The Effects of *Staphylococcus Aureus* Biofilm conditioned media on 3T3 fibroblasts

Presented by: Goh Jin Ying, Jarryl Tsai, Shannon Chin
Supervised by: Prof David Becker

Introduction

Chronic wounds: Wound such as diabetic foot ulcers and pressure ulcers heal poorly due to a persistent inflammatory state, reduced ECM, senescent fibroblasts.

Bacterial biofilm: These are found in 80% of chronic wounds. They release substances that negatively affect the cells crucial to the healing process.

Staphylococcus aureus (SA): SA is commonly found in chronic wounds. It reduces the rate of re-epithelialization and granulation tissue formation, delaying wound healing.

Biofilm Secretions and Wound Healing: Existing research shows that bacterial biofilms negatively impact in vitro wound healing responses, specifically affecting fibroblasts.

However, a detailed understanding of how these secretions affect specific cellular processes within wound healing is lacking.

As such, our **research questions** are:

1. How do secretions from SA biofilms affect the cell biology of fibroblasts during wound healing?
2. What elements of the cell biology of wound healing processes are affected by biofilm secretions?

Our **hypotheses** are:

1. SA biofilm may affect cell viability, movement, and senescence (aging), potentially altering fibroblast function.
2. The biofilm or bacteria-produced substances might change the environment around fibroblasts, leading to alterations in cellular processes.
3. SA biofilm could induce markers of aging or stress in fibroblast cells, suggesting an immune or stress-related response.

Methods

Biofilm-conditioned media was prepared by growing S. aureus (ATCC 29213) biofilms on polyester fleece submerged in tryptic soy broth. The media was replaced with Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum to collect biofilm secretions, which were sterile-filtered to create BCM. Fibroblasts were treated with varying concentrations (25–100%) of BCM, and their responses were evaluated through several assays.

Cell viability and metabolic activity were assessed using propidium iodide (PI) staining and the MTT assay. Scratch wound assays were conducted to measure migration, and immunofluorescence was used to analyze cytoskeletal and adhesion protein changes. Cell cycle progression was examined with flow cytometry, and senescence was quantified using β-galactosidase staining. Quantitative data were analyzed using ANOVA with appropriate post hoc tests.

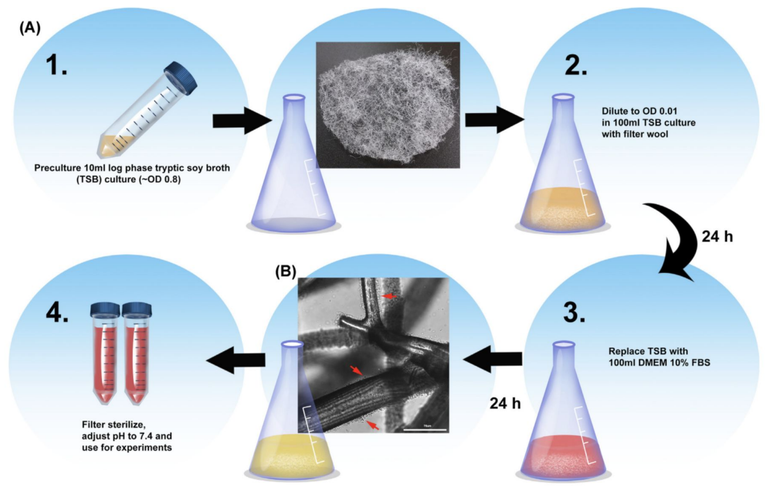

Fig. 1 Biofilm Preparation. (A) Workflow of BCM production. (B) A representative image of an area of filter wool with SA growth as a biofilm layer before BCM collection. Scale bar represents 75 µm.

