

Processing and Preservation Methods Impact Osteogenic Potential of Amnion–Chorion Membranes In Vitro

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Background

- Amnion chorion membranes (ACMs), derived from placental tissue, are gaining widespread use in various dental applications. ACMs' bioactive extracellular matrix is rich in growth factors, which makes them highly effective in soft and hard tissue regeneration.^{1,2}
- Commercial ACMs are processed differently; therefore, they poses diverse structural, chemical and biological differences. Intermediate (IL) and trophoblast layers of placental structure are believed to contain critical signaling components for tissue regeneration, thus, their preservation or removal during processing of ACMs may impact their clinical outcomes.³⁻⁵

Aim

- We aim to investigate how different processing methods affect the in vitro cell response to ACMs, focusing on the adhesion and proliferation of human bone marrow–derived mesenchymal stem cells (hBMSCs).

1.Agrawal, R.; Sinha, A.; Chauhan, S. S.; Sharma, S. Harnessing the Power of Amnion-Chorion Membrane in Periodontal Therapy: A Comprehensive Review. J. Oral Res. Rev. 2024, 16 (2), 157.

2.Gulameabasse, S.; Gindraux, F.; Catros, S.; Fricain, J.-C.; Fenelon, M. Chorion and Amnion/Chorion Membranes in Oral and Periodontal Surgery: A Systematic Review. J. Biomed. Mater. Res. B Appl. Biomaterial. 2021, 109 (8), 1216–1229.

3.Moreno S, Masee M, Campbell S, Bara H, Koob TJ, Harper JR. PURION® processed human amnion chorion membrane allografts retain material and biological properties supportive of soft tissue repair. Journal of Biomaterials Applications. 2024;39(1):24-39.

4.Roy A, Griffiths S. Intermediate layer contribution in placental membrane allografts. J Tissue Eng Regen Med. 2020; 14: 1126–1135.

5.Wetzell B, Ork B, Softic D, et al. Characterization of a full-thickness decellularized and lyophilized human placental membrane for clinical applications. Int Wound J. 2024; 21(5): e14888.

Materials & Methods

Three differently processed amnion chorion membranes (test groups) and one pericardium membrane (PCM - control group) were compared.

Membranes	Manufacturer	Membrane Tissue Structure	Preservation Method	Source	Abbreviation
Puros®	RTI Surgical, DBA Evergen (Marketed by ZimVie)	Intact pericardium	Tutoplast®	Pericardium	PCM
Biotivity™	Surgenex (Marketed by ZimVie)	Intact placental membrane Amnion + IL + Chorion (never separated)	Excellion®	Placenta	ACM1
BioXclude®	Solvita	Amnion + Chorion (separated and re-assembled)	Purion®	Placenta	ACM2
AmnioExcite™	LifeNet Health (Marketed by Salvin)	Full thickness placental membrane Amnion + IL + Chorion + Trophoblast	Matracell®	Placenta	ACM3

Characterization

- Surface morphology:** examined by a scanning electron microscope (SEM, JEOL-JSM-IT500HR).
- Surface roughness:** mapped with a Keyence microscope (VK-X1050) on a total of 4 areas (144 µm x 108 µm) per membrane. Each area's arithmetical mean height (Sa) was assessed with a MultiFile Analyzer (Keyence).
- Osteogenic cell growth:** characterized by culturing 2.5×10⁴ hBMSCs on the membranes at 37°C, and the cell growth was quantified with a cell counting kit (Dojindo® Molecular Technologies) at 1, 3, and 7 days (n=3). Adherent cell growth on the membranes was also visualized by staining with LIVE/DEAD™ Viability/Cytotoxicity Kit (Invitrogen) and observed with a fluorescent microscope (Olympus BX53) at 1, 3, and 7 days.

Statistical analysis

- One-way ANOVA with Tukey multiple comparison tests was performed to determine statistical differences between groups (GraphPad Prism 10). The p<0.05 was considered significant.

