Title: *In vitro* analysis of matrix-filler interactions in Gellan Gum/Bioactive Glass hydrogels

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Abstract
3D cell culture, for disease modeling as an example, requires complex bioactive cell culture mimicking the target tissue. Here we aim to study the chemical and physical interactions between Gellan Gum (GG) and Bioactive Glass (BAG) to optimize the design and fabrication of this composite biomaterial. We investigate how varying BAG content (10–50 wt%) and the interactions between BAG and GG affects the composite's microstructural and mechanical properties, gelation kinetics, and degradation behavior. Using the calcination tests and X-ray microcomputed tomography (μCT), we assess the loading and distribution of BAG within GG matrix. Interestingly, our findings reveal that compressive strength peaks at 20–30 wt% BAG loading due to competition for crosslinking sites, rather than at maximum loading. Additionally, *in vitro* experiments show that the chemical interactions between the glass and the hydrogel inhibit enzymatic degradation. Ions released from the glass, in both PBS and SBF, led to the precipitation of hydroxyapatite, indicating bioactivity.
We also explore the effects of pH and preparation temperature on gelation kinetics, elasticity, stiffness, and viscosity, continuously monitoring these parameters during the gelation of GG/BAG. Gelation kinetics were assessed through tube tilt and rheological time sweep tests.

In conclusion, tailoring the BAG loading and understanding the chemical interactions with GG enhances mechanical properties and bioactivity while demonstrating resistance to enzymatic attack. These advancements make the composite promising for 3D cell culture and organ-on-chip applications, with ongoing investigations into the impact of pH and temperature on material properties.