Results

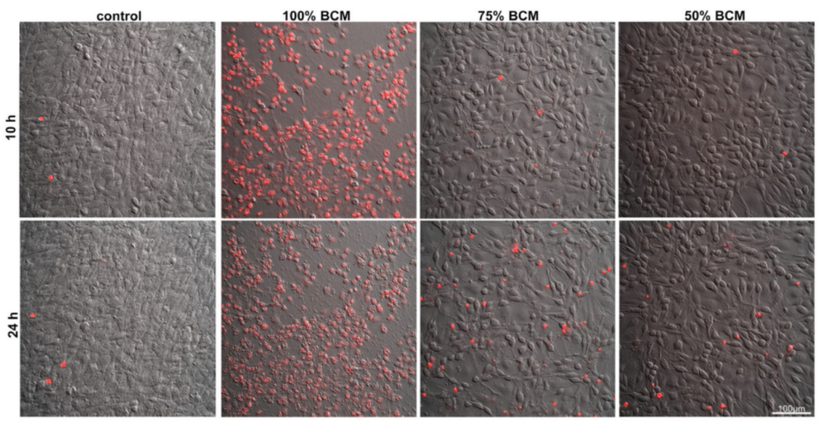

Fig. 2. PI staining of 3T3 fibroblasts treated with varying dilutions of BCM was performed at 10 and 24 hours post-treatment. PI, a positively charged molecule, penetrates porous cell membranes and binds to DNA and RNA, emitting fluorescence. The results indicate that treatment with 100% BCM caused significant cell death, while lower concentrations (75% and below) resulted in markedly reduced cell death, showing no significant difference from untreated controls.

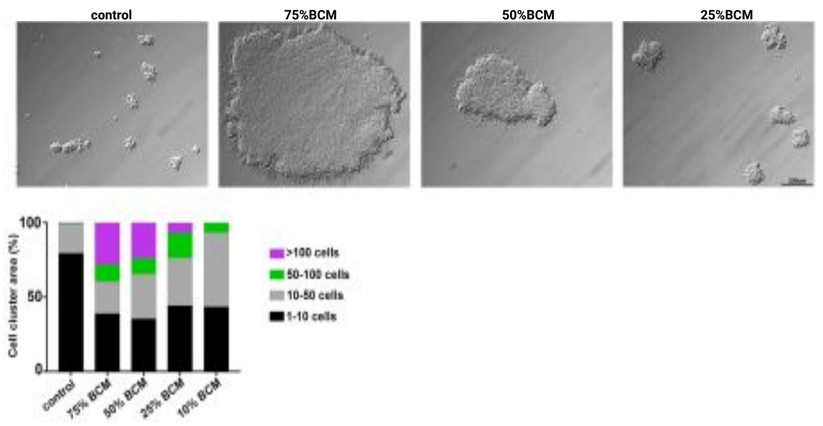

Fig. 3 Images depict 3T3 fibroblasts in a hanging drop assay following a 4-hour treatment with BCM and 10 cycles of trituration, with cell cluster areas quantified. The results reveal a BCM concentration-dependent increase in cell-cell adhesion.

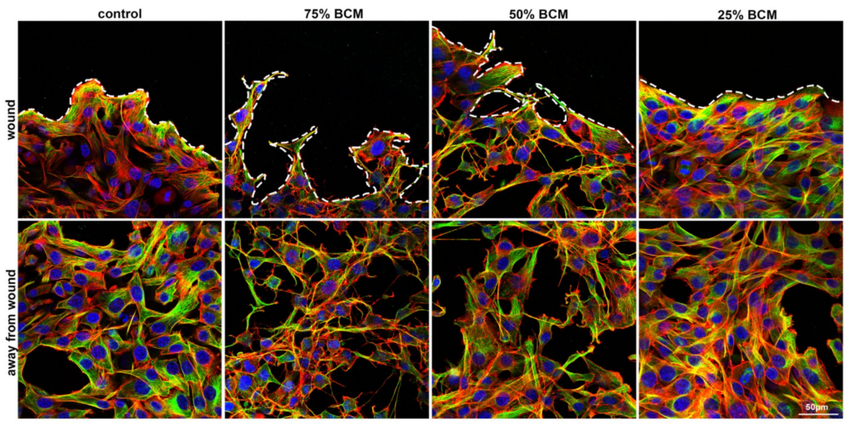

Fig. 4 3T3 fibroblasts located at and away from the wound were stained with DAPI for nuclei (blue), phalloidin for the actin cytoskeleton (red), and α-tubulin (green). Cells treated with 25% BCM exhibited morphology and actin-tubulin distribution similar to control cells. In contrast, treatment with 50% and 75% BCM resulted in fibroblasts with contracted cell bodies and elongated cellular extensions, showing intense staining for F-actin and tubulin.

