

SeedEZ™ Interdigitated Electrodes and Multifunctional Layered Biosensor Composites (MLBCs): A Paradigm Shift in the Development of *In Vitro* BioMicrosystems

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Abstract—We developed a new technology for the realization of composite biosensor systems, capable of measuring electrical and electrophysiological signals from electrogenic cells, using SeedEZ™ 3D cell culture-scaffold material. This represents a paradigm-shift for BioMEMS processing; ‘Biology-Microfabrication’ versus the standard ‘Microfabrication-Biology’ approach. An Interdigitated Electrode (IDE) developed on the 3D cell-scaffold was used to successfully monitor acute cardiomyocyte growth and controlled population decline. We have further characterized processability of the 3D scaffold, demonstrated long-term biocompatibility of the scaffold with various cell lines and developed a multifunctional layered biosensor composites (MLBCs) using SeedEZ™ and other biocompatible substrates for future multilayer sensor integration. [2020-0092]

Index Terms—BioMicrosystems, Cellular Biocompatibility, interdigitated electrodes (IDEs), makerspace microfabrication, multilayer biosensors, SeedEZ™ 3D cell scaffold.

I. INTRODUCTION

CELL culture devices which are microfabricated in a conventional manner, typically use materials which must be functionalized for *in vitro* settings. This becomes necessary due to biocompatibility requirements, and often such fabrication strategies can be categorized into the ‘Microfabrication-

Biology’ line of thought, which treats the biological relevancy of the microfabrication materials as secondary to microfabrication considerations. A strategy traditionally employed in this manner is to treat the substrate material with coatings which tailor the device for their eventual end applications. These coatings in various combinations can serve dual purposes with respect to Microelectrode Arrays (MEAs) and Interdigitated Electrodes (IDEs) assays, where the coating material becomes both the cellular interface and an electrical insulation material [1].

In turn, microfabrication of such devices can become unnecessarily complex, adding several additional steps and increases the production time for the microfabricated devices. Additionally, the durability of these coatings is frequently time-limited leading to toxicity, altered phenotypes, and potentially uncertain biological outcomes [2]. Microfabrication approaches, especially for biosensors, could benefit from a shift in this currently available fabrication paradigm and thought process.

Traditional MEMS devices are typically fabricated in a cleanroom manufacturing environment, which necessitates special training, is cost and time prohibitive and imposes material restrictions. The advent of makerspace environments [3] has meant that more non-traditional materials such as polymers, epoxies, and biomaterials (e.g. porous cell-scaffold materials) and methods (3D Printing, Screen Printing etc.) may be incorporated into novel device approaches especially *in vitro* microsystems and other biological microdevices.

Herein we demonstrate microsensor fabrication on one such cell-scaffold material for *in vitro* electrogenic cell assays, which dramatically alters the microfabrication paradigm to a ‘Biology-Microfabrication’ approach, where the biologically approved material is the starting point, and microfabrication/packaging strategies are tailored around it.

The basis for this platform is a 3D cell culture scaffold: SeedEZ™ (Lena Biosciences, USA) with known cytocompatibility [4] across cell types, including HepaRG hepatocytes (28 days in culture; Figure 1A). The SeedEZ™ material is an inert, transparent, and hydrophilic glass microfiber scaffold which is optimized for long-term cell growth and drug testing [4], [5]. In practical application, the scaffold is thin (~500 μm), and can be handled much like paper. Cell populations that are pipetted onto the scaffold in medium or

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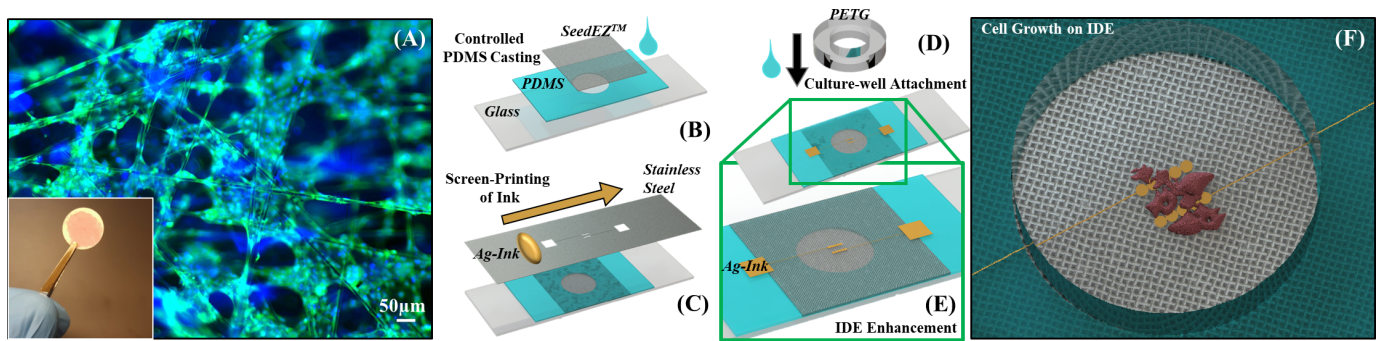


