P19 Fluoxetine delivery through an integrated bioelectronic device promotes wound healing in swine

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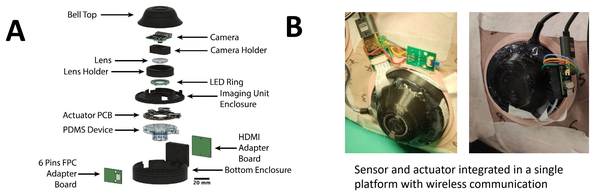
Abstract

Epidermal wound healing, including hemostasis, inflammation, cell proliferation, cell migration, and tissue remodeling, is a highly coordinated biological process. To optimally promote wound closure by a temporally and spatially controlled drug delivery system, a team of bio- and electrical engineers, computer scientists and wound specialists collaborated to create this iontophoresis bandage device with an actuator for drug delivery, and a sensor for wound monitoring. Previously we have demonstrated that this bioengineered device with programmable bioelectronic ion pumps to release protonized fluoxetine in negligible amount of solution, can increase re-epithelialization and decrease macrophage M1/M2 ratio on mouse wounds. Here we further integrated an actuator, the fluoxetine iontophoresis bandage, and a sensor, a microscopic camera with LED lights, onto a single platform with on-board PCB electronics and wireless communication on the device for the in vivo swine tests. Full-thickness, 20mm circular wounds were created on the back of Yorkshire pigs and the fluoxetine device with camera was applied to the wounds. Fluoxetine was targeted to deliver 0.45mg/wound/day during the daily delivery program. On the post-operative days 3 or 7, wound tissue was harvested to examine healing. On day 7, the treatment with fluoxetine device showed a trend of improved re-epithelialization by 50.4% compared to standard of care (n=6-8 wounds, p=0.056). The anti-inflammatory macrophage M2 subtype also increased with the fluoxetine device treatment, which results in decreased ratios of M1/M2 by 77.0 % on day 3 and by 62.5 % on day 7. Another indication of the tissue repair is innervation to the wound site. By using the expression of MAP2, Microtubule-Associated Protein 2, as a surrogate marker for neuronal ingrowth and dendrite extension into the wound, we demonstrated that the relative gene expression of MAP2 for fluoxetine-treated wounds on day 7 was 0.9 compared to 0.2 for the control, a 4.5-fold increase on wound edge. The integrated bioelectronic device with fluoxetine delivery has a great potential for wound treatment by reducing the burden for daily drug application, and possibly increasing patients' compliance. It also demonstrates that the fluoxetine released from the device retains its reparative biological activity to promote healing. In the future, we hope to further optimize the device design for the next stage of development for device commercialization and clinical use.

Introduction

Device design: An integrated, bioelectronic bandage device for chemical delivery, imaging and sensing



Sensor and actuator integrated in a single platform with wireless communication

C. Fluoxetine device: Fluoxetine delivery from device

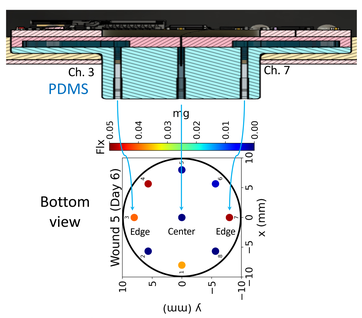
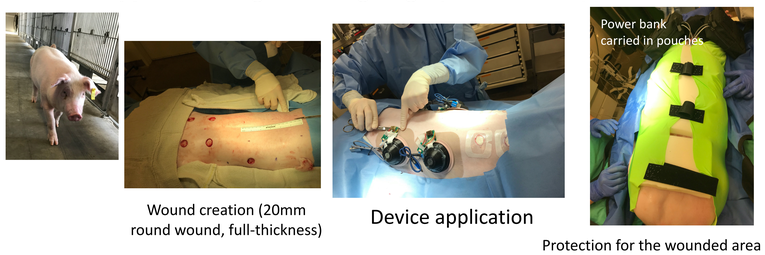


Fig. 1. Fluoxetine delivery from a bioelectronic device.