Results

Morphology (SEM) & Surface Sa Roughness

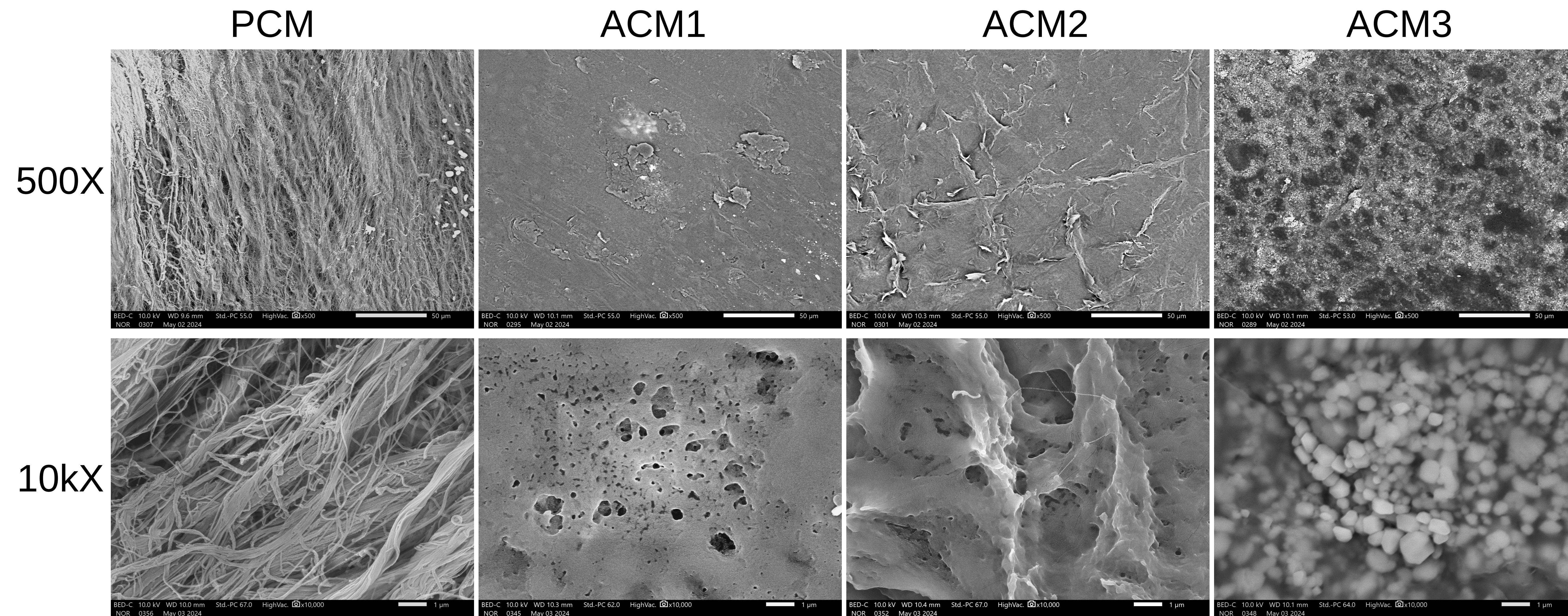


Figure 1: SEM images of the membrane surfaces taken at low magnification (top row, scale bar = 50μm) and high magnification (bottom row, scale bar = 1μm).