Fig. 1. SeedEZ™-IDE Fabrication Schematic. (A) Fluorescence image of HepaRG cells cultured in the SeedEZ™ scaffold for 28 days. Stains are Calcein AM Green and Hoechst 33342. The inset is a photograph of the SeedEZ™ scaffold seeded with cells. (B-F) Schematic of the SeedEZ™-IDE microfabrication. (B) PDMS was controllably cast over the matrix on a glass slide. (C) Ag-Ink was screen-printed on the matrix using a stencil mask. (D) A culture well was attached and sealed with PDMS. (E) Close-up of (D), highlighting the microelectrodes of the IDE. (F) Further close-up schematically illustrating how cells will grow between the IDE electrodes to change signaling in a culturing environment.

in a sol-state hydrogel are wicked into the scaffold interior (Figure 1A). SeedEZ™ allows cells to utilize both their cadherin (cell-cell) and integrin (cell-extracellular matrix (ECM)) receptors that are vital for tissue-representative cell behavior and drug responses. ECM proteins may be used to coat the scaffold, and cells may also be delivered with sol-state ECM to further promote cell-matrix interactions.

The demonstrated paradigm-shift in this work is essential as future electrogenic on-a-chip models will be required to perform like *in vivo* systems. In this work, IDE microfabrication strategies were developed on this novel cell scaffold and HL-1 rat cardiomyocytes were cultured to demonstrate not only the inherent biocompatibility of the device, but also the IDE biosensor's ability to measure the growth and controlled decline of the cellular population. HL-1 cells were chosen as an excellent demonstration vehicle since they adhere, grow, and reach confluency rapidly when compared to other electrogenic cell lines (needing only 4-5 days on average, depending on culturing density).

Additionally, these cells exhibit spontaneous activity in culture, allowing for passive electrical recordings to be performed [6]. These features made them the ideal cell line for a preliminary growth assay, that demonstrates the capabilities of the SeedEZ™ IDE microsystems. Functional metrics emanating from the BioMicrosystems include a simple impedance spectroscopy assay coupled with a Cell Index (CI) calculation for the assay.

Further to this paradigm-shifting microfabrication approach, we demonstrate the creation of a prototype Multifunctional Layered Biosensor Composites (MLBC), reflecting the potential for integrating 3D cellular-scaffold centrally into novel BioMicrosystems. Although the MLBC application demonstrated here is a "proof-of-concept" device, the microfabrication and packaging strategies lend themselves to large scale integration of biosensors with the potential for these substrates to act as biological Printed Circuit Boards (bPCBs). Additionally, long-term culturing capabilities and metabolism enzymatic studies with the SeedEZ™ scaffold are reported.

II. MATERIALS AND METHODS

A. Processability and SeedEZ™ IDE Impedance Biosensor

IDE microfabrication (Figures 1B-F) was performed first with casting of 10:1 PDMS onto a microscope slide, to affix

and insulate a 25mm by 25mm sheet of the SeedEZ™ scaffold. The construct was then cured at 60°C for 1 hour. An IDE pattern was designed in Solidworks 2019 3D CAD (Dassault Systems, France), using a "circle-in-line" geometry, with a diameter of 500μm, and a finger pitch of 900μm. A deposition mask was laser micromachined using 12.5μm thick Kapton® (DuPont, USA), using the IDE pattern designed using Solidworks (Laser Micromachining conditions - wavelength: 355nm; repetition rate: 50Hz; power: 3.6 mJ; spot size: 25μm). The IDE mask was applied to the cured substrate, and a class-VI biocompatible Ag-Ink (EP3HTSMED; MasterBond®, USA) was screen printed through the laser micromachined mask to approximately 50μm thickness, and subsequently cured at 100°C for 6 hours.

A Polyethylene Terephthalate-Glycol (PETG) culture well, with a diameter of 12mm and height of 10mm, was affixed over the exposed matrix device with 10:1 PDMS and cured at 90°C for 1 hour.

