

Artificial Interstitial Fluid Synergistically Interacts with Triblock Polymers to Reduce Purinergic Signaling in an Engineered Skeletal Muscle Implant Model

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Background

- This study was devised to identify temporal cellular triggers or damage-associated molecular patterns (DAMPs) released from stressed or damaged cells that induce sterile inflammation during the surgical repair of skeletal muscle tissue damage. ATP in particular is a DAMP that triggers the innate immune system through purinergic signaling.
- The Yost laboratory has developed scaffold-free pre-vascular endothelial-fibroblast constructs (SPECs), which display the biophysical properties and histological characteristics of vascular networks, can rapidly anastomose with host vasculature, and activate host satellite cells.
- All bioengineered implants, including the SPECs, remain susceptible to the host innate immune response. SPEC survival after three days is poor.

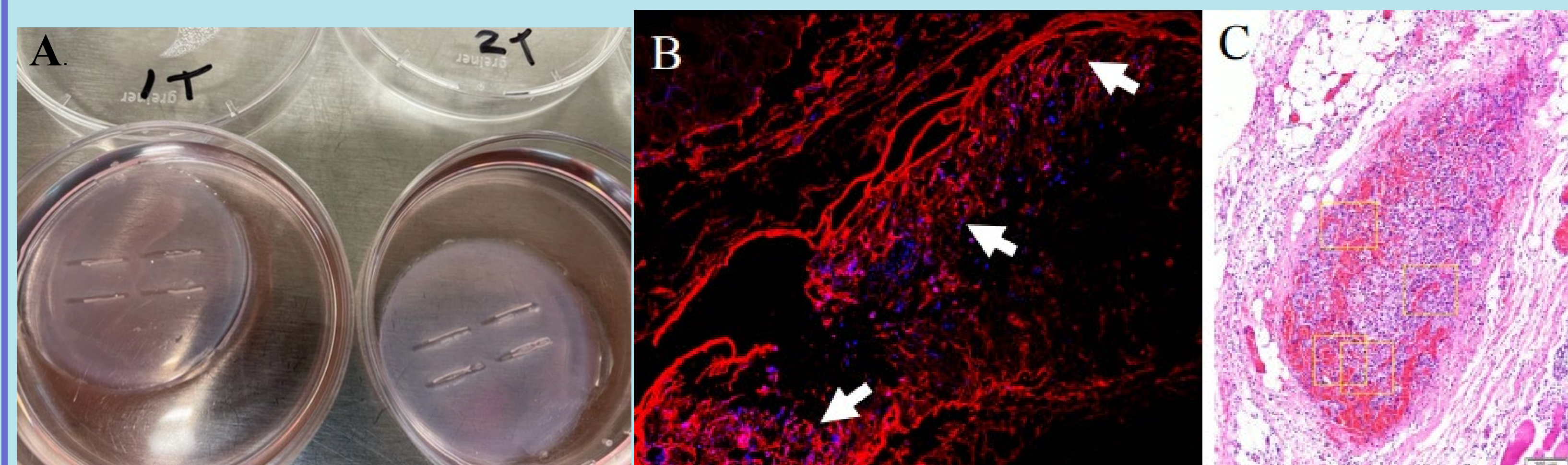
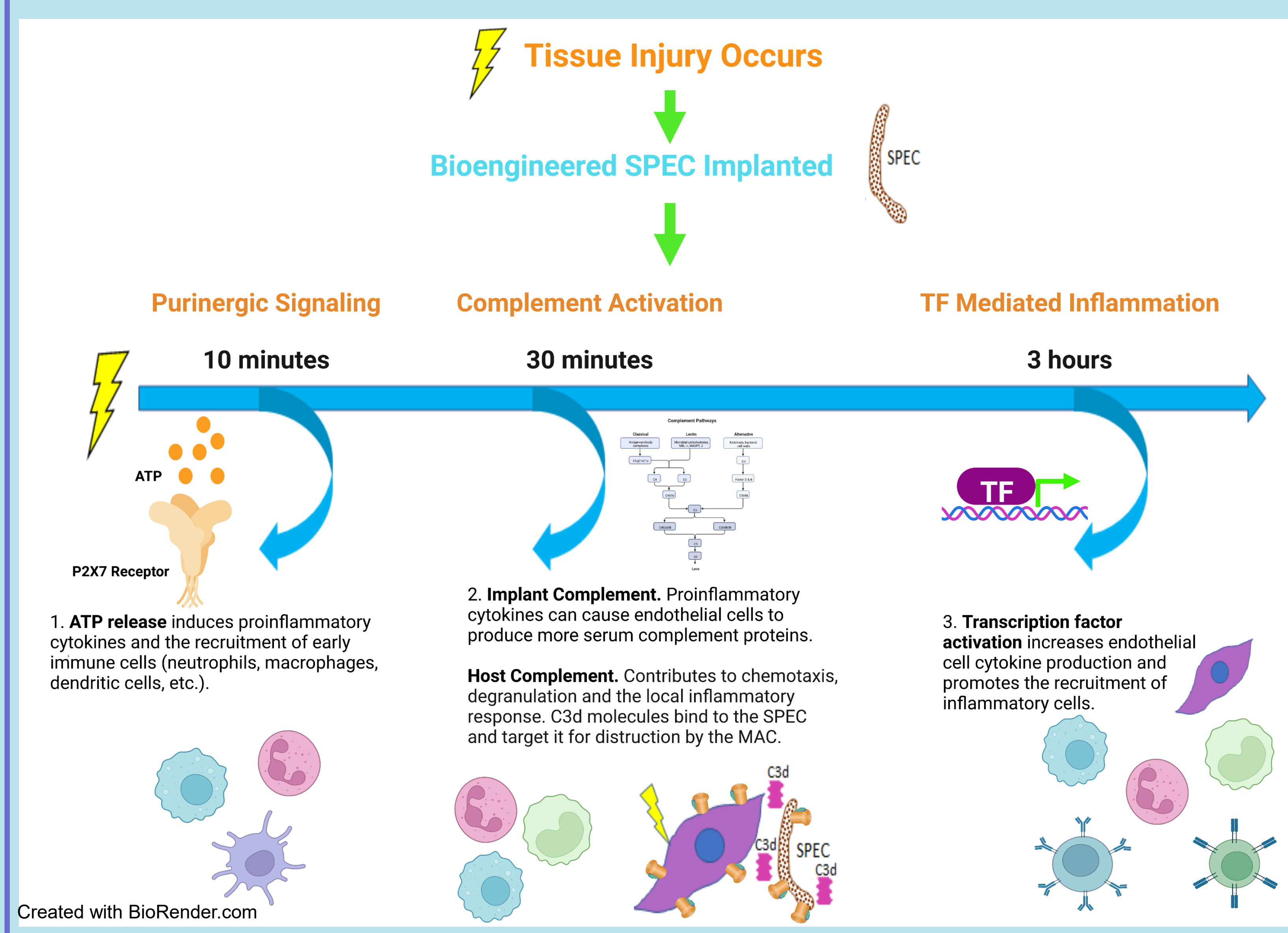


