Double-Layered Amnion in Burn Care
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Introduction: Burn injury remains a major global burden. Split-thickness skin grafting is the gold standard but limited by the donor-site availability, while commercial skin substitutes are cost-prohibitive in most Lower - Middle Income Country (LIMC) settings. Acellular amnion bilayer (AB) offers a low-cost scaffold alternative but degrades early and yields a thicker, less mature epidermis than native skin. This program evaluated whether seeding autologous keratinocytes and human amniotic epithelial cells (hAEC) onto AB improves outcomes acutely and long-term.

Methods: A translational program spanning a preclinical rat model, a human pilot study, a randomized single-blind confirmatory trial (n=20; acellular AB vs. AB co-cultured with keratinocytes+hAEC) assessing epithelialization, epidermal thickness, histology, and gene expression (TGF-β3, CTNNB-1, MMP-2, MMP-9) over 14 days, and a cross-sectional follow-up (same cohort, n=20) at 2–5 years assessing VSS, POSAS, and pilot histology.

Results: Co-culture accelerated epithelialization (Day 5 vs. 7, p=0.048) and produced a thinner, more native epidermis (69.6 vs. 115.9 μm) with higher histology scores. TGF-β3 rose significantly with co-culture (p=0.007); CTNNB-1 and MMP-9 increased significantly within co-culture alone (p=0.01, p=0.005); MMP-2 improved in both arms without distinguishing them. At ≥12 months, co-culture showed lower VSS (1.5 vs. 3.0, p=0.030) and POSAS-Observer scores (9.0 vs. 14.6, p<0.001), driven largely by pigmentation, while patient-reported scores were comparable (p=0.138).

Conclusion: Co-culturing keratinocytes and hAEC on amnion bilayer accelerates epithelialization, improves acute architecture and anti-fibrotic signaling, and yields durable long-term scar quality gains, while acellular AB alone remains a viable, lower-cost option.