Gelatins are widely used in the biomedical and tissue engineering fields, due to their bio-compatibility, biodegradability and cell-interactivity. Gelatin can be chemically crosslinked to originate hydrogel constructs that are stable at body temperature. However, to achieve predictable hydrogel strengths several important factors need to be considered. Starting with consistent production, to ensure consistent molecular weight (MW) and degree of modification (DoM) levels. Next, the resin formulation needs to be on point; the interplay of (bio)polymer concentration, salt concentration, photo-initiator (PI) type and concentration, are crucially important. Because gelatin is thermo-responsive, temperature control in the pre-phase and during hydrogel production, is essential. The interplay is diverse, but mappable and insightful decisions can be made early in the research phase.

### MATERIALS AND METHODS

Four GelMA types (90p40; 90p60; 160p60; 160p80) show that the hydrogel strength, as represented by the storage modulus (G'), is greatly affected by the temperature at which the GelMA is being cured into a biopolymeric network, e.g., the GelMA-hydrogel. The crosslinking rates too are affected by temperature, e.g., faster rates at decreased temperature (3°C). In this example, the 4°C, 21°C and 37°C temperatures might be most interesting to consider in the biological context. Typically, hydrogel preparatory work is performed at room temperature (21°C), and bioprinting has been reported in a wide range of temperatures across this spectrum (cells are already present in the bio-ink formulation, e.g., VAT-based or extrusion-based printing). Furthermore, regarding volumetric printing, in which the GelMA is rapidly cooled to induce physical gelation, the curing kinetics and hydrogel are further affected by the temperature (4°C). The crosslinking rate increases substantially and GelMA molecular weight distributions within the network will affect the G' (bottom panel). Moreover, at 4°C, the synergy phenomenon will play a dominant role in hydrogel formation. Syneresis is the spontaneous shrinkage of the hydrogel and resulting expulsion of liquid from the (bio)polymer network (i.e., GelMA hydrogel).

In conclusion, the temperature at which photo-crosslinking is performed can cause a 95% difference in hydrogel strength depending on the temperature in the preparatory phase, the concentrations and types of PI, the MW, the DoM, and GelMA concentration. It is important to understand that these hydrogels will induce dissimilar cellular responses, as hydrogen strength and stiffness are affected by the temperature during the curing process.

With consistent GelMA molar mass and degree of modification, hydrogel strength becomes predictable. However, the applied protocols greatly affect hydrogel outcomes, as demonstrated herein through the temperatures applied in the preparation and the photo-curing phases. In short, the interplay between the various components of a GelMA resin is diverse, but it is mappable and hence insightful decisions can be made, greatly improving reproducibility and results for biomedical scientists.

### RESULTS

The photo-rheological data, presented in the top 4 panels (from left to right: 90p40, 90p60, 160p60 and 160p80) show that the hydrogel strength, as represented by the storage modulus (G'), is greatly affected by the temperature at which the GelMA is being cured into a biopolymeric network, e.g., the GelMA-hydrogel. The crosslinking rates too are affected by temperature, e.g., faster rates at decreased temperature (3°C). In this example, the 4°C, 21°C and 37°C temperatures might be most interesting to consider in the biological context. Typically, hydrogel preparatory work is performed at room temperature (21°C), and bioprinting has been reported in a wide range of temperatures across this spectrum (cells are already present in the bio-ink formulation, e.g., VAT-based or extrusion-based printing).

In conclusion, the temperature at which photo-crosslinking is performed can cause a 95% difference in hydrogel strength depending on the temperature in the preparatory phase, the concentrations and types of PI, the MW, the DoM, and GelMA concentration. It is important to understand that these hydrogels will induce dissimilar cellular responses, as hydrogen strength and stiffness are affected by the temperature during the curing process.

With consistent GelMA molar mass and degree of modification, hydrogel strength becomes predictable. However, the applied protocols greatly affect hydrogel outcomes, as demonstrated herein through the temperatures applied in the preparation and the photo-curing phases. In short, the interplay between the various components of a GelMA resin is diverse, but it is mappable and hence insightful decisions can be made, greatly improving reproducibility and results for biomedical scientists.