Fig. 1 – A. SPECs in culture prior to use. B. and C. SPECs 24 hours post implantation. B. shows significant endothelialization of the SPEC (red, arrows), Von willibrand (vWF) immunostaining (Red) and nuclei (Blue). C. Hematoxylin and eosin stain SPEC appears fully perfused with blood from the host Sprague Dawley rat.

Hypothesis

Implantation of a bioengineered tissue graft triggers the innate immune response over time by three different mechanisms, purinergic signaling, the complement system, and transcription factor mediated cytokine release.

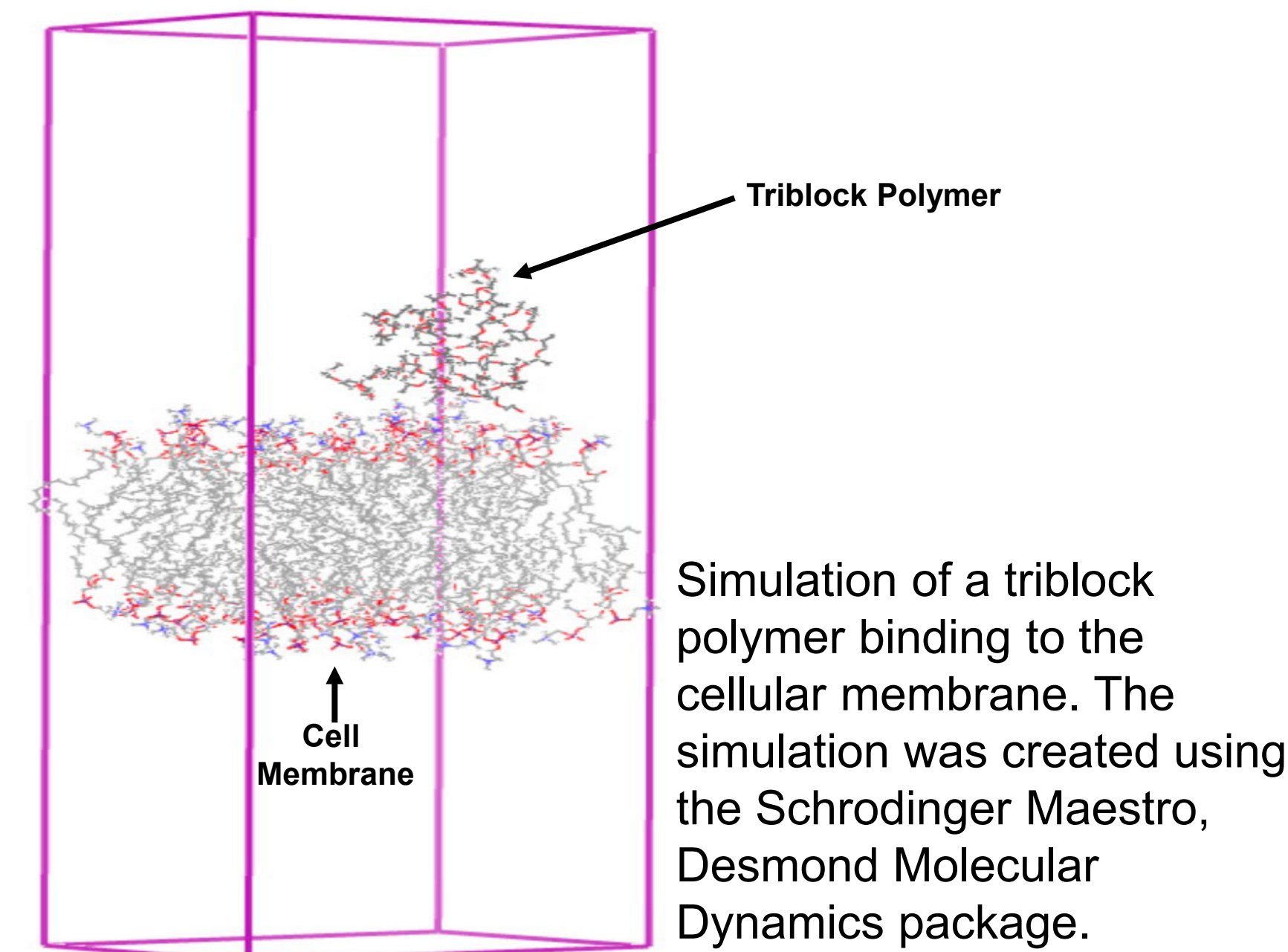
Proposed Timeline of the Innate Immune Response after Tissue Injury and Surgical Repair