In order to evaluate the solvent and media compatibility of the SeedEZ™ cell scaffold, 25mm by 25mm sheets of the scaffold material were first weighed dry, subsequently hydrated for 12 hours and weighed, and lastly subjected to an evaporation process overnight followed by another mass measurement (average of $N = 3$, results in Figure 2A). Solvents used included: De-Ionized (DI) Water (H_2O), Acetone (C_3H_6O), Isopropanol (C_3H_8O), Ethanol (C_2H_6O), and Fetal Bovine Serum (FBS). Scanning Electron Microscopy (SEM) images were obtained to confirm any relevant changes in the structure of the scaffold material, which would impact its processability. For capillary action characterization, Polydimethylsiloxane (PDMS) (Sylgard-184; Dow Corning, USA) and 353ND two-part epoxy (Epo-Tek®, USA) were mixed in three differing ratios (10:1, 5:1, and 1:1; polymer to curing agent respectively). As a next step ~2.5μg each of the polymer mixtures were applied to one end of a 5mm by 30mm sheet of the SeedEZ™ scaffold ($N = 3$). Each sample was equilibrated for 30 minutes at a temperature of 20 - 25°C, to allow for uniform capillary action. The materials were then cured at 60°C overnight to achieve the final composite material properties. The controlled capillary action was subsequently measured by identifying the length and breadth of the material (PDMS and 353ND) penetration into the cell scaffold (results in Figure 2B).

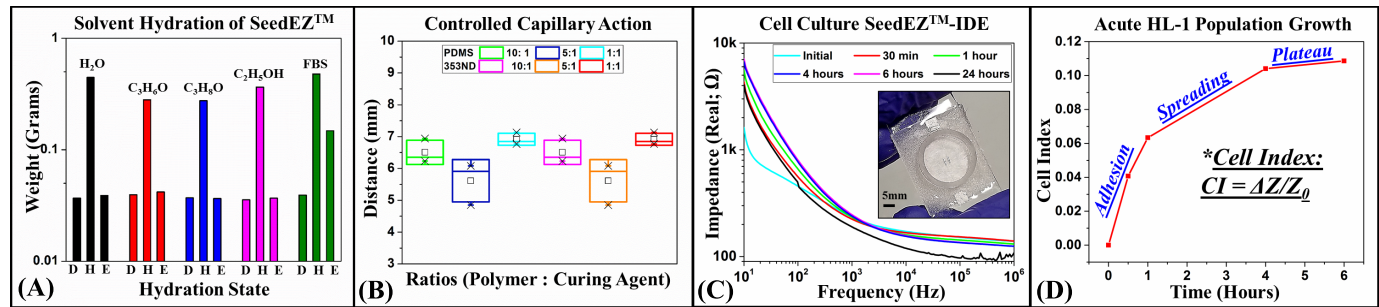


Fig. 2. Characterization of the SeedEZ™ scaffold, and fabricated IDE sensor. (A) Solvent hydration states of the matrix material Dry “D,” Hydrated “H,” and Evaporated “E.” Solvents used included DI water, fetal bovine serum (FBS), and 70% concentrations of isopropanol, ethanol and acetone ($N = 3$). FBS is the only solvent changing the weight significantly, due to protein deposits, demonstrating the robust nature of this material to be processed and sterilized. (B) Controlled capillary action characterization for PDMS and 353ND epoxy ($N = 3$). The “x” in each indicates the min/max, the “□” indicates the mean, and the inner solid colored line indicates the median. (C) Full spectrum impedance of the IDE sensor (values represent average of $N = 4$). The increase in initial impedances of the sensor up until the 6-hour mark indicate the adherence and then pre-confluence of a dense cell culture. There is a noticeable reduction in impedance at the 24-hour mark, which represents the controlled population decline. The inset shows an optical image of the fully fabricated SeedEZ™ IDE sensor platform. (D) Graph of the calculated Cell Index of the culture, derived from the 1kHz impedance values ($N = 4$). An expected growth profile [10] is observed, from adhesion (0.0-0.06335), to spreading (0.06335-0.10407) and plateau (0.10407-0.10860). The reduction in 1kHz impedance and confirmation cell index of -0.14932 is expected for controlled population decline, as demonstrated in literature.

