Supplementary Information

for

A Ready-to-Use, Versatile, Multiplex-able Three-Dimensional Scaffold-Based Immunoassay Chip For High Throughput Hepatotoxicity Evaluation

Xiaojun Yan[a], Jingyu Wang[a], Lu Zhu[a], Jonathan Joseph Lowrey[d], Yuanyuan Zhang[e], Wei Hou[c], Jiahong Dong[b] and Yanan Du*[a]

[a]Department of Biomedical Engineering, School of Medicine, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Tsinghua University, Beijing 100084, China

[b]Department of Hepatopancreatobiliary Surgery, Medical Center of Hepato-Pancreato-Biliary, Beijing Tsinghua Changgung Hospital, Affiliated Hospital of Tsinghua University, Beijing 102218, China

[c]Tianjin Second People's Hospital and Tianjin Institute of Hepatology, Tianjin 300192, China

[d]Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, Maryland, MD 21205, USA

[e]Chemistry Department, The State University of New York at Stony Brook, Stony Brook, New York, NY 11794-3400, USA

Prof. Y. N. D. Corresponding Author

School of Medicine, Tsinghua University, Beijing 100084, China

E-mail: (duyanan@tsinghua.edu.cn)

PEGDA Synthesis

Dry PEG4000 was acrylated with acryloyl chloride (TCI, Japan) and triethyl amine, TEA (Acros) in anhydrous dichloromethane under inert condition for 16-24 h at 1:6 PEG4000:acryloyl chloride and 1:3 PEG4000:TEA molar ratios. Resulting solution was concentrated by vacuum pumping to half the volume and washed with 2 M K_2CO_3. It was allowed to separate into aqueous and organic phases before precipitating PEGDA, in the organic phase, in diethyl ether. PEGDA was then dissolved in water, lyophilized and stored as fine powder at -30^{o}C.

Buffer Recipes

Washing buffer: 50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0
Blocking buffer: 50 mM Tris, 0.14 M NaCl, 5% BSA, pH 8.0
1%-BSA dilution buffer: 50 mM Tris, 0.14 M NaCl, 1% BSA, 0.05% Tween 20, pH 8.0
Protectant buffer: 5mM Tris, ~43 µM sucrose (for 10 µg/mL antibody of 140 kDa molecular weight), pH 7.0

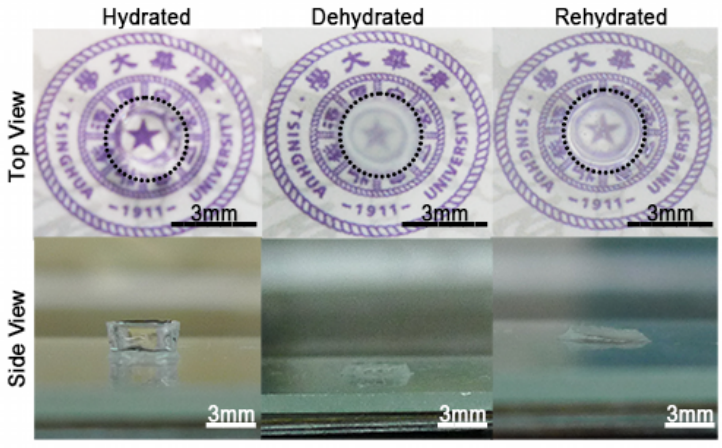

Fig. S1. A hydrogel in 4 mm diameter and 1.5 mm height is highly transparent, allowing clear text to show through. The hydrogel loses its transparency and height when dehydrated and these properties cannot be well restored even when rehydrated.

