SHORT COMMUNICATION

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Collagen gel delivery of Tgf-β_3 non-viral plasmid DNA in rat osteoblast and calvarial culture

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Abstract

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Different forms of collagen as a carrier for naked plasmid DNA have shown potential as vehicles for therapeutic gene delivery and tissue engineering. The objective of this study was to determine the suitability of a dense collagen gel as a vehicle for sustained delivery of plasmid DNA in cell and organ culture. Plasmid DNA encoding Tgf-β_3 was combined with collagen gel. DNA released into the media was measured by Pico-Green spectrophotometry. Results showed that DNA was released from the collagen gel at a gradual rate for up to 14 days. To evaluate collagen-mediated transfection in tissue, calvariae were exposed to collagen containing plasmid encoding GFP or DsRed. Transfection was visualized by fluorescence localized to tissue adjacent to the vehicle. To evaluate protein production, fetal rat calvarial osteoblasts were cultured with a collagen/Tgf-β_3 plasmid mixture or in media containing plasmid alone. Media was collected at various time points to measure Tgf-β_3 protein production. ELISA assays showed that collagen-transfected osteoblasts demonstrated an elevated Tgf-β_3 protein production for up to 14 days. Therefore, collagen delivery of viable plasmid DNA created a sustained transient transfection of calvarial osteoblasts resulting in prolonged and elevated growth factor production. Together, these results suggest that use of collagen gel as a vehicle may provide a strategy to achieve localized and controlled, non-viral gene delivery *in vivo*.

Key words: cranial suture; craniosynostosis; collagen gel; non-viral plasmid delivery; osteoblast; Tgf-β_3

DNA delivery from biomaterials or polymers is currently being applied to the multidisciplinary science of gene therapy and tissue engineering. Collagen gels are primarily used for injectable, controlled delivery systems and have the advantage that it can be introduced in a minimally invasive manner. Previous studies have clearly shown the ability of the collagen gel to deliver therapeutic proteins *in vitro* and *in vivo* (1,2). Studies directed toward using different forms of collagen as a carrier for naked plasmid DNA and standard vectors have shown the potential of these carrier matrices in therapeutic gene delivery and tissue engineering (3,4). This current study was designed to determine the suitability of a dense collagen gel as a vehicle for sustained delivery of plasmid DNA in calvarial osteoblast and organ culture.

Materials and methods

In vitro plasmid DNA release study

Transforming growth factor beta3 (Tgf-β_3)-pCMV6-XL5 plasmid DNA, 5 μg (Origene Technologies, Rockville, MD, USA) was mixed 1:1 volume with collagen gel (32 mg/ml, NeuColl, Inc., Campbell, CA, USA) and placed in eight-well chamber slides. Media was added to immerse the gel mixture and samples were collected at various time-points. The PicoGreen ds-DNA Quantitation kit (Molecular Probes, Eugene, OR, USA) was used to detect DNA release from collagen. The samples were measured using a fluorescent spectrophotometer (Fluorskan II; Labsystems, Helsinki, Finland).

In vitro transfection study using pEGFP-C2 and pDsRed2

Calvariae were dissected from 15-day-old rats as described by Moursi et al. (2). The day prior to transfection, EGFP plasmid or DsRed plasmid DNA (*pEGFP-C2*, *pDsRed2*; Clontech, Palo Alto, CA, USA) was mixed with collagen (1:1) and GenePorter transfection reagent (Gene Therapy Systems, San Diego, CA, USA). On the day of transfection, the EGFP/collagen or DsRed/collagen mixture was loaded into a syringe and implanted superficial to the posterior frontal suture by injection under the periosteum. After 14 days then fluorescent signal was visualized by Zeiss 510 META Laser Scanning Confocal microscope (Carl Zeiss Advanced Imaging Microscopy, Jena, Germany).

In vitro transfection study using plasmid DNA encoding Tgf-β_3

Fetal rat osteoblasts were grown at 37°C in an atmosphere of 5% CO_2 in DMEM supplemented with 10% fetal calf serum and antibiotics. On the day prior to addition of gels loaded with plasmids, cells were seeded at a density of 50 000 cells/well into 48-well plates giving rise to an almost confluent cell layer on the day of transfection. Plasmid DNA encoding Tgf-β_3 (3 μg/well) combined with Geneporter was mixed with collagen gel. On the day of transfection, collagen alone (control) or collagen with DNA was added to the wells then supernatants were collected at various time-points up to 14 days. Transfection was evaluated by measurement of Tgf-β_3 production using ELISA (Duo-Set ELISA kit; R&D Systems, Minneapolis, MN, USA).

Results

DNA release studies showed that plasmid release gradually increased at each time point with the total collected over 14 days equal to approximately 20% of the total loaded (Fig. 1). After 14 days, 80% of the loaded plasmid DMA remained in the gel.

Transfection studies demonstrated that released EGFP plasmid and DsRed plasmid were able to transfect cells in calvarial organ culture and express fluorescent proteins for up to 14 days (Fig. 2).

In the primary osteoblast culture system, released Tgf-(53 plasmid was able to transfect the osteoblasts and express Tgf-β_3 protein (Fig. 3). Tgf-β_3 protein production was considerably higher than endogenous production (control) and increased at each time point up to 14 days.

