Bone Morphogenetic Protein-2 Non-viral Gene Therapy In A New Screening Model For Orthotopic Bone Formation In The Goat

Loek Loozen, M.D.1, Cumhur F. Oner1, Wouter JA Dhert, PhD, MD1,2, Moyo C. Kruyt, PhD, MD1, Jacqueline Alblas1.
1University Medical Center Utrecht, Utrecht, Netherlands, 2Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.


Introduction: Treatment and reconstruction of large bone defects, delayed unions and non-unions is challenging and has resulted in an ongoing search for novel tissue-engineered therapies. Bone morphogenetic protein-2 (BMP-2) has shown to be an excellent stimulus for bone growth. BMP-2 gene therapy is a promising strategy to provide a sustained production of protein locally, omitting the need for the extremely high dosages of protein currently used in clinical applications. In previous work we developed a non-viral alginate hydrogel-based gene therapy with the BMP-2 gene (pBMP-2). In vitro this gene therapy resulted in an increased bioactive protein production by mesenchymal stromal cells (MSCs) over a period of several weeks. When implanted subcutaneously in mice, abundant bone formation was observed.[1] To further translate this technology towards larger preclinical models, important issues remain to be investigated, such as the necessity of seeded cells as a target for gene therapy. To screen multiple conditions simultaneously in a clinically relevant environment we developed a screening model in the iliac crest of goats. To minimize crossover effects when soluble factors such as BMP-2 are investigated, we deliberately kept the implants well separated at a distance of at least 5 mm.[2] The model was used to investigate the feasibility of BMP-2 gene therapy in a setting with bone contact and the possibility for gene expression in non cell-seeded constructs.

Methods: Four cylinder shaped implants were placed in the iliac crests of ten goats. An inert plastic tube around each implant prevented bone ingrowth from the sides and only allowed bone ingrowth from the bottom (Fig. 1). Unloaded control biphasic calcium phosphate (BCP) scaffolds were compared to scaffolds containing pBMP-2 in alginate hydrogel either combined with seeded MSCs or as cell-free construct. An empty tube was included to evaluate spontaneous bone ingrowth and an autologous bone condition served as an indicator of bone function with respect to survival and resorption. Fluorochromes were administered at three, six and nine weeks after surgery to assess onset of bone formation. Histomorphometry was performed after termination at twelve weeks. Immunohistochemistry was used to quantify BMP-2 presence at twelve weeks.

Results: All constructs remained firmly in place. Microscopic analysis of the BCP did not show signs of degradation. Fluorochrome markers showed that pBMP-2 with MSCs resulted in an earlier onset of bone formation and larger ingrowth distance of bone within the implanted period compared to the controls. The empty controls showed low levels of spontaneous bone ingrowth and implantation of autologous bone did not result in complete healing of the defect, but indicated proper bone function with respect to survival and resorption. Histomorphometry at twelve weeks showed that the condition with pBMP-2 combined with MSCs resulted in increased bone formation compared to control condition of only BCP. This was not observed for the unseeded constructs with pBMP-2 (Fig 2). Immunohistochemical analysis
revealed the presence of BMP-2 protein in all pBMP-2 containing constructs. All bone containing implants showed BMP-2 in two distinct cell types: multinucleated cells aligning the scaffold material and osteoblasts aligning the newly formed bone.

**Discussion:** The current study demonstrates a relatively easy model for screening multiple conditions for bone regeneration in a clinically relevant environment. By shielding the sides of the cylinders nutrient supply and tissue ingrowth could only occur from the top and the bottom, preventing rapid chamber filling. The model allows dynamic assessment of bone formation and assures full separation of the experimental conditions. The BCP cylinders showed osteoconduction from the underlying bone, with a significant increase of bone formation when adding BMP-2 plasmid DNA combined with seeded MSCs.

**Significance:** This work introduces a large animal-screening model in an orthotopic location for studying bone formation. The model appeared robust, showed no neighboring effects and seems suited for the screening of multiple new bone formation inducing strategies including gene therapy in a clinically relevant environment.

Fig. 1: Overview of the surgical procedure, section plane and histology. Two implants (6.4 mm diameter and 10 mm high) per iliac wing (a). Intraoperative image of the holes (b) and implants press fit in situ (c). Implants consisted of porous ceramic cylindrical scaffolds surrounded by a PCL tube (blue), preventing bone ingrowth from the sides (d). Microscopic image of a stained construct (methylene blue/basic fuchsin) and a pseudo-colored version to highlight the bone (red) and the scaffold (green) (e). Scale bar = 2 mm.
Fig. 2: Bone formation expressed as bone contact% and bone area%.
The results are represented as mean ± SD. The cell-seeded implants show a significantly higher bone contact% compared to the non-cell seeded implant (*p<0.05).

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