ELU511: A NOVEL SMALL-MOLECULE BACTERIOSTATIC WNT SIGNALING INHIBITOR THAT PROMOTES REGENERATIVE REPAIR AND IMPROVES TENSILE STRENGTH FOLLOWING FULL-THICKNESS CUTANEOUS INCISIONAL WOUNDS IN YORKSHIRE PIGS

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CHANGING THE WORLD OF WOUND HEALING

ABSTRACT

BACKGROUND: The canonical Wnt signaling pathway is quiescent in many mammalian organs and becomes activated in response to injury. Wnt signaling promotes fibrotic wound healing (including scarring) following acute cutaneous injury. Eluciderm Inc. has manufactured a potent Wnt signaling inhibitor, ELU511, with bacteriostatic properties. Application of the topical "spray-on" patented formulation of ELU511 promoted regenerative cutaneous repair with increased tensile strength in acute full thickness suture wounds.

METHODS: In this study, we utilized a porcine model to analyze wound repair following a surgical full thickness suture wound. Six (6) full-thickness 1 cm acute full thickness wounds were created on the backs of Yorkshire pigs. ELU511, a novel, potent, aqueously soluble, bacteriostatic, topical small molecule Wnt signaling inhibitor, was applied three (3) days a week (200μL) up to Day 30. The animals were allowed to heal for another 30 days before being sacrificed at Day 60. Histopathological analyses were performed on excised tissues.

RESULTS: Topical application of the novel small molecule Wnt signaling inhibitor, ELU511, significantly promoted regeneration of tissue, as evidenced by the presence of restored skin architecture with adnexal structures and restoration of well-organized cross-linked collagen assessed by trichrome blue staining and polarized microscopy. Wounds treated with ELU511 demonstrated 105-fold improvement in the tensile strength compared to saline-treated wounds.

CONCLUSIONS: Small molecule Wnt signaling inhibitors used as research tools have typically been limited for therapeutic usage due to their poor aqueous solubility. We have created ELU511, a water-soluble small molecule Wnt signaling inhibitor, in spray-on form. It is a non-toxic potential drug that is easy to apply to full thickness suture wounds. Our study reveals its positive properties of being a stable, potent, bioavailable, bacteriostatic small molecule Wnt signaling inhibitor that has strong pharmacological potential for state-of-the-art therapeutic usage in surgical wounds.

ELU511: First in a generation of novel, potent, aqueously soluble, topical small molecule Wnt signaling inhibitors for surgical wounds[1]

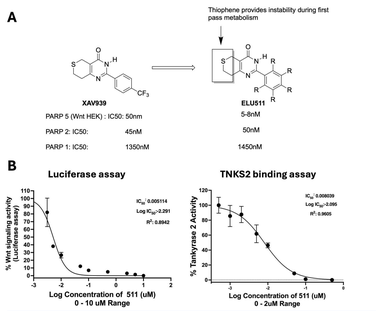

Figure 1. Creation of ELU511: a portable, bacteriostatic, and topical Wnt signaling inhibitor for surgical wounds. XAV939 has been shown to promote regenerative repair and reduce fibrosis in cutaneous wounds, and to promote cartilage regeneration in wounded ears[2]. **A.** Building on the positive results observed with XAV939, XAV939 has served as a template to design ELU511. The thiophene moiety of XAV939 was retained to ensure a short systemic half life for ELU511. The trifluorophenyl moiety was modified to improve aqueous solubility and potency. **B.** Luciferase and Tankyrase binding assays were used to determine the IC_{50} of ELU511, which was in the range of 3-8 nM. PARP1 and 2 inhibition remained unaffected following XAV939 derivatization.

