Estetrol Dampens Inflammation and Accelerates Wound Repair *In Vitro* and *In Vivo*

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Introduction

1. Wound healing and the menopause

Menopause is associated with a rapid decline in circulating 17β-estradiol (**E2**) and decrease in E2 receptor expression throughout the skin. Low E2 following the menopause impacts the structure and function of the skin, with over half of post-menopausal women reporting dermatoses [1]. These issues result from the loss of extracellular matrix proteins (**ECM**) , including collagens, proteoglycans and glycosaminoglycans, contributing to age-associated delayed human wound healing [2].

2. Estrogen: a key mediator of would healing and repair

We have shown that E2 administration restores the reparative capacity of murine skin [2,3]. These effects are mirrored in the clinic, where E2 is administered as hormone replacement therapy [**HRT**; 4].

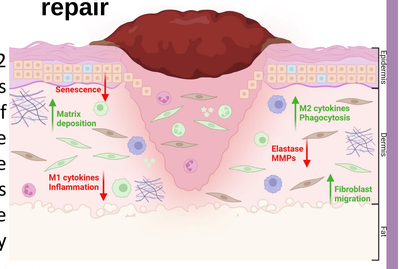


Figure 1. E2 protects against age-related delayed wound healing following the menopause (adapted from [3]).

3. A role for estetrol?

Estetrol (**E4**) is a native estrogen produced exclusively by the human fetal liver during pregnancy. E4 is currently in late-stage clinical development as HRT and presents a favorable safety profile to E2 [5]. E4 could provide an effective alternative to E2 in HRT to aid recovery, however no studies to-date have the evaluated the effects of E4 on wound healing.

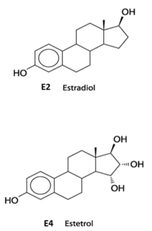
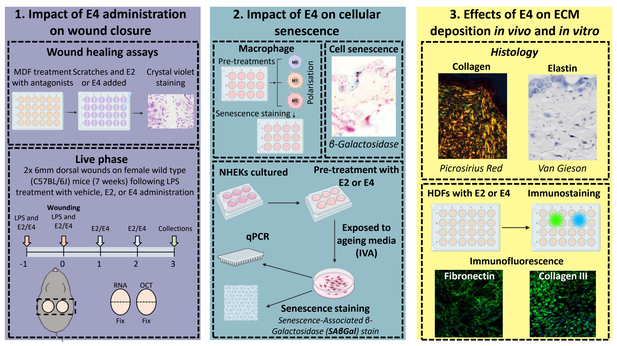


Figure 2. E2 and E4 chemical structure [5].

Aims

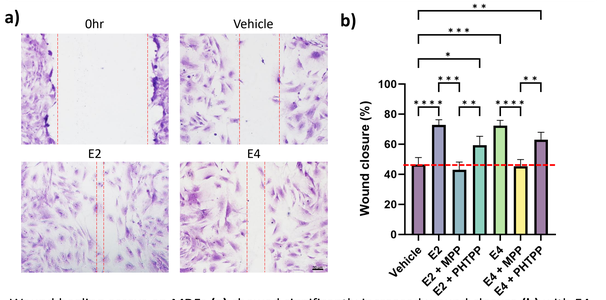
- Evaluate the impact of E4 administration on wound closure *in vitro* and *in vivo.*
- Investigate the impact of E4 treatment on cellular aging (senescence).
- Evaluate E4 effects on elastin fibre synthesis and ECM deposition.

