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BMP-2 plasmid loaded PLGA/HAp composite scaffolds for treatment of bone defects in nude mice

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ABSTRACT

We studied three different types of scaffolds, encapsulating bone morphogenetic protein-2 (BMP-2) plasmid, in terms of their performances in bone regeneration in nude mice. The plasmid was loaded into fibrous matrices in three different ways: coating of naked DNA (Group A) or DNA/chitosan nanoparticles (Group B) onto scaffolds after fiber fabrication by dripping, and encapsulation of DNA/chitosan nanoparticles into scaffold by mixing them with PLGA/DCM solution before fiber fabrication (Group C). Their individual performances were examined by soft X-ray observation, histological analysis and immunostaining of bone tissue. In addition, the BMP-2 protein concentration and alkaline phosphatase (ALP) activity in serum were monitored. The results revealed that the bioactivity of BMP-2 plasmid released from all three kinds of scaffolds was well maintained; this eventually helped improve the healing of segmental defects *in vivo*. Interestingly, the three kinds of scaffolds released DNA or DNA nanoparticles in different modes and their performances in bone healing were diverse. These observations demonstrate that the *in vivo* performance of these newly developed DNA delivery devices correlates well with their *in vitro* release profiles.

1. Introduction

Bone is one of the most commonly treated and repaired organs in the human body. Existing treatment modalities include autogenous, allogenic and synthetic bone grafts, with autograft being the clinically preferred choice of bone grafting material [1–6]. However, disease transfer and histo-incompatibilities are very likely to occur in the case of allografts. Autografts are not limited by risks of disease transfer and histo-incompatibilities, but the supply of autograft tissue is physically limited [7–9]. On the other hand, synthetic grafts based on metals and some ceramics, like hydroxyapatite and tricalcium phosphate fail to provide similar mechanical properties of human bone tissue, often resulting in implant failure and revision surgery [10,11].

Due to the aforementioned limitations associated with bone grafts, engineered biomaterials combined with growth factors have emerged as a new treatment alternative in bone repair and regeneration. A number of different growth factors, including bone morphogenetic proteins (BMPs), transforming growth factor β, platelet-derived growth factor, fibroblast growth factor and insulin growth factor have been shown to stimulate bone growth, collagen synthesis, and fracture repair both *in vitro* and *in vivo* [12–16]. In particular, BMPs are osteoinductive proteins originally identified in demineralized bone [17], which are known to facilitate bone healing without transferring bone tissues. Among this group of proteins, BMP-2 has been particularly known to induce healing in segmental bone defects. Aebli and Saito [18,19] reported that BMPs improve bone regeneration *in vivo*, and BMP-2 has been found to induce healing of segmental bone defects. However, a single exposure to an exogenous growth factor may not be sufficient to stimulate and sustain adequate bone growth [18,19]. Lucas et al. [20] showed that the incorporation of a water-soluble BMP mixture into a synthetic polymer matrix promoted chondrogenesis and osteogenesis when implanted ectopically *in vivo*. However, when the BMP mixture was administered alone, this effect was not found [20]. These results suggest that the biological activity of BMP may be strongly influenced by the properties of the carrier system utilized for its delivery. Moreover, the use of BMP-2 protein alone requires large amounts of protein because of its short half-life.

Gene transfection is a powerful and promising technique that involves the *in vitro* or *in vivo* incorporation of exogenous genes into cells for experimental and therapeutic purposes. Successful bone regeneration by gene transfer into hMSC (human mesenchymal

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stem cells) has also been reported [21–23]. Here, a retrovirus or adenovirus vector carrying human BMP-2, -4, or -7 was used as the therapeutic gene and found to be effective in the formation of new bone. However, considering the immunological and safety issues of viral vectors, necessity in the development of non-viral vector systems has been increasingly important [24]. Conventionally DNA was used in combination with marrow stem cells for the expression of corresponding proteins, which might cause unexpected immune-response from the host. Interestingly, Huang and colleagues obtained promising results by using DNA/polymer composite as implantation dose [6]. In the present work, DNA would be introduced into nude mice in a similar way, without foreign MSC being introduced.

In the recent years, many controlled release dosage forms have been developed for drug, protein and DNA delivery, for instance, nanoparticles and microspheres. However, this type of devices is known to exhibit a large burst during the early period of release. To tackle this drawback, fiber is chosen in the present study as the release dosage form because its moderate surface/volume ratio may produce a relatively constant controlled release of DNA with inhibited initial burst as compared to that of nanoparticles and microspheres [25,26]. Moreover, in comparison to microspheres, compact fibrous scaffolds give cell stable three-dimensional growth environments and may provide newly generated bone enough support. On the other hand, hydroxyapatite (HAp), which is a major component of the bone, can be used as a subsidiary in the bone generation. In addition, HAp has another advantage of being able to bind directly to the bone since both of them have similar chemical structures [27]. Therefore, polymer/HAp composite scaffolds are promising as a substitute for bone graft. In a previous study [28], PLGA/HAp composite scaffolds with different HAp contents (0%, 5% and 10%) were fabricated by electrospinning method and DNA was incorporated into the scaffolds in three ways (i.e. coating of naked DNA or DNA/chitosan nanoparticles on scaffolds after fiber fabrication by dripping, and encapsulation of DNA/chitosan nanoparticles into scaffold by mixing them with PLGA/HAp solution before fiber fabrication) (see Fig. 1a). The results showed that BMP-2 plasmid loaded PLGA/HAp composite scaffolds could maintain the integrity of encapsulated BMP-2 plasmid, enhance cell attachment with negligible cytotoxicity. In the present study, the main objective was to investigate the bone regeneration capability of these PLGA/HAp composite fibrous scaffolds *in vivo*. The hypothesis is that different loading methods of BMP-2 plasmid and different HAp contents in scaffolds will alter the release profiles of BMP-2

