

## **Title**

Estrogen Enhances Host-Pathogen Interactions in an *in vitro* Model of Age-Related Impaired Healing.

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## **Abstract**

Chronic wounds are difficult to treat and often become colonised by bacteria, leading to substantial morbidity and mortality in the elderly. Impaired healing in the elderly is mediated changes in steroid hormones, particularly declining levels of estrogen with increasing age. The aim of this study was to determine the effect of aging (estrogen deprivation) on the ability of inflammatory cells to eliminate bacteria via phagocytosis. An *in vitro* host-pathogen assay was developed to determine the ability of U937 macrophages to internalise methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* under conditions that model endogenous levels of estrogen found in the elderly (*estrogen deprivation*;  $1 \times 10^{-9}$ M estradiol or an absolute absence of estrogen), during youth ( $1 \times 10^{-8}$ M estradiol) and following estrogen supplementation (*suprabasal levels*;  $1 \times 10^{-7}$ M estradiol). To confirm phagocytosis was taking place, co-localisation of fluorescence from GFP-labelled *S. aureus* SH1000 and mCherry-labelled *P. aeruginosa* PAO1 with macrophages was measured on a fluorescent cell counter and host-pathogen interactions were visualised by confocal microscopy, epifluorescence microscopy and scanning electron microscopy (SEM).

Findings indicated estrogen ( $1 \times 10^{-8}$ M and  $1 \times 10^{-7}$ M) significantly ( $P < 0.05$ ) increased the amount of phagocytosis (i.e. decreased bacterial recovery) in a concentration-dependent manner compared to estrogen deprivation. Co-localisation of fluorescent signal from GFP-labelled *S. aureus* and mCherry-labelled *P. aeruginosa* with macrophages was significantly ( $P < 0.05$ ) enhanced by estrogen ( $1 \times 10^{-8}$ M and  $1 \times 10^{-7}$ M) compared with estrogen deprivation. Host-pathogen interactions, visualised by microscopy, confirmed estrogen-mediated internalisation of bacteria by macrophages.

In conclusion, elevated levels of estrogen found in youth or following estrogen supplementation result in increased phagocytosis of both gram-positive *S. aureus* and gram-negative *P. aeruginosa* by U937 macrophages. The findings suggest estrogen may promote the resolution of wound infections during youth but this protection may decline with increasing age as estrogen levels decline, resulting in increased propensity and progression of wound infections in the elderly.