Ion-Induced Changes in Regenerated B. Mori Silk Fibroin Self-Assembly

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Silk fibroin is a sustainable biopolymer with extraordinary mechanical properties derived from its hierarchical self-assembly into ß-sheet nanostructures. Biological silk spinning leverages a pH gradient alongside lyotropic salts to induce Liquid-Liquid Phase Separation (LLPS); however, the independent contributions of Hofmeister series ion effects and pH on assembly remain unresolved. This study decoupled lyotropic ion effects from pH by treating 10% regenerated B. mori silk fibroin solutions with salts of varying kosmo- and chaotropicity, benchmarked against zwitterionic MES buffer controls at matched pH values. Spectrophotometric turbidity measurements at 550nm revealed that kosmotropic anions (PO4^3-, SO4^2-, CO3^2-) substantially promoted assembly while chaotropic anions (SCN^-, NO3^-) inhibited ß-sheet crystallization. Pearson correlation analysis confirmed a strong relationship between ion concentration and turbidity ($r=0.93$, $p<0.01$), with no significant association between pH and turbidity ($r=0.23$, $p>0.05$) across tested concentrations. NaCl and KCl solutions exhibited significantly greater turbidity than pH-matched MES buffer controls ($p<0.05$), isolating ion-specific assembly effects from pH-driven ones. Confocal microscopy with Texas Red-IgG immunofluorescence staining validated the highly tunable filament microstructure of the resulting silk matrices. These results establish that lyotropic ion selection, independent of pH, governs silk fibroin self-assembly, enabling physiological-condition processing for targeted drug delivery and cell-compatible bioprinting applications.

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