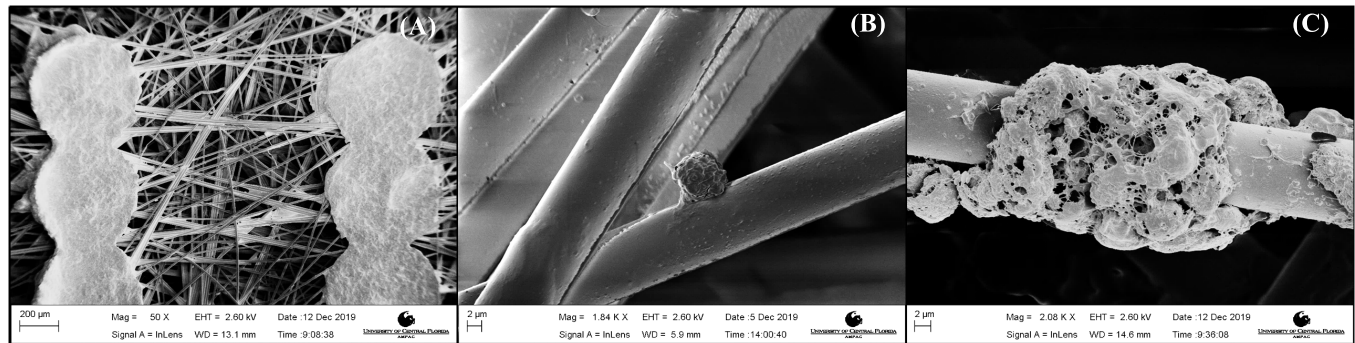


Fig. 3. SEM imaging of the matrix sensor. (A) The IDE electrodes are interspaced in the matrix, making for ideal contact with cell populations. (B) SEM image of a single HL-1 cell demonstrating a more spherical morphology, owing to a non-planar, 3D environment (C) Close up of the acetone-fixed HL-1 cell networks, showing the spreading of the cell population on the scaffold. These cells have their cell body intact, but are in the process of losing their ability to retain connections to neighboring cells due to both the controlled decline of the population and the acetone-fixing process.

For the cell culture experiments, the assembled IDE devices were pre-coated with a gelatin/fibronectin ECM mixture, and then HL-1 rat cardiomyocyte cells were cultured using a standard protocol [7]. Full spectrum impedance spectroscopy ($N = 4$) was performed with alligator clip connections, across the IDEs using BODE 100 (Omicron Labs, Austria) with the original media to measure unaltered cellular conditions over the course of 24 hours. Single channel, impedance measurements were obtained at representative time points ($T = 0, 0.5, 1, 4, 6$ and 24 hours) (results in Figure 2C). Physiologically relevant [8] 1 kHz impedance values (real part of impedance) were extracted and used in the calculation of the Cell Index (C.I.) (Equation 1 [9]; Figure 2D). In Equation 1, the Delta Z is the overall change in impedance, and Z_0 is the original baseline impedance. Upon the completion of the assay, cells were fixed on the scaffold using 70% acetone, and sputter-coated with 70nm of Au (Quorum Q150T; Quorum, UK) and imaged under SEM (Ultra 55; Zeiss, Germany) (results in Figure 3 A-C).

$$C.I. = \frac{\Delta Z}{Z_0} \quad (1)$$

A custom MLBC design (Figure 4) was created by sandwiching the SeedEZ™ cell scaffold between two 50μm thick stainless-steel (SS) layers (316L; Trinity Brands, USA) and multiple layers of Pyralux® adhesive lamination (DuPont, USA) and performing lamination at 200°C for 1 hour. A custom “UCF Pegasus” trace pattern, and associated vias were then defined in the MLBC substrates using micromilling (Quick Circuit J5; T-Tech, USA).

B. Long-Term SeedEZ™ Cell Culture Protocols

For all long-term studies presented in this work (Figure 5), cells were intracellularly labelled by Calcein AM (AnaSpec Inc., USA) and imaged at 10X using a Nikon Eclipse 80i upright microscope (Nikon, Japan) equipped with an Optronics MicroFIRE camera (Optronics, USA). Human hepatocellular carcinoma (HepG2 (ATCC)) cells were seeded into poly-D-Lysine (Sigma) coated scaffolds and cultured in MEM (+) Earle’s salts (+) L-Glutamine +1 % NEAA + 1% Sodium Pyruvate + 10% FBS (HyClone Lab Inc., USA). Mouse osteoblastic precursor cells (MC3T3-E1) were seeded into poly-D-Lysine (Sigma) coated scaffolds and cultured in Alpha

