Pre-clinical Identification of Dose-Dependent Cardiac Toxicity

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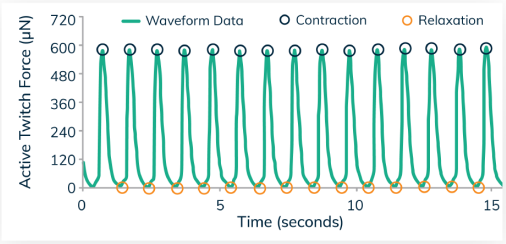
Highlights

- Turnkey scalable production of human 3D engineered heart tissues.
- Label-free automated capture and analysis of functional data across 24 tissues in parallel.
- *In vitro* validation of clinical drug toxicity and efficacy using human cell models.
- Identification of optimal dosing regimens to minimize functional side-effects.



Summary

Cardiac toxicity poses a major risk for patients' safety and for the sustainability of drug development, highlighting the need to identify cardiotoxic side-effects early in the development pipeline. Human 3D engineered heart tissues (EHTs) can enhance the predictiveness of *in vitro* pre-clinical cardiotoxic screening and enable screening for structural Type II cardiotoxicity, as they provide functional and structural data across an extended experimental time frame. Streamlining and scaling production and data analysis from EHTs is critical for incorporating 3D tissue testing in standard drug development practices. Here, we present a standardizable, scalable method using human 3D EHTs to validate the dose-dependent cardiotoxicity of the well-established chemotherapeutic agent, Doxorubicin, and BMS-986094, a hepatitis C drug that failed clinical trials due to unanticipated Type II cardiotoxic side effects.

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Introduction

The drug development pipeline is a lengthy and resource-intensive journey that spans on average a decade and incurs expenditures exceeding $2 billion[1]. Of the thousands of candidate compounds that enter the pipeline, only a handful make it to the clinical phases, and just one may eventually reach the market. The success of the pipeline and the safe use of the marketed therapy are essential for the safety and well-being of patients and paramount to the interests of stakeholders. Nevertheless, 24% and 32% of drugs are impacted by safety issues in the clinical[2] and post-market[3] phases respectively, with unanticipated cardiotoxicity being one of the leading issues[4, 5]. To protect patients from such unforeseen side-effects, the FDA has issued detailed guidelines on the use of pre-clinical models for safety evaluation. However, existing *in vitro* toxicity screening methods have historically struggled to replicate clinical failures, as they do not accurately represent the complexities of the human body and the diverse patient population encountered in the clinical and market phases of the development pipeline.

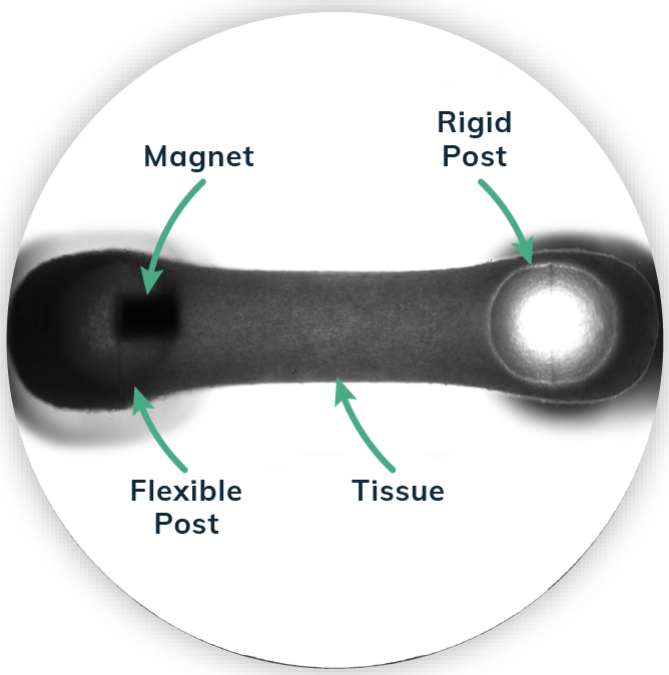

Figure 1: *3D engineered tissues are a promising alternative to existing pre-clinical models. Pictured above is a 3D engineered tissue connected to flexible post with magnet (left) and rigid post (right).*

Human induced pluripotent stem cells (iPSC) presents a promising solution to diversify the cell resources available for *in vitro* testing. It is now possible to create cell lines across a range of donors by reprogramming easily accessible adult cells into iPSCs and differentiating them into select cell types. These cells can then be assembled into 3D tissues, allowing for multi-lineage cultures and tissue architectures that better mimic the natural composition and properties of *in vivo* tissues. Moreover, the 3D matrix enables functional, *in vitro* cardiac testing with clinically relevant, non-terminal outputs, such as contractile force and kinetics. Recent advancements in 3D heart tissue assembly have significantly improved this technology, and the next frontier lies in the scalable use and automated data extraction from these 3D EHTs.

