Induced cellular senescence disrupts vascular organisation in an in vitro vascularised tendon model

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Background

***Figure 1**. Schematic illustration of tendon vasculature.*

- Tendon injuries and diseases are common, especially with ageing, but difficult to treat.
- Blood vessels localise to the tendon interfascicular matrix (**Fig. 1**).
- With ageing, tendon vascularisation decreases, and tendon vascular cells become senescent[1,2].
- Targeting the vascular network represents a promising therapeutic avenue for age-related tendon injuries.
- Horse superficial digital flexor tendons (SDFTs) were used as a functionally and clinically relevant model for human Achilles tendon.

AIM: to establish the effects of endothelial cell senescence on the tendon vasculature *in vitro* using a 3D tenocyte-endothelial cell co-culture model.

Methods

Co-culture model development

- Tenocytes and endothelial cells were isolated from equine forelimb SDFTs (age: 2-5 years, n=4).
- Co-culture was performed for 14 days with fibroblast growth factor (10 ng/ml) and vascular endothelial growth factor (0.5 ng/ml) to promote vessel formation (**Fig. 2**).

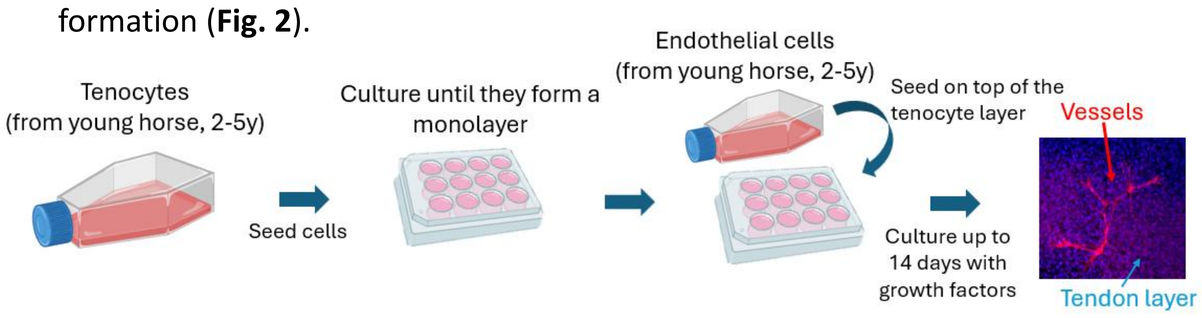


***Figure 2**. Overview of tenocyte-*endothelial cell *co-culture model development.*

Cellular senescence induction

- Hydrogen peroxide (H_2O_2, 600µM; 2 hours; two consecutive days) was used to promote stress-induced senescence in endothelial cells prior to co-culture and then untreated or senescent endothelial cells were seeded onto the tenocyte layer.

Quantitative analysis

- Senescence induction in endothelial cells was confirmed through immunostaining for p16, p21 and beta-Galactosidase (Gal) staining.
- Vessel, branch and segment (immature branch, length < 50µm, **Fig.3**) formations were measured in co-cultures immunostained for the endothelial cell marker CD146.
- Differences in vessel formation due to endothelial cell senescence were measured.

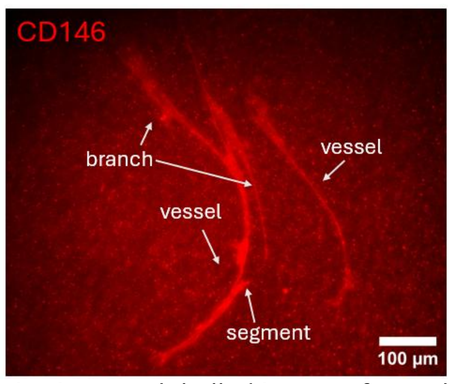


***Figure 3**. Representative immunolabelled image of vessel, branch and segments.*

Discussion & Conclusion

- This study developed a novel *in vitro* 3D tenocyte-endothelial cell co-culture model, which supported vascular network formation.
- H_2O_2 successfully induced endothelial cell senescence.
- With induced endothelial cell senescence, shorter, more immature vessel formation was observed.
- This 3D co-culture model provides a valuable platform for investigating the impact of senescence on the tendon vasculature and for screening therapeutic compounds.

