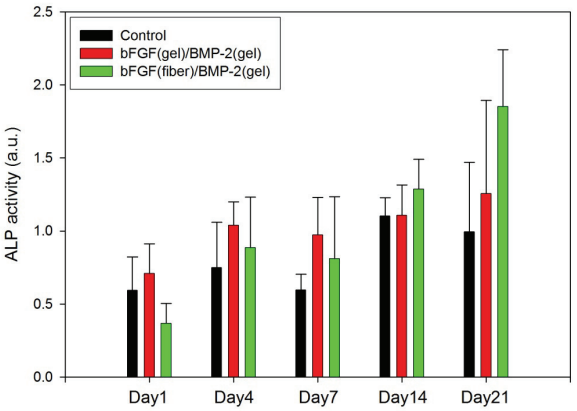

Figure 6. ALP activity of hMSC. Black; no growth factors, Red; simultaneous release of bFGF and BMP-2, Green; sequential release of bFGF and BMP-2.

To estimate osteogenesis capability of scaffold, hMSCs were cultured and the effectiveness of independently controlled release of two growth factors was demonstrated from the differentiation of hMSCs. The cell proliferation and activities were measured by MTT assay (Fig. 5). The values which represent cell numbers and viability increased until day 14, and then decreased in all cases. When the hMSCs are cultured on the growth factors-free

scaffold, the increasing amount of value was the highest. On the contrary, scaffold that release bFGF and BMP-2 sequentially had the lowest increasing. This could be expected as a result of brisk differentiation of hMSC to the osteoblast in the sequential release system, because cell differentiation has the tendency to slow down the cell growth. In other words, the MTT result of growth factors-free scaffold was high due to the relatively low differentiation rate. The result of alkaline phosphatase (ALP) activity which is one of the major markers of osteogenesis supports the explanation (Fig. 6). Without growth factors, certain evidence derived from ALP activity was not observed because there were no significant differences among values. On the other hand, the sequential release system, showing low increase and fast decrease in MTT assay, represents definite increase of ALP activity with time. In case of simultaneous release of bFGF and BMP-2, better osteogenesis capability was determined than growth factor-free system but not as much as sequential release system. So, we could know the fact that sequential released system of bFGF and BMP-2 is more attractive for the effective osteogenesis of hMSC than simultaneous released system of two growth factors.

CONCLUSION

In summary, independently controlled release capability of two growth factors was incorporated to the three dimensional nanofiber scaffolds which consist of electrospun nanofiber and PEG hydrogel. The nanofiber and hydrogel were used for the sustained release of bFGF for early short period and BMP-2 for long period, respectively. And the simultaneous release of two growth factors can also be achievable via insertion of both growth factors in hydrogel. Osteogenesis of hMSC demonstrated the capacity as multifunctional cell scaffold which includes some advantages, such as 3D structure, spatial control by hydrogel pattern, distinct growth factors release. The induction of differentiation was most effective in sequential release system, although the simultaneous release system shows better result than growth factor-free scaffold.

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REFERENCES

[1] A. Abbott, *Cell culture: Biology’s new dimension*, Nature, **424**, 870, (2003).
[2] K. Lee, E. A. Silva, D. J. Mooney, *Growth factor delivery-base tissue engineering: general approaches and a review of recent developments*, Journal of Controlled Release, **125**, 193 (2008).
[3] T. P. Richardson, M. C. Peters, A. B. Ennett, D. J. Mooney, *Polymeric system for dual growth factor delivery*, Nature Biotechnology, **19**, 1029 (2001)
[4] N. J. Shah, M. L. Macdonald, Y. M. Beben, R. F. Padera, R. E. Samuel, P. T. Hammond, *Tunable dual growth factor delivery from polyelectrolyte multilayer films*, Biomaterials, **32**, 6183 (2011)

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