# Development of Inhaled Platelet-based Therapy to Repair Damaged Lungs

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## Background

**Platelets & Related Bio-products**

Platelets are crucial in the body's natural healing process and contain abundant growth factors and other immune system components. Platelet rich plasma (PRP) is derived from whole blood containing a supraphysiological concentration of platelets in plasma. PRP and its derivates, especially platelet extract, show regenerative and anti-inflammatory roles in musculoskeletal medicine and are being used clinically.

**Damaged Lungs & Acute Lung Injury**

Acute lung injury (ALI) is characterised by the disruption of the capillary endothelial and alveolar epithelial barriers and presents clinically as respiratory failure. The morbidity and mortality of ALI is high due to diagnostic uncertainty and lack of specific pharmacologic therapy.

**Pulmonary Delivery & Mesh Nebulizer**

Pulmonary delivery is an effective method to deliver medicines to the lung directly and produce rapid drug action. Most biologics are delivered in a liquid form by nebulization in early development. Mesh nebulizers have lower requirement of volume in the reservoir and produce lower shear stress than traditional jet nebulisers.

## Introduction

After lung injury, it is critical to recover a healthy epithelial barrier. Platelet extract may contribute to lung homeostasis through cell proliferation and migration. The potential for development into an inhaled medicine will also be investigated.

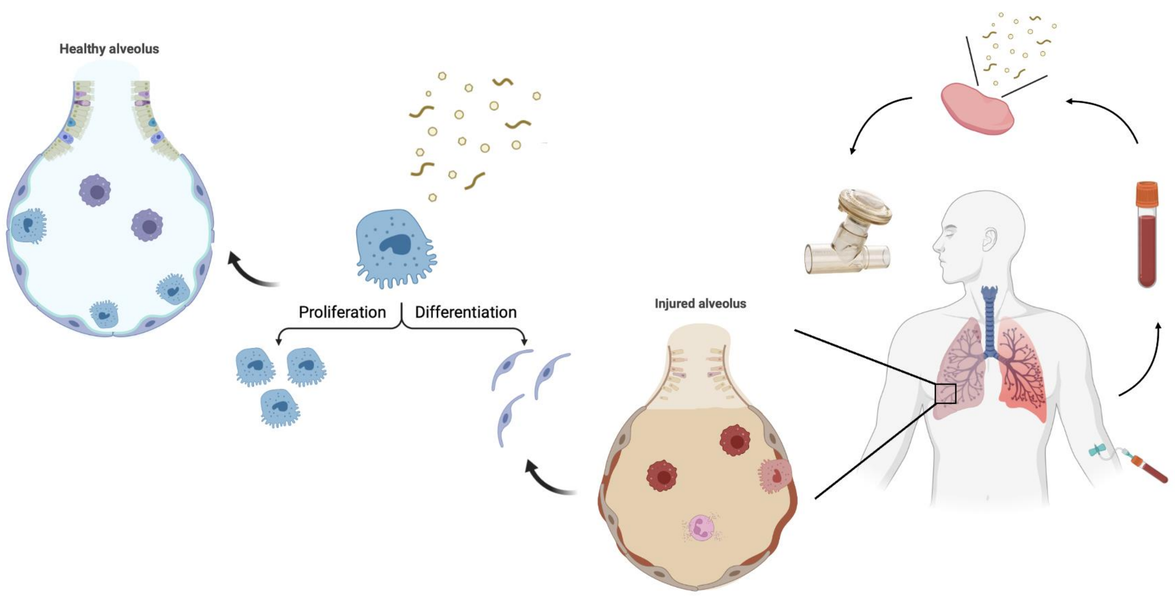


Fig. 1. Description of how to develop inhaled platelet extract to treat acute lung injury

## Aims

- To prepare platelet extract effectively
- To explore the regenerative role of platelet extract in vitro
- To prove the potential of delivering platelet extract via inhalation

