Elucidating and Modulating Macrophage – Fibroblasts Crosstalk in Response to Nanoclay for Chronic Wounds

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Background

Non healing wounds are a worldwide healthcare problem and estimated to cost the medical system over US$25 billion per year, affecting around 6.5 million patients[1]. Prolonged inflammation is the fundamental cause of chronic wounds[2]. During wound healing, macrophages are activated and adopt context-dependent phenotypes to facilitate the transition of wounds from inflammation to repair and regeneration. Macrophages can be broadly categorized into pro-inflammatory macrophages (M1) and anti-inflammatory macrophages (M2) phenotypes[3].

The failure of phenotype transition causes persistent inflammatory signalling, resulting in non-healing wounds[4]. With advances in understanding macrophage phenotypes and their roles in tissue regeneration, there is growing interest in strategies to modulate the immune microenvironment surrounding implants.

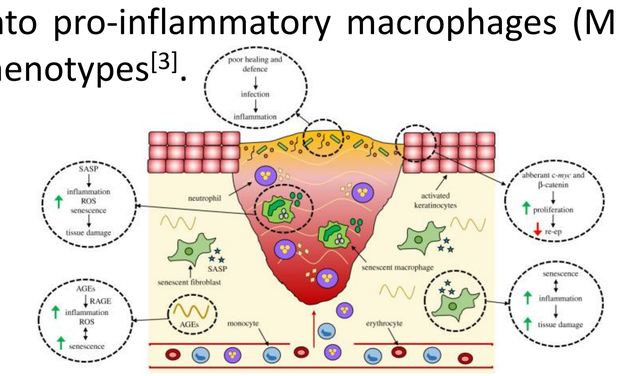


Figure 1. Schematic illustration of chronic wound stuck in the inflammatory phase (right), adapted from Holly and Matthew[5].

Why Laponite?

Laponite (LAP), a synthetic smectite clay, has been proven to be internalised by dendritic cells, resulting in migration and endocytosis[6]. And the degradation products of Lap, such as Mg2+, Si(OH)4, Li+, which are nontoxic and can be easily absorbed by the body, even control a variety of cell processes. In addition, according to our previous studies[7], Laponite can stimulate greater re-epithelialisation and hair follicle anagen. This is consistent with the potential M2 polarisation induced by Laponite.

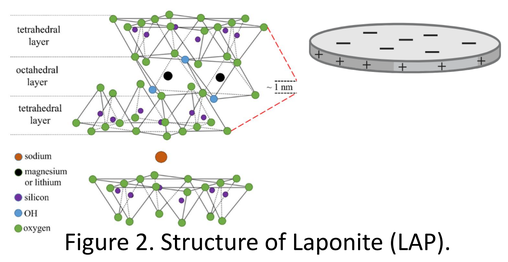


Figure 2. Structure of Laponite (LAP).

However, the mechanism by which Laponite stimulates immune cells and other wound-related cells to modulate the inflammatory response and new tissue growth has not yet been elucidated.

