Biomimetic 3D printed scaffolds promote mesenchymal stromal cell chondrogenesis for cartilage regeneration

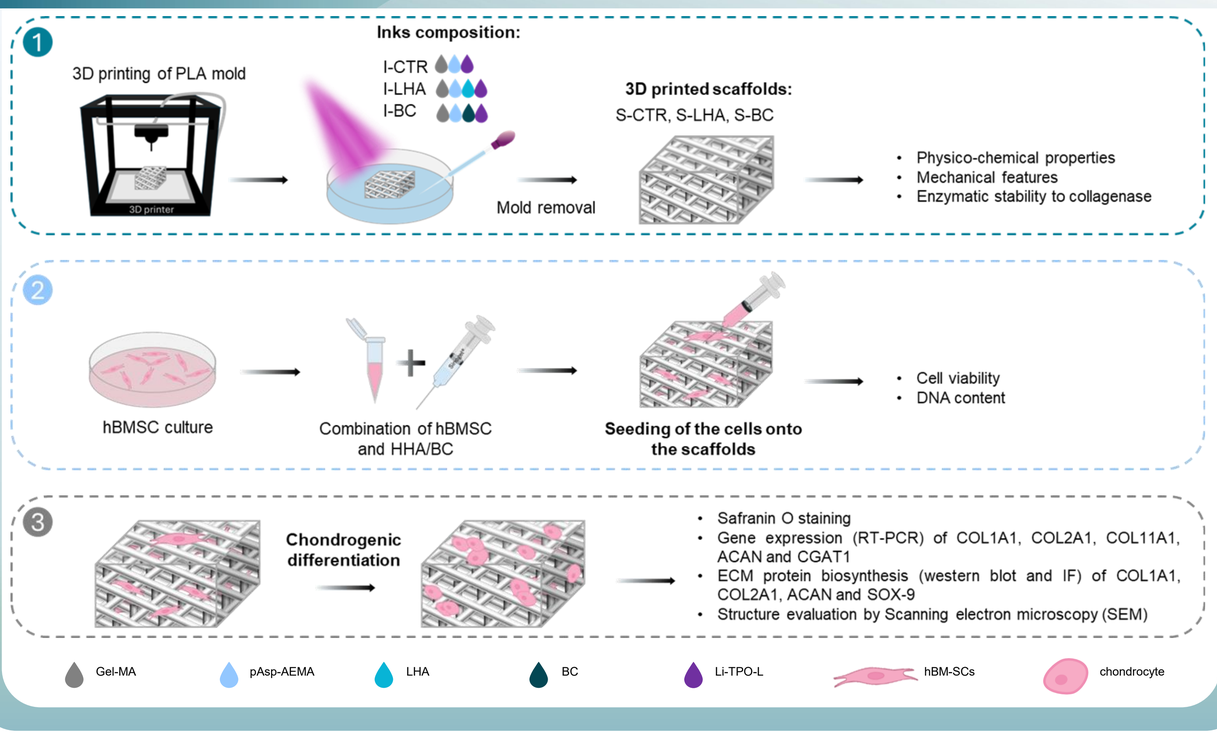
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INTRODUCTION

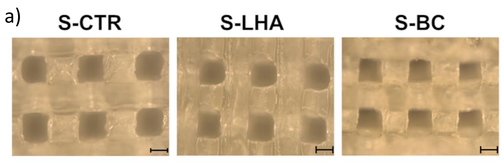
Articular cartilage has limited self-repair capacity, making its regeneration a major clinical challenge. Tissue engineering strategies using 3D biomimetic scaffolds can support cell growth and differentiation. In this study, methacrylated gelatin (GelMA) and methacrylated poly(aspartic acid) (pAsp-AEMA) scaffolds, enriched with biofermentative glycosaminoglycans, namely low molecular weight hyaluronic acid (LHA) and unsulfated chondroitin (BC) were developed to enhance mesenchymal stromal cell chondrogenesis and promote the formation of cartilage-like tissue.

