School of Dentistry / Yr Ysgol Ddeintyddiaeth

Modulatory Effects of Novel Epoxy-Tiglianes on Dermal Fibroblast-Myofibroblast Wound Healing Responses Mediate Their Enhanced Anti-Scarring Properties

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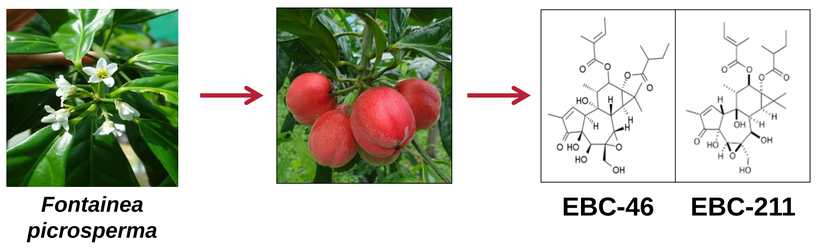
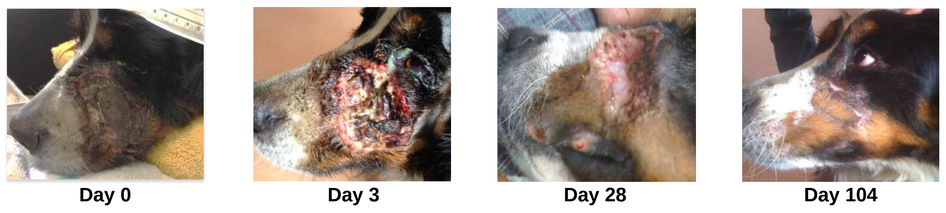
Introduction

- **The novel epoxy-tiglianes, 12-tigloyl-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tigliane-3-one (EBC-46) and a less active related compound, 12-tigloyl-13-(2-methylbutanoyl)-5,6-epoxy-4,5,9,12,13,20-hexahydroxy-1-tigliane-3-one (EBC-211), occur within seeds of the Fontain's Blushwood Tree, indigenous to Queensland's tropical rainforest[1].**
- **EBC-46 is currently being developed as an anti-cancer agent by Australian biotechnology company, Qbiotics (*www.qbiotics.com/*), for the intra-lesional treatment of cutaneous & sub-cutaneous tumours in humans & animals[2].**
- **In veterinary clinical trials, exceptional dermal wound healing responses, characterised by accelerated re-epithelialisation, closure & reduced scarring, have been consistently observed following tumour ablation by EBC-46[2].**

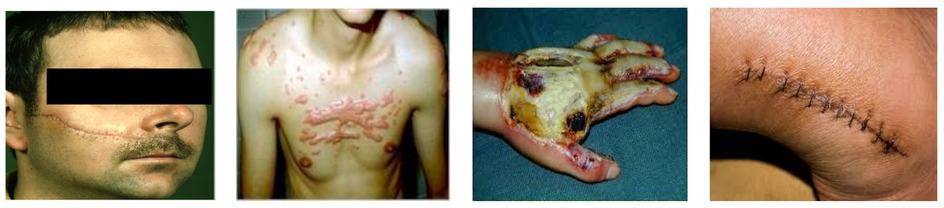


Fontainea picrosperma



Deep necrosing facial wound that had been unresponsive to 3 months treatment with current standards of veterinary wound care, resolved following treatment with EBC-46.

- **This suggests that EBC-46 & EBC-211 could offer treatments that abrogate normal & excessive dermal scarring (fibrosis), evident during clinical situations such as burn injuries, surgical / non-surgical lacerations & hypertrophic / keloid scarring.**
- **Indeed, as existing clinical therapies are acknowledged to be unsatisfactory for use in the prevention or attenuation of excessive scar formation, there is a huge clinical need to develop effective therapies which arrest or prevent fibrosis[3-4].**



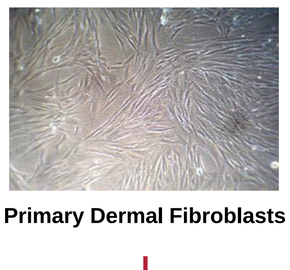
Oro-facial scar | Hypertrophic scar | Full-thickness burn | Surgical laceration

Examples of clinical conditions associated with normal or excessive scarring (fibrosis), which could benefit from the development of anti-scarring therapies, such as EBC-46 & EBC-211.

