



The Effect of Amnion-Chorion Membrane on Bone Marrow Derived Stem Cells

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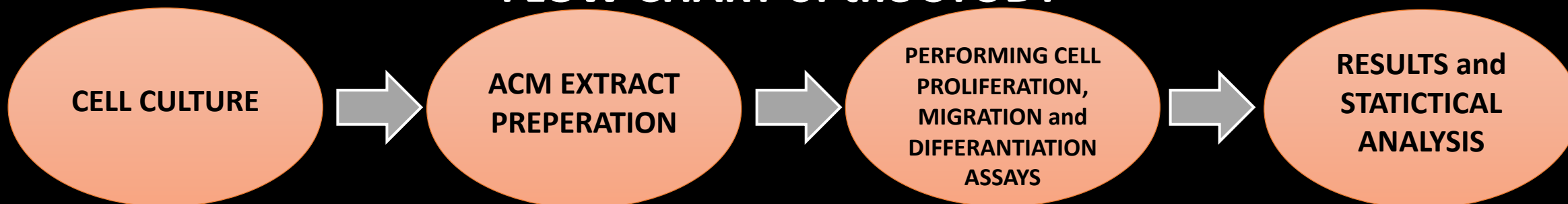


INTRODUCTION

- Amnion-chorion membrane (ACM) has been used successfully as a biomaterial source of growth factors during regeneration procedures. However, there are no studies to evaluate ACM regarding the effects on stem cell proliferation, migration, and differentiation/mineralization.
- Therefore, the aim of this study was to evaluate effects of ACM on
 - Cell proliferation
 - Cell migration
 - Cell differentiation/mineralizationof bone marrow derived stem cells (BMSCs).