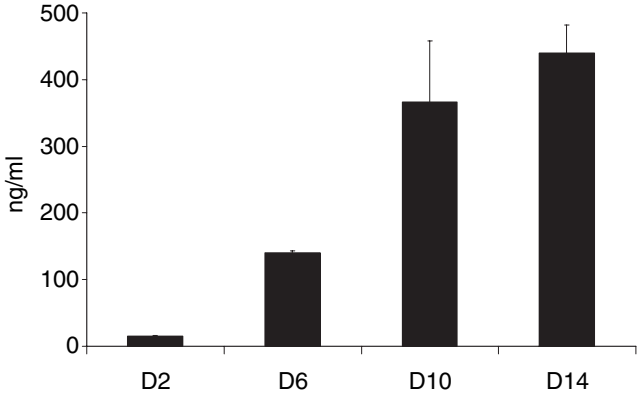

Fig. 1. Tgf-β_3 plasmid DNA release from collagen vehicle.

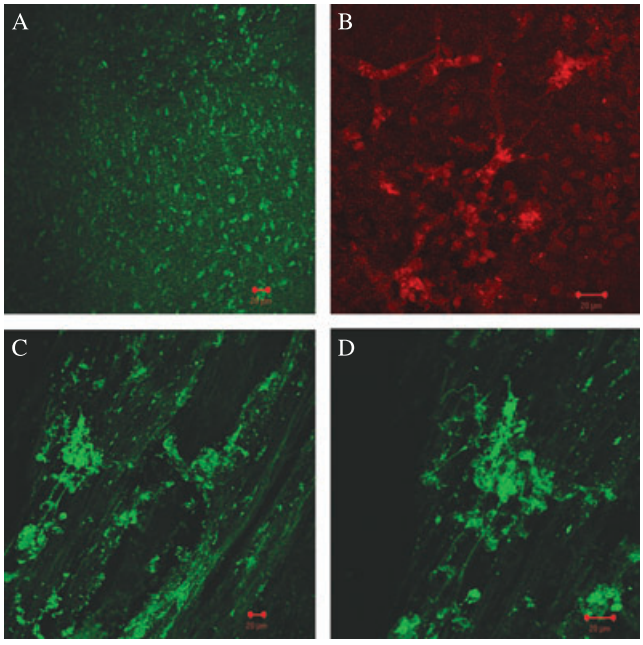

Fig. 2. Plasmid DNA, delivered via a collagen gel, transfects calvarial tissue. (A) EGFP (low magnitude), (B) DsRed (high magnitude), (C) EGFP (low magnitude) and (D) EGFP (high magnitude). Visualized by Laser Scanning Confocal microscope.

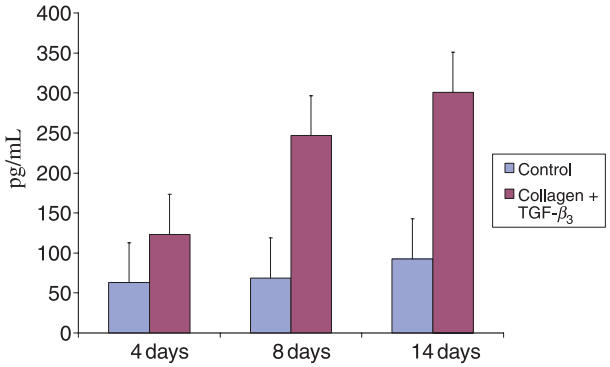

Fig. 3. Plasmid-encoded Tgf-β_3 protein production as measured by ELISA.

Discussion

DNA release increased at each time-point with the total collected over 14 days equal to approximately 20% of the total loaded. These data suggest that, given the amount of DNA present on day 14, subsequent transfection and recombinant protein production could continue for a prolonged period. Successful transfection was seen in both osteoblasts and calvarial tissue. The results indicate that the collagen vehicle was able to deliver the plasmid intact to the cells for uptake, transfection and production of the protein. Tgf-β_3 plasmid released from the collagen gel was able to transfect and generate Tgf-β_3 protein in calvarial osteoblasts. Tgf-β_3 production continued to increase through day 14. This result correlated well with DNA release data (Fig. 2) and suggests that Tgf-β_3 production could remain elevated for an extended period. Therefore, this approach may have therapeutic potential to achieve localized and controlled, non-viral gene delivery *in vivo*.

Conclusions

1. Collagen gel can provide sustained release of plasmid DNA which maintains a substantial transfecting capacity.
2. Non-viral mediated, sustained release of plasmid resulted in prolonged osteoblast transfection and elevated growth factor production.
3. Collagen gels can be formulated to provide sustained release of a viable plasmid DNA carrying a potential therapeutic gene *in vivo*.

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References

1. Chong SL, Mitchell R, Moursi AM, Winnard PL, Losken HW, Bradley J et al. Rescue of coronal suture fusion using transforming growth factor-beta 3 (Tgf-beta3) in rabbits with delayed-onset craniosynostosis. *Anat Rec* 2003;**274A**:962–71.
2. Moursi AM, Winnard PL, Fryer D, Mooney MP. Delivery of transforming growth factor-beta2-perturbing antibody in a collagen vehicle inhibits cranial suture fusion in calvarial organ culture. *Cleft Palate Craniofac J* 2003;**40**:225–32.
3. Fang J, Zhu YY, Smiley E, Bonadio J, Rouleau JP, Goldstein SA et al. Stimulation of new bone formation by direct transfer of osteogenic plasmid genes. *Proc Natl Acad Sci USA* 1996;**93**: 5753–8.
4. Scherer F, Schillinger U, Putz U, Stemberger A, Plank C. Nonviral vector loaded collagen sponges for sustained gene delivery in vitro and in vivo. *J Gene Med* 2002;**4**:634–43.