Future work

- Apply therapeutic compounds such as senolytics to the senescent cultures to determine if they can reverse endothelial cell senescence.

References

1. Zamboulis (2024) doi:10.14336/AD.2023.0425-1.
2. Iwasaki (2026) doi: 10.1111/acel.70293.

Results

- Expression levels of p16, p21 and beta-Gal were higher in H_2O_2-treated endothelial cells than in untreated cells (**Fig.4**), showing successful induction of senescence.

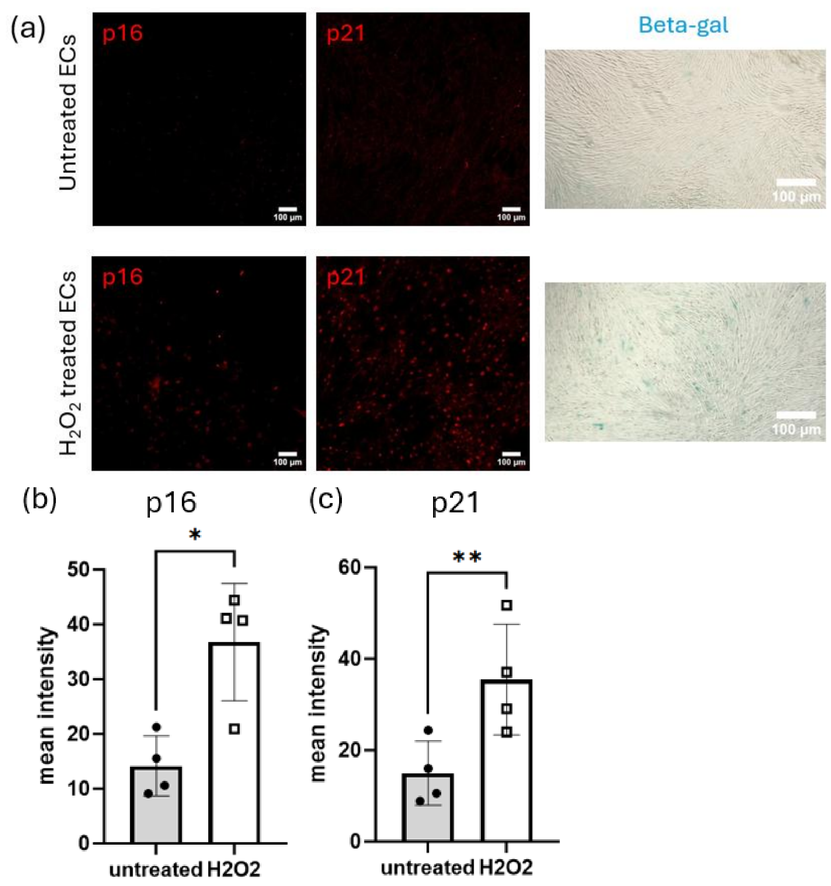


***Figure 4**. (a) Representative images of untreated and H_2O_2 treated endothelial cells labelled for p16, p21 (red) and beta-Gal (blue) activity (b&c) Mean fluorescent intensity of (b) p16 and (c) p21 in untreated and H_2O_2 treated endothelial cells. Data are presented as mean ± SD (n = 4). Paired t-test was used to calculate the differences between groups. *p<0.05 **p<0.01.*

- Vessel-like structures were formed after 14 days (**Fig.5a**).
- In senescent cultures, vessel length decreased by 34% (**Fig.5b**) and a trend towards a decreased number of vessels was observed (**Fig 5c**, p=0.09).
- Fewer branches and more segments were observed per vessel in senescent cultures (**Fig. 5 d&e**), and the branch length decreased significantly (**Fig. 5f**).

