**Statement of Purpose:** Glioblastoma multiforme (GBM) is the most prevalent malignant brain tumor with dismal patient outcome. To advance treatment options for GBM, there is a critical need to develop physiologically relevant GBM disease models to elucidate GBM biology and for drug screening. Using hydrogels as 3D tumor models, previous work has shown stiffness and degradation of the tumor niche to be important modulators of GBM progression. The brain tissue is viscoelastic and exhibits stress relaxation. However, most hydrogels used for modeling GBM are elastic. Using physically crosslinked alginate hydrogels as a model, recent studies have shown breast cancer phenotype is modulated by viscoelasticity. However, how viscoelasticity modulates GBM fate and drug response in 3D remains largely unknown. The goal of this study is to develop PEG-based adaptable hydrogels with tunable stress relaxation for growing GBM cells in 3D, thus uncovering the role of viscoelasticity on GBM fates and drug responses. Outcomes analyses include GBM cell spreading, migration, phenotype, and drug response.

**Methods:** Hydrogels with tunable stress relaxation (fast, medium, and slow) were fabricated using hydrazone-crosslinked PEG. Specifically, 8-arm PEG (10k MW) was functionalized with aliphatic hydrazine, which react with PEG functionalized with either aliphatic aldehyde (aHz) or benzaldehyde (bHz). Collagen-I was also incorporated to provide cell adhesion. Rheological testing was performed to characterize stress relaxation. A patient-derived GBM xenograft cell line (D270 MG) was used for all studies. Western blot was done on samples at day 3 for GBM marker characterization. Confocal microscopy was used to assess cell morphology at day 3, and live-cell imaging was performed to track cell motility. Imaris Bitplane surface analysis was used to calculate the probability of cell migration. Drug response studies were conducted with drug treatment with chemotherapeutic temozolomide (TMZ) for 3 days after encapsulation, followed by drug screening that would be missed using elastic hydrogels. Drug response was measured using PrestoBlue cell viability assay.

**Results:** Bulk rheology testing confirms PEG-based adaptable hydrogels exhibit tunable stress relaxation (Fig. 1A,B), with fast group exhibiting a stress relaxation profile similar to that of mouse brain (positive control) (Fig. 1A,B). All hydrogels exhibit comparable stiffness (~ 1 kPa) that is similar to brain. Increasing stress relaxation led to higher expression of Nestin and glial fibrillary acidic protein (GFAP) (Fig. 1C), two markers associated with higher invasive phenotype of GBM and poor patient outcome. Further, GBM cells in the fast stress relaxation group displayed robust cell spreading; whereas, decreased cell spreading was observed in hydrogels with slow stress relaxation (Fig 1D). Live-cell imaging further shows high GBM cell migration in fast gels, and reducing stress relaxation led to significantly less cell migration (Fig. 1E). Finally, GBM cells exhibit enhanced drug sensitivity to TMZ with increased stress relaxation (Fig 1F). We further showed that stress relaxation-induced changes in GBM response are modulated through cytoskeleton and requires TRPV4 expression (data not shown).

**Conclusion:** Here we report a PEG-based 3D GBM model with tunable stress relaxation. We demonstrate that viscoelasticity plays a critical role in promoting GBM cell spreading, migration, and expression of GBM related markers associated with invasion and poor patient prognosis. Furthermore, our 3D in vitro model enables interrogation of the specific mechanisms that may be at play in how GBM cells respond to stress relaxation that cannot be studied in animal models. Finally, GBM cells in hydrogels with brain-mimicking stress relaxation (fast gels) exhibited increased drug sensitivity, highlighting the importance of using gels with viscoelasticity for GBM drug screening that would be missed using elastic hydrogels.

**References:**

**Acknowledgements:** Thank you to the following funding resources including Stanford Child Health Research Institute Faculty Scholar Award (F.Y.), Bio-X seed grant (F.Y.), NIH Biotechnology training grant (S.S), NIH F31 fellowship (S.S), SIGF fellowship (M.A.) and NSF predoctoral fellowship (S.J.).