(A) An integrated bioelectronic device for wound treatment with a mini camera and LED lights, an actuator PCB board, PDMS for ion delivery, and wireless communication. (B) The integrated device has been applied to to swine wounds in this study. (C) Spatial delivery of fluoxetine (FLX) from the PDMS side of the device. Fluoxetine is loaded and delivered from the 8 micro-channels at the edges to the wound, but not from the center. Daily dose of 0.45mg/wound fluoxetine is targeted for the wound treatment.

Methods

In vivo application: Pig wounding surgery and device application



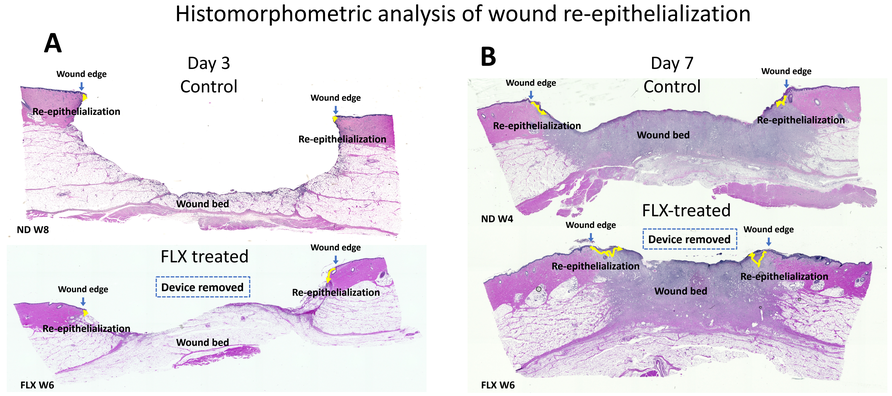
Wound creation (20mm round wound, full-thickness)

Device application

Protection for the wounded area

Results

Histomorphometric analysis of wound re-epithelialization



Treatment with FLX-device demonstrates a trend of improved re-epithelialization at day 10

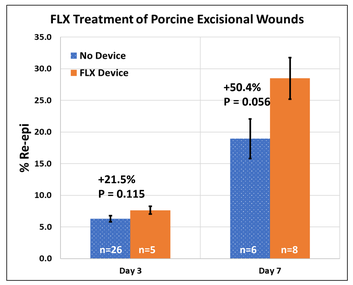


Fig.2. H&E staining for measuring wound re-epithelialization.

(A and B) Wound tissue was harvested after the FLX-device treatment, formalin-fixed, paraffin-embedded, and sectioned to 5µm slides and H&E stained. Wound imaged was taken with a Keyence BioRevo microscope at 2x, and re-epithelialization is measured by the extension of epidermis from wound edge to wound bed (yellow lines). (C) On both post-op day 3 and 7, the treatment with fluoxetine device showed a trend of improved re-epithelialization compared to standard of care (dressing only).

Immunohistostaining for macrophages in pig wounds

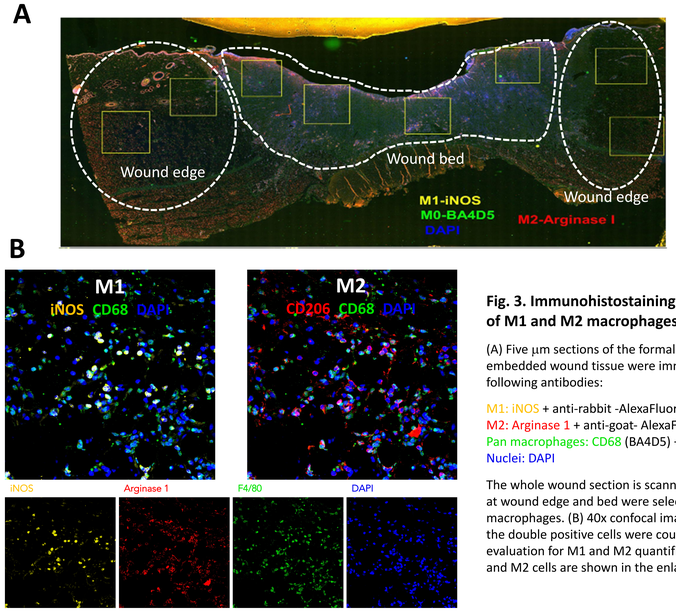


Fig. 3. Immunohistostaining and Quantification of M1 and M2 macrophages in wounds.