FLOW CHART of the STUDY

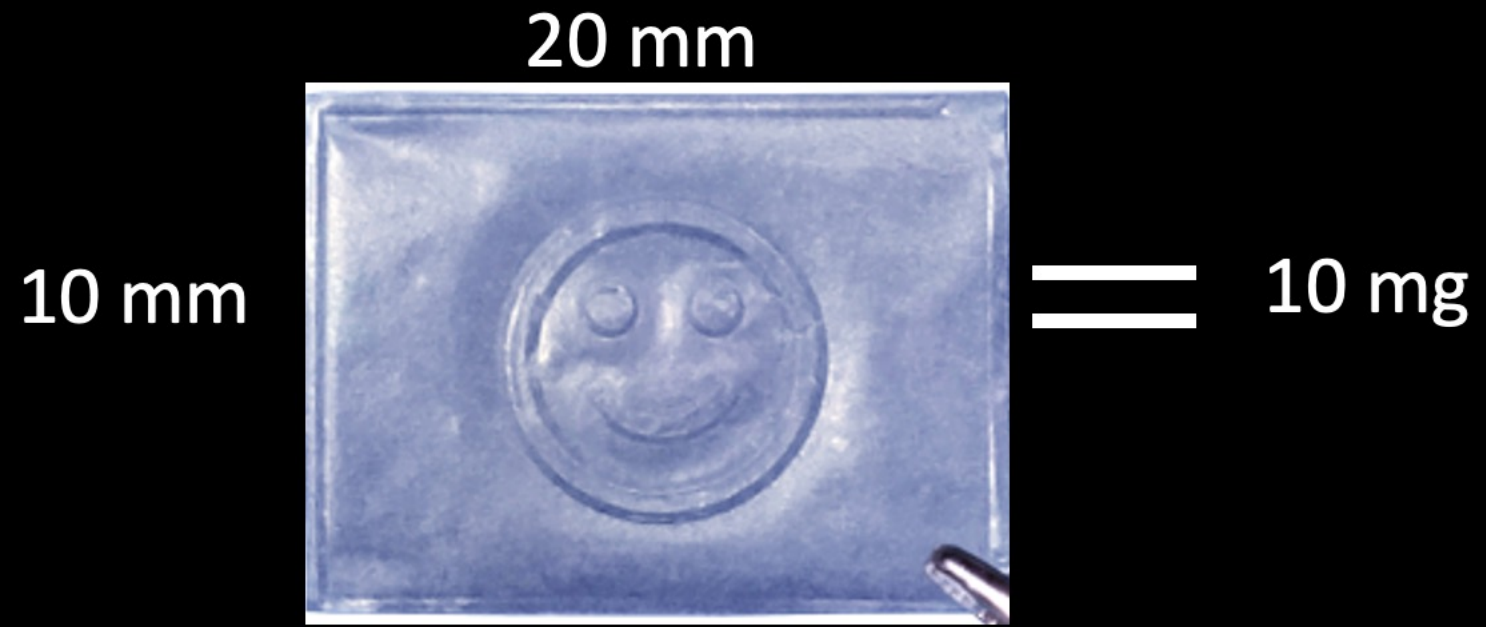


METHODS

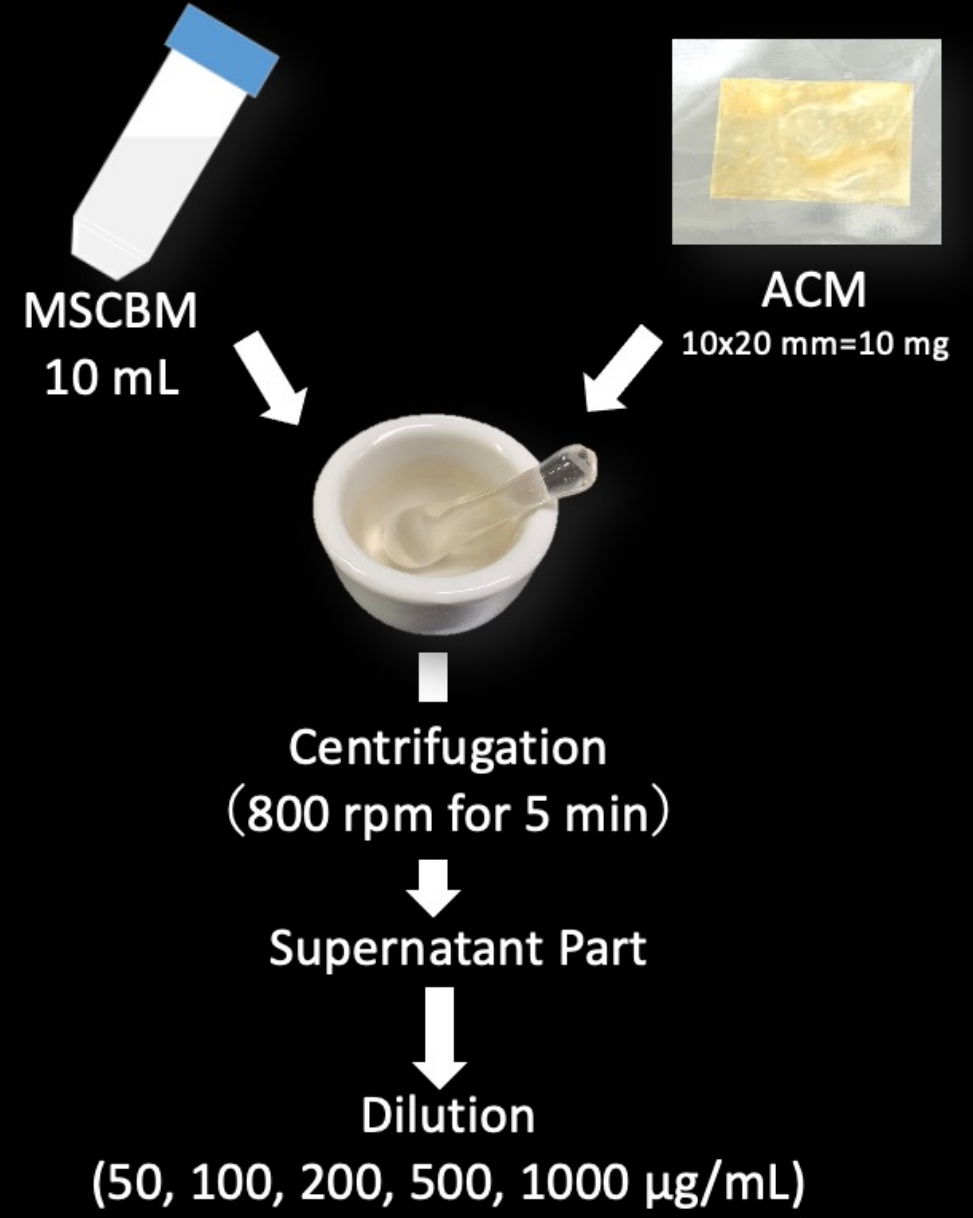
CELL CULTURE

Commercially available BMSCs were utilized for this study. BMSC growth media was prepared following the manufacturer instructions. Following media preparation, the BMSCs were thawed and cultured.

ACM (BioXclude[®], Snoasis Medical, Denver, CO) was used for the study.