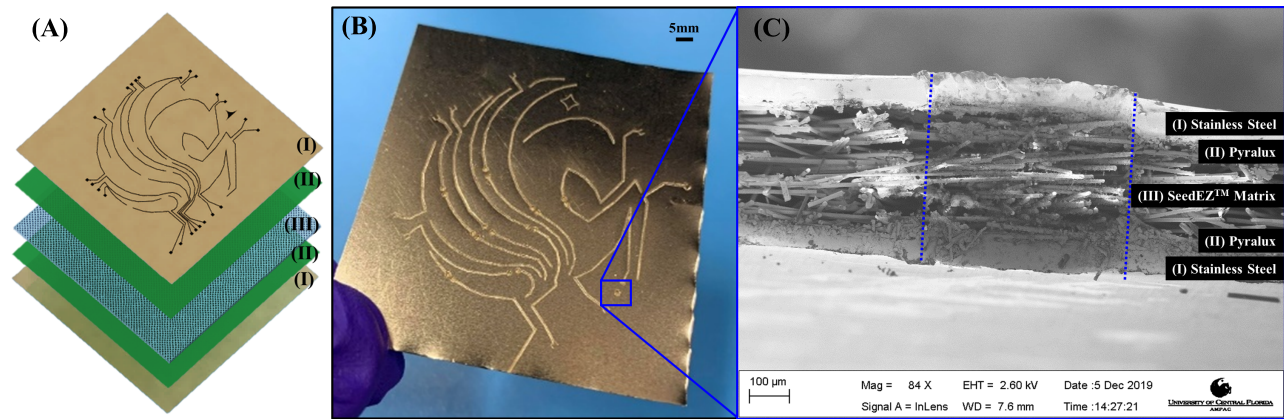


Fig. 4. MLBC fabrication and via/traces images. (A) Schematic image of the MLBC biomicrosystem platform. The layers indicated are Stainless Steel (I), Pyralux (II), and the scaffold (III). (B) Optical image of the fabricated design, with a custom micromachined “UCF Pegasus” trace design, and adjacent microdrilled through-vias. (C) SEM image, showing a closeup of the layered MLBC stack, with the precision microdrilled via cross-sectioned. The indicated via is highlighted with the blue dashed lines, and each layer component is denoted.

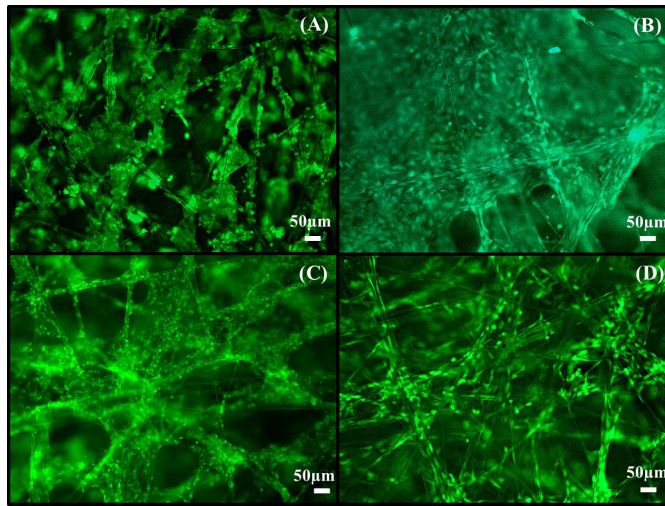


Fig. 5. Fluorescence images of various cell types cultured long-term in the SeedEZ™ scaffold. All cells were labeled using Calcein AM and imaged as described in text. (A) Human hepatocellular carcinoma (HepG2 (ATCC)) cells after 24 days in culture (N = 3). (B) Mouse osteoblastic precursor cell (MC3T3-E1) after 28 days in culture (N = 3). (C) Rat brain tri-culture with endogenous immune cells after 28 days in culture (N = 6). (D) Mouse fibroblasts (NIH-3T3) after 32 days in culture (N = 3).

MEM (Invitrogen, USA) + 10% FBS (HyClone Lab Inc., USA). Rat brain tri-cultures with endogenous immune cells were generated by combining cells from two cortical tissue dissociations (postnatal day-0 (P0) and embryonic day-18 (E18); BrainBits® LLC., USA). Astrocytes and microglia from the P0 dissociation were expanded in DMEM/F12 (Invitrogen) supplemented with 10% FBS (HyClone). After 2 weeks, the cells were combined with neurons from the E18 dissociation, seeded on scaffolds in 8 mg/mL Matrigel (Corning Inc., USA) and cultured in Neurobasal medium supplemented with B-27 Supplement and GlutaMAX (Invitrogen) for 28 days. Mouse fibroblast cells (NIH-3T3) were seeded into an uncoated scaffold and cultured in DMEM (high glucose; Life Technologies, USA) + 10% newborn calf serum (NBCS) + 1% Pen/Strep.

C. SeedEZ™ Fluorescence and Luminescence Assays

NoSpin HepaRG Cells (TRL/Lonza Group, Switzerland) human hepatocytes and biliary cells, in identical numbers, were delivered to the scaffolds in 8 mg/ml ice-cold Matrigel (Corning Inc.) and were cultured in MH100 media (TRL/Lonza). After the incubation, the 6 days *in vitro* (DIV) survival was measured using the AlamarBlue assay (Bio-Rad Labs, USA) following the manufacturer protocol. A volume of 200 μ L of media from each sample was transferred to a 96-well plate and fluorescence (ex/em 545/590 nm) read on a Biotek Synergy 4 plate reader (Biotek, USA).