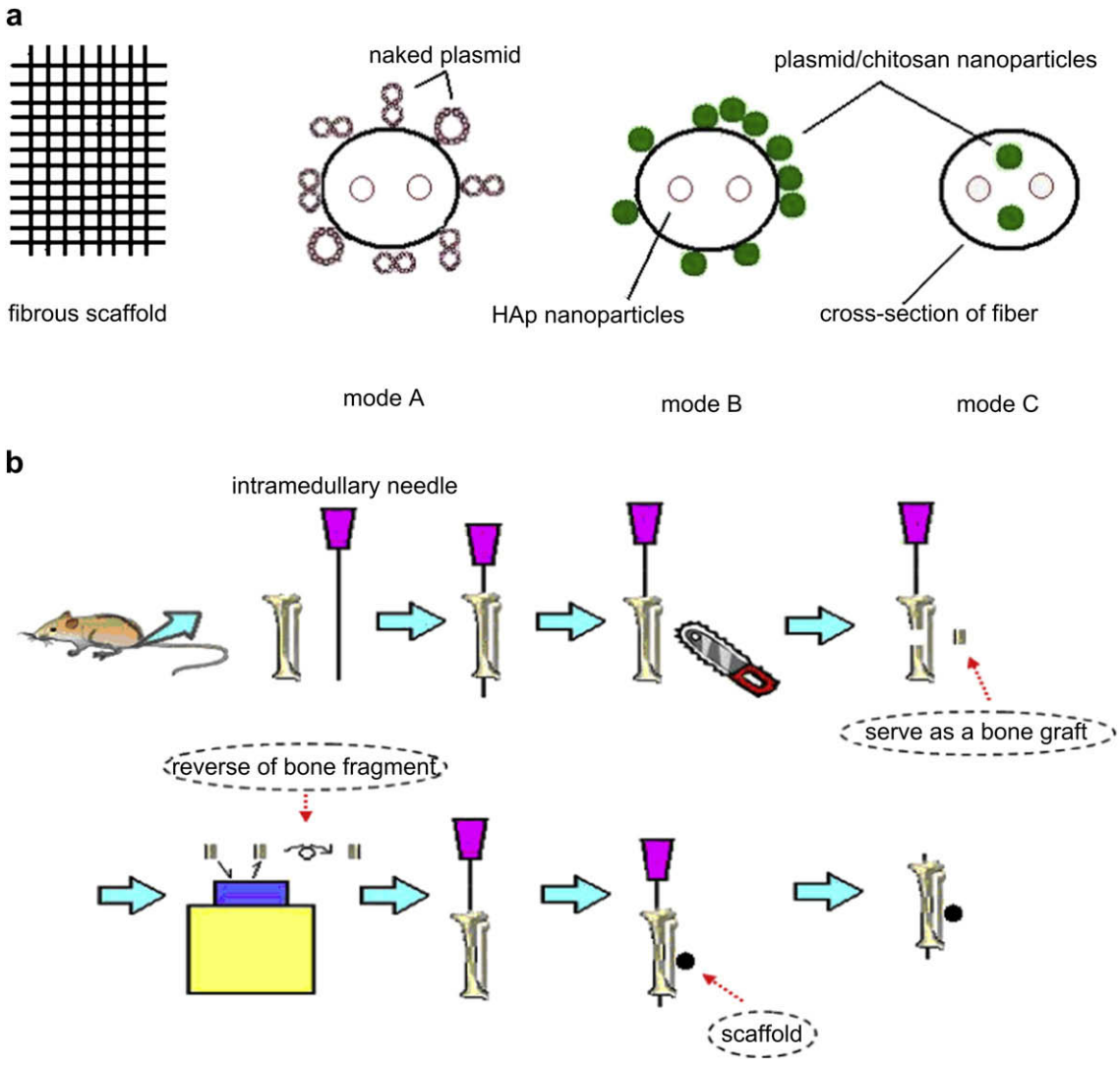

Fig. 1. (a) Illustration of three plasmid loading modes in the present work. Schematic drawing modified from reference [28]. (b) Schematic diagram depicting the construction of tibia bone defect model.