BTX Applications

Tech-Trends

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In vivo electroporation induces bone regeneration

Bone Regeneration Induced by Combining In Vivo Electroporation of an Osteogenic Gene and Human Mesenchymal Stem Cells

Introduction

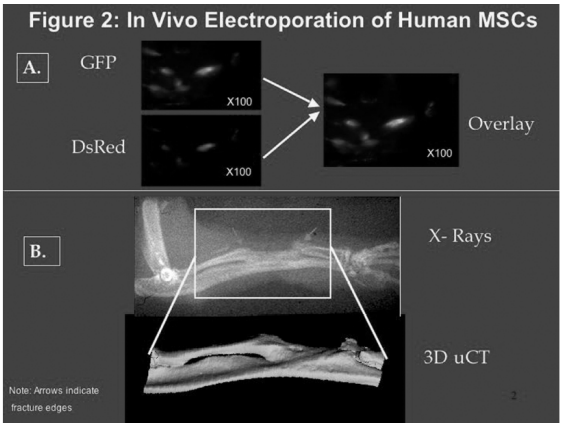
Recent studies indicate that *in vivo* electroporation (EP) is a safe, simple, and effective method. This study hypothesized that combining *in vivo* EP of an osteogenic gene with implanted human mesenchymal stem cells (MSCs) would induce bone regeneration.

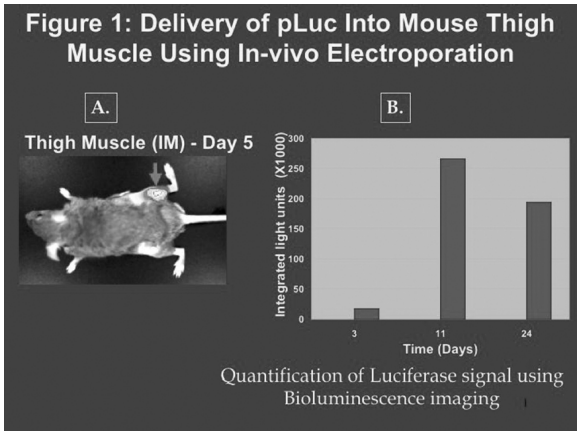
Methods

12 µg of luciferase plasmid were injected into the thigh muscles of C3H/Hen mice and transcutaneous electric pulses were applied using an ECM 2001 Electro Cell Manipulator (BTX). A bioluminescence imaging system was used to monitor noninvasively and quantitatively luciferase activity *in vivo*. In the second phase, human (h)MSCs were obtained from bone marrow. To demonstrate that hMSCs implanted *in vivo* can be efficiently transfected using EP, we implanted hMSCs engineered to overexpress the GFP gene and embedded in fibrin gel into the thigh muscles of NOD/SCID mice. Three days later we injected a plasmid encoding for red fluorescent protein (pDsRed) into the implantation site and *in vivo* EP was performed. On Day 10 frozen sections of thigh muscle were prepared. In the third phase, to demonstrate bone regeneration, 2 ×10^6 hMSCs embedded in fibrin gel were implanted into a 2.5-mm defect created in radii of NOD/SCID mice. Three days later the defect site was injected with 12 µg of bone morphogenetic protein–9 plasmid and the EP protocol was repeated. The injury site was monitored for bone formation by using quantitative micro–CT analysis.

Conclusion

We observed successful gene transfer to the skeletal muscle. Our results indicate for the first time that bone in a nonhealing defect can be regenerated by the combination of *in vivo* EP of a therapeutic gene and human MSCs.





We observed successful gene transfer to the skeletal muscle (Fig. 1A). Quantification of the bioluminescent signal demonstrated high levels of luciferase expression up to Day 24 (Fig. 1B). Using a fluorescence microscope we identified coexpression of the GFP and DsRed reporter genes in the implanted hMSCs (Fig. 2A). Moreover, the bone formation had led to defect closure in the radii (Fig. 2B).

ECM 2001 Dual Wave Electroporation/Electrofusion System
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Source: G. Pelled,[1] A. Lazarus,[1] Yoram Zilberman,[1] E. Zeira,[2] H. Yotvat,[2] E. Galun,[2] J. Li,[3] G. A. Helm,[3] D. Gazit.[1]
[1]Skeletal Biotech Laboratory, Hebrew University Hadassah Medical Center, Jerusalem, Israel; [2]Faculty of Medicine and Hadassah Gene Therapy Center, Hebrew University Hadassah Medical Center, Jerusalem, Israel; [3]Departments of Neurological Surgery and Biomedical Engineering, University of Virginia Health System, Charlottesville, VA.

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