Cryo-plateable primary human hepatocytes (10-donor pool; BioIVT) were cultured in on the SeedEZ™ in 8 mg/ml Matrigel ECM and in 2D with InVitroGro CP medium (BioIVT). After 5 DIV, 200 μ L of medium from each sample was transferred to a 96-well plate and fluorescence (ex/em 560/584 nm) read on a Biotek Synergy 4 plate reader, to measure the activity of CYP45 drug metabolizing enzymes. CYP1A and CYP3A4 activities were measured using Ethoxyresorufin-O-deethylase (EROD; AnaSpec Inc.) assay and P450-Glo™ CYP3A4 (Luciferin-PFBE; Promega, USA) respectively. For the EROD assay, the cells were exposed to 7-ethoxyresorufin (10 μ M) and salicylamide (inhibitor of phase II metabolism of resorufin, 1.5mM; MP Biomedicals, USA) for 4 hours at 37 °C. For the Luciferin assay, cells were exposed to Luciferin-PFBE (40 μ M) for 4 hours at 37 °C. After the incubations, 200 μ L of medium from each sample was transferred to a 96-well plate and luminescence read on a Biotek Synergy 4 plate reader.

III. RESULTS AND DISCUSSIONS

A. Processability and SeedEZ™ IDE Impedance Biosensor

Solvent compatibility and controlled capillary action results are depicted in Figures 2A-B and provided the basis for the necessary device handling and processing. Among the solvents used, all materials demonstrated an order of magnitude increase in hydrated weight and returning to approximately the original scaffold weight after drying. No significant changes

in the scaffold material were observed by SEM (not depicted) during this test. Hydrating in FBS was the only condition which demonstrated any significant change in weight for both hydrated and dried conditions. This result was expected due to absorption of protein deposits in the SeedEZTM matrix after evaporation. These results indicate that the cell scaffold matrix is easily able to be processed and sterilized using water or other common alcohols used in BioMEMS fabrication. Enhanced protein absorption with media exposure further indicates the advantage of the SeedEZTM material as biological material ideal for cell culture applications.

The controlled capillary action test served a dual purpose: first, it allowed for the observation of the integration of a commonly used biocompatible polymer (PDMS) and an epoxy (353ND) into the SeedEZTM matrix, and composite material fabrication upon curing; second, it allowed for the comparison of mixing ratios of the polymer/epoxy and curing agent for such an integration and provided information on the controllability of the capillary action process in the resultant composite. PDMS and 353ND samples of comparable mixing ratios performed similarly. The 1:1 ratio of polymer/epoxy to curing agent integrated into the material rapidly, was the most consistent in terms of length (measured as distance traveled) of the resultant composite and demonstrated an approximate viscosity of <2000 cPs (calculated with values from datasheets of the materials). The 5:1 ratio was the least reproducible in terms of composite material length and demonstrated approximate viscosities near ~3000 cPs for both materials. The 10:1, which is typically the standard ratio used for both PDMS and 353ND, demonstrated the highest apparent viscosity (~3500 cPs for both materials), and the demonstrated higher consistency in resultant composite material size when compared to the 5:1 ratio. PDMS was ultimately chosen due to its known cytocompatibility, and its good optical clarity when cast under partial vacuum conditions. Moving forward, the 10:1 ratio was thus chosen, due to its prevalence as the widely used standard, as well as due to the lack of available evidence in literature for the long-term cytotoxic effects of higher polymerization catalyst ratios on electrogenic cells.

The microfabricated IDE sensor demonstrated high fidelity impedance recordings to changes to the HL-1 population over the entire assay (average of $N = 4$ measurements in Figure 2C). Initial recordings ($T = 0$ hours) were procured immediately following the seeding of HL-1 cells on the construct. The average recorded 1 kHz impedance (electrophysiologically significant) was 221 Ω . At $T = 0.5$ hours, the 1 kHz impedance was observed to increase to 230 Ω . Consecutive further increases in this value were observed at $T = 1$ hour, 4 hours and 6 hours, with 1 kHz values of 235 Ω , 244 Ω , and 245 Ω respectively. Finally, after 24 hours under static media conditions, the 1 kHz impedance was recorded at 188 Ω , signaling the expected controlled population decline.

Cell Indices [9] were calculated from 1kHz, real part of the impedance values, reinforcing the clear aforementioned pattern [10] (Equation 1; Figure 2D). Equation 1, calculates the growth curve based on the Cell Index using the changes in 1 kHz impedance, relative to the initial IDE impedance with freshly seeded cells. The resulting curve demonstrates

the expected phases of adhesion, spreading and culminates in a plateau (pre-confluence) of the cells measured until the 6-hour time mark. HL-1 cells require daily media change, thus static media conditions (equaling altered pH) resulted in expected, controlled population decline [11]. The data point corresponding to the controlled population decline was omitted from Figure 2D to better highlight the calculated growth curve.