MATERIALS & METHODS

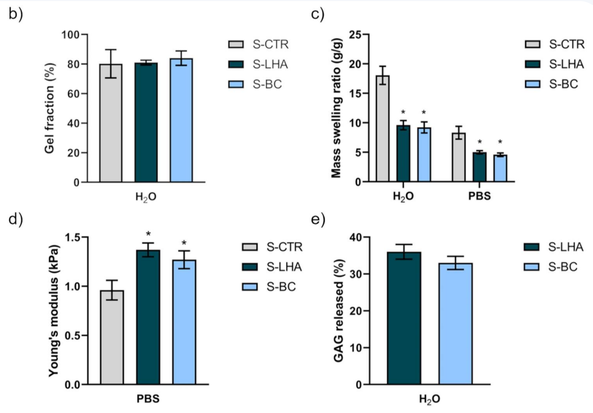


RESULTS

Scaffold Characterization

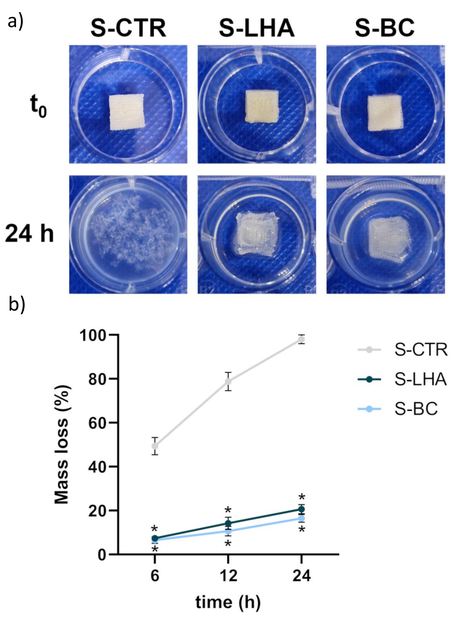


Structure of the scaffolds by optical microscopy (a): 250 µm average pore size



Physico-chemical properties: gel fraction (b) and swelling (c) after 3 days in H_2O and PBS; Young's modulus in PBS swollen condition (d); GAG release evaluated through capillary electrophoresis after 3 days in H_2O (e). mean ± SD, n=3; *p<0.05 vs S-CTR.

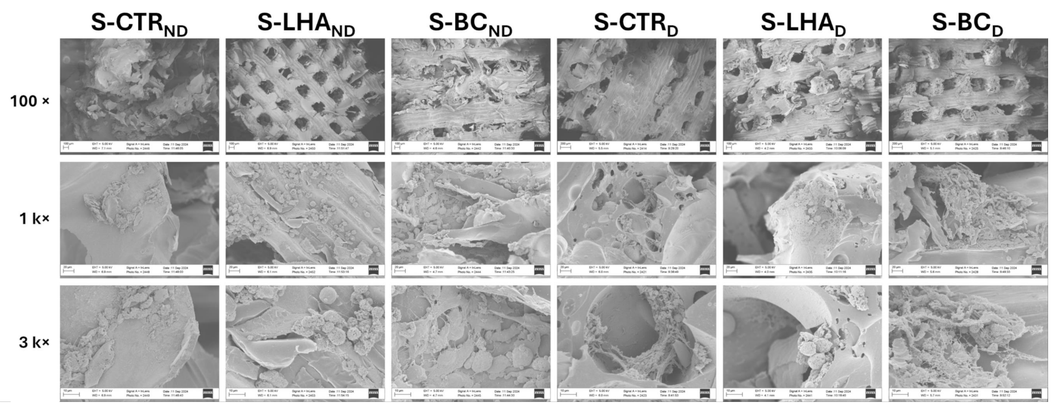
Enzymatic Degradation



Type 1 collagenase-mediated degradation: images before/after 24 h of incubation with 10 U/mL of enzyme, and mass loss over time. mean ± SD, n=3; *p<0.05 vs S-CTR.