Aims & Objectives

As fibroblasts are pivotal to dermal healing responses, wound closure & scarring, fibroblasts & the scar-forming myofibroblasts represent viable targets for the anti-fibrotic properties of epoxy-tiglianes. Therefore, this study examined the effects of EBC-46 & the lesser active analogue (EBC-211), on fibroblast proliferation, migration & transforming growth factor-β_1 (TGF-β_1)-driven fibroblast-myofibroblast differentiation *in vitro*.

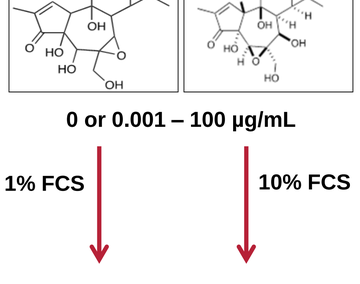
Materials & Methods



Primary Dermal Fibroblasts

Serum-starvation (24h) → **DMEM, antibiotics, L-glutamine (2mM)**

EBC-46 **EBC-211**



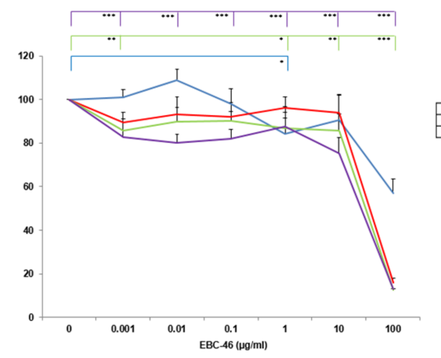
0 or 0.001 – 100 µg/mL

- **1% FCS** → **Automated Time-Lapse Confocal Microscopy (48h)** → **ImageJ Analysis of *in vitro* Scratch Wound Migration**
- **1% FCS** → **MTT Assay (24 – 168h)** → **Proliferation & Viability**
- **10% FCS** → **Flow Cytometry (0 – 36h)** → **Cell Cycle Analysis**
- **0% FCS** → **α-Smooth Muscle Actin (α-SMA) Immunocytochemistry & RT-qPCR (72h, + 10ng/mL TGF-β_1)** → **TGF-β_1-driven Fibroblast-Myofibroblast Differentiation**

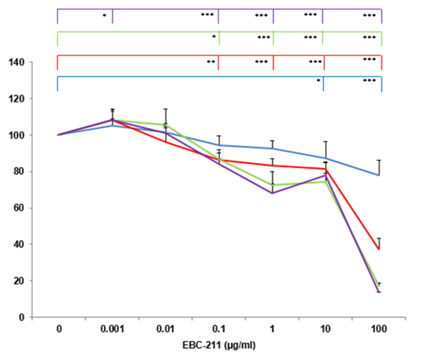
Results

- **Both EBC-46 & EBC-211 are cytotoxic to fibroblasts at 100 µg/mL.**
- **EBC-46 significantly reduced fibroblast proliferation at 1-10 µg/mL.**
- **EBC-211 significantly reduced fibroblast proliferation at 0.1-10 µg/mL.**

Effects of EBC-46 on Fibroblast Proliferation & Viability (Average +/- SE, N=3)

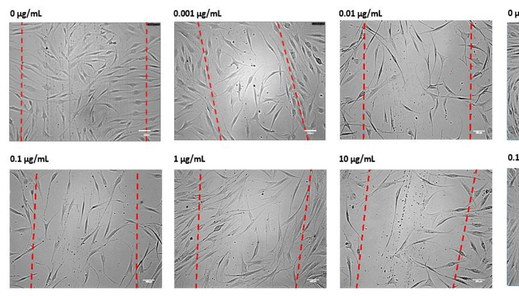


Effects of EBC-211 on Fibroblast Proliferation & Viability (Average +/- SE, N=3)