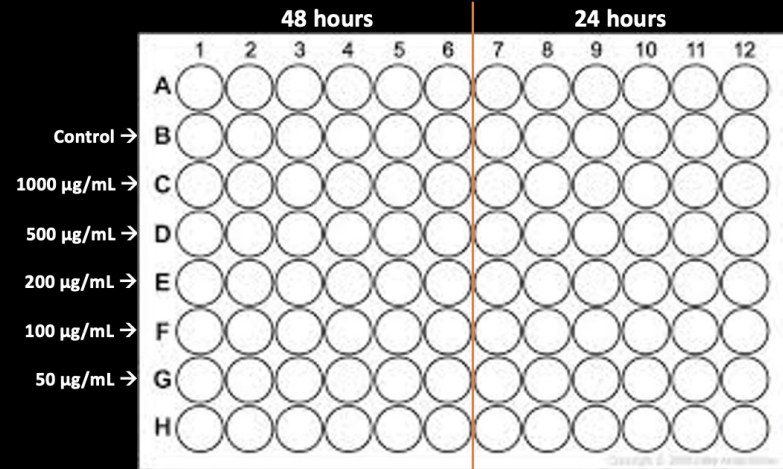


ACM EXTRACT PREPERATION



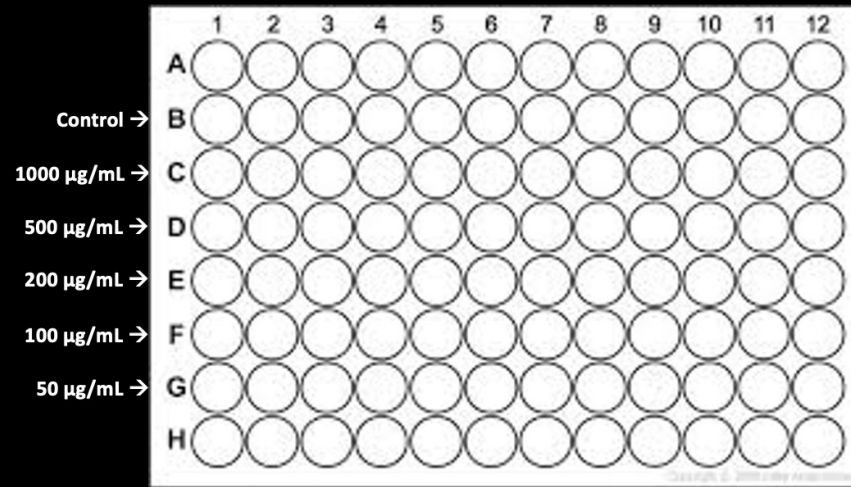
METHODS

CELL PROLIFERATION ASSAY



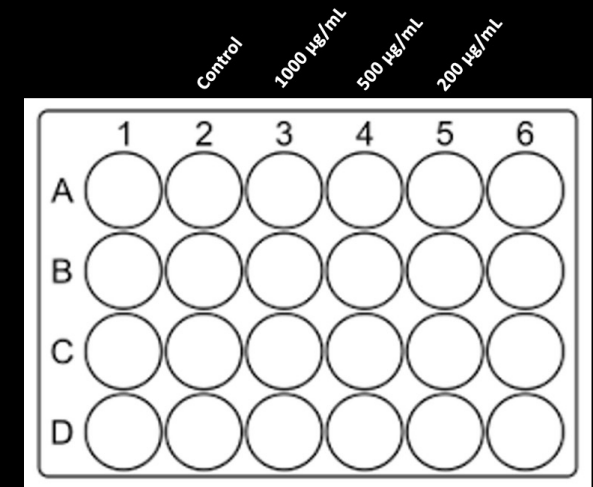
- BMSCs were seeded on a 96-well plate (2500 cells/well) and were cultured with ACM at different concentrations for 24 and 48 hours.
- As a control, basal medium was used. All media was changed every other day.
- A WST-1 assay (Roche) was used to analyze BMSC proliferation.

CELL MIGRATION ASSAY



- BMSCs were seeded on a 96-well plate (2500 cells/well) using medical-grade silicone cell seeding stoppers to prevent cell seeding and create cell-free zone on the center of each well.
- After 3 days of incubation, silicone stoppers were removed, and cells were treated with ACM at different concentrations for 24 and 48 hours.
- As a control, basal medium was used.
- The interval images were used to compare migration compared to the baseline.

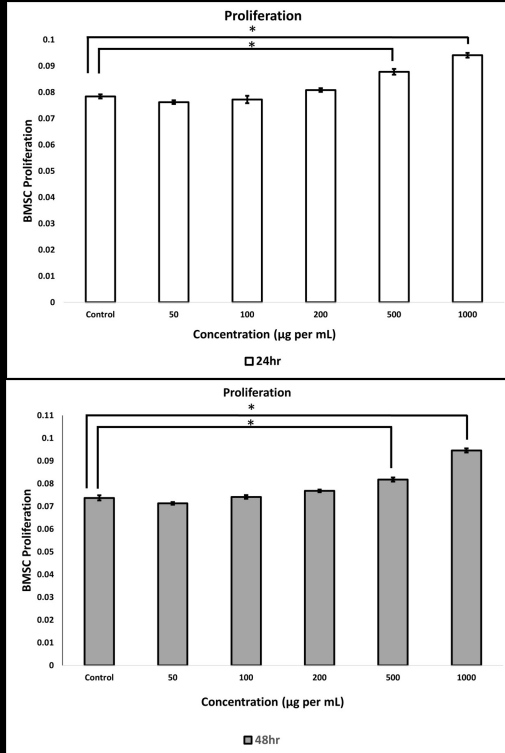
CELL DIFFERENTIATION ASSAY



- BMSCs were seeded on a 24-well plate (25x10³ cells/well) and incubated for 3 days. After incubation, cells were treated with ACM extract prepared using osteogenic differentiation medium at different concentrations for 7, 14, and 21 days.
- As a control, osteogenic differentiation medium was used.
- Osteogenic cell differentiation of BMSCs, and subsequent mineralization, was determined using Alizarin Red S staining. BMSCs were fixed in 4% paraformaldehyde and stained with 2% Alizarin Red S solution and mineralization nodules were quantified.