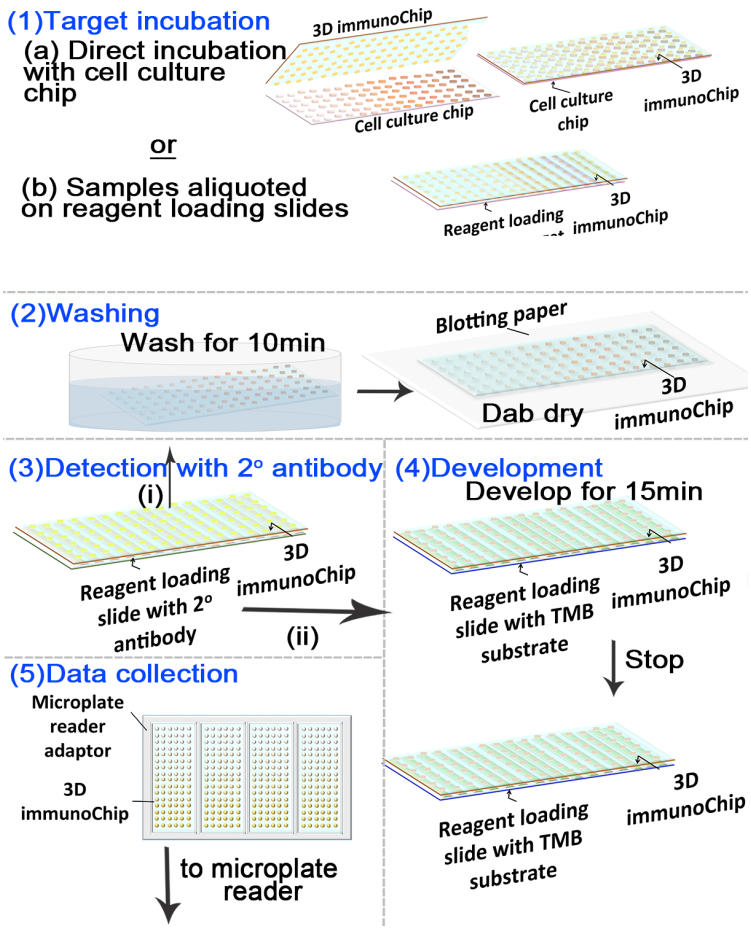

Fig. S2. Schematic of assay protocol using 3D immunoChip. Samples can be auto-loaded onto 3D immunoChip by direct insertion into reservoir wells of 3D cell culture chip or incubation with reagent loading slide (1). Unbound molecules are then washed off by submerging in a dish of fresh washing buffer for 3 times, 10 min each (2). ImmunoChip is then dabbed dry on blotting paper (2) before incubation with secondary antibody (3). After washing off unbound secondary antibody and dabbing dry, immunoChip is incubated with TMB substrate for colour development and stopped after 15 min (4). Finally, 3D immunoChip is slotted into a microplate reader adapter for data recording in microplate reader (5).

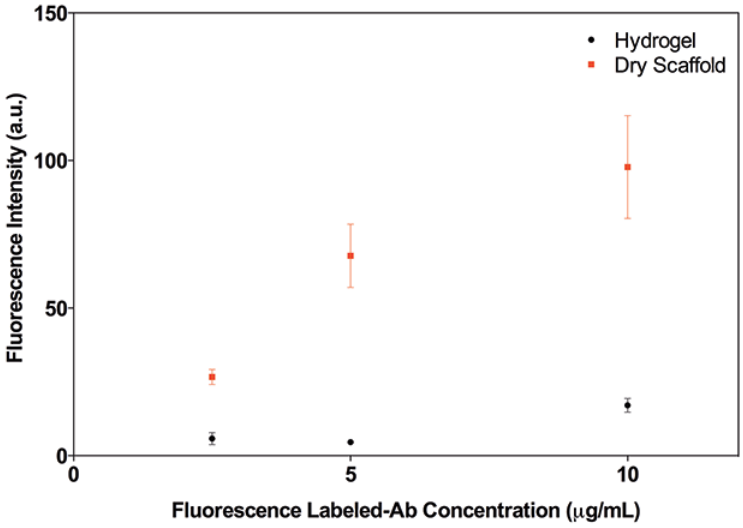

Fig. S3. Lesser antibodies are conjugated to hydrogel than dry scaffold within the same reaction time. This can be attributed to higher mass transfer resistance from, and sample dilution by, the large volume of water present in hydrogel.