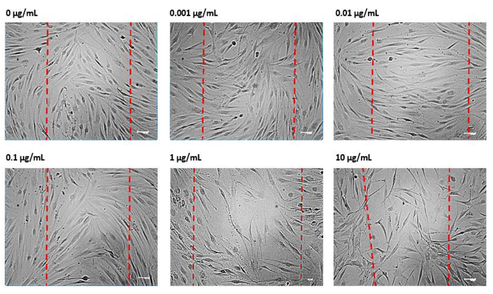


- **EBC-46 & EBC-211 exert no significant effects on dermal fibroblast migration & scratch wound repopulation.**

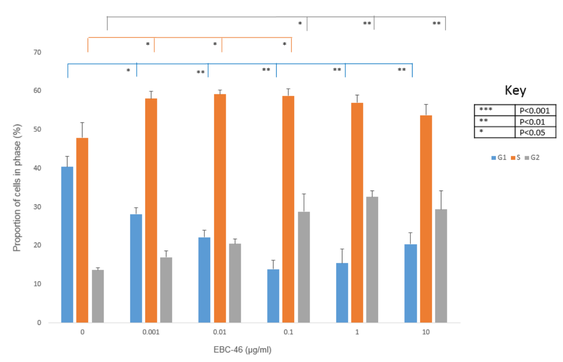
Effects of EBC-46 on Fibroblast Wound Repopulation (N=3)

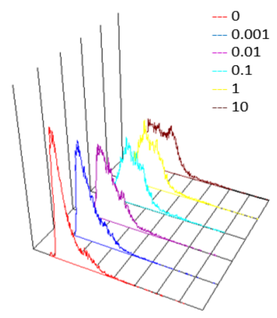


Effects of EBC-211 on Fibroblast Wound Repopulation (N=3)

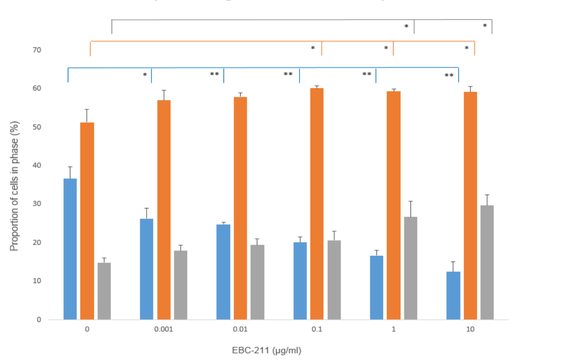


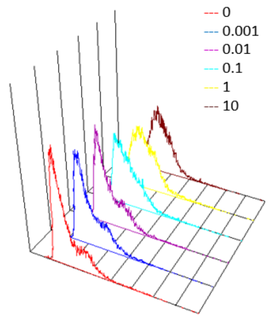
Effects of EBC-46 on Fibroblast Cell Cycle at T29 (Average +/- SE, N=3)





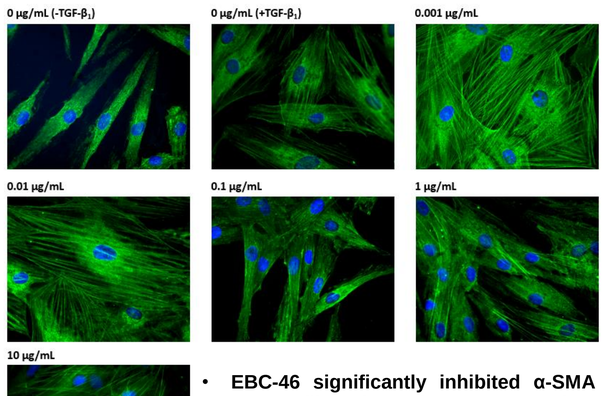
Effects of EBC-211 on Fibroblast Cell Cycle at T29 (Average +/- SE, N=3)