Materials and Methods

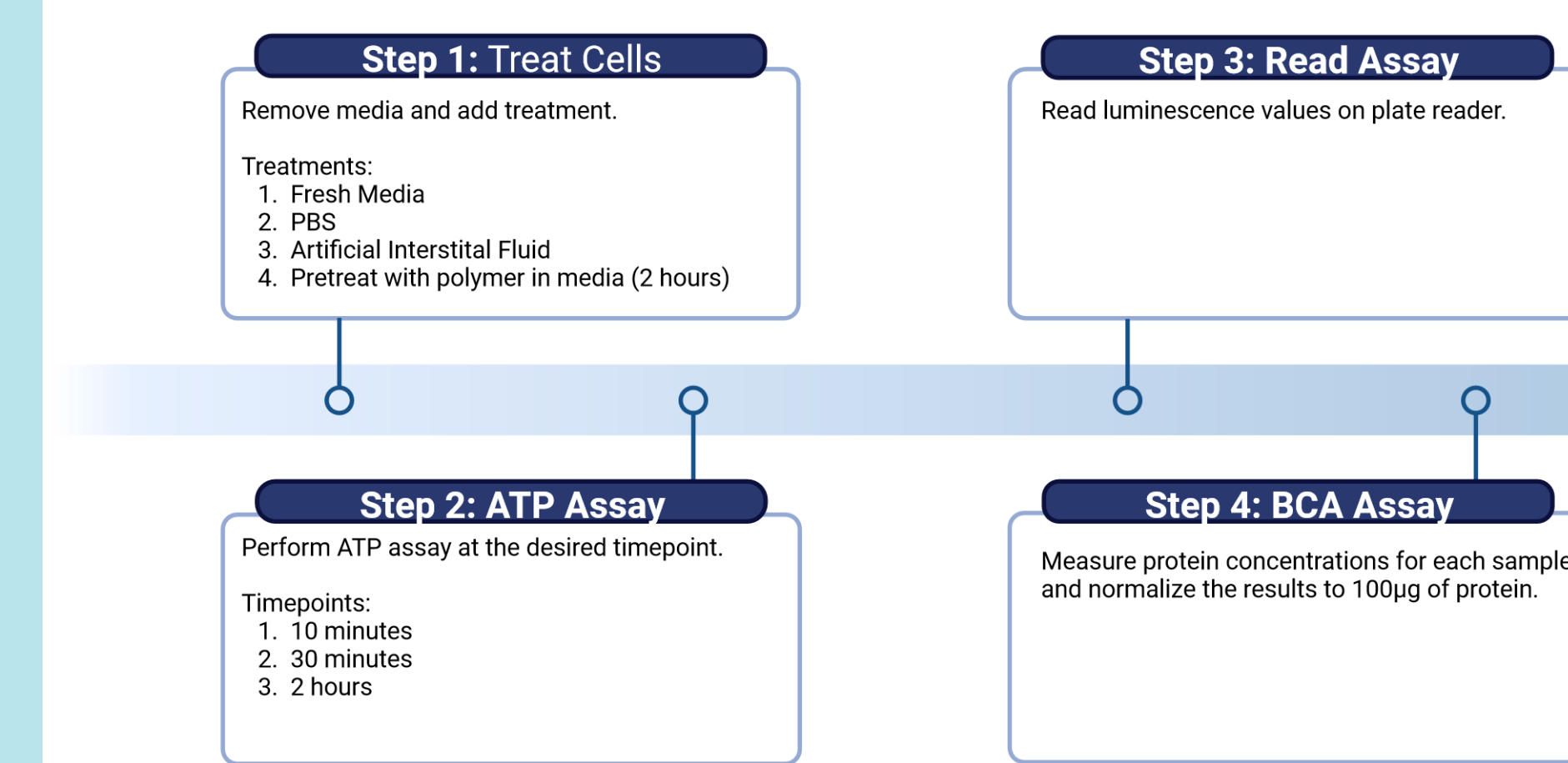
List of Salts Added to Deionized Water to create Artificial Interstitial Fluid (AIF)

| |
|--|
| MgSO ₄ * 7 H ₂ O |
| MgCl ₂ |
| CaCl ₂ |
| KH ₂ PO ₄ |
| NaHCO ₃ |
| NaCl |
| NaOH |



Structure of the F127 triblock polymer

SPECs are transferred to a 96 well plate in fresh endothelial cell growth media.