Scaffold Morphology

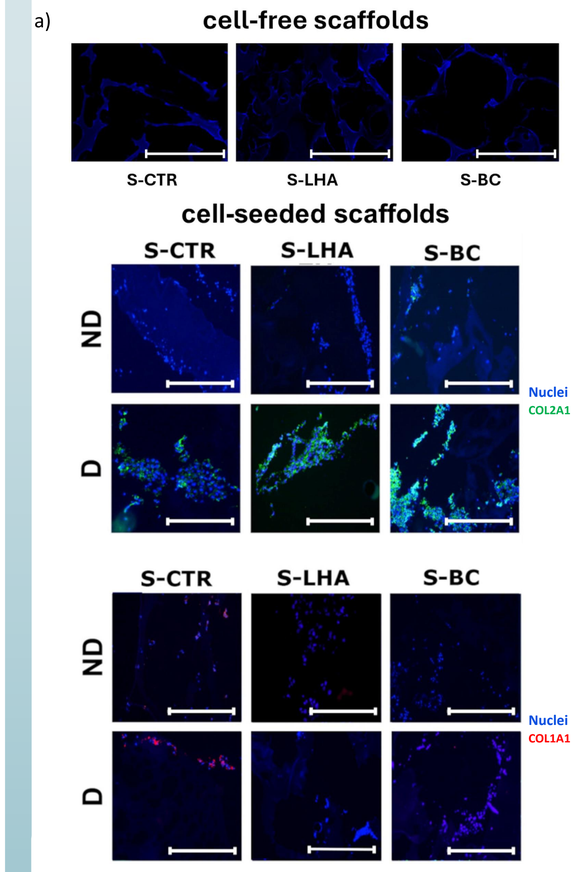
Observation of the structural stability and chondrogenic response of the scaffolds after long-term culture (21 days) in proliferating (ND) and chondrogenic differentiating (D) medium by SEM.

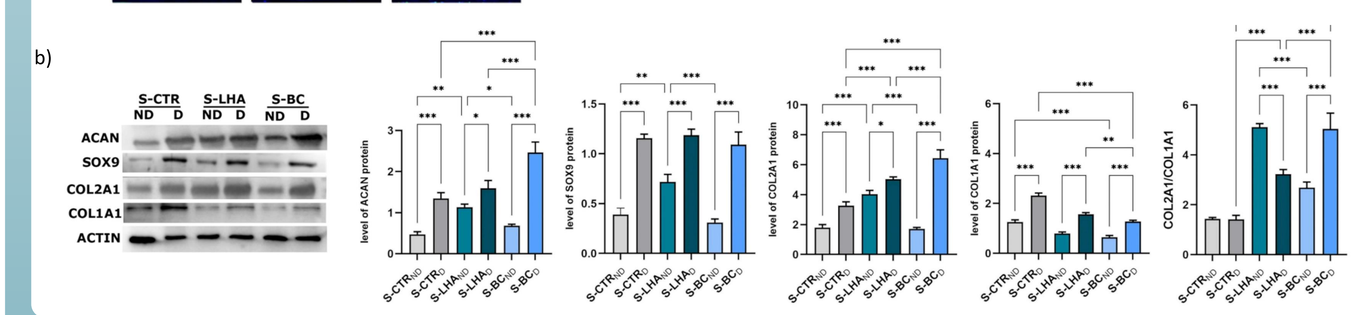


ECM production was observed in cavities where the original matrix was replaced by cell-secreted components.

Protein Expression

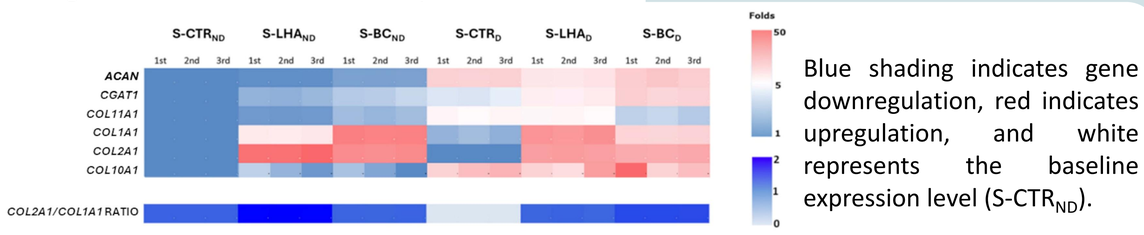
Chondrogenic markers after 21 days: COL2A1 and COL1A1 fluorescence (a) and western blot quantification of COL2A1, ACAN, SOX9, COL1A1 (b). mean ± SD, n=3; *p<0.05.