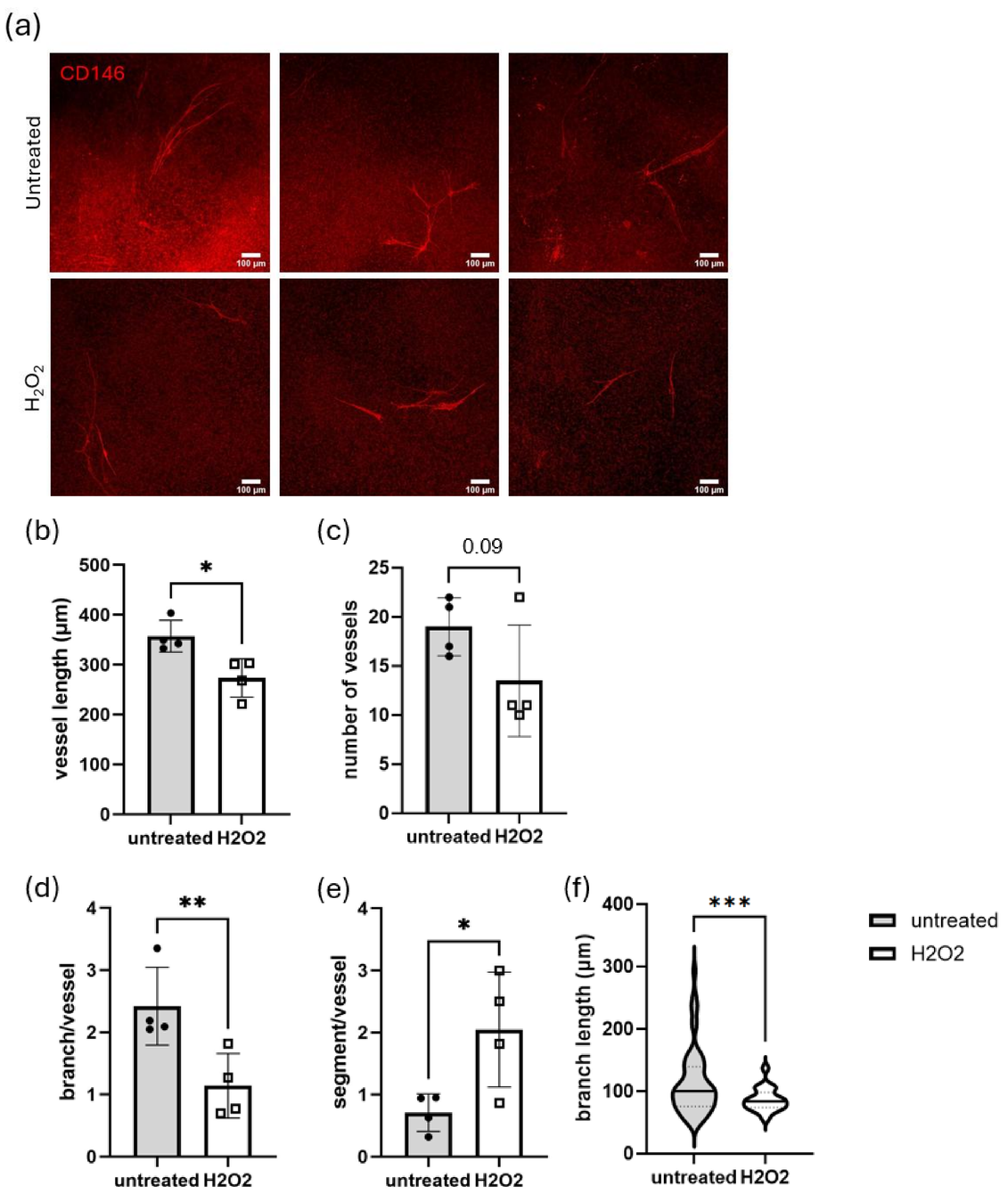


***Figure 5**. (a) Representative immunofluorescent images of untreated and H_2O_2 treated tenocyte-endothelial cell co-culture labelled with CD146. Quantitative analysis of (b) vessel length, (c) number of vessels, (d) number of branches per vessel, (e) number of segments per vessel and (f) branch length in untreated and H_2O_2 treated tenocyte-endothelial cell co-cultures. Data are presented as mean ± SD (n = 4). Paired t-test was used to calculate the differences between groups. *p<0.05 **p<0.01 ***p<0.001.*

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