## Method

### Ⅰ Preparation

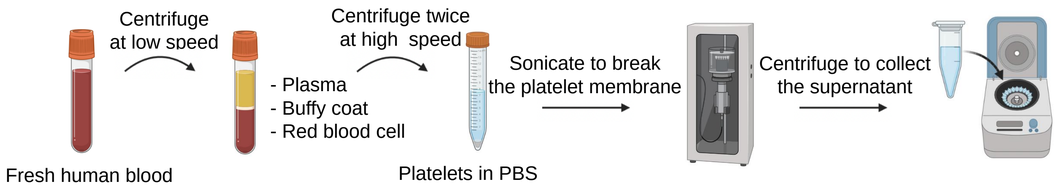


Fig. 2. Preparation routines of sonicated platelet extract

### Ⅱ Cell Study

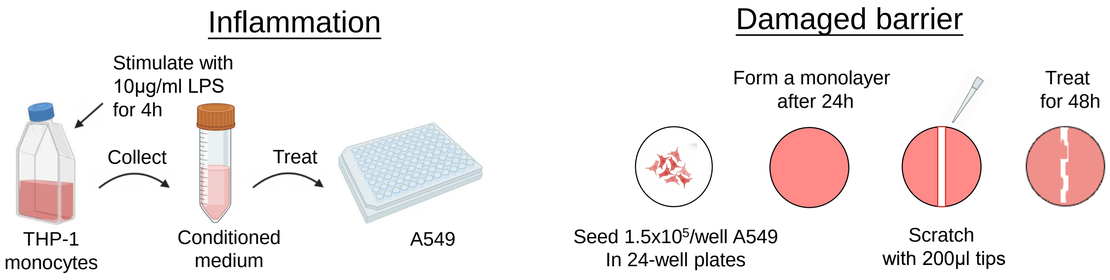


Fig. 3. Cell model of lung injuries using A549 cells

### Ⅲ Pulmonary Delivery

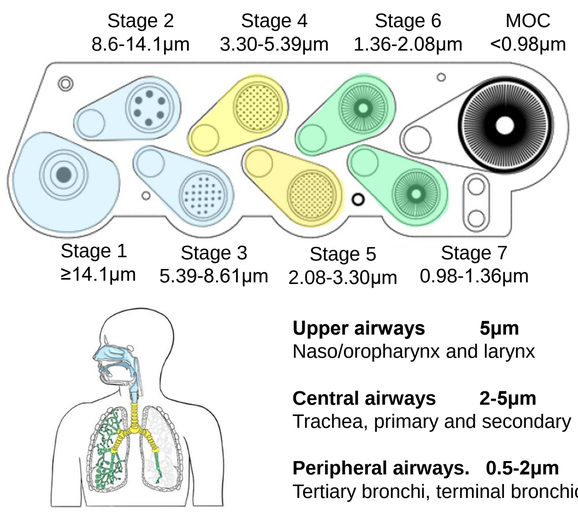


Fig. 4. Correspondence of the different stages of next generation impactor to airway generations [2]

## Results

### Ⅰ Preparation

Decreased optical density and increased protein level indicated sonication disrupted platelets and released their content.

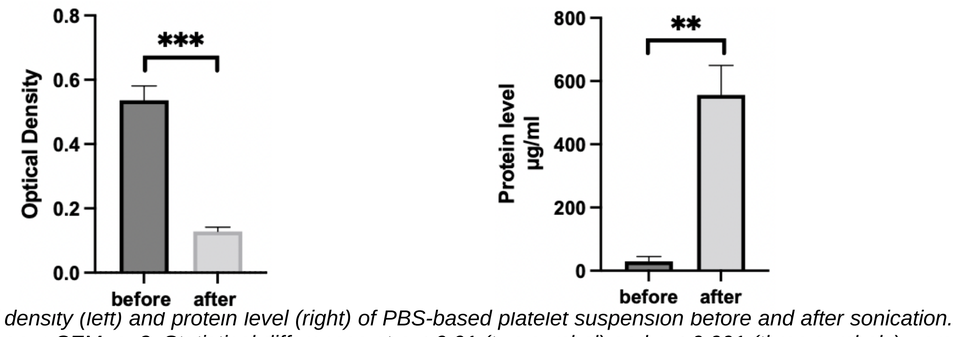


*Fig. 5. The optical density (left) and protein level (right) of PBS-based platelet suspension before and after sonication. Data represent mean ± SEM, n=3. Statistical differences at p < 0.01 (two symbol) and p < 0.001 (three symbols) were determined by unpaired student's t test.*