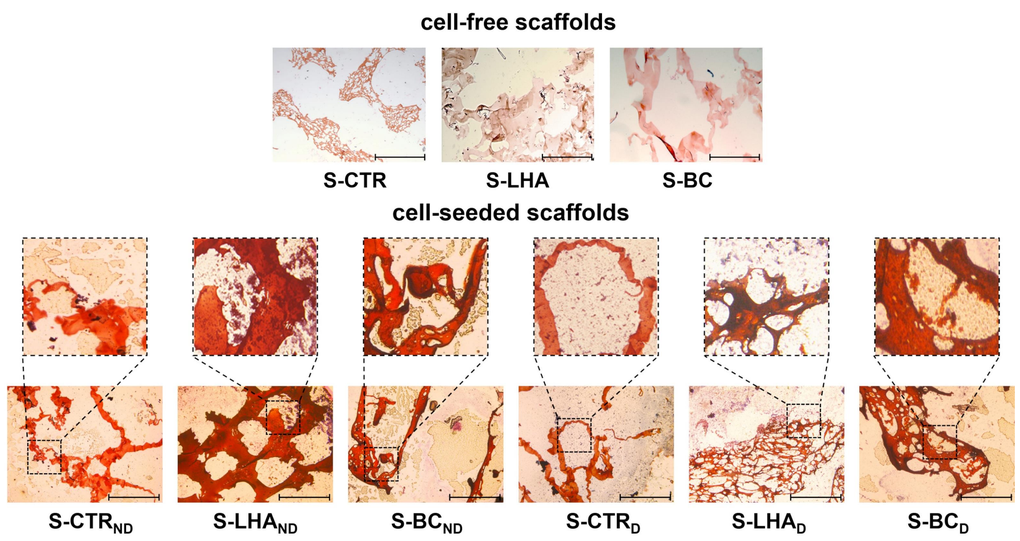
Chondrogenic Differentiation

The heat map shows gene expression profiles related to the chondrocyte phenotype detected by qRT-PCR of cells grown on the scaffolds for 21 days.



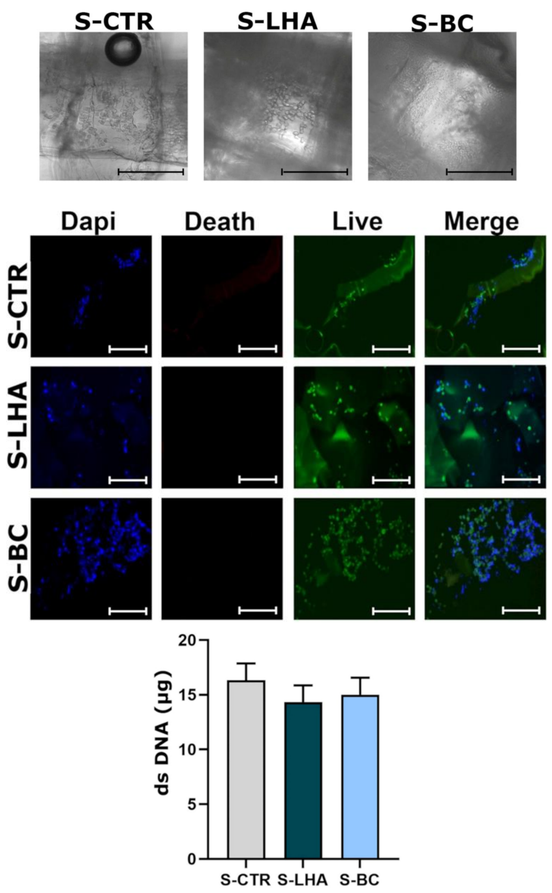
Blue shading indicates gene downregulation, red indicates upregulation, and white represents the baseline expression level ($S\text{-}CTR_{ND}$).

Safranin O staining after 21 days in proliferating (ND) and chondrogenic differentiating (D) medium.



Cell Viability

Cell adhesion and proliferation after 7 days: microscopy, Live/Dead staining, and DNA quantification. mean ± SD, n=3.



No statistically significant differences in hMSC proliferation among the samples.
The scaffolds provided a comparable and favourable environment for cell growth.

REFERENCES

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CONCLUSIONS

Development of novel 3D-printable bioinks for cartilage engineering, incorporating fermentation-derived GAGs to enhance regeneration while reducing the ethical impact of animal sourcing, achieving high structural fidelity to CAD designs, and demonstrating strong biocompatibility with effective promotion of chondrogenesis.