SPECs Release ATP 10 Minutes After Being Exposed to Saline

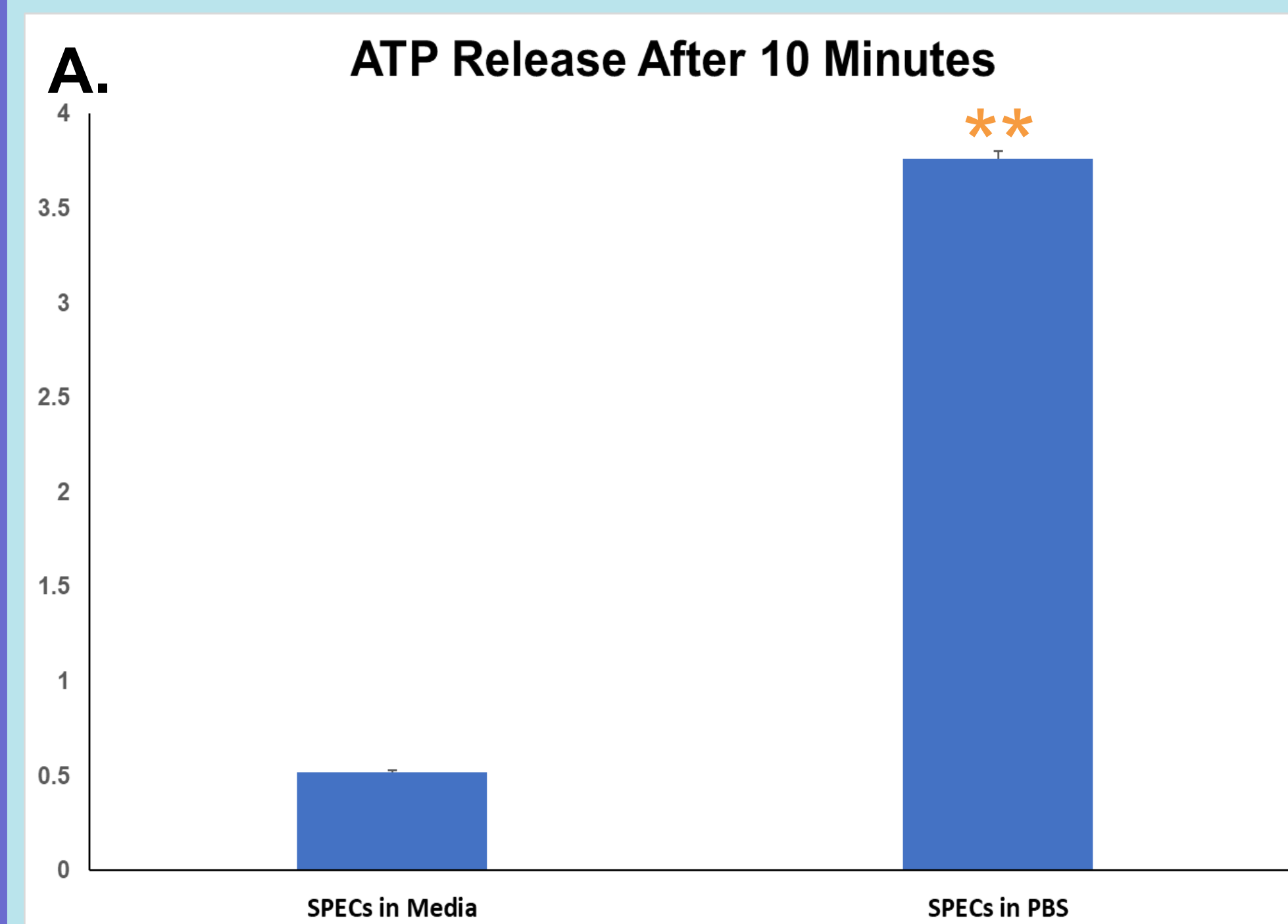
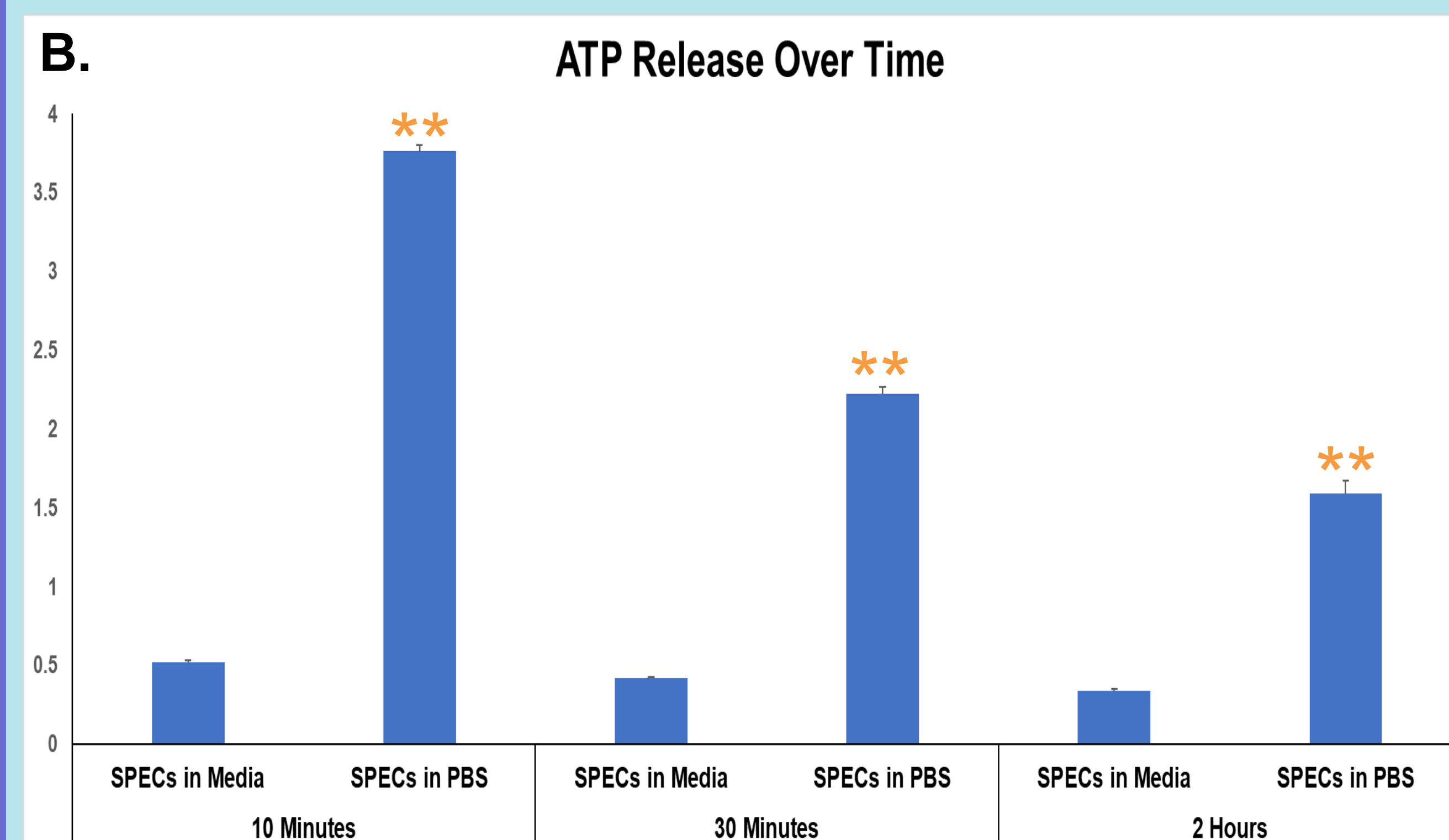