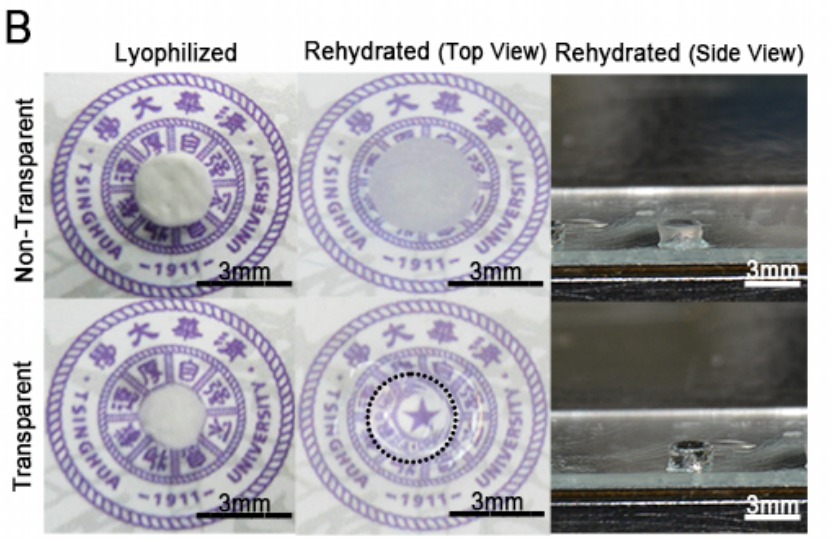

Fig. S4. Chemically cross-linked scaffolds made with or without glycerol mediation (i.e. transparent and non-transparent). Transparent scaffold is able to regain transparency upon rehydration, but not non-transparent scaffold.

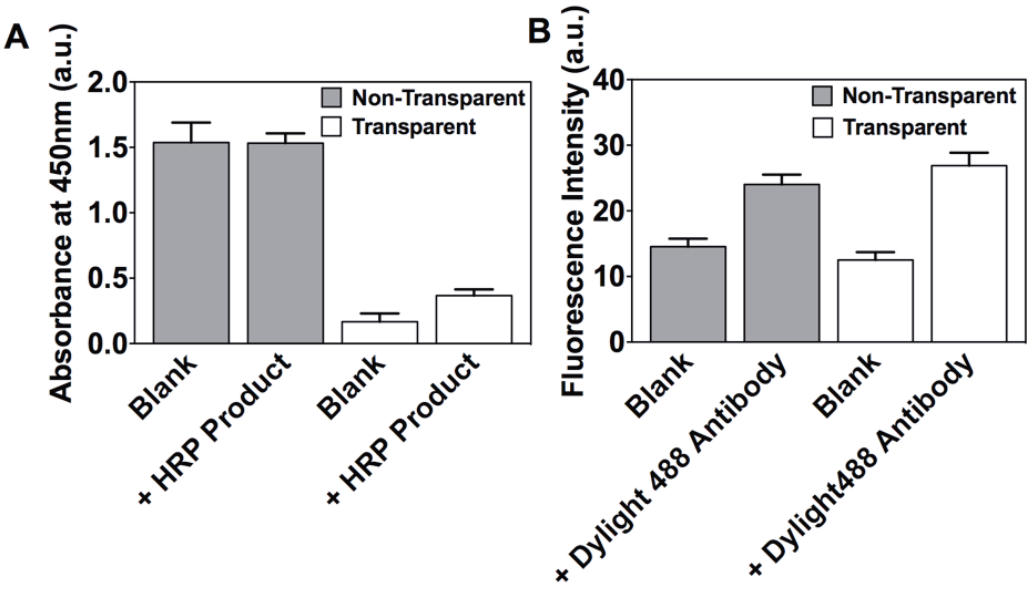

Fig. S5. Absorbance at 450 nm (A) and fluorescence intensity (B) for blank T and NT scaffolds and corresponding scaffolds absorbed with HRP converted TMB product and fluorescence-labeled antibody.