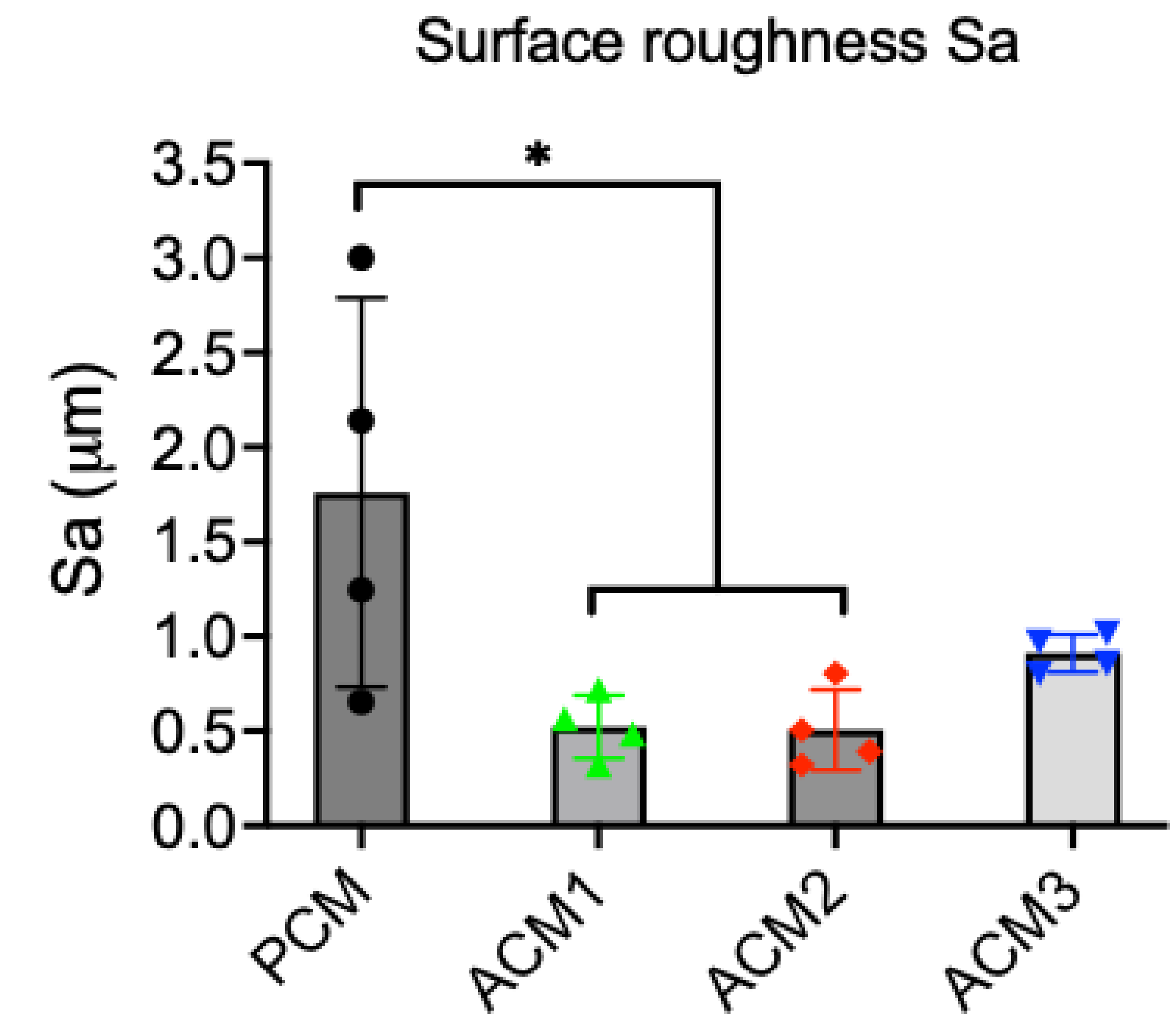


Figure 2: Surface roughness (Sa values) of membranes as measured by Keyence microscope.

- SEM images showed highly intact collagen structure and surface complexity on PCM, whereas ACMs showed relatively smooth surfaces, except for ACM3, which was covered by substantial retention of salt crystals throughout, obliterating the underlying topography. While no considerable morphological differences were observed between ACM1 and ACM2, the latter exhibited more folds than the former (Figure.1).
- Consistent with SEM observations, significantly higher Sa roughness values were measured on PCM as compared to ACM1 and ACM2, but no significant differences were observed in Sa roughness values between ACMs (Figure.2).