(A) Five µm sections of the formalin-fixed, paraffin-embedded wound tissue were immunostained with the following antibodies:

M1: iNOS + anti-rabbit -AlexaFluor 647
M2: Arginase 1 + anti-goat- AlexaFluor 568
Pan macrophages: CD68 (BA4D5) + anti-rat- AlexaFluor 488
Nuclei: DAPI

The whole wound section is scanned in (A). Multiple areas at wound edge and bed were selected to quantify macrophages. (B) 40x confocal images were captured and the double positive cells were counted with blind evaluation for M1 and M2 quantification. Examples of M1 and M2 cells are shown in the enlarged images.

Results

M2 macrophages increases on day 3 resulting in a decrease of M1/M2 ratio on wound edge with FLX treatment

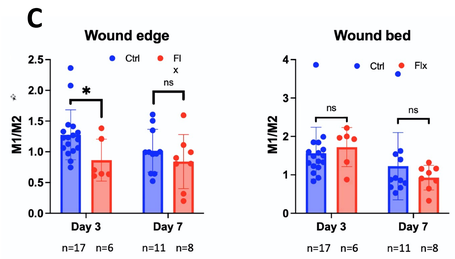
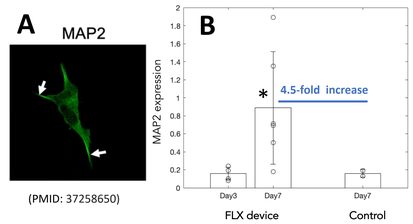


Fig. 3. Immunohistostaining and Quantification of M1 and M2 macrophages in wounds.

(C) Ratios of M1/M2 macrophages were compared at wound edge (left) and wound bed (right). The number of M2 macrophages increases on day 3 on wound edge, resulting in a decrease of M1/M2 ratio with with FLX treatment. (n= 6-17 wounds, *p<0.05)

MAP2 gene expression increases on day 7 with FLX treatment



(PMID: 37258650)

Fig. 4. MAP2 gene expression in wounds.

(A) MAP2 (Microtubule-associated protein 2) is a neuron-specific, microtubule-crosslinking protein found mostly in dendrites. GFP-MAP2 is found to express in dendrites of cultured neuron (PMID: 37258650).

(B) We examined MAP2 expression in the wound as a surrogate marker for neuronal ingrowth and dendrite extension into the wound. Control and FLX-treated wound tissue was collected in RNALater, and mRNA was extracted for qPCR with Qiagen RT² qPCR primer (GeneGlobe ID: PPS02696A-200, catalog #330001). The relative expression level of MAP2 for FLX-treated wounds on day 7 increased by 4.5-fold from 0.2 in control to 0.9 (n= 2-6 wounds, p<0.05).

Conclusion

1. An integrated bioelectronic device with fluoxetine delivery, wound imaging, and wireless communication has been developed for in vivo application.
2. Wound re-epithelialization demonstrates a trend of improvement on both day 3 and day 7 with FLX treatment.
3. M2 macrophages increases on day 3 resulting in a decrease of M1/M2 ratio on wound edge with FLX treatment.
4. The relative expression level of neuronal marker MAP2 for FLX-treated wounds on day 7 increased by 4.5-fold.
5. Fluoxetine released from the device retains its reparative biological activity to promote healing, and the integrated device has a great potential for wound treatment by reducing the burden of daily drug application.

Acknowledgements

UC Davis Large Animal Surgery Lab for performing pig surgery
Amy Lesneski, Victoria Hammitt, and Kirstie Tully
UC Davis Campus Vet Services for research support
Dr. Betty Ma and all the staff
UC Davis TRACS for pig transfer and housing
Juan Cabrera and all the staff

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