### Ⅱ Cell Study

Sonicated platelet extract increased time and concentration-dependent proliferation of A549 cells and could still improve the proliferation in the inflammation environment.

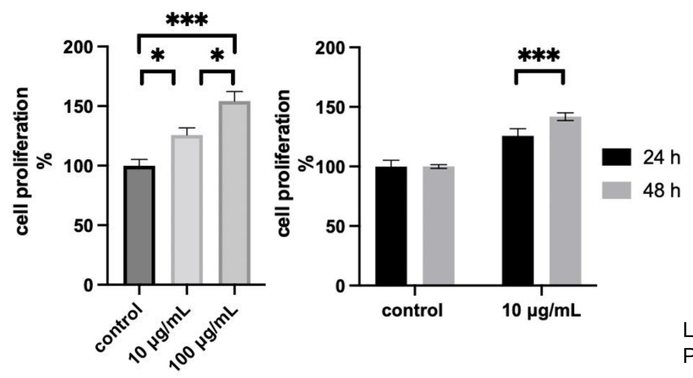


*Fig. 6. The proliferation of A549 cells after treatment with different concentrations of sonicated platelet extract (left) for different periods of time (right). A549 cells proliferation was tested by Alamar-Blue assay. Data represent mean ± SEM, n=5. Statistical difference at p < 0.05 (one symbol) and p < 0.001 (three symbols) was determined by unpaired one-way ANOVA and unpaired student's t test.*

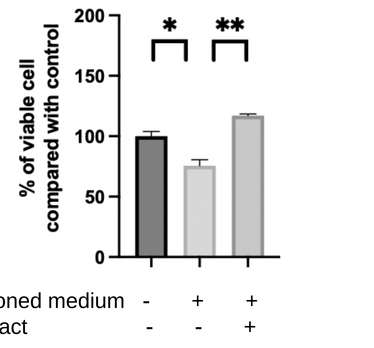


*Fig. 7. The proliferation of A549 cells after treatment with LPS–stimulated conditioned medium of THP-1 and sonicated platelet extract The viable cell level was tested by Alarm-Blue and compared with control group. Data represent mean ± SEM, n=3. Statistical difference at p < 0.05 (one symbol) and p < 0.01 (two symbols) was determined by unpaired student's t test.*

Sonicated platelet extract demonstrated in vitro wound healing capacity.