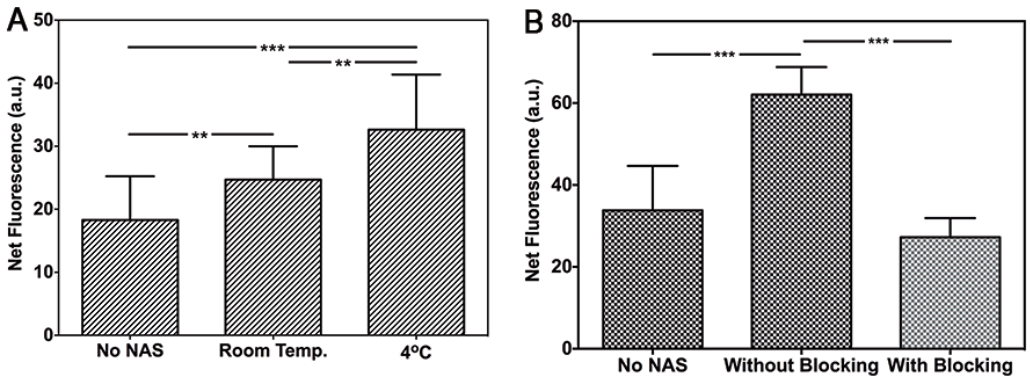

Fig. S6. NAS hydrolysis was reduced when hydrogel array chip was washed in cold water (A). Blocking of immunoChip with 5% BSA effectively passivates remaining NAS (B) as lesser fluorescence-labeled antibody were able to bind to scaffolds that are blocked. The difference between scaffold without NAS and scaffold with NAS blocked with 5% BSA is insignificant.

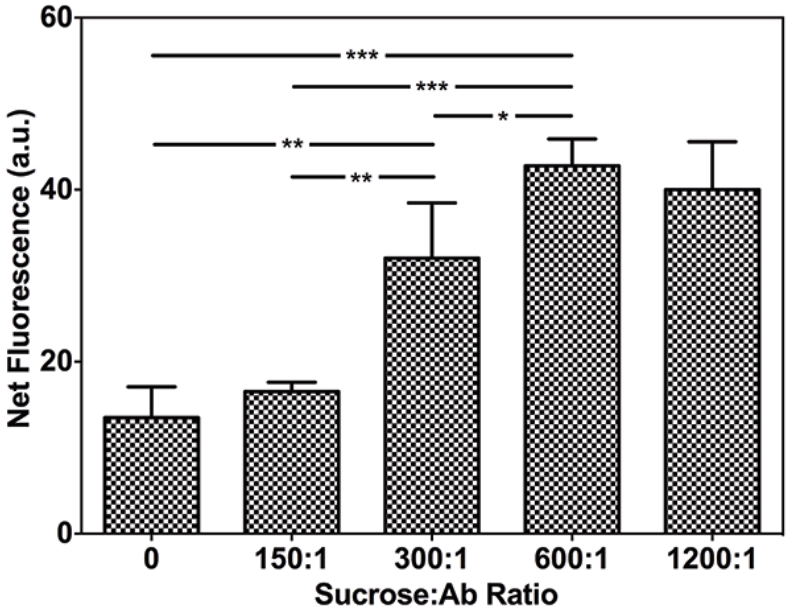

Fig. S7. Sucrose is a substitute for water molecules in hydrogen bonding for protein structure maintenance. Sucrose supplemented at 600 molecules to 1 molecule of antibody is sufficient to maintain rabbit-anti-goat IgG activity as they captured the most Dylight488 conjugated goat IgG.

