Eggshell-derived ACP Scaffold Prefilled with Eggshell Membrane Proteins Hydrogel for Sustainable Repair of Critical-sized Bone Defects *In-vivo*

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INTRODUCTION: Critical-sized bone defects require sustainable grafts with ideal spatial supporting properties. We developed a porous amorphous calcium phosphate (ACP) ceramic scaffold derived from eggshells and evaluated its early bone-regeneration performance alone and combined with flexible adjunct materials in an *in-vivo* model.

METHODS: ACP scaffolds with approximately 60% porosity were fabricated by a novel pressure-densification using ice microspheres as space holders. Bilateral critical-sized femoral and calvarial defects were created in New Zealand White rabbits. The defects were reconstructed with: (1) Sham, (2) ACP scaffold (ACP), (3) ACP + hyaluronic acid gel/P2 peptide (HA/P2), or (4) ACP + HA/P2 + soluble eggshell membrane proteins (SEPs). Before implantation, scaffolds were vacuum-infiltrated with HA gel to preserve their amorphous phase and minimize internal voids. Bone regeneration was assessed using micro-CT and histology after 6 weeks of healing.

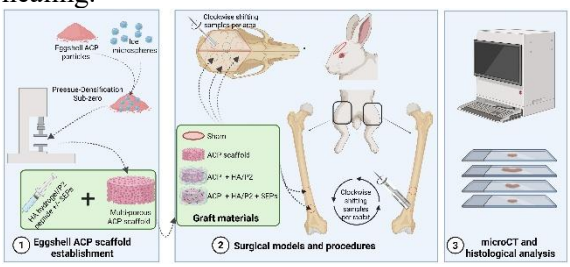

Fig. 1 Flowchart of research design in materials preparation, surgical operation and data analysis.

RESULTS: All ACP-containing groups showed greater defect filling and mineralization than Sham. Region of interest (ROI)-based micro-CT analysis revealed that the ACP + HA/P2 + SEPs group exhibited the highest bone volume, trabecular number, and the lowest trabecular separation. Histology confirmed more mineralized collagenous tissue deposition and a more integrated bone–material fusion zone formed in ACP + HA/P2 + SEPs group. Notably, in femur samples with graft displacement, ACP scaffolds induced bone bridging between the scaffold and cortical bone inside the bone marrow, most evident in the ACP + HA/P2 + SEPs group.

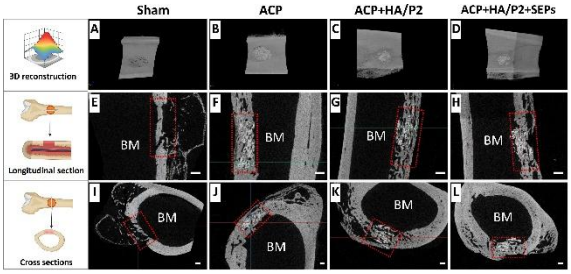

Fig. 2 Micro-CT images and virtual sectioning of critical bone defects in rabbit femurs at 6 weeks post-operation. (A-D) Reconstructive images of bone regeneration in rabbit femur defects; (E-H) Bone regeneration and mineralization of longitudinal section of femur defects; (I-L) Bone regeneration and mineralization of cross section of femur defects. Scale bar = 1.0 mm; Red dotted frame: original bone defect area.

DISCUSSION & CONCLUSIONS: Eggshell-derived ACP scaffolds, particularly when combined with SEPs, enhanced early regeneration in rabbit critical-sized bone defects. Upcycling eggshell constituents into a synergistic, multi-dimensional graft system offers a sustainable approach for repairing critical-sized bone defects, with high clinical potential.

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