Figure 3A represents SEM of the SeedEZTM IDE, which highlights the cellular scaffold and the silver-ink IDE structures in a “circle-in-line” configuration. The use of such an IDE design [12] allows for a more controlled casting on a porous substrate such as the SeedEZTM scaffold, with minimal bleeding of conductive ink during the screen printing process. Figure 3B shows a more spherical cell morphology in a 3D scaffolding environment. In general, cells typically demonstrate a flatter morphology in a typical 2D well plate or a planar IDE environment. HL-1 cells in particular depict flat and oblong morphology in a dish, with protrusions necessary to adhere to the substrate and also to connect with neighboring cells [13]. But as observed in this figure in an acetone-fixed state, a single HL-1 cell has a more spherical morphology common to cells in 3D environments. In Figure 3C, a more complete view of the HL-1 cell network is observed. As predicted through the Cell Index growth modelling, Figures 3B-C suggest that the nature of the 3D scaffold led to a unique HL-1 cell spreading architecture, and presumably a pre-confluent network, not typically observed in 2D. Desiccation of the cells through the necessary acetone-fixing for SEM imaging, reveals gaps in the cellular network, which would have otherwise been coherent contact between cells in this culture.

Figure 4 demonstrates advanced integration of the SeedEZTM cell scaffold into multifunctional layered biosensor composite, designed around a biological PCB concept. Figure 4A illustrates the breakdown of the layered micro-fabrication strategy, which was assembled using a lamination process. Such a design demonstrated a proof of concept multilayer sensor with the seamless integration of the SeedEZTM scaffold accomplished using traditional micromachining methods for the creation of complex, multi-layered BioMicrosystems. Figure 4B serves to illustrate the physical fabrication of this MLBC design, complete with micromilled vias and traces. The vias themselves can be micromachined with a high degree of precision (Figure 4C), allowing access to the cell scaffold for electrophysiological measurements of any given cell population seeded in the scaffold.

B. Long-Term Cell Studies in SeedEZTM

As a pilot study, results in Section A focused on a short-term assay for feasibility of the composite biosensor, however the SeedEZTM scaffold has been well characterized for its long-term culturing capabilities. Several examples of long-term cultures and co-cultures in SeedEZTM are depicted in Figure 5. Figure 5A shows human hepatocellular carcinoma cell line, HepG2 (ATCC), after 24 days in culture. Figure 5B shows mouse osteoblastic precursor cells, MC3T3-E1 cultured for 28 days. Figure 5C shows rat brain tri-culture with endogenous immune cells (microglia) after 28 days in culture. Lastly,

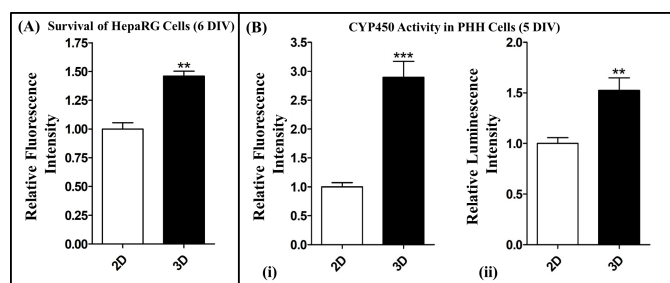


Fig. 6. Fluorescence and luminescence data of cells seeded on the SeedEZTM scaffold for viability and enzymatic activity respectively. (A) Survival of terminally differentiated human hepatobiliary cells (HepaRG). 3D SeedEZTM (N = 6) demonstrated higher survivability than standard 2D culture (N = 3). The data is normalized to 2D; ** $p < 0.01$ two-tailed t-test. The cells cultured in 3D in the SeedEZTM scaffold had over 46% higher survival than did the cells cultured in 2D. (B) P Cytochrome enzymatic activity assays. (i) CYP1A EROD assay (N = 6) and (ii) CYP3A4 P450-GloTM assay (N = 6), after 5 DIV. Data is normalized to 2D; *** $p < 0.001$, ** $p < 0.01$ two-tailed t-test. The assays measured baseline cell activity without drug inducers. The primary human hepatocytes exhibited significantly higher extended activity of CYP450 drug metabolizing enzymes in the SeedEZTM scaffold versus the 2D cell cultures.

Figure 5D demonstrates mouse fibroblasts, NIH-3T3, after 32 days in culture on uncoated SeedEZTM scaffolds.

The extended cultures are also vital for drug testing because they allow for the application of multiple drug doses to assess repeat-dose efficacy and toxicity *in vitro*. Commonly used cell lines allow only a small number of population doublings in a dish before cells become contact-inhibited and senescent. Depending on the doubling time, 2D studies are therefore time-limited to a few days to about a week. When cells are cultured in 3D, they have more room to grow and divide. They reach confluence much later than do the cells in a dish, thus enabling long-term mechanistic studies and repeated dose responses. For translatable results, cell culture health in 3D is important; i.e. cell viability for cell lines, and the survival of post-mitotic and terminally differentiated primary cells. As shown in Figure 6A, terminally differentiated human hepatobiliary cells (HepaRG; TRL/Lonza), had 46% higher survival in SeedEZTM than in 2D after only 6 days in culture.

