Angiogenesis is Enhanced by Sustained Delivery of rhGM-CSF from Porous Collagen-Chitosan Scaffolds
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Introduction
Local delivery of GM-CSF (granulocyte-macrophage colony-stimulating factor) has been has been used for treating human chronic skin ulcers and deep second-degree burns wounds [1, 2], with good therapeutic effects. As a pleiotropic cytokine, GM-CSF regulates the proliferation, differentiation and survival of hematopoietic cells [3], by mobilizing and recruiting hematopoietic and endothelial stem cells from the bone marrow [4]. GM-CSF secreted by keratinocytes, mediates epidermal cell proliferation in an autocrine manner [5]. Moreover, many other cells involved in wound healing, such as macrophages, lymphocytes, fibroblasts, endothelial cells, and dendritic cells also synthesize GM-CSF and/or are GM-CSF targets [6]. Nevertheless, continuous injection or external application is another big issue restricting better utilization in clinical practice. Thus, in the present study, we integrated recombinant human (rh) GM-CSF into a heparinized collagen-chitosan scaffold to control local and sustained release of the cytokine, as previously described [7], considering the slow angiogenesis of artificial three-dimensional (3D) scaffolds during repair of full-thickness skin defects. We tested the hypothesis that a heparinized collagen-chitosan scaffold combined with rhGM-CSF accelerates angiogenesis for tissue engineering.

Materials and Methods
A cytokine-containing porous collagen-chitosan scaffold was fabricated by incorporating rhGM-CSF using freeze-drying and further cross-linking treatment with EDC/NHS and heparin. *In vitro* release kinetics was investigated by ELISA. Scaffolds with/without heparin (Ø6mm) were loaded with rhGM-CSF (10μg/cm²) overnight, and then incubated in PBS at 37°C. Supernatants were replaced with fresh PBS every 24h and stored at -20°C until assayed. But at day 11 they were replaced with fresh PBS supplemented with 1mg/ml collagenase (Type I).

*In vivo* biological activity of local, sustained rhGM-CSF release was accessed by a subcutaneous implantation experiment in SD rats. Three groups of scaffolds were fabricated: heparinized scaffolds loaded with/rhGM-CSF and blank unheparinized scaffolds. Tissue specimens were harvested at different time points after implantation for histopathological observation. Three rats were treated in parallel.

Results
*In vitro*, there was a burst release from both types of scaffolds during the first day of the study, but no significant difference was observed between the two groups (*p* = 0.311). The unheparinized scaffold tended to release more rhGM-CSF than the heparinized scaffold until the collagenase was applied, indicating that heparin immobilization contributed to the controlled release of rhGM-CSF. (Fig.1).

As shown in Fig.2, the scaffold containing rhGM-CSF had a significantly faster rate of neovascularization than all other groups, especially at prior phase (~14 days). The same result could be confirmed by counting vessels (Fig.3). In another aspect, there were more regenerative tissues formed in the rhGM-CSF group as well.

Discussion and Conclusions
The heparin treatment improved the binding affinity of the scaffold for rhGM-CSF. An *in vivo* study showed that the rhGM-CSF the release of rhGM-CSF enhanced the angiogenic potential of the heparinized scaffold and accelerated tissue regeneration into the scaffold. In summary, a heparinized porous collagen-chitosan scaffold loaded with rhGM-CSF could be valuable in tissue engineering and regenerative medicine to develop a well-vascularized artificial dermal substitute.

References