Adnina Rudaiba-MS Thesis Defense

Digital Light Processing (DLP) 3d bioprinting of engineered *E. coli* MJK2 for in situ biomineralization in PEGDA hydrogels

Conventional manufacturing of rigid materials such as cement and ceramics demands high-temperature, energy-intensive processing that contributes significantly to global carbon emissions while offering limited flexibility for complex internal architectures. One alternative is to embed microorganisms within 3D-printed hydrogel scaffolds to enable post-print mineralization under ambient conditions. This study explores this concept by combining digital light processing (DLP) bioprinting with microbially induced calcium carbonate precipitation to create biomineralized engineered living materials. Engineered ureolytic *Escherichia coli* MJK2 was immobilized within photocurable hydrogels composed of poly (ethylene glycol) diacrylate (PEGDA), lithium phenyl-2,4,6-trimethylbenzoylphosphinate and tartrazine, and evaluated for compatibility with DLP processing, post-print survival, and biomineralization behavior and compatibility with complex printed architectures. Cells remained viable following resin exposure and were recoverable from printed constructs for at least seven days across all tested conditions (~10^8–10^9 CFU/mL). Notably, *E. coli* MJK2 outperformed the conventional biomineralizing organism *Sporosarcina pasteurii* under identical photopolymerization stresses, establishing it as the more print-compatible microbial system. Although mineralizing environments reduced viability relative to growth-only conditions, cells retained sufficient metabolic activity to drive biomineralization. Confocal imaging showed mineral formation initiating near hydrogel surfaces and progressing inward over 240 h, indicating transport-limited mineral growth. X-ray Diffraction confirmed crystalline vaterite specifically in cell-containing samples, while thermogravimetric analysis and scanning electron microscopy further supported biomineral formation through increased mineral-associated residual mass and observation of mineral deposits embedded within the printed hydrogel matrix. Finally, complex gyroid architecture remained structurally intact and supported biomineralization within 72 hours of post-print incubation. Together, these findings establish a proof-of-concept for engineered living materials that begin soft and printable, then stiffen biologically, merging geometric design freedom with ambient-temperature mineral formation for applications in sustainable manufacturing and adaptive material systems.

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