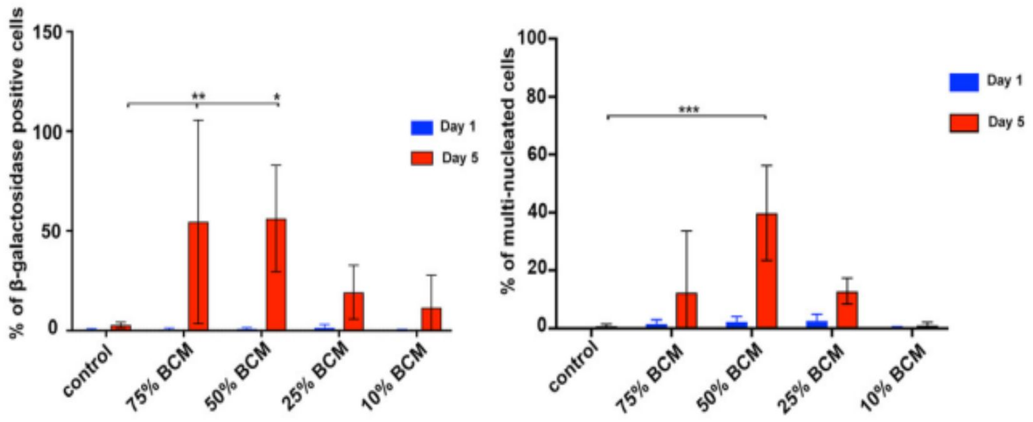

Fig. 5 The graph illustrates the percentage of cells stained for β-galactosidase and the proportion of multinucleated cells on days one and five following BCM treatment. On day one, cells treated with 75% and 50% BCM showed minimal β-galactosidase expression, which increased significantly ($P < 0.05$–$P < 0.001$) by day five.

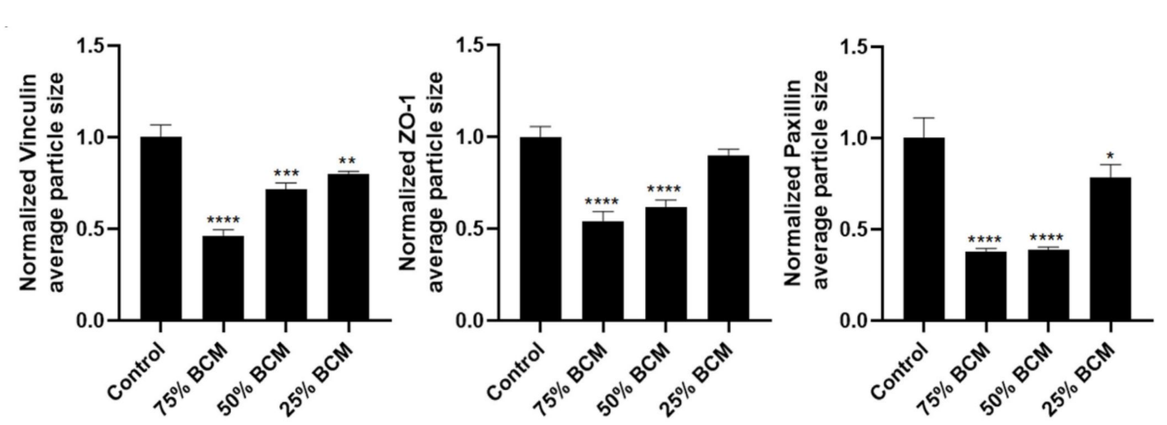

Fig. 6 Quantification of average vinculin, ZO-1, and paxillin particle sizes revealed significant differences between BCM-treated cells and controls at cell-plastic contact points. This suggests that BCM treatment influences cell-substrate attachment.

Discussion

Bacterial biofilm secretions alone are sufficient to induce senescence in fibroblasts. It has been suggested that wounds will not heal in the presence of more than 15% senescent fibroblasts in chronic wound edges (Harding, Moore and Phillips 2005). Our findings suggest that biofilms, which are prevalent in chronic wounds, may inhibit the healing by inducing senescence in fibroblasts.

BCM of SA 29213 contains fructose bisphosphate aldolase (ALDOA) which can bind directly to the actin cytoskeleton, modulating its polymerization and lamellipodial formation. Epidermal cell differentiation inhibitor (EDIN), also found in SA 29213, inactivate Rac and Rho GTPase by ADP-ribosylation activity (Sugai et al. 1992) influencing the cytoskeletal activities. These molecules within the BCM could negatively influence cell shape and migration.

Conclusion

SA BCM significantly impairs fibroblast functions critical to wound healing by inducing cell cycle arrest, senescence, and disrupting cytoskeletal dynamics, migration, and adhesion. These findings highlight the need for effective management of biofilm infections in chronic wounds to restore normal healing processes.

Future Work

In-vivo studies on animal wound models

Explore potential treatments targeting the biofilm-secreted factors to restore fibroblast function

Investigate the molecular components in the BCM responsible for inducing senescence, disrupting cytoskeletal dynamics, and impairing cell functions.