Bone Formation Increases with Higher Gap Junction Intercellular Communication

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Statement of Purpose

Many cell-based tissue engineering strategies are limited in the ability to regenerate large volumes of tissue. This limitation is due in part to transport blockage caused by the formation of a shell at periphery of the construct. Increasing the rate of bone formation and enabling cell-cell communication may help overcome these limitations. Gap junctions are aqueous conduits that are formed by the docking of two hemichannels in juxtaposed cells. These channels have been hypothesized to play an important role in the coordination of bone remodeling (Stains, BioChem. BioPhys. 2005, 1719:69). In particular, gap junctions formed by Connexin 43 (Cx43) hemichannels are necessary for proper ossification. A 7 base pair deletion in the Cx43 gene (Cx43Δ7) can produce a connexin structure without transport functionality (Kizana, Gene Ther. 2006; 1-5). This study aimed to quantify the effects of gap junction intercellular communication (GJIC) in bone marrow stromal cells (BMSCs) overexpressing Cx43 (with and without overexpression of BMP-7), as well as the ability to enhance bone regeneration in-vivo following transplantation of BMSCs overexpressing Cx43.

Methods

BMSCs from the femoral and tibial cavities of 5 week old male C57BL/6 mice were cultured in α-MEM with 10% fetal bovine serum (FBS) and antibiotics (100 µg/ml penicillin G-streptomycin) at 37°C in 5% CO_2/95% air. Cx43 infection via lentivirus LV-GFP-Cx43 or LV-Cx43Δ7 was performed by culturing the cells with 8 ml of virus-containing media with 10 µg/ml protamine sulfate for 12 h (infection), followed by replacing with fresh media for 12 h (recovery). BMP7 was incorporated by 24 hr *ex vivo* transfection (AdCMVBMP-7, multiplicity of =200, Krebsbach, Hum Gene Ther. 2000; 1201-10).

Fluorescent dye transfer studies were performed to assess GJIC. Calcein-AM (10um) and Vybrant-DiI were used to label donor cells (DC) grown to confluence. As a negative control, 50uM of the gap junction uncoupler alpha-glyccirrhetinic acid (AGA) was used. DCs were added to recipient cells in 12-well plates, at 1:8 ratio. Cells were harvested for quantitative assessment of GJIC by flow cytometry.

For the in-vivo studies, cells were trypsinized and seeded in Gelfoam scaffolds (D=5mm, thickness =0.2mm; Gelfoam®; Pharmacia & Upjohn). On the second passage, two million cells were collected, suspended in 50 µl medium, and loaded onto each sponge by capillary action. 5 sponges were used for each group and time period (4 and 8 weeks). The implants were transplanted into a calvarial defect created in nude mice (n=4). After the time periods, the ossicles were harvested and scanned by µCT (MS8X130-EVS). Ossicles were analyzed on the basis of bone volume fraction (BVF).

Results/Discussion:

A 2-fold increase in calcenin-AM transfer was noted in BMSC-Cx43 cells after 4 hrs (fig 1, $p<0.001$, vs. all groups). Cells that contain AGA and those expressing the mutant Cx43Δ7, showed significantly less transfer than BMSCs and BMSC-Cx43 ($p<0.001$, for both), indicating GJIC dependent transfer.

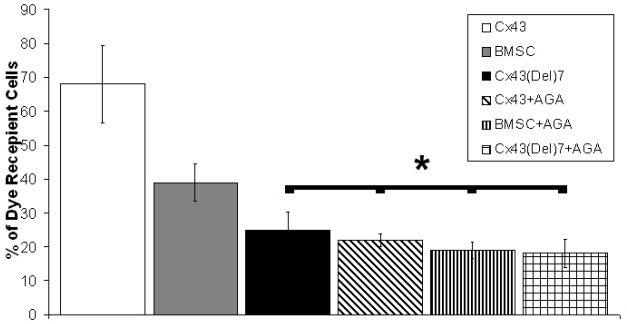

Fig 1: 4 hr transfer fraction of Cx43-BMSC, BMSC, Cx43Δ7 and negative controls (all groups significantly different except *).

In-vivo (fig. 2), cells overexpressing Cx43 produced more bone than BMSCs alone ($p=0.003$) after two months. Transplantation of cells expressing Cx43Δ7, resulted in a lower BVF than all other groups (5.0+/-3.2%, $p<0.001$). A synergistic effect is observed in ossicles formed from cells co-transfected with Cx43 and BMP7 ($p=0.041$ vs. Cx43 alone).

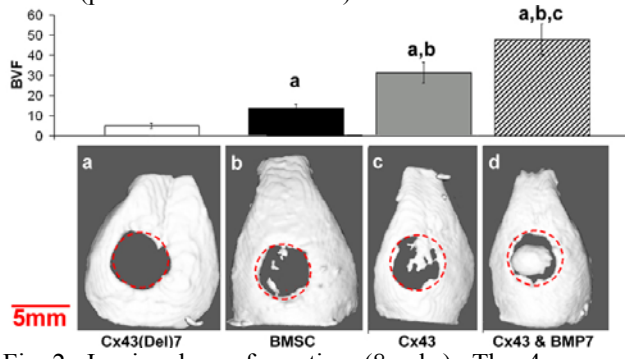

Fig 2: In-vivo bone formation (8 wks). The 4 groups showed different BVF. Red circles show defect region. Overexpression of Cx43 increased the BVF compared to BMSCs. Cells co-transfected with Cx43 and BMP7 generated the highest BVF (47.1+/-6.5%). Cells expressing the mutant Cx43Δ7 gene produced significantly less bone than BMSCs ($p=0.012$).. Letters indicate significant differences.

Conclusions:

Transduction of Cx43 facilitated cell-cell communication by providing more GJIC. Overexpression and inhibition of GJIC alter the amount of bone formed in-vivo. These results demonstrate the importance of high levels of GJIC in tissue engineering strategies.

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