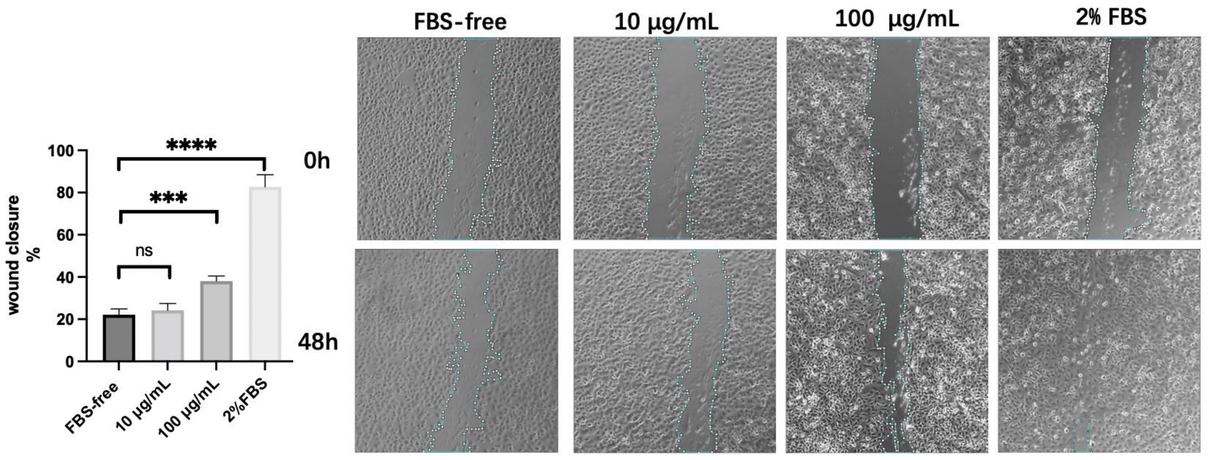


*Fig. 8. Effect of platelet extract on wound closure of scratched A549 cell monolayers over 48 h presented as '% wound closure' (left) and representative images (right). Data are expressed mean ± SEM, n=3. Statistical differences at p < 0.001 (three symbol) and p < 0.0001 (four symbols) were determined by unpaired student's t test.*

### Ⅲ Pulmonary Delivery

Nebulization did not influence the bioactivity of sonicated platelet extract in the respirable fraction produced by vibrating mesh nebulizer.

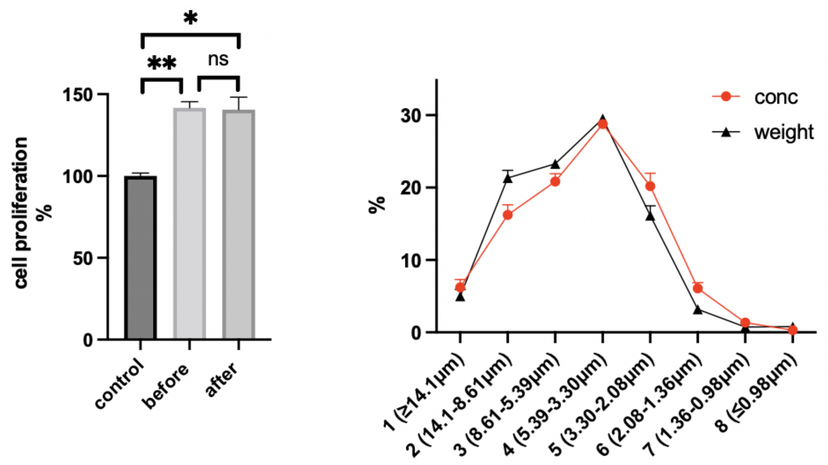


*Fig. 9. The effect of sonicated platelet releasate on cell proliferation before and after nebulization (left) and deposition profile in the Next Generation Impactor (Right). A549 cells proliferation was tested by Alamar-Blue assay. Data represent mean ± SEM, n=4. Statistical differences at p < 0.05 (one symbol) and p < 0.01 (two symbols) were determined by unpaired student t test between each group. The deposition of the nebulized platelet releasate in the Next Generation Impactor was measured by weight or protein quantification Data represent mean ± SEM, n=5.*

## Conclusion

Sonicated platelet extract promoted cell proliferation in a concentration & time manners. It can promote cell growth in the inflammation environment and induce wound healing on alveolar epithelial cells, which meant this biologic could play a regenerative role on lungs. It has potential to be developed into treatment for diseases such as acute lung injury. Sonicated platelet extract was still effective after nebulization. In-vitro studies showed the aerosol sizes were small enough to reach deep lung and around half of inhaled dose could be delivered successfully with mesh nebulizers. Inhalable platelet extract has potential as an inhaled treatment to repair damaged lungs.

References:
[1] Johnson E R, Matthay M A. Acute lung injury: epidemiology, pathogenesis, and treatment[J]. Journal of aerosol medicine and pulmonary drug delivery, 2010, 23(4): 243-252.
[2] Bianco F, Salomone F, Milesi I, et al. Aerosol drug delivery to spontaneously-breathing preterm neonates: lessons learned[J]. Respiratory Research, 2021, 22(1): 1-31.