Methods



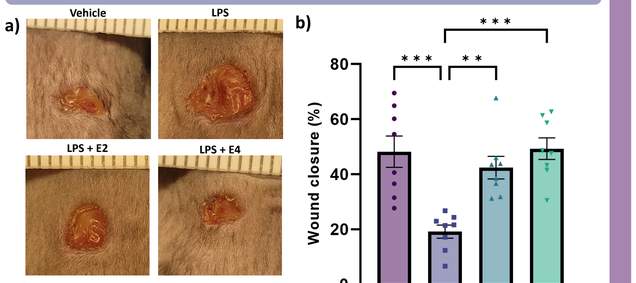
Results

E4 accelerates wound closure *in vitro*…



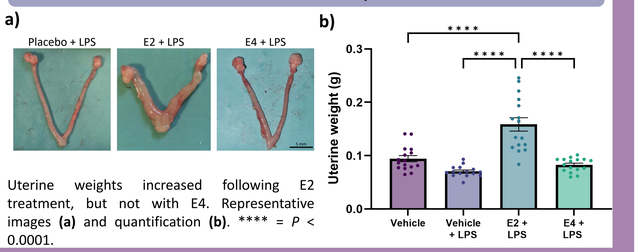
Wound healing assays on MDFs **(a)** showed significantly increased wound closure **(b)** with E4 treatment, which was ameliorated when cells were treated with ERα antagonist (MPP). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$.

…and LPS-stimulated wounds *in vivo..*



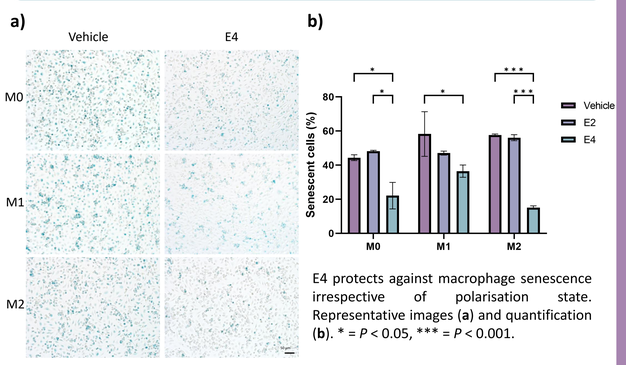
Dorsal wounds **(a)** showed a trend to increased wound closure **(b)** when treated with E2 and E4.

…with limited uterotrophic effects



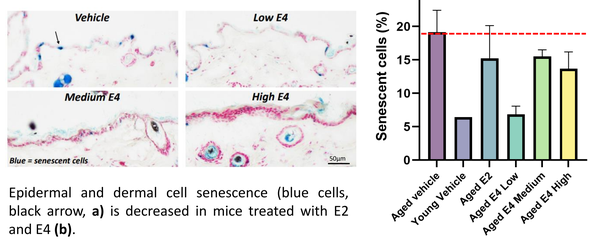
Uterine weights increased following E2 treatment, but not with E4. Representative images **(a)** and quantification **(b)**. **** = $P < 0.0001$.

E4 protects against senescence in polarised macrophages isolated from wounded mice



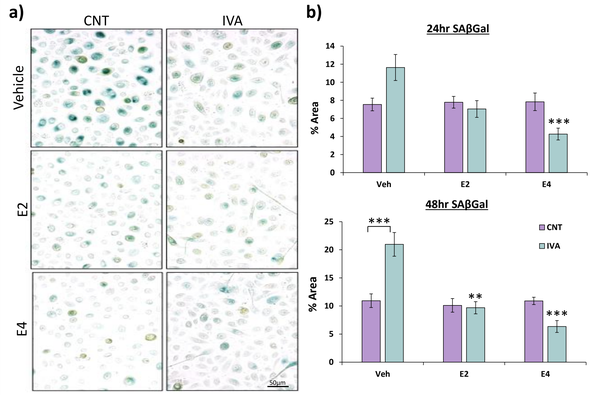
E4 protects against macrophage senescence irrespective of polarisation state. Representative images (**a**) and quantification (**b**). * = $P < 0.05$, *** = $P < 0.001$.

E4 protects against senescence in aged mouse skin…



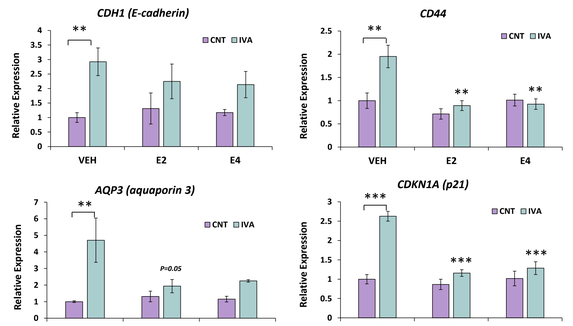
Epidermal and dermal cell senescence (blue cells, black arrow, **a)** is decreased in mice treated with E2 and E4 **(b)**.