Results

Osteogenic Cell Growth on Different Membranes

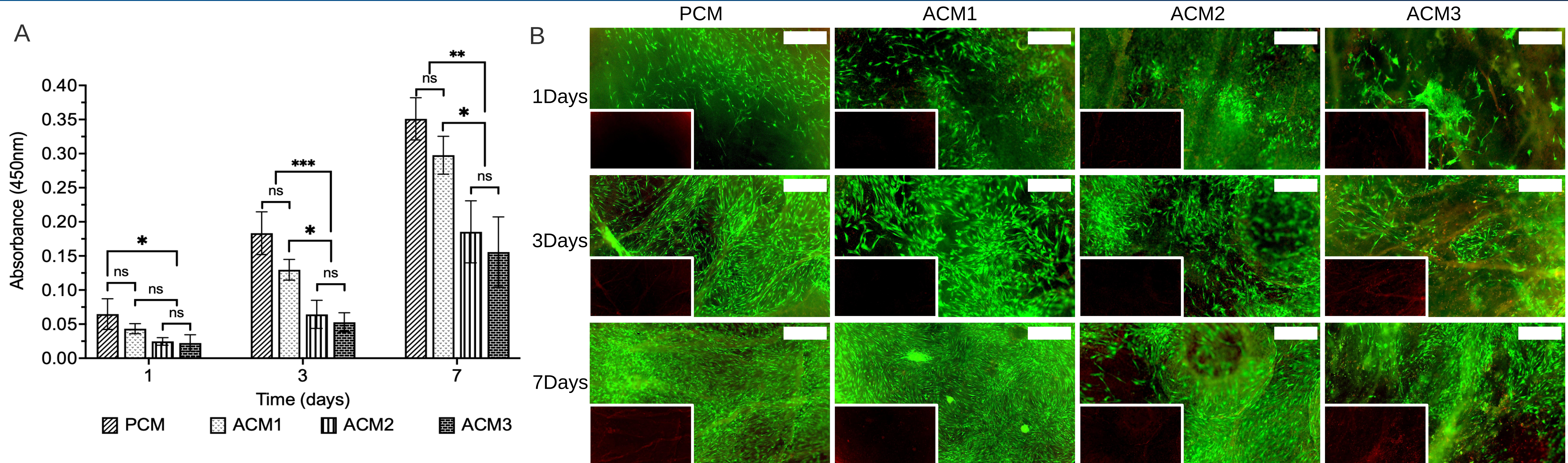


Figure 3: (A) Quantitative analysis of hBMSCs growth on different membranes at 1,3 and 7 days. (B) Fluorescence LIVE (green) / DEAD (red) images of hBMSCs growth on different membranes at 1,3 and 7 days. (Scale bar = 500µm)

- ACM1 demonstrated an effective hBMSCs growth potential over time up to 7 days similar to the highly collagenous PCMs.
- However, ACM2 and ACM3 showed significantly lower hBMSCs compared to ACM1 at 3 and 7 days, and PCM at all time points (Figure.3A).
- Fluorescent LIVE/DEAD staining showed more live cells (green) on PCM and ACM1 compared to ACM2 and ACM3 at all time points. Considerably more dead cells (red) on ACM3 were observed at all time points (inset images). At 7 days, only PCM and ACM1 were fully confluent with live hBMSCs (Figure.3B).

Discussion & Conclusion

- Processing of ACMs, particularly the preservation or removal of the intermediate and trophoblast layers, affects their structural integrity and growth factor composition, influencing their tissue regeneration potential in dental applications.
- Findings of this study showed significant differences in the *in-vitro* cell response to ACMs with different tissue structures as a result of their manufacturing process.
- ACM1 demonstrated a favorable hBMSC response comparable to PCM (positive control), despite the absence of collagen complexity, indicating the preservation of the intermediate layer and manufacturing processing method may have retained growth factor content more effectively.
- Within the limitations of this study, ACM1 offers a more favorable cell response *in vitro* compared to ACM2 and ACM3 and thus may show improved clinical performance in dental procedures such as ridge preservation and bone augmentation.