Overall design

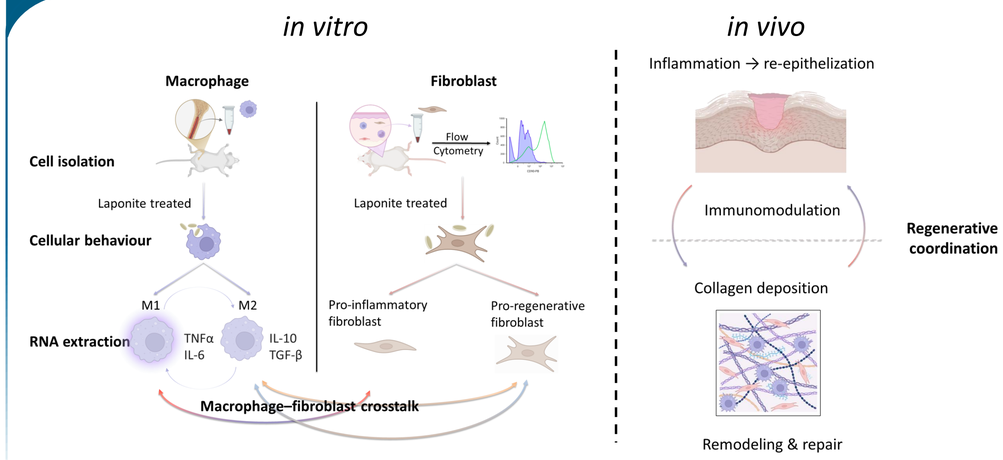


Figure 3. Schematic overview of the *in vitro* and *in vivo* experimental design and possible mechanisms of macrophage–fibroblast crosstalk as influenced by Laponite.

Aim

To elucidate the interaction during chronic wound healing process between the polarization of immune cells, especially macrophages, and to explore the effects of laponite induced immune microenvironment on cells associated with skin regeneration.

Hypothesis: Laponite gels promote healing of chronic wounds by modulating macrophage phenotype transition

Results

1. Laponite gels promote macrophage migration and phagocytosis

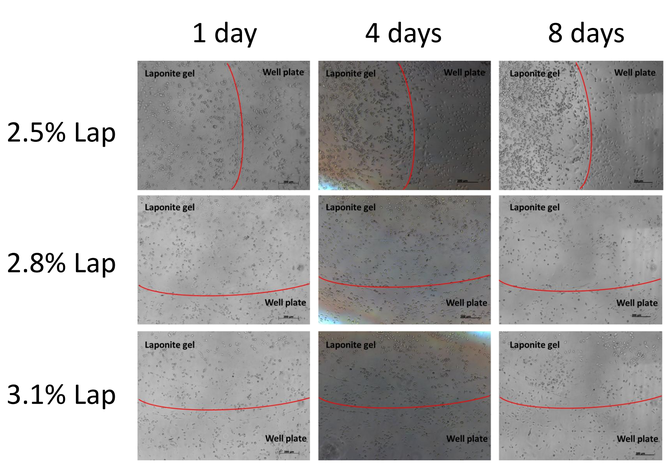


Figure 4. Bone marrow-derived macrophages tend to move and aggregate more on the laponite hydrogel.

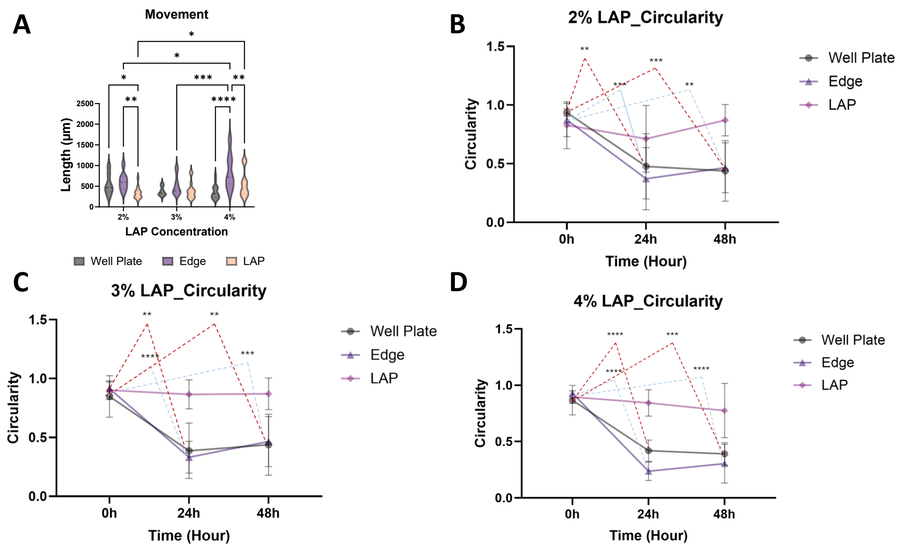


Figure 5. Different locations will affect the cell migration (A) and morphology (B, C, D), but different concentration of LAP seems like only affect the movement, and the morphology seems similar.

