**Substrate Stiffness Affects Cell Responsiveness to Cytotoxic Compounds**

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**Statement of Purpose:** Prompted by high costs and failures during clinical trials, a need for predictive *in vitro* drug screening platforms has emerged. Biomaterials hold great potential to address this immediate need by providing more *in vivo*-like cell microenvironment. Indeed, current research suggests that cells respond differently to cytotoxic compounds when seeded into a biomaterial scaffold as opposed to a standard tissue culture polystyrene (TCP), yet the underlying reasons are largely unknown. The focus of our work is on understanding the effect of substrate stiffness, both in a two-dimensional (2D) and three-dimensional (3D) context, on cells' response to cytotoxic compounds. Our hypothesis is that biomaterial stiffness, which greatly affects overall cell fate, would also affect cell responsiveness to cytotoxic compounds. This work establishes the basis towards our overall goal in determining if and when physiologically-relevant stiffness would be a prerequisite for improving the predictive capacity of drug screening platforms.

**Methods:** To test cell responsiveness to drugs on 2D, we developed a 96-well plate stiffness assay that employed polyacrylamide (PAA) gels of 1, 10, and 100 kPa Young's modulus (encompassing the range for most soft tissues), coated with collagen Type I to elicit cell attachment. Cells were seeded for 24 h, prior to 72 h of drug exposure. Cell viability was measured with an MTS metabolic activity assay. $IC_{50}$ (50% inhibitor concentration) was calculated using a 4-paramter Hill equation from dose-response curves. Ten different cell lines were screened. To test cell responsiveness to drugs in 3D, the cells were seeded in alginate gels. The stiffness of alginate gels in particular was varied by changing alginate concentration, 0.25% alginate corresponded to ~0.9 kPa and 1% to ~9.0 kPa Young's modulus. Drug screening was performed as described above. TCP or collagen-coated TCP were used as controls. For all conditions, cell proliferation and cell spreading area were also determined. Cell spreading was measured using microscopy cell images. Gel stiffness was measured via atomic force microscopy and rheology.

**Results:** Using our newly-developed 2D PAA-based stiffness assay[1], we performed a small scale screen of 10 human cancer cell lines. Most cell lines exhibited higher proliferation and larger spreading area on the stiff 100 kPa PAA gels as compared to the soft 1 kPa gels. However, when the same cells were treated with the chemotherapeutic paclitaxel, a microtubule stabilizing agent, only half of the cell types showed differential responses on soft versus stiff substrates. Moreover, as opposed to the clear unidirectional trend for both proliferation and cell spreading in response to stiffness, drug responses were cell type-dependent: two cell lines had higher $IC_{50}$ on soft substrates, three cell lines on stiff substrates, and five cell lines had $IC_{50}$ independent of stiffness (Figure 1).

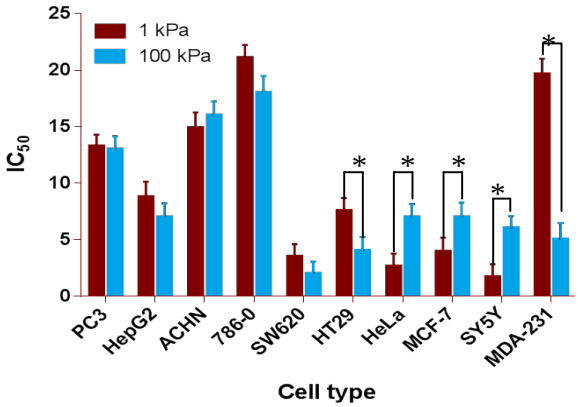


Figure 1. Cells respond differentially to paclitaxel when seeded on gels of varying stiffness. Cancer cell lines: PC3 – prostate adenocarcinoma; ACHN and 786-0 – renal cell adenocarcinoma; HT29 and SW620 – colorectal adenocarcinoma; HeLa – cervical adenocarcinoma; MCF-7 and MDA-MB-231 – mammary gland adenocarcinoma; SY5Y neuroblastoma; and HepG2 – hepatocellular liver adenocarcinoma. Asterisks designate significant differences ($p$<0.05).

To understand the role of 3D gel stiffness, neural glioblastoma U-87 cells were encapsulated in alginate gels of 0.9 and 9 kPa stiffness and treated with four different cytotoxic compounds. While the cells exhibited the highest $IC_{50}$ on the TCP control for all compounds, the encapsulated cells also had higher $IC_{50}$ in the stiff as opposed to the soft gels. Through the further use of constitutively active mutant RhoA U-87 cells (CA RhoA is a strong activator of actomyosin contractility and stress fiber formation), we established that increasing RhoA activity, enhances the cell tolerance to the cytotoxic compounds on the soft gels. As a result, no difference in $IC_{50}$ towards acrylamide and quinidine toxins between soft and stiff gels was observed for the CA RhoA U-87 cells, implying that mechanotransduction might play a role in drug resistance in the stiffer gel environment.

**Conclusions:** In conclusion, our results demonstrate that stiffness affects cell responsiveness to cytotoxic compounds both in 2D and 3D microenvironments. We also showed that stiffness-dependent drug responses are cell-specific and, at least in the case of 3D, dependent on RhoA GTPase activity. While more targeted drug screening, including cells of various origins as well as a larger library of compounds is due, our preliminary data suggests that targeting physiological stiffness might be an important parameter in the development of more predictive drug screening platforms.

**References:** [1]Zustiak SP. Biotech&Bioeng. 2013; In Press.