C. Future Perspectives for IDE-Biosensor Applications

Electrogenic cell biosensors can be used for time-resolved measurements in excitable tissues such as heart, brain and muscle to assess long term drug effects *in vitro*. In addition, impedance data can be multiplexed with capacitance data to provide pharmacokinetics including drug metabolism insights by measuring parent drug deletion, for example. As previously found, primary human hepatocytes cultured in SeedEZTM scaffolds consistently exhibited higher activity of Cytochrome P450 (CYP450) drug metabolizing enzymes than did the cells cultured in 2D [14].

In this study (Figure 6B[i-ii]), cryo-plateable primary human hepatocytes (10-donor pool; BioIVT, USA) were cultured in 3D in the SeedEZTM scaffold for 5 days *in vitro* (DIV). As can be seen, Cytochrome P450 1A (CYP1A) activity was over 2.5X higher in the SeedEZTM scaffold than in

2D, and Cytochrome P450 3A4 (CYP3A4) activity approximately 1.5X higher than in 2D. This is significant because the CYP3A4 drug metabolizing enzyme, metabolizes over 50% of all FDA-approved drugs, and the SeedEZTM scaffold was able to maintain this activity elevated long-term compared to the activity of cells cultured in 2D. Identifying human drug metabolites *in vitro* is important to prevent toxic metabolites from reaching human trials. Five out of seven drug metabolizing enzymes that collectively metabolize over 90% of approved drugs have different isoforms in humans and animals, and their metabolites are generally different [15]. By using SeedEZTM in future IDE-based assays, both cell-based readouts and drug metabolites can be identified.

IV. CONCLUSION

The rapidly emerging “Organ-on-a-Chip” and “Human-on-a-Chip” technologies require microfabrication and packaging experts to modify their thinking from a ‘Microfabrication-Biology’ approach to a ‘Biology-Microfabrication’ paradigm. Towards such a paradigm-shifting BioMEMS strategy, we demonstrate, the novel creation of a biological scaffold material-based Interdigitated Electrode (IDE) platform, using a SeedEZTM scaffold, as well as advanced microfabrication and packaging for continued development of complex sensors using a Multifunctional Layered Biosensor Composites (MLBC) concept. For the IDE platform, its defined fabrication was characterized through a controlled capillary action assay, as well as a solvent compatibility test. Within the controlled capillary action characterizations, PDMS and 353ND epoxy were tested, and 10:1 PDMS was preferred as the material composite with SeedEZTM scaffold demonstrating consistent performance and optical clarity. The solvent testing provided insight into the effects of common lab alcohols and protein containing media which impact the processability and functionality of the cell scaffold. HL-1 rat cardiomyocytes, with a rapid adhesion to confluency rate, were seeded onto the fabricated IDE-matrix construct and full spectrum impedance values were measured for 24 hours. Using the biologically relevant 1 kHz values, growth pattern of the culture was ascertained. The Cell Index of the culture at time points from 0 – 24 hours were calculated from these impedances, and a standard growth curve (adhesion, spreading, plateau) was observed. Controlled population decline was additionally observed by a static media protocol, until a predetermined 24-hour timepoint. SEM imaging suggested a 3D conformation of the HL-1 cell culture on the scaffold. Preliminary MLBCs were demonstrated, providing a pathway for the SeedEZTM scaffold to be the central component for large area biological Printed Circuit Boards (bPCBs).

Additionally, the collective long-term studies reported here showed that both survival of primary cells and the maintenance of differentiated phenotypes (in the context of drug metabolism *in vitro*) were significantly higher in the SeedEZTM scaffold than in 2D culture benchmarks. Such a scaffold is ideally suited for use in both impedance-based biosensor applications

(electrogenic cell growth impedance sensor shown herein) and capacitance-based biosensing (affinity-based sensors), for example, to measure metabolism enzyme kinetics. In this work, impedance measurements were used to provide basal cell health, however both impedance and capacitance-based sensing will be built into this device in the future for broad sensing applications in disease modelling, functional evaluation, and safety / toxicity assessments. Additionally, electrodes may be functionally coated for disease / cellular damage markers and thus multiplexed with basal cell sensing capabilities. This work therefore opens new avenues for mechanistic studies and, as an example, cardiotoxicity testing in physiologically relevant 3D environments. Future microsensor developments include multilayer biosensors, 2.5-3D Micro-electrodes, advanced neural and cardiac assays paving the way for future Human-on-a-Chip sensing systems.

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