Development of a Compliant Cell Culture System for Improved Correlation Between *in vivo* and *in vitro* Testing

James McMasters, Jamie Brugnano, Alyssa Panitch.
Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN

Statement of Purpose: Our lab has designed and reported on a class of cell penetrating peptides that inhibit a kinase important in inflammation, mitogen-activated protein kinase activated protein kinase 2 (MK2). The MK2 inhibitor, YARAAARQARAKALARQLGVAA (YARA) has been shown to be effective at inhibiting inflammation in both *in vitro* cell culture models and *in vivo* animal studies [1]. However, *in vivo* data also indicated that the concentration required for efficacy was 1-10% of what was required for *in vitro* models. Additionally, animal data suggested that a single dose of YARA had a long-term effect, with inflammation being inhibited for up to a week after dosing [2]. We hypothesized that the differences in effective dose from *in vitro* and *in vivo* models was due to the stiffness of the cellular substrate, as tissue culture plastic is significantly stiffer than soft tissue [3]. Thus, we used polyacrylamide (PA) gels to develop a more biologically relevant cell culture substrate to test the effect of stiffness on MK2 inhibitor peptide efficacy, and allow for better prediction of the results obtained with *in vivo* animal models. In addition, we examined the therapeutic time course for a single dose of YARA to determine if a more biologically relevant substrate stiffness could replicate the long-term response observed *in vivo*.

Methods: PA gel were made from a protocol modified from Tse and Egler [4]. 18 mm glass coverslips were etched with 0.1 N sodium hydroxide at 60°C overnight. Coverslips were reacted with (3-aminopropyl)triethoxysilane and crosslinked with 0.5% gluteraldehyde. 10% PA gels were crosslinked with 0.01-1.0% bis acrylamide (bis) were formed on the coverslips under a nitrogen tent. Finally, the substrates were rinsed and incubated with 0.14 mg/mL fibronectin at 4°C. To determine the mechanical properties of the gels, frequency and stress sweeps were performed using an ARG2 rheometer (TA instruments, New Castle, DE). To determine the functional timecourse of a single dose of YARA, human pleural mesothelial cells (80,000 cells/well) were cultured on either tissue culture plastic or substrates for 120 hours. Cells were then treated with 1 ng/mL IL-1β and varying concentrations of YARA. After treatment, supernatant was collected at various time points and the concentration of TNF-α and IL-6 were measured with a MSD multispot cytokine assay (Meso Scale Discovery, Rockville, MD).

Results: Depending on the amount of bis, the PA substrates had a storage modulus that varied between 2.5 kPa and 25 kPa. We selected the 0.3% bis gels as our substrate, as they showed a storage modulus of 4kPa, which is similar to the stiffness of soft biological tissue [3]. Results of the cytokine assay show that cells cultured on tissue culture plastic produce significantly higher levels of IL-6. In addition, both 30-minute and 6-hour treatment times were shown to be effective on tissue culture plastic, whereas the soft substrates only showed efficacy in the 30-minute YARA exposure. In addition, compared to TNF-α, IL-6 was shown to be a better reporter of inflammatory inhibition in cells cultured on either surface (data not shown).

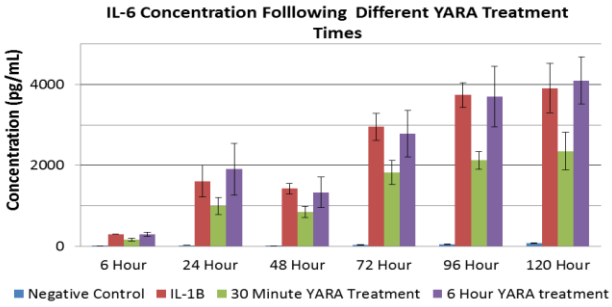

Figure 1. IL-6 concentrations from cells cultured on PA substrates and treated with IL-1β and 100 μM YARA for 30 minutes and 6 hours

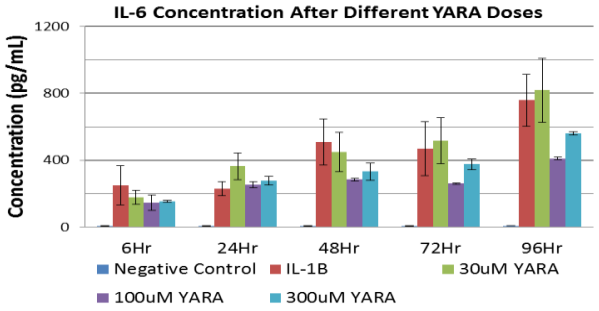

Figure 2. IL-6 concentrations from cells cultured on PA substrates and treated with IL-1β and 30, 100, 300 μM YARA for 30 minutes

As shown in Figure 1, Measurements of IL-6 concentration show that a single 30 minute dose of 100 μM YARA retains its therapeutic effectiveness for over 120 hours. Conversely, figure 2 shows that a 300 μM dose begins to lose effectiveness after 48 hours, and a 30 μM dose is ineffective after 6 hours.

Conclusions: We have successfully used a soft PA substrate that has a modulus much lower than tissue culture plastic, and is better able to better mimic the stiffness that cells would experience *in vivo*. The importance of more closely mimicking the *in vivo* stiffness is demonstrated by the different IL-6 levels that were observed in cells cultured on tissue culture plastic versus our soft substrates, with the soft substrates showing lower levels of IL-6 production, and showing inhibition with shorter exposure to YARA. Using the soft substrates, we were also able to demonstrate that a single 100 μM dose of YARA retains therapeutic effectiveness for over 120 hours. We have found the soft substrates to provide a better model of *in vivo* cellular response, and should provide a more accurate prediction of cellular response during drug screening, which will reduce the costs associated with drug screening and development.

References: 1. Brugnano, J. J Control Release, 2011; 155: 128-133. **2.** Muto, A. et al. Vasc Pharmacol, 2012; 56: 47-55. **3.** Engler, A.J. et al. Cell, 2006; 126: 667-689 **4.** Tse, J.R. et al, Curr Protoc Cell Biol, 2010; unit 10 16