Chronic inflammation as a driver of pain after total knee replacement

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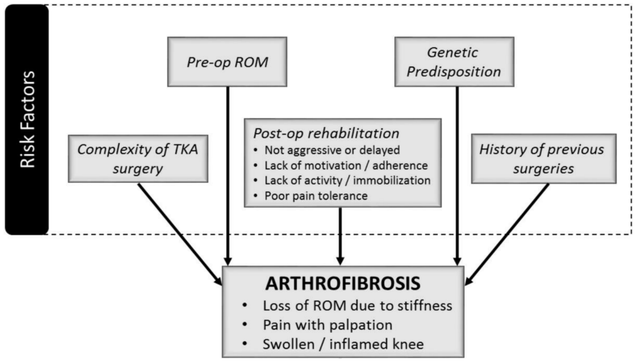
1. Introduction

Fibrosis is the final stage of a chronic inflammatory response; defined as the excessive deposition of proteins such as collagen. Total knee arthroplasty (TKA) is a common surgical procedure used to remove diseased tissue and bone, before inserting an artificial joint.

Figure 1 | Risk Factors of Arthrofibrosis (1)



Around 20% (~ 20,000) of TKA patients (UK) develop joint fibrosis with chronic pain (2).

This study aims to elucidate the inflammatory role of the Interleukin 1 family (IL-1) in the post-TKA knee and test IL-1 inhibitors as potential treatments using fibroblast media in the **synovial fluid (SF) environment** via an ***in vitro*** model.

2. Methods

The project involved reanimating 3 human fibroblast cell lines and testing their response within the environment of 23 different synovial fluids (SF). The fibroblasts were analysed for **pro-inflammatory cytokines Interleukin 8 & 6 (IL-8/6) and Collagen.** This was then correlated with other pro-inflammatory cytokines Interleukin 1 alpha and beta (IL-1a/b) that was previously measured from the SF via Meso Scale Discovery Multi-array (analysis method)

The **treatments include**; **(1)** Interleukin 1 alpha (IL-1a) Neutralising antibody (Nab), **(2)** SF+ IL1 beta (IL-1b) Nab, **(3)** SF + IL1a + IL1b Nab, **(4)** SF+ IL1 receptor blocker/antagonist (Ra), **(5)** SF+ IL1Ra (in house).

OBJECTIVES;
1. What is the difference in inflammation between primary and revision (w/pain) surgeries?
2. Is there a correlation between inflammation and pro-inflammatory markers?
3. What are the effects of treatments that block the IL-1 inflammatory signaling pathway?

Figure 2 | Reanimation and treatment of fibroblasts
Human synovial membrane fibroblasts (n=3) were reanimated and seeded into T75 flasks transferred to 96-well plates and spiked with various treatments as described above.

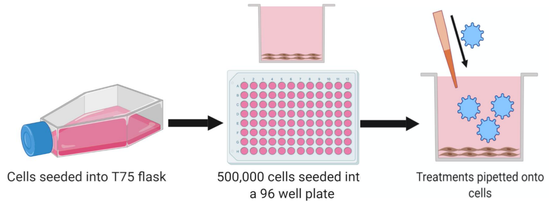


Figure 3 | Media harvesting
The cells were then suspended in SF (n=23); the media harvested and quantified using **Enzyme linked Immunosorbent assay (ELISA)** and the signal was read using a plate reader.

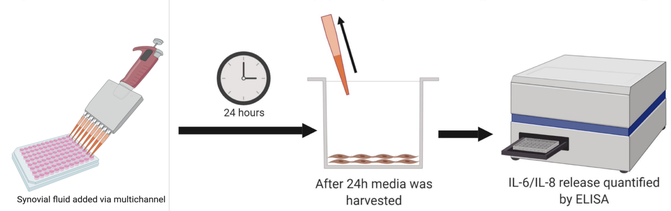
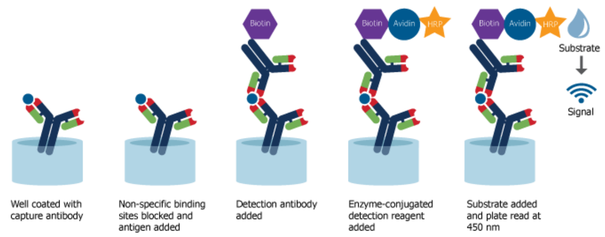


Figure 4 | Enzyme linked Immunosorbent assay analysis (3)
The sandwich ELISA protocol involved using specific capture and detection antibodies to facilitate enzyme-conjugate (horse-radish peroxidase) mediated marker detection from fibroblasts.



