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## ACCEPTED MANUSCRIPT

#### A chitin film containing basic fibroblast growth factor with a

#### chitin-binding domain as wound dressings

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#### ABSTRACT

Basic fibroblast growth factor (bFGF) can stimulate wound healing. However, consistent delivery of bFGF has many disadvantages. To decrease their instability and diffusible, we introduced chitin-binding domain (ChtBD) into bFGF. Two expression plasimids were constructed. The first one (named bFGF) contained bFGF (154 amino acids), the second (named ChtBD-bFGF) contained bFGF and the ChtBD (54 amino acids). ChtBD was derived from chitinase A1 (ChiA1) of Bacillus circulans WL-12. The recombinant protein ChtBD-bFGF had the same biological activity as bFGF in promoting fibroblast proliferation. Chitin powder was dissolved in 11 wt% NaOH and 4 wt% urea aqueous solution via the freezing/thawing method. A chitin solution was spread on a glass plate and coagulated with anhydrous alcohol. The chitin binding ability of ChtBD-bFGF was 11.4-fold higher (up to 286  $\mu$  g/cm<sup>2</sup>) than bFGF *in vitro*. The immunofluorescence data indicated that the ChtBD-bFGF@chitin film promoted cell adhesion and proliferation. The ChtBD-bFGF@chitin film and bFGF@chitin films were implanted subcutaneously. Histological analysis showed that ChtBD-bFGF promoted vascularization at the implanted site more

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