A NOVEL APPLICATOR FOR ENDOSCOPIC GENE ELECTROTRANSFER

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ABSTRACT

Intratumoral gene delivery is a novel immunotherapy showing promise in cutaneous cancers such as melanoma. However, the broad application of this technology is limited by delivery efficiency and tumor accessibility. Efficient gene delivery requires intimate association of DNA and cells and a coincident electric field. Due to the low diffusion of plasmid DNA combined with the single bolus injection provided by a standard syringe, conventional electrogene transfer yields a small volume of transfected tissue. Our novel use of a helical delivery needle provides the dual benefit of increased DNA-tissue interface and anchoring of the device necessary for endoscopic use. This device has yielded efficient and repeatable delivery of cytokine-encoding plasmid DNA and reporter genes in several tumor models. Anchoring associated with the action of helical needle attachment ensures co-localization of the electric field with the injected tissue volume. The small form factor of this device makes it compatible with standard medical devices, including trocars, endoscopes and other catheter based devices, enabling intratumoral gene immunotherapy of previously inaccessible cancers, including breast cancer, lung cancer, and hepatocellular carcinoma.

INTRODUCTION

Immunotherapeutic intratumoral gene delivery of cytokines is an effective anti-tumor therapy for cancer, including melanoma, Merkel cell carcinoma, and head and neck squaumous cell carcinoma. Plasmid containing human IL-12 delivered to cutaneous tumors promotes immune recognition and attack of both treated tumor and distant metastasis.[1,2] This technology requires direct access to tumors, limiting treatment to cutaneous disease. To access visceral tumors, alternative intratumoral gene delivery approaches are required. Conventional devices have been used for electroporation mediated gene delivery to internal organs,[3–5] however these devices require surgical procedures, resulting in longer recoveries and an increased risk of complications. Alternatively, endoscopic electroporation therapies have been developed, but not in the context of gene delivery.[6,7]

Our approach to intratumoral treatment of non-cutaneous solid tumors incorporates DNA delivery and electroporation into a single, small-profile device for minimally invasive procedures. The device presented here anchors to target tissues, injects plasmid DNA, and electroporates via a 2.5mm diameter trocar to a maximum depth of 10cm. The helical injection needle both anchors the device to the tumor and enhances gene delivery by increasing surface area for tissue-DNA interaction, overcoming the low electrophoretic mobility of plasmid DNA in tissue8. This enhanced DNA distribution co-localized within the electroporation site yields improved gene delivery to a broad range of tumor types and improved control of tumor growth in the aggressive B16F10 melanoma model.

ADVANTAGES

HELICAL NEEDLE BENEFITS:
Helical injection needle anchors device to target tissue for reliable gene delivery in various tissues.

Circuitous injection channel increases injectate retention and DNA/tissue interaction within the treatment area, improving gene delivery efficiency.

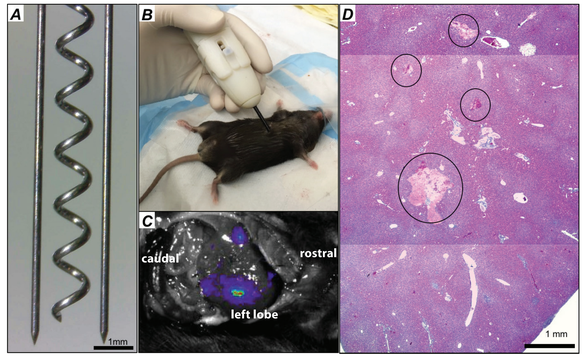
INTEGRATED DEVICE BENEFITS:
Integrated injection and electrodes increase procedure reliability and reduce user error.

Small form factor allows deep tissue access via trocar or other medical devices.

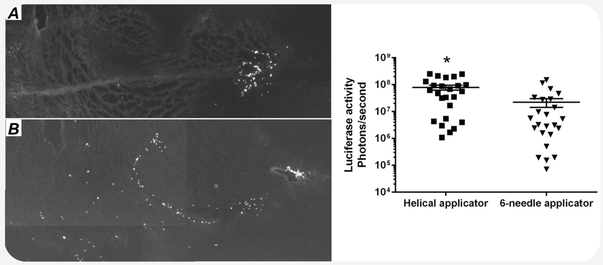
METHODS

- **Syngeneic Tumors:** Six-week-old albino B6 mice were injected with 1x10^6 murine colon adenocarcinoma cells (MC-38) or B16 f10 Melanoma cells in subcutaneous tissue on both flanks. Tumors were monitored by caliper measurements until volumes reached approximately 100 mm^3.
- **Tumor regression:** Six-week-old C57Bl6 mice were injected with 1x10^6 B16 f10 Melanoma cells in subcutaneous tissue on both flanks. Tumors were monitored by caliper measurements until volumes reached approximately 100 mm^3. Primary (left) tumors were injected with 1µg of PVUMVC3_IL-12 plasmid and electroporated. Both primary and contralateral (untreated) tumors were measured by calipers until tumor volume reached 1000mm^3.
- **Applicator:** An applicator capable of co-locating the electric field and injectate was used for these experiments. This applicator has two 30-gauge depth adjustable stainless steel electrodes separated by 1.6 mm and a 30-gauge helical injection lumen with adjustable depth centered between the electrodes. The conventional applicator used 6-needles in a hexagonal configuration with a 5mm electrode separation.
- **Electroporation:** Helical applicator electroporation was performed using the with the BTX ECM830 (Harvard Apparatus) at 350 V/cm for 10ms with a 1 Hz duty cycle for each of the 8 pulses. Conventional electroporation used 1500 V/cm for 100µs at 4 Hz, with 6 pulses with a rotating field polarity normal with each the side of the hexagon.
- **Plasmids:** pUMVC3_Luc2p, pUMVC3_mIL-12_IRES, pUMVC3.
- **Expression:** A Spectral Instruments Lago imaging system was used to quantify luminescence at 48 hours. To perform imaging 200 µl of a 15 mg/ml solution of D-luciferin was administered by intraperitoneal injection. Luminescent images were acquired 20 minutes after injection.
- **Data Collection and Statistics:** Biological data gathered was analyzed using a Kruskal-Wallis nonparametric one-way analysis of variance with a post-hoc Dunn's test. A significance level of 0.05 was selected for this analysis.
- **Needle insertion force:** Penetration and removal testing was measured in 20% gelatin maintained at 4˚C. penetration force was measured using the Chatillon DFS-II force gauge and LTCM-100 motorized test stand.