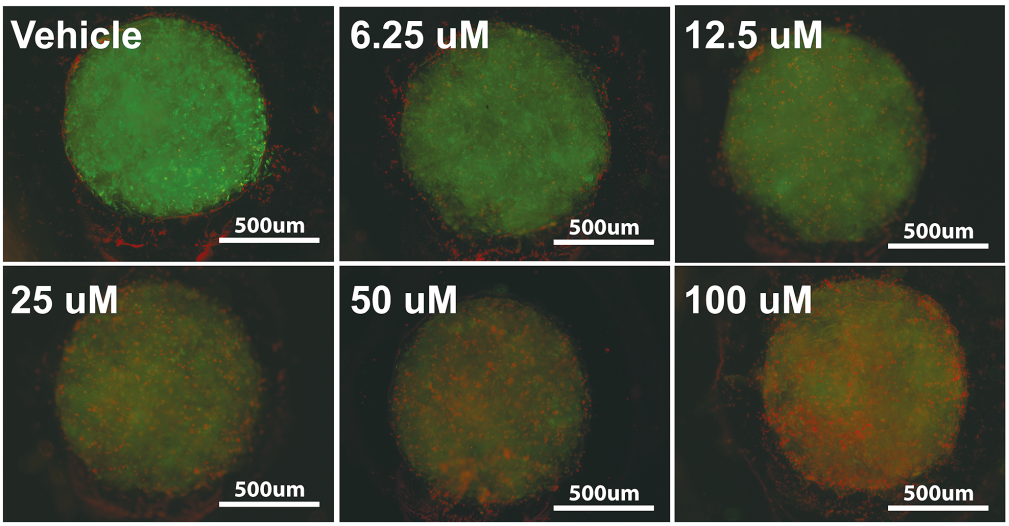

Fig. S8. Live/dead images of HepaRG cells in 3D cell culture chip after been treated with different concentrations of DOX for 24 h.

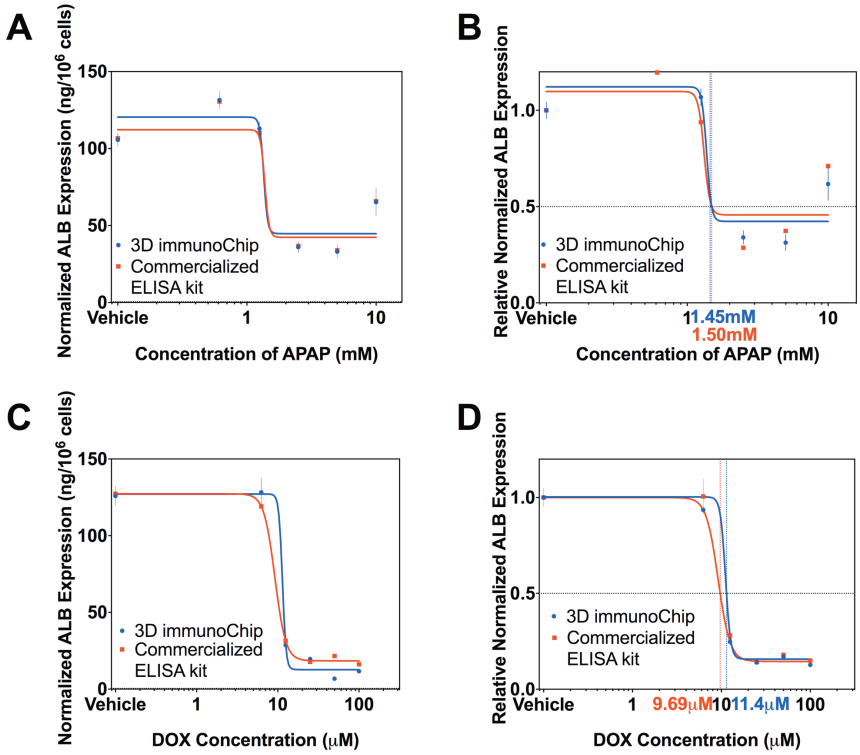

Fig. S9. Drug hepatotoxicity evaluation based on normalized ALB expression. (A) and (C) are ALB expression in ng normalized to 10^6 HepaRG cells after treatment with different concentrations of APAP and DOX respectively. (B) and (D) plot relative normalized ALB expressions for treatment at different concentrations of APAP and DOX respectively. Relative normalized ALB expression is defined as normalized ALB production by cells treated with drugs as a fraction of normalized ALB production by

cells treated with vehicle only, where IC50 is the drug concentration at which relative normalized ALB expression is 0.5. All four graphs show data obtained from 3D immunoChip (blue) and commercialized ELISA kit (red).