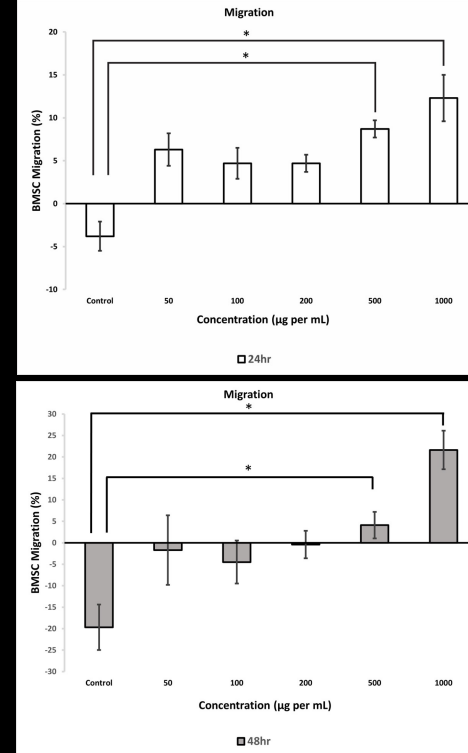
RESULTS

CELL PROLIFERATION



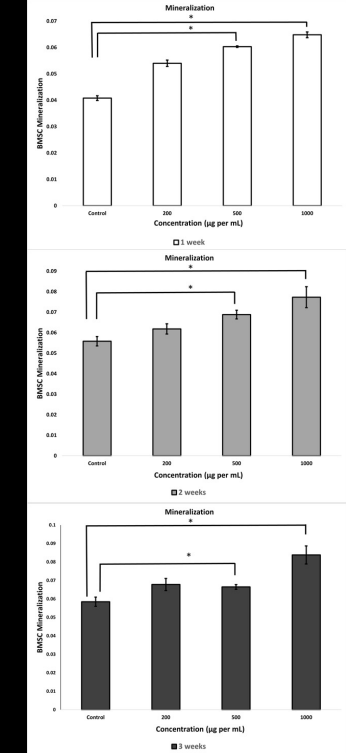
After both 24-hours and 48-hours, control, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, and 200 $\mu\text{g/mL}$ group exhibited significantly lower cell proliferation than 500 and 1000 $\mu\text{g/mL}$. Additionally, the 500 $\mu\text{g/mL}$ group displayed significantly lower cell proliferation compared to 1000 $\mu\text{g/mL}$.