Wnt signaling pathway is activated following injury

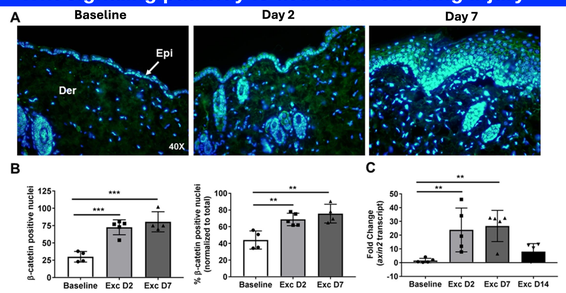

Figure 2. Wnt signaling is activated following acute injury in mice. A 1 x 1 cm^2 full thickness excisional wound was made on C57Bl6 mice. Uninjured mouse tissue was used as a baseline control. Mice were sacrificed on Day 2, 7, and 14. Immunostaining of β-catenin was performed to assess canonical Wnt signaling and DAPI was used for nuclear staining. **B.** Quantification of β-catenin positive nuclei was normalized to the total number of cells. β-Catenin expression was increased by Day 2 and peaked at Day 7. **C.** A semiquantitative RT-PCR analysis identified Wnt signaling downstream where target gene *axin2* was upregulated at Day 2 and Day 7 and declined by Day 14 following injury. Statistical significance: * indicates **p≤0.005; ***p≤0.001; N=5.

ELU511 promotes functional healing following full thickness incisional wounds in Yorkshire porcine model

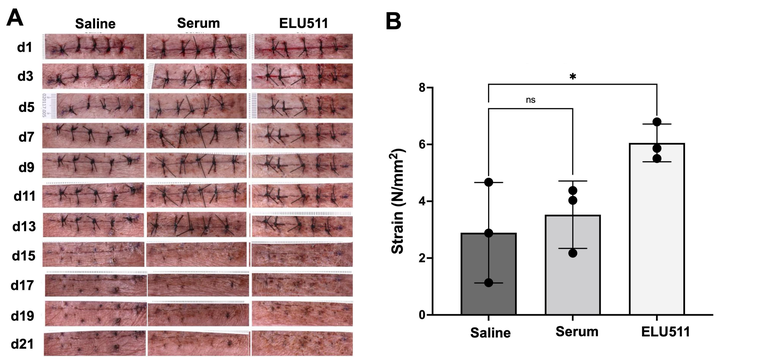

Figure 3. ELU511 promotes functional healing and improved cosmesis following full thickness suture incision in porcine wounds. A. 5 cm × 0.5 cm full thickness fusiform (spindle shaped) wounds were created on Yorkshire pigs in a paraspinal column/each side. Each wound had **5 simple interrupted sutures** with a 0.5-1 cm interval between sutures. **A.** A photograph of each wound site was taken after suturing and prior to dose administration. Topical applications of 0.3 mLs of ELU511 (0.25mg/mL) and controls (saline and serum) were applied every other day for 21 days. ELU511 decreased scar formation and improved cosmesis by Day 15 as compared to controls. These results show that ELU511 reduces scarring and improves cosmesis (N=8 wounds per treatment; N=3 pigs). **B.** At Day 22, each wound site (dose site) was collected and evaluated for tensile strength. ELU511 treatment significantly improved tensile strength as compared to controls. Statistical significance: * indicates * p≤0.05; N=3.

ELU511 promotes mature reticular collagen infiltration in the scar in full thickness sutures in Yorkshire porcine model