In this application note, we present the Mantarray™ system, a label-free, magnet-enabled platform designed for tracking and analyzing the contractile function of engineered muscle tissues[6]. The system is highly scalable, enabling data capture and analysis from 24 tissues in parallel. Here, we show functional evidence of the acute and prolonged cardiotoxicity of two compounds: Doxorubicin, a well-known cardiotoxic cancer drug, and BMS-986094, a hepatitis-C therapy that failed in phase II clinical trials due to cardiotoxicity.

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Methods

3D engineered heart tissues were generated using the commercially available Mantarray casting kit and analyzed using the Mantarray instrument, as described in our video guide[7] and in Figure 2. In summary, human iPSC-derived cardiomyocytes and stromal cells were combined with a fibrin-based extracellular matrix and mixed together in the Mantarray tissue casting mold. The cell mixture compacted around 2 microposts in each well of the Mantarray plate forming 24 individual, spontaneously beating 3D tissues, with >95% casting success rate. One of the two posts features an embedded magnet, and upon tissue contraction, the deflection of the post can be tracked by the Mantarray instrument, to automatically measure the tissue force amplitude and kinetics. The instrument output was automatically processed using the Pulse 3D™ analysis platform.

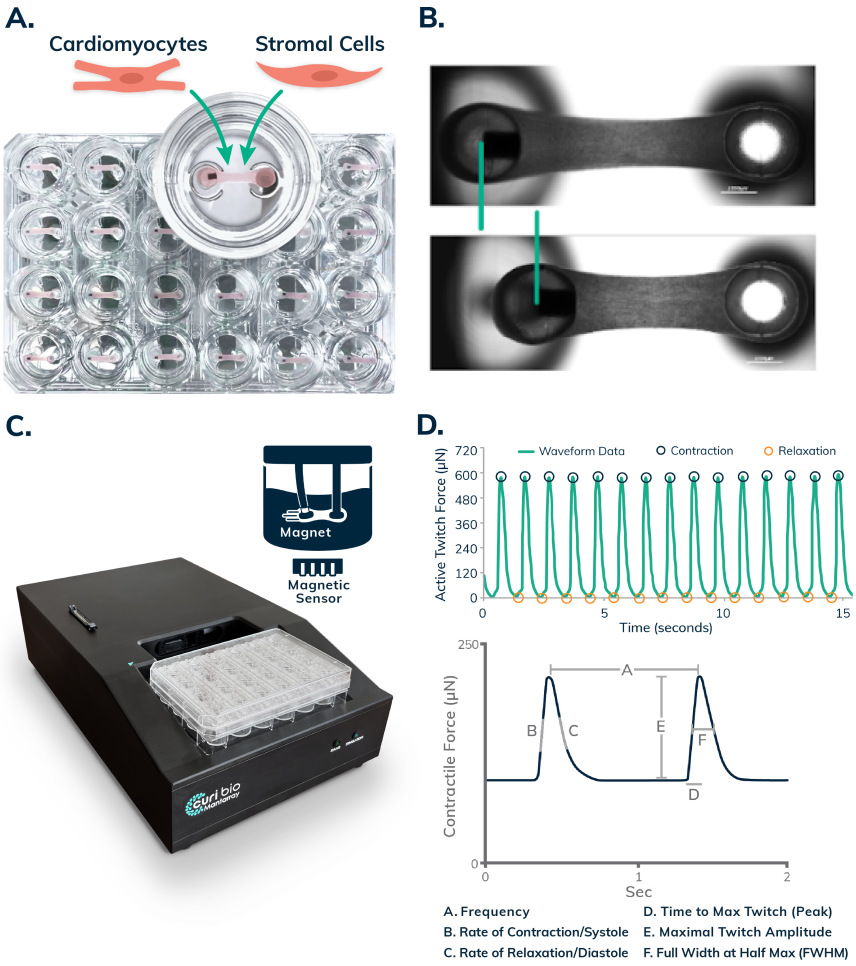

Figure 2: ***A.*** *Cells assemble into tissues in the Mantarray casting kit.* ***B.*** *Tissues contract and move the embedded magnet.* ***C.*** *The Mantarray instrument records the contractions.* ***D.*** *The contraction waveform is automatically analyzed.*

Tissues were cultured for up to 30 days, and were exposed to various concentrations of Doxorubicin (Dox) and BMS-986094 through direct addition of the compounds in the tissue medium. Tissue medium was changed every 3 days in all experiments. Doxorubicin was either applied continuously at every media change or as a bolus at days 0, 12 and 24. The BMS-986094 compound was administered in a continuous manner only.

