Incorporating 3D Bioprinting with the Alginate Immobilization of Metabolic Enzymes Platform to Retrofit *In Vitro* Assays with Metabolic Competence

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Introduction

- Most *in vitro* systems lack the biotransformation capabilities of intact *in vivo* systems, which raises the possibility of under- or overestimating the hazard of compounds that are rapidly transformed to more or less active metabolites.
- The Alginate Immobilization of Metabolic Enzymes (AIME) method retrofits bioassays with phase I metabolic competence using rat hepatic S9 fraction with minimal cytotoxic and assay interference effects compared to free S9 assays.
- The current AIME platform consists of custom 384-well microplate peg lids with encapsulated S9-alginate microspheres attached to solid supports:

Stamp lid in Matrigel | **Stamp in S9-alginate** | **Stamp in $BaCl_2$** | **Stamp in PLL** | **Ready for assay**

- Accessibility of the lid-based AIME platform is limited by proprietary custom materials and a complex workflow, so we aim to adapt it to a 3D bioprinter.

Objectives

- Address operational limitations of the lid-based AIME method with 3D bioprinting
- Incorporate phase I (cytochrome P450 = CYP) and II (UGT, GST, SULT) hepatic metabolism through the addition of requisite cofactors:
  - I: Reduced nicotinamide adenine dinucleotide phosphate (NADPH)
  - II Uridine 5'-diphosphoglucuronic acid (UDPGA)
  - II: Glutathione (GSH)
  - II: Adenosine 3'-phosphate 5'-phosphosulfate (PAPS}

Methods

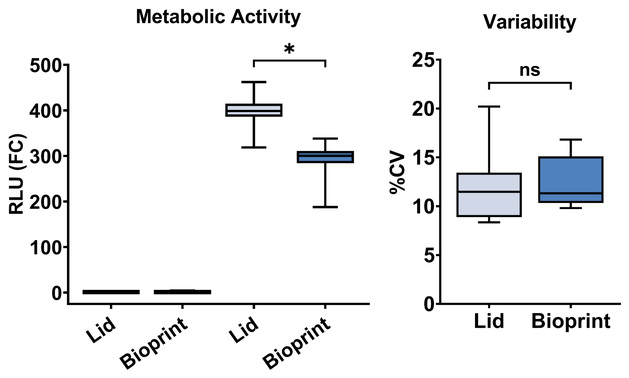
Compare lid-based & bioprinter methods using a common CYP enzyme

Verify common CYP activity using bioprinter method with requisite cofactors

Measure glucuronidation (UGT) activity with bioprinter method & cofactors

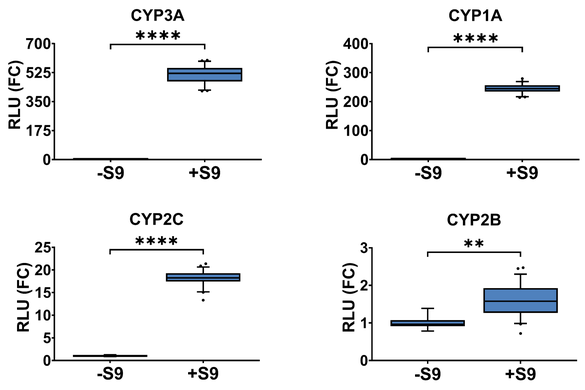
Key Findings

AIME Method Comparison



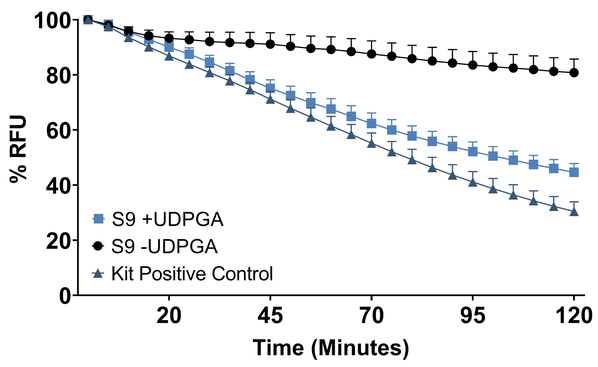
- Promega P450-Glo™ Luciferin-IPA was used to measure CYP3A activity with rat S9-alginate using lid-based (Lid) and Bioprinter methods.
- Medium was supplemented with NADPH then conditioned with lid/bioprinted S9-alginate for 2h.
- Treatment activity was higher than -S9 controls (black). The bioprinter method (dark blue) had lower activity than lid (light blue) but comparable precision (n = 4, * = $P < 0.05$, ns = no significance, %CV = coefficient of variation).

Phase I CYP Enzyme Activity



- Promega P450-Glo™ Luciferin substrates were used to measure CYP activity with (+S9) and without (-S9) rat S9-alginate.
- Medium was supplemented with phase I and II cofactors then conditioned with bioprinted S9-alginate for 2h.
- Significant activity was shown for all substrates tested with bioprinted S9-alginate (n = 3-5, **** = $P < 0.0001$, ** = $P < 0.01$).

Phase II Glucuronidation Activity



- BioVision UGT Activity Assay determined activity by tracking the loss of fluorescence as the substrate was glucuronidated.
- Medium supplemented with and without UDPGA was added to bioprinted S9-alginate (S9 ± UDPGA) and fluorescence measured in kinetic mode for 2h at 37°C.
- Glucuronidation increased in a time-dependent manner ($P < 0.0001$, n = 3).

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Print alginate with BIOX | **Crosslink with liquid handler** | **Ready for assay**

Luminogenic CYP Substrates

CYP Substrate	Human CYP	Rat CYP
IPA	CYP3A4	CYP3A1
ME	CYP1A2	CYP1A2
H	CYP2C9	CYP2C6
2B6	CYP2B6	CYP2B1

Conclusions

- The lid-based AIME method was adapted to an automated 3D bioprinter platform.
- The bioprinting method expands the functional capacity for hepatic phase I and phase II metabolic enzymes.
- Proof-of-concept application of this new approach method is the next step toward incorporating metabolic competence into high-throughput *in vitro* assays.

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