CELL MIGRATION



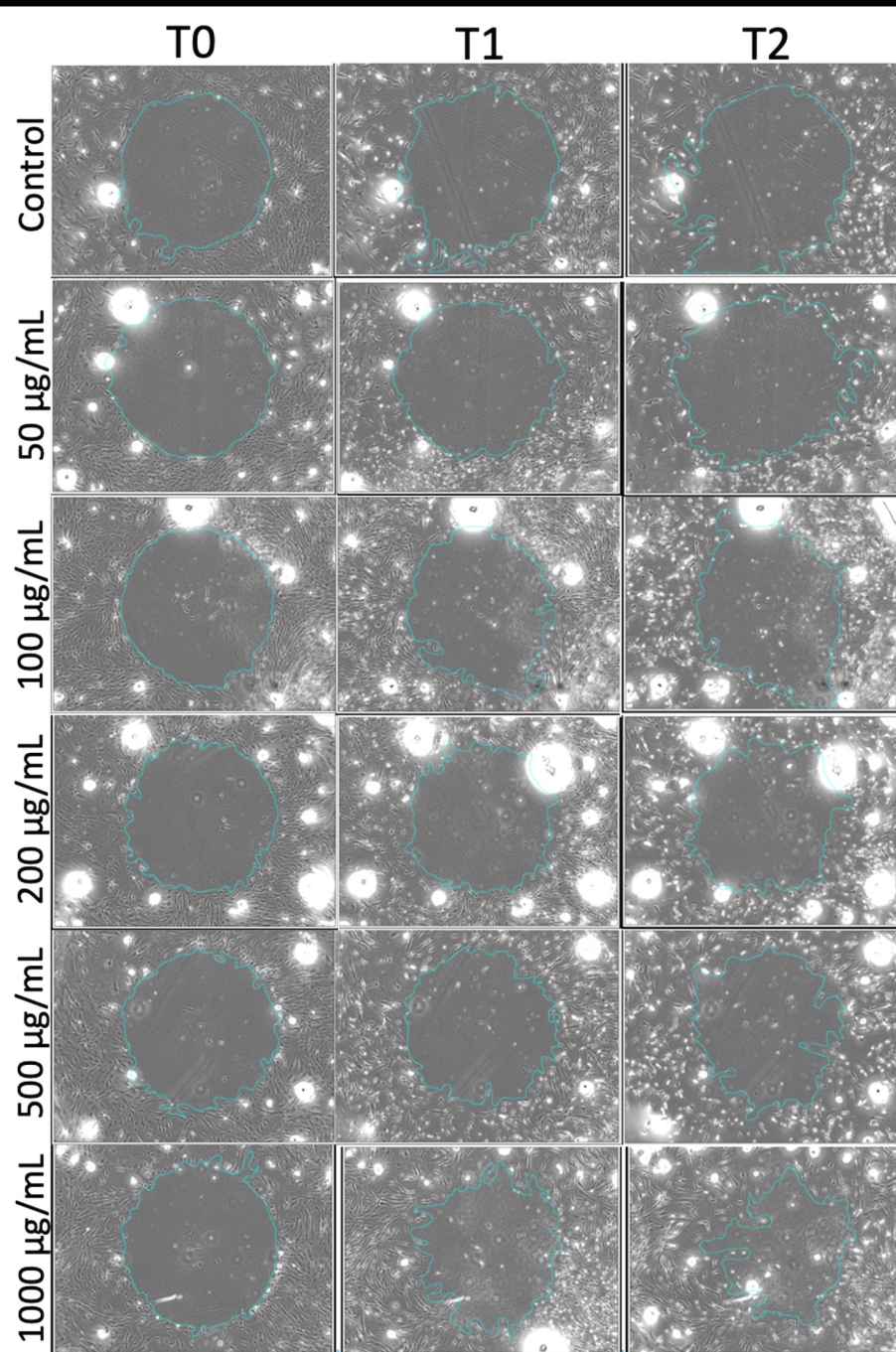
At the 24-hour mark, the control displayed significantly lower cell migration compared to all other groups. Additionally, the 100 $\mu\text{g/mL}$ group and 200 $\mu\text{g/mL}$ group showed significantly lower cell migration compared to 500 and 1000 $\mu\text{g/mL}$ groups. At 48 hours, the control group demonstrated significantly lower cell migration compared to all other groups except the one treated with 50 $\mu\text{g/mL}$. Moreover, the groups treated 50, 100, 200, and 500 $\mu\text{g/mL}$ all exhibited significantly lower cell migration compared to 1000 $\mu\text{g/mL}$ group.

CELL DIFFERENTIATION



At 1 week, the control group showed significantly lower cell mineralization compared to all other groups. The 200 $\mu\text{g/mL}$ group also exhibited significantly lower cell mineralization than 500 and 1000 $\mu\text{g/mL}$. Similarly, 500 $\mu\text{g/mL}$ group demonstrated significantly lower cell mineralization compared to 1000 $\mu\text{g/mL}$. At the 2-week mark, both the control group and 200 $\mu\text{g/mL}$ group exhibited significantly lower cell mineralization compared to 500 and 1000 $\mu\text{g/mL}$ groups. At the 3-week point, the control group displayed significantly lower cell mineralization compared to other groups. Moreover, 200 and 500 $\mu\text{g/mL}$ groups showed significantly lower cell mineralization compared to 1000 $\mu\text{g/mL}$ group.

CELL MIGRATION PICTURES



LIMITATIONS of the STUDY

- In vitro study
- Differences in clinical application, including blood supply
- Growth factor content and concentration in the ACM membrane or extract were not measured.

CONCLUSION

Within its limitations, the present study showed that the use of amnion-chorion membrane for regenerative procedures can positively effect proliferation, migration, and mineralization of the stem cells due to growth factors present in the membrane. However, animal studies and high-quality RCTs are needed to confirm the results of the current in vitro study.

Acknowledgement

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Conflict of Interest Statement

All authors declare no conflict of interest.