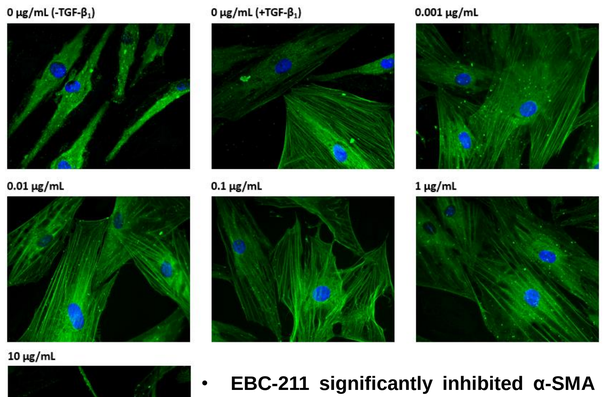
- **EBC-46 & EBC-211 significantly delay dermal fibroblast progression through the cell cycle, supporting the MTT proliferation data (above).**

Effects of EBC-46 on Myofibroblast Differentiation (+TGF-β_1)



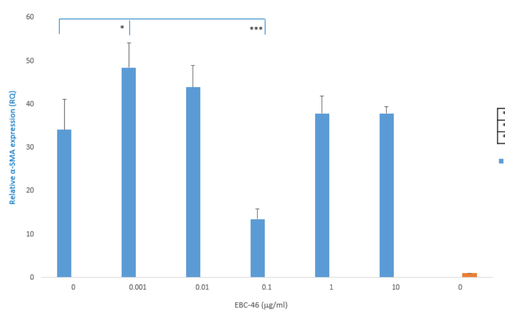
- **EBC-46 significantly inhibited α-SMA stress fibre formation at 0.1 µg/mL only, with cells retaining normal unstimulated dermal fibroblast morphology.**

Effects of EBC-211 on Myofibroblast Differentiation (+TGF-β_1)

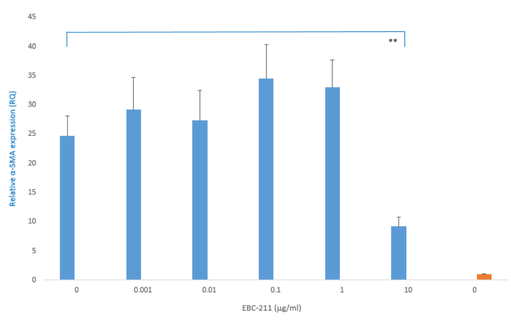


- **EBC-211 significantly inhibited α-SMA stress fibre formation at 10 µg/mL only, with cells retaining normal unstimulated dermal fibroblast morphology.**

α-SMA Expression in EBC-46-Treated Fibroblasts (+TGF-β_1)



α-SMA Expression in EBC-211-Treated Fibroblasts (+TGF-β_1)



- **EBC-46 and EBC-211 significantly inhibited α-SMA gene expression at 0.1 µg/mL & 10 µg/mL, respectively.**

Conclusions

- **Both EBC-46 & EBC-211 significantly inhibit fibroblast proliferative responses & TGF-β_1-driven, fibroblast-myofibroblast differentiation *in vitro*.**
- **EBC-46 & EBC-211 have no significant effects on scratch wound repopulation.**
- **Therefore, findings suggest that epoxy-tiglianes primarily induce anti-scarring responses by attenuating fibroblast proliferation & TGF-β_1-driven, myofibroblast differentiation; & highlight the potential of epoxy-tiglianes as novel therapeutics for excessive dermal scarring & fibrosis.**
- **Further studies are elucidating the mechanisms by which epoxy-tiglianes induce these anti-scarring effects.**

References

1) Dong L *et al.* Anticancer agents from the Australian tropical rainforest: Spiroacetals EBC-23, 24, 25, 72, 73, 75 and 76. *Chem Eur J* (2009); **15:**11307-18.
2) Boyle GM *et al.* Intra-lesional injection of the novel PKC activator EBC-46 rapidly ablates tumours in mouse models. *PLoS One* (2014); **9:**e108887.
3) Gauglitz *et al.* Hypertrophic scarring and keloids: Pathomechanisms; and current and emerging treatment strategies. *Mol Med* (2011); **17:**113-25.
4) Editorial. Anti-scarring pharmaceuticals: Lost in translation? *Wound Rep Regen* (2014); **22:**293-4.

Acknowledgements

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