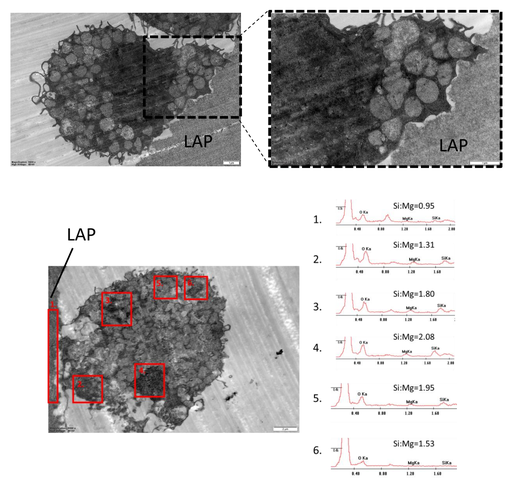


Figure 6. Observation of macrophage phagocytic activity on Laponite gel. EDX was used to locate and quantify Laponite, significant changes in the ratio of Si2+ to Mg2+ ions at different sites, which may suggest that laponite is internalized inside the macrophages.

2. Laponite gels promote re-epitheliasation in *db/db* mice

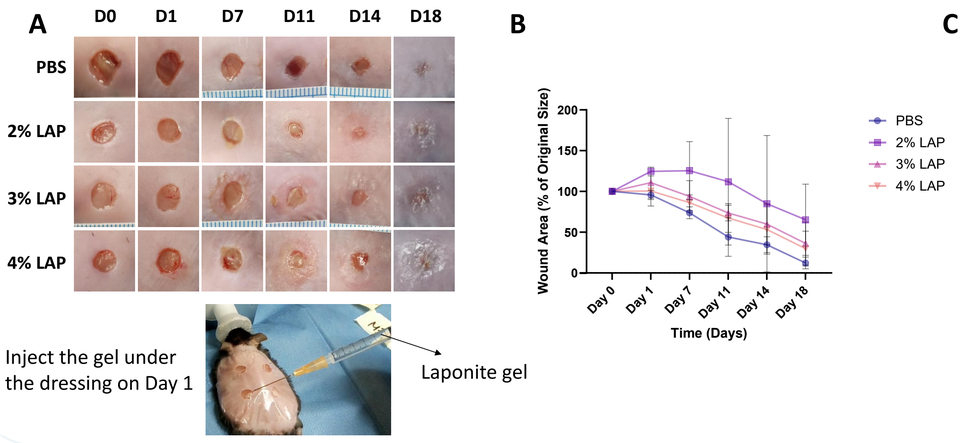


Figure 7. Analysis of wound healing following topical Laponite treatment in db/db mice. (A) Macroscopic images of each treatment group are shown over time (Top), and an example of the gel application (Bottom). The wound area difference (%) plotted over time measured from all wound photos (B). Although the wound area went up at day 1 in Laponite treatment group and 2% Laponite showed a trend of delayed wound closure compared to other treatments, no statistically significant differences were observed between groups. H&E staining reveals cellular invasion and better re-epitheliasation in Laponite treatment groups (C).

Conclusion

in vitro:

- Macrophages exhibited preferential adhesion to Laponite, with morphological changes, increased activity, and Laponite internalisation, suggesting a potential differentiation toward an M2 phenotype.
- A stable RNA extraction method from Laponite-based culture was established, enabling downstream gene expression analysis.

in vivo:

- *in vivo* application of Laponite did not significantly accelerate macroscopic wound closure by day 18.
- Laponite improves re-epithelisation in db/db wounds. These findings suggest that Laponite gel promotes the progression from the inflammatory to the proliferative phase of wound healing, supporting further investigation of macrophage-fibroblast crosstalk *in vivo.*

Reference

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