Fig. 2 – Histograms of ATP assay results from at least three separate experiments normalized to 100µg of protein. A. Results depicted for the 10 minute timepoint (n=21). B. Compiled results for three separate experiments at three distinct timepoints (10 minutes n=21, 30 minutes n=16, 2 hours n=7 for media and n=9 for PBS). Statistical significance was calculated using a two tailed Student's T-test assuming unequal variances, double asterisks indicate a p > 0.01.



Artificial Interstitial Fluid Reduces ATP Leak from SPECs

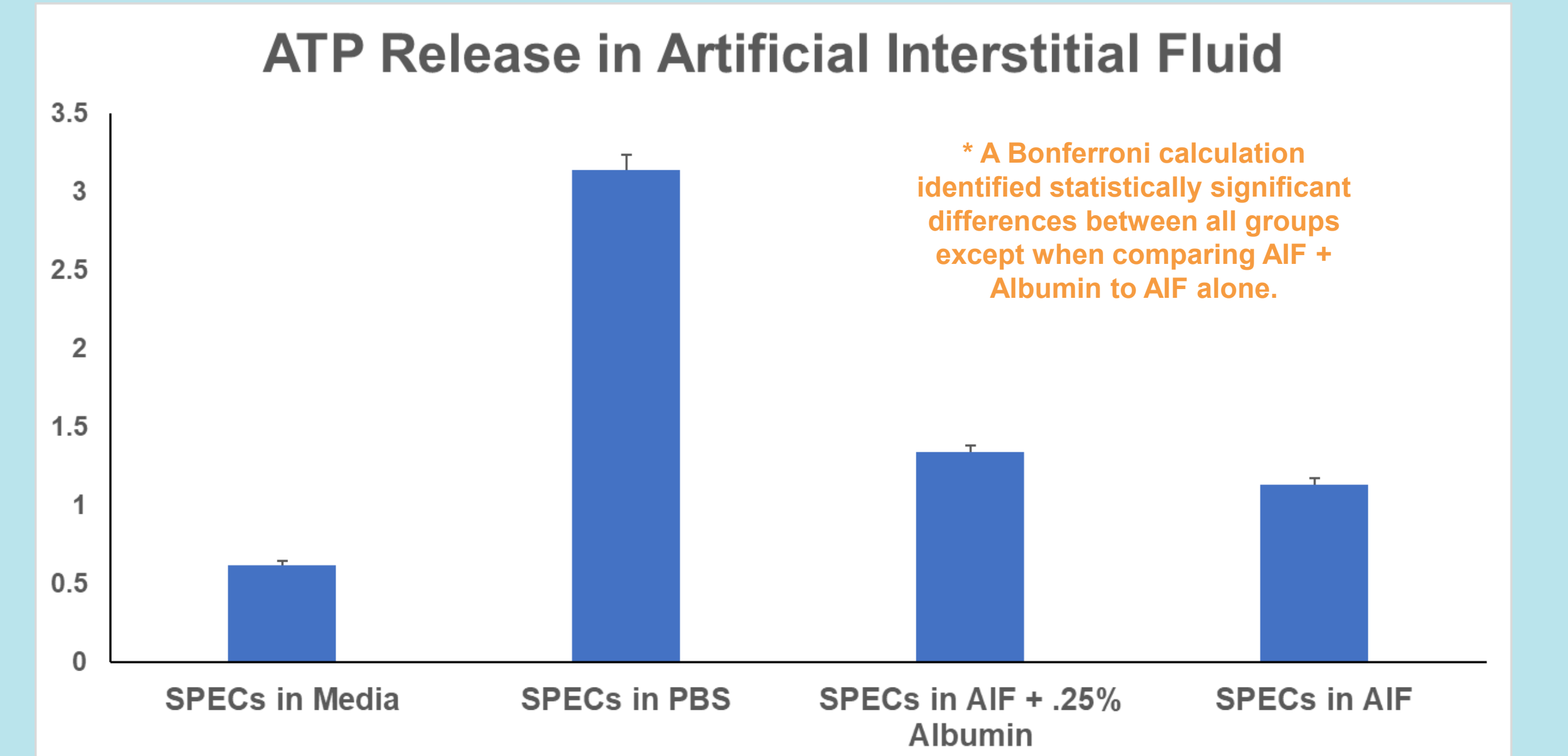


Fig.3 – SPECs in artificial interstitial fluid (AIF) for two hours demonstrates a significant reduction in ATP release (n=16). ATP assay results were normalized to 100µg of protein.