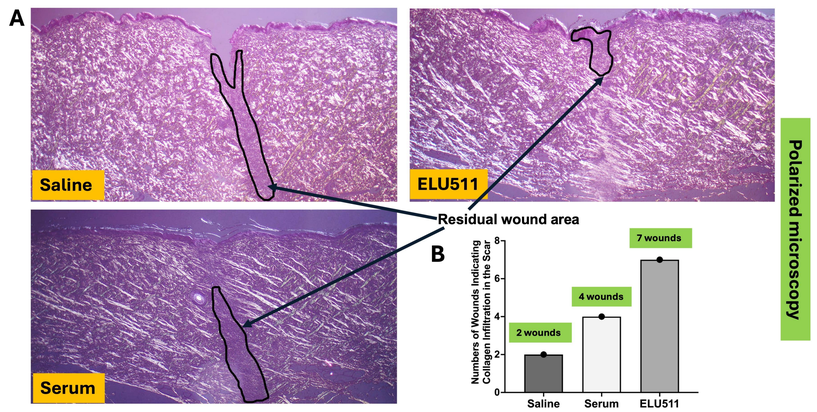

Figure 4. ELU511 promotes regenerative healing and reduces scarring in full thickness incisional suture wounds. 5 cm × 0.5 cm full thickness fusiform (spindle shaped) wounds were created on Yorkshire pigs in a paraspinal column/each side. Each wound had **5 simple interrupted sutures** with a 0.5-1 cm interval between sutures. ELU511 and controls (saline and serum) were applied topically every other day for 21 days. At Day 22, the entire wound site (dose site) was collected separately for each site and analyzed for histology. Plane polarized microscopy followed by H & E staining was performed to assess collagen organization and infiltration into the scar area. Increased collagen infiltration in the scar area was identified following ELU511 treatment. Conversely, a large scar area was present in the groups treated with saline and serum. **B.** The bar graph represents the number of wounds with collagen infiltration in the scar area. N=8 wounds per treatment.

ELU511 is bacteriostatic against a broad spectrum of aerobic and anaerobic, GNB (gram (-) bacteria) and GPB (gram (+) bacteria

Strain	24-Hours Log Reduction	72-Hours Log Reduction
C. acnes (ATCC 11827)	>2.0	>2.0
E. coli (ATCC 8739)	>3.2	2.5
E. coli (ATCC 25922)	>3.1	>3.1
E. faecalis (ATCC 19433)	0.3	>2.5
MRSA (ATCC 33593)	0.1	1.2
P. aeruginosa (ATCC 15442)	>3.1	>3.1
S. aureus (ATCC 6538)	0.1	1.5
S. epidermidis (ATCC 14990)	0.8	2.3
A. baumannii (ATCC 19606)	>3.0	>3.0
E. coli 0157:H7 (ATCC 12900)	>1.9	>1.9
K. pneumoniae (ATCC 10031)	>2.5	>2.5
P. aeruginosa (ATCC 9027)	>3.0	>3.0
P. vulgaris (ATCC 8427)	>3.6	>3.6
S. maltophilia (ATCC 13637)	>2.8	>2.8
S. pyogenes (ATCC 19615)	>3.2	>3.2

Figure 5. ELU511 has robust bacteriostatic effects. The bacteriostatic properties of the ELU511 serum (0.25mg/mL) were tested against a panel of bacterial strains comprised of aerobic and anaerobic, GNB (gram (-) bacteria) and GPB (gram (+) bacteria). ELU511 and a control sample were spiked with 0.5mL of the appropriate organism, such that the final concentration in the tube was ~1 x 10^6 CFU/mL. Negative sample controls, containing neat sample with no organism spike (one for the sample and one for the vehicle) and positive organism controls, containing 1 mL saline and 1mL of the 1 x 10^6 CFU/mL organism were used. ELU511 exhibited robust bacteriostasis across all tested bacterial strains.

CONCLUSIONS

- Wnt signaling pathway is upregulated following full thickness excisional wounds.
- Eluciderm Inc. has developed ELU511, a first-in-a-generation, novel, potent, bacteriostatic, **topical small molecule** Wnt signaling inhibitor.
- ELU511 promotes regenerative healing, cosmesis, and reticular collagen infiltration, and improves **tensile strength**, and reduces scar formation (fibrosis).
- ELU511 exhibits **bacteriostatic activity** against both aerobic and anaerobic Gram-negative bacteria (GNB) and Gram-positive bacteria (GPB).
- **ELU511 is anticipated to make a significant impact on procedural outcomes due to its combination of bacteriostasis, wound strengthening, and scar reduction with functional healing, thus reducing post-procedural complications such as infections, wound dehiscence, and scarring.**

ACKNOWLEDGEMENTS

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