3. Results

What is the difference in inflammation between fluids from primary and revision (w/pain) surgeries?
- The fibroblast measurement yielded a higher presence of inflammatory markers Il-6 and Collagen in the environment of the painful revision TKA fluids.
- Thus more inflammation is observed in comparison to the primary.

Is there a correlation between inflammation and pro-inflammatory markers within the SF?
- The previously measured levels of Inflammatory IL-1a and IL-1b in the SF is correlated with the pro-inflammatory IL-6 measured in the fibroblasts.

What are the effects of treatments that block the IL1 signaling pathway?
- Although some treatments had a minor effect; there was no significant reduction in inflammation (IL-6) measured from the fibroblasts; when the IL-1 signaling pathway was blocked in both revision and primary samples.

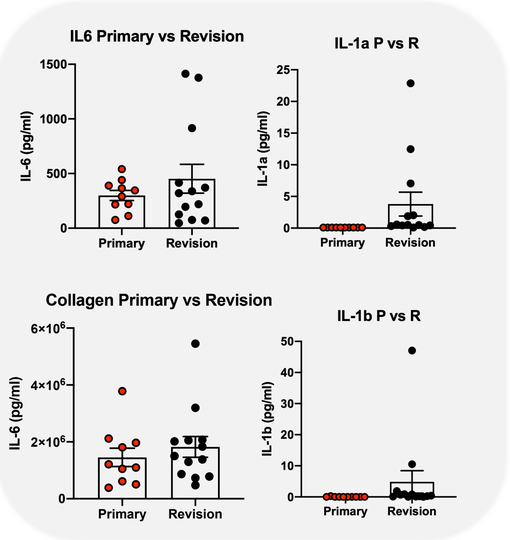


Figure 5 | *Left to right box 1: IL-6 & Collagen* ***(in fibroblasts),*** *IL-a and b in primary and revision* ***(measured in SF)****.*

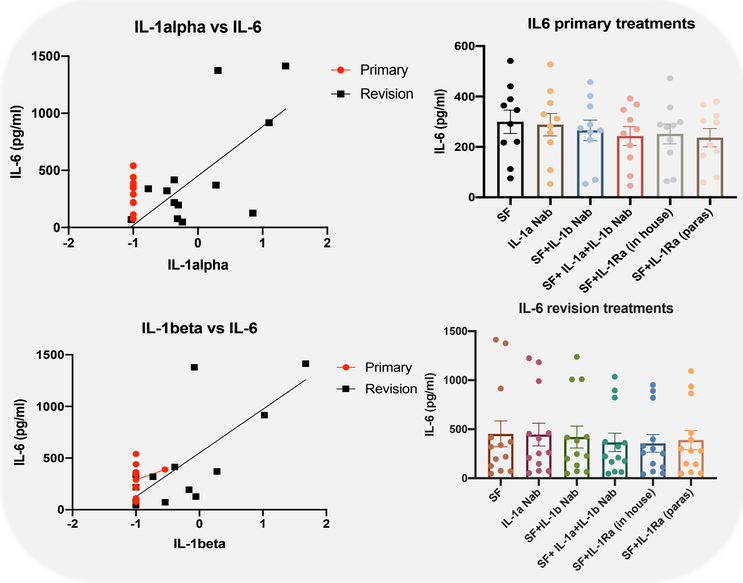


Figure 6 | *IL-1a and b from SF correlates with pro-inflammatory marker IL-6 measured from fibroblasts and lastly, the effect of treatments on SF IL-6 levels.*

4. Conclusion

1. Inflammation is correlated with the increases in IL1-α and β as there is a higher concentration in revision TKA (SF) than primary.
2. *In vitro* IL1 signalling inhibition of fibroblasts that suspended in the synovial fluid and does not have a significant effect.
3. Although there is a correlation between the IL1- α/β and pro-inflammatory marker IL-6/collagen it may not be causative.
4. IL-1 (acute injury communication molecule) peaks at damage induction; this requires measurements at different time points of the disease progression. (Proteomics may be used to look for another driver of pain).

5. References & Acknowledgements

(1) | Cheuy, V. A. *et al.* Arthrofibrosis Associated With Total Knee Arthroplasty. *Journal of Arthroplasty* **32**, 2604–2611 (2017). Wylde.
(2) | Wylde V, Beswick A, Bruce J, Blom A, Howells N, Gooberman-Hill R. Chronic pain after total knee arthroplasty. EFORT Open Reviews. 2018;3(8):461-470.
(3) | https://rockland-inc.com/ELISA-Kits.aspx

Placement: Newcastle University Fibrosis Group
Funding: Newcastle University Vacation Scholarships