..and aged human keratinocytes…



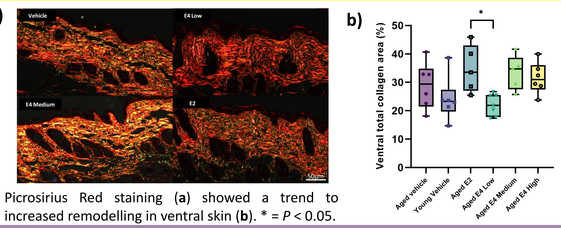
Area coverage of SAβGal positive cells (blue, a) showing E2 and E4 protect against IVA-induced senescence in keratinocytes **(b)**. ** = $P < 0.01$, *** = $P < 0.001$.

...which is mediated at the transcriptional level



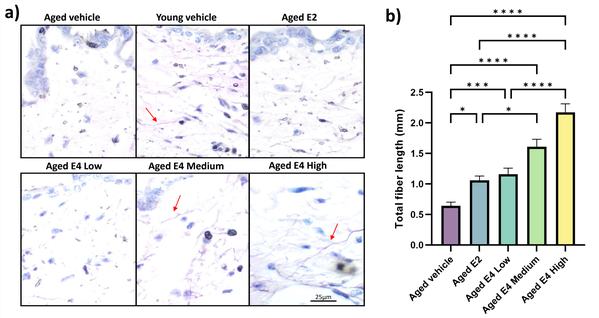
E2 and E4 protect cells from IVA-induced transcriptional changes in genes associated with cellular aging (*CDH1, CD44,* and *AQP3*) and senescence (*CDKN1A*). ** = $P < 0.01$, *** = $P < 0.001$.

E4 increases collagen turnover in mouse skin



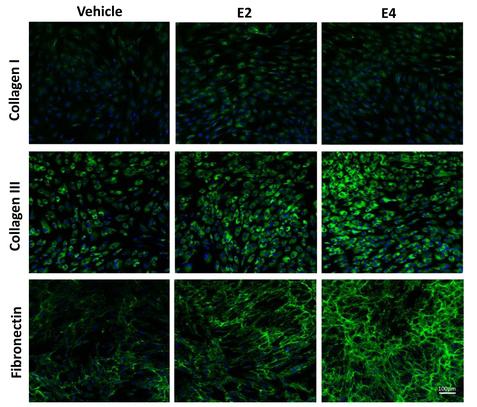
Picrosirius Red staining (**a**) showed a trend to increased remodelling in ventral skin (**b**). * = $P < 0.05$.

E4 induces elastin fibre synthesis in mouse skin



Elastin fibres (red arrows, **a**) are significantly increased following E2 and E4 treatment in aged mouse skin. Total fibres and number of branching fibres (**b**). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$.

E4 increases ECM deposition in aged HDFs



Confocal imaging of fluorescently stained ECM components in aged (>65 years) human dermal fibroblasts showing an increased trend in Collagen I, Collagen III and fibronectin following E2 and E4 administration.

Summary

1) Administration of E4 increases wound closure *in vivo* and *in vitro*
2) E4 protects macrophages, fibroblasts and keratinocytes from senescence.
3) E4 substantially increases ECM turnover (*in vivo*) and ECM deposition (in aged fibroblasts).

We show that E4 protects the skin against multiple characteristics of ageing and age-related delayed wound healing, suggesting that E4 could provide a suitable therapeutic alternative to E2 in HRT.

Acknowledgements

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References

[1] Kamp E et al. 2022.. *Clin Exp Dermatol*. 47(12):2117-2122
[2] Hardman MJ, Ashcroft GS. 2008. *Genome Biol*. 9:1-7.
[3] Wilkinson HN, Hardman MJ. 2017. *Maturitas*. 103:60-64
[4] Sator et al. 2007. *Climacteric*. 10(4):320-34.
[5] Gérard C et al. 2022. *Expert Rev Clin Pharmacol*. 15(2):121-37.