Results

Doxorubicin was administered in 3 concentrations (low, medium, high) and in two patterns (continuous, bolus), as shown in Figure 3. The high concentration led to the immediate and terminal cessation of tissue contractions, unequivocally revealing Doxorubicin's toxicity. However, the medium and low concentrations reveal opportunities for Doxorubicin's administration method despite its toxicity. While tissue contractility terminally ends with the continuous medium concentration, it does recover to control levels after the end of

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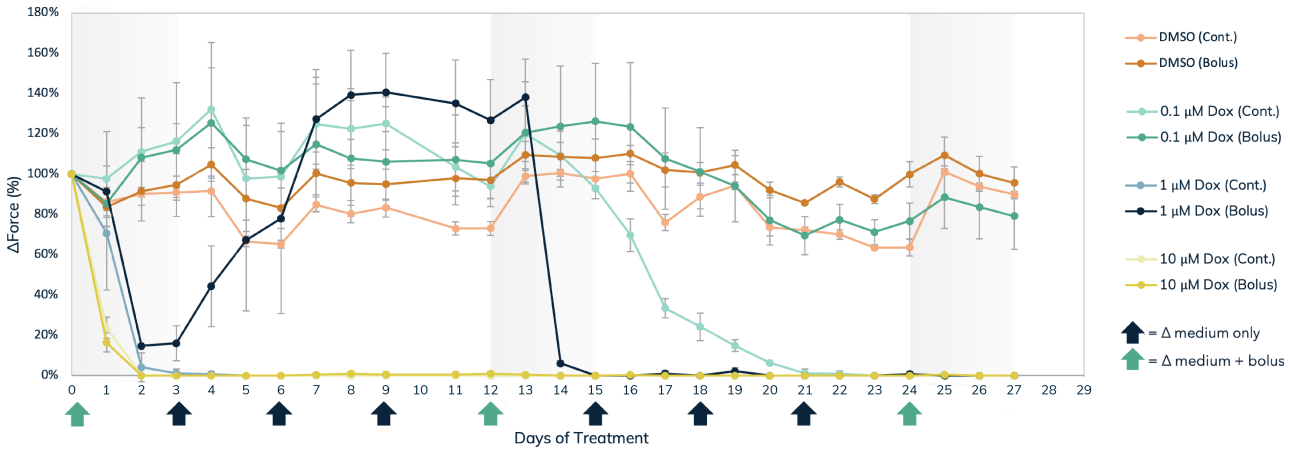

Figure 3: *Relative Twitch Force After Doxorubicin Treatment.*

the first medium bolus concentration. Likewise, while the contractility terminally decreases with the low continuous concentration starting day 16, the low bolus concentration condition is indistinguishable from the controls. This highlights the utility of the Mantarray contractility platform in identifying not only cardiotoxic compounds, but also patterns of administration that minimize toxicity to functionally acceptable levels.

When BMS-986094 was administered to Mantarray tissues, contractility dropped for all concentrations within the first 2 days (Figure 4). The decrease in twitch force in all concentrations is in stark and statistically significant ($p<0.05$) contrast to the preserved force of controls. Furthermore, terminal cessation of contractility ensued in a dose-dependent manner, as shown by the twitch frequency, with the highest concentration reaching cessation early in 4 days, and the lowest concentration later in 7 days. These results highlight that access to 3D engineered tissues can help identify otherwise missed toxicity and derisk drug development.

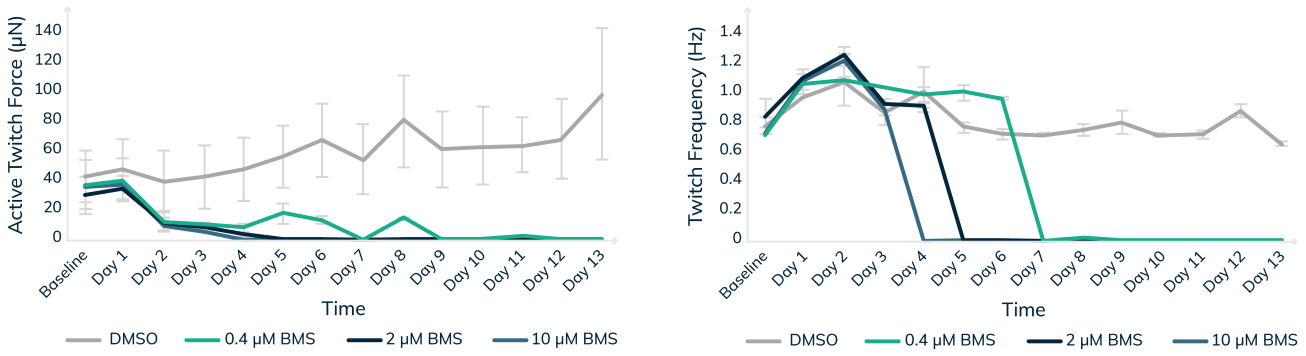

Figure 4: Left: *BMS-986094 Active Twitch Force.* ***Right:*** *BMS-986094 Twitch Frequency.*

Conclusions

This study demonstrated that the scaled *in vitro* pre-clinical testing of compounds using human 3D EHTs can uncover subtle nuances in the concentration- and time-dependent nature of cardiotoxicity. The prolonged experimental time frame and functional output of our approach also propose administration patterns for the safer use of toxic compounds. The Mantarray platform's automated monitoring capabilities provide invaluable insights into the dynamic aspects of cardiotoxicity for more informed decision-making in drug development.

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