The Triblock Polymer F127 Further Reduces ATP Leak from SPECs

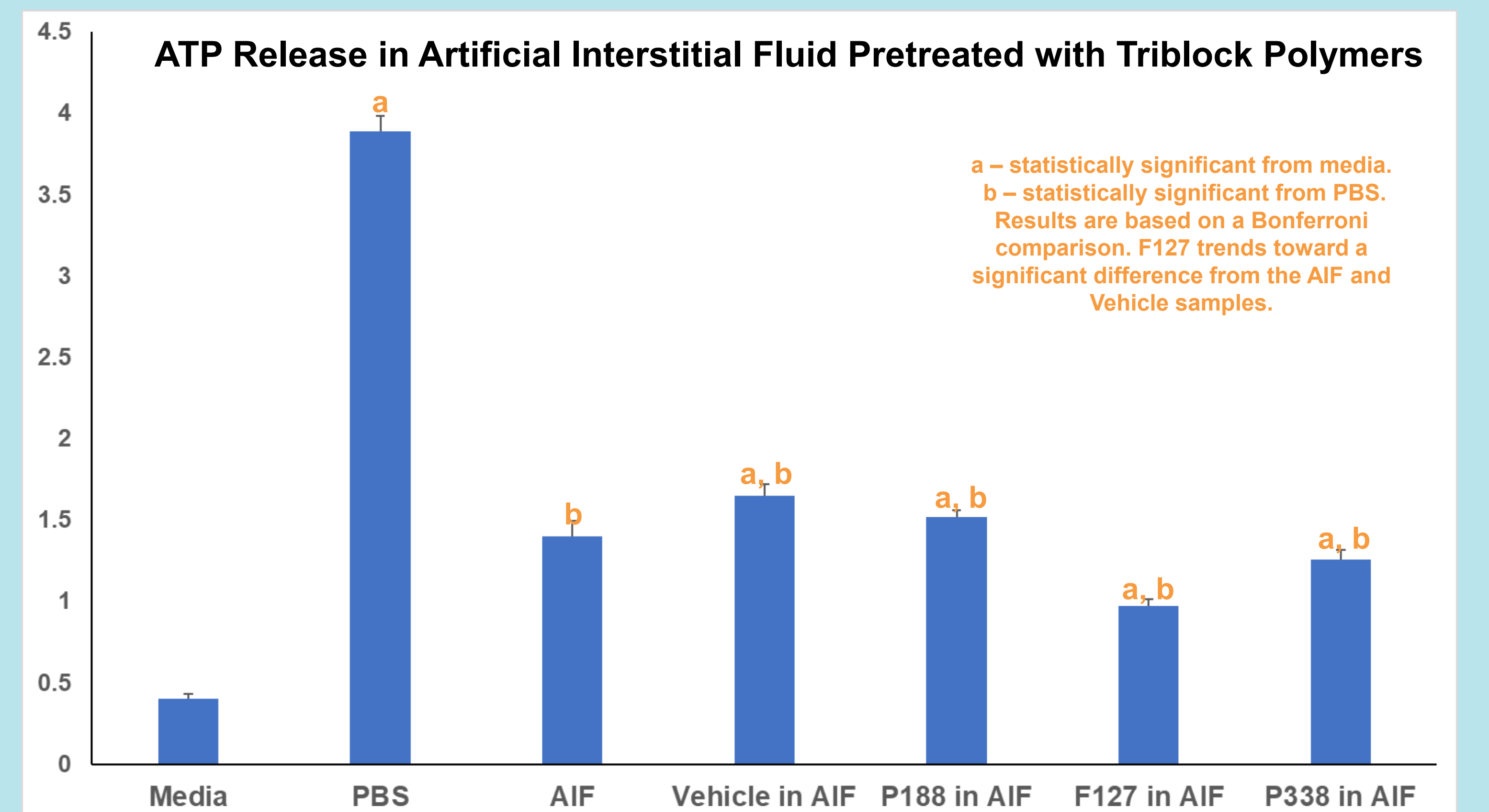


Fig.4 – Pretreatment for two hours with the triblock polymer F127 indicates further protection from ATP leak in the SPECs after only 10 minutes. ATP assay results from four separate experiments were normalized to 100µg of protein (n = 7-10 samples).

Conclusions

- A significant ATP release rapidly occurred in SPECs within 10 minutes of exposure to saline, indicating cellular stress.
- ATP release continued to be detected at a significant level at the two hour timepoint, although not as strongly. This suggests that ATP is likely being degraded by ectoenzymes in a linear manner over time.
- ATP release can be significantly ameliorated by switching to an artificial interstitial fluid made up of salts typically found in the bodies interstitial fluid, denoting the importance of balanced salts to cellular health.
- The F127 triblock polymer binds to the cellular membrane in a rapid on/off fashion that further helps to prevent leakage of ATP from the cell membrane.
- These findings may have a profound impact on the survival of bioengineered tissues used in the wound healing process. By attenuating triggers of the innate immune response over time, bioengineered tissues, like SPECs, have a greater chance of survival, which may lead to improved vasculature and skeletal muscle tissue regeneration. Thus preventing some of the loss of muscle function, scarring, and permanent disfigurement often impacting patients who have suffered traumatic injury, burns, tumor resection, or infection.

Impact for Surgery Patients

This study has identified simple methods, backed by science, that are generally recognized as safe, to improve surgeries. By switching how we pre-process bioengineered implants we can reduce sterile inflammation from the innate immune system at the surgical site. This procedure can be used in wound healing, tissue grafts, burns, and other surgical procedures.