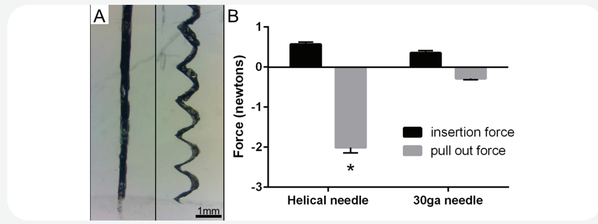
RESULTS



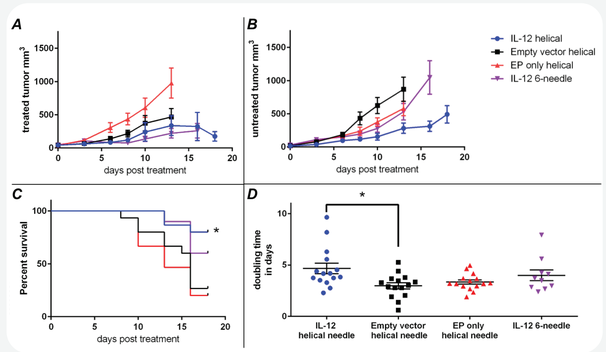
INTEGRATED ELECTROPORATION APPLICATOR WITH HELICAL INJECTION NEEDLE. **A.** Novel electroporation applicator features a helical injection lumen and parallel independently deployed electrodes. **B.** Small form factor of integrated device allows minimally invasive treatment of internal organs, yielding targeted liver expression **C.** and minimal tissue damage **D.** Circles denote helical needle path through tissue.



HELICAL INJECTION NEEDLE ENHANCES DNA DISTRIBUTION AND GENE DELIVERY. **A,B.** Cross-section of MC38 tumor electroporated with Plasmid DNA solution + fluorescent 1µm microparticles. **A.** 30ga needle or **B.** helical electroporator. The fluorescent microparticles distribution in indicates the relative distribution of DNA in tissue. **C.** Luciferase expression in MC38 tumors electroporated via Helical applicator or 6 needle cutaneous applicator. (n= 24, * p<0.001)



HELICAL INJECTION NEEDLE ANCHORS TUMORS. **A.** 30ga needle & helical needle in gelatin. Helical needle creates a 3-fold increase in surface area for DNA-tissue interaction. **B.** Peak forces of needle insertion and pull out in 20% gelatin. Helical needle has similar insertion force and 4-fold greater pullout force. (n= 4, * p<0.001)



HELICAL ELECTROPORATION APPLICATOR improves immunotherapeutic IL-12 treatment of B16 F10 melanoma. Mice were injected with B16f10 melanoma cell line, 1E6 cells the primary (left) flank, and 5E5 cells on the contralateral (right flank). 8 days post injection, the primary was treated by a single electroporation of IL-12 using the helical applicator, 6-needle cutaneous applicator, DNA only, or no treatment. Tumor volumes and survival were monitored for 18 days post treatment. Treatment groups: ● IL-12 electroporation with Helical applicator (n = 15), ■ Empty plasmid electroporation with Helical applicator (n = 15), ▲ no DNA injection electroporation with Helical applicator (n = 15), ▼ IL-12 electroporation with 6-needle applicator (n = 10). N = 15 mice per group **A.** Primary tumors were controlled by both the helical applicator and the 6-neede cutaneous applicator. **B.** Contralateral tumors showed a strong control of tumor growth by the Helical applicator, where as the 6-needle cutaneous applicator was ineffective with a single treatment. **C.** Survival of IL-12 treated mice. The helical applicator showed a significant increase in mouse survival (p< .01) , where as the 6-needle cutaneous applicator treated mice was not significantly different. **D.** The calculated tumor doubling time for each tumor after IL-12 treatment. The helical applicator strongly controlled tumor growth, where as the 6-needle cutaneous applicator was not statistically different from controls (p<.01).

CONCLUSIONS

The Helical integrated applicator is a novel device which allows the immunotherapeutic gene delivery of tumors up to 10cm into tissue through minimally invasive means, such as a trocar or endoscope. The helical injection needle provides the dual benefits of increased DNA distribution for more effective gene delivery and tumor anchoring for reliable treatment of compliant tumors. These characteristics yield enhanced efficacy of IL-12 plasmid electroporation in an aggressive B16F10 melanoma model, with improved tumor growth control and survival with a single treatment. This device will allow the application of intratumoral IL-12 gene immunotherapy to a broad range of deep tissue cancers.

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