# Human Engineered Tissue: Medical Countermeasures For Public Health & CBRN Emergencies

**White Paper Report**

**Defense Strategies Institute**

**August 2014**

DSI does not take specific policy positions. Recommendations suggested within this report are derived from the summaries of comments and discussions that occurred during our Joint Civil & DoD CBRN Symposium, March 2014 & independent research.

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*"Developing and providing medical countermeasures for CBRN threats, pandemic influenza, and emerging infectious diseases by product development, stockpile acquisition/building, manufacturing infrastructure building, and product innovation"*

# INTRODUCTION

With the rise of Asymmetric and non-traditional homeland threats to the National Security apparatus , priorities for chemical, biological, radiological, nuclear (CBRN) and Improvised Explosive Devices (IEDs) efforts have been transformed in ways untested before.

A national strategy for chemical, biological, radiological, nuclear and explosives (CBRNE) countermeasures is based upon assuring continued responsiveness to these threats. With processes and policies in place through multiagency standardization, maintaining national security against such threats in new way must be prioritized

These new processes of advancing CBRN response, defense & technology readiness were directly addressed at Defense Strategies Institute's 2014 Joint Civil & DoD CBRN Symposium. The medical portion of the event revolved on equipping multi-mission forces and specialized CBRN units on establishing a new national medical countermeasures (MCM) pipeline to assistance the federal governments response to and recovery from CBRN biothreats.

Today, even with the rollout of state of the art vaccines and therapeutics, multiple, ongoing threats may evade current countermeasures. Therefore, it is critical to build up rapid detection capacities to protect the health, well-being, and safety of the nation. In the development of medical countermeasures for CBRNE, the main challenge over the next 5-10 years will be demonstrating direct effectiveness in humans. For now, the use of animal models is the standard approach. Thus, an ongoing strategy for continued development of human engineered tissue is needed for greater CBRN countermeasures effectiveness.

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1. National Strategy for Biosurveillance, http://www.fda.gov/downloads/EmergencyPreparedness/MedicalCountermeasures/UCM314532.pdf

# Human Engineered Tissues in DoD Applications

New methods in tissue engineering technology for drug discovery and safety analysis have taken the spotlight, notwithstanding limited success to-date. Human engineered tissues could provide a potential solution for detecting unknown biological weapons and accurately testing and developing countermeasures. Currently, there are only a few methods that have been widely adopted for these purposes while a strong interest continues for the faster development of effective therapies. However, with a limited number of human subject studies and approved follow up studies, it is difficult to assess countermeasure toxicity profiles. To thoroughly test these profiles, the use of human engineered tissues can be applied during the preclinical stage.

U.S. government agencies including the National Institute's of Health (NIH) and Food and Drug Administration (FDA) seek to fund and guide the development of new drugs and diagnostics while reducing costs and time to market. Such is also the interest of the DoD Chemical and Biological Defense Program, wherein technologies are being identified to fill capability needs. Time-to-market and overall costs are critical issues for rapidly responding and preparing for biological threats. However, safety assessment requirements still remain in force, not only in general pharmaceutical development but for the countermeasures (prior to approval for first-in-human studies). At present, efforts are underway to experiment with innovative approaches and rapidly arrive at a streamlined solution to find safe and effective countermeasures.

While current countermeasures development programs mainly focus on therapeutics immediately after incidence, very few address mid to long-term effects that lead to life-threatening conditions. Skin and lung damage normally occur first, but the heart is also exposed to the effects of biothreat agents and radiation. Recent studies confirm that such heart injuries are terminal. Until this was made known, vulnerability to the heart was underestimated. The citation reveals a heart injury treatment strategy proposed by InvivoSciences, Inc. to develop countermeasures and effective treatments using engineered human heart tissues.

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2 Medical countermeasures to protect humans from anthrax bioterrorism, Dimitrios G. Bouzianas, Review Trends in Microbiology Vol.17 No.11

3 Martinou, M. and A. Gaya, *Cardiac Complications After Radical Radiotherapy.* Seminars in oncology, 2013. **40**(2): p. 178-185, Jaworski, C., et al., *Cardiac complications of thoracic irradiation.* J Am Coll Cardiol, 2013. **61**(23): p. 2319-28..

# Pluripotent Stem Cell Tissue Engineering

The use of human engineered tissues is not new. In fact, Dr. Y.C. Fung of the University of California at San Diego introduced the term "Tissue Engineering" in the mid '80s. Later, tissue engineering was defined as an interdisciplinary field between the engineering and life sciences by Doctors R. Langer and J. Vacanti to develop organs and tissues for clinical applications. Despite significant progress in research and development, the commercial activities in this area reached a breakeven point ($3.5B sales and $3.6B spending) in 2011. One of the challenges of industry growth in this field is the difficulty in securing a stable source of human cells for manufacturing engineered tissues. The 2006 discovery of reprogramming human fibroblasts into pluripotent cells (i.e., potential to become any kinds of cells), however, resolves this limitation.

Accordingly, unlimited numbers of patient-specific pluripotent stem cells can now be used for regenerative purposes in damaged organs and tissues. In addition to the engineered tissue applications, there is a growing interest in the drug discovery industry to apply this process for discovering effective and safe drugs. The application of induced pluripotent stem cell (iPSC) technology in tissue engineering enables scientists to develop laboratory grown human models to mimic healthy and diseased organs and tissues. For example, laboratory grown heart tissues are developed via cells isolated from a patient who develops a hereditary heart disease. The model will support the analysis to determine disease mechanisms and to screen potential drug candidates. Since the patient's heart failure will be recapitulated by the tissues, there is a high likelihood that a drug reversing disease phenotype in the tissue can successfully treat heart condition in patients. This concept establishes a disease model in laboratory grown tissues and translates well for use in DoD applications. This will then lead to a more personalized disease treatment, (i.e., developing drugs for specific patients). Thus, a patient-specific approach for precision medicine is the answer for rare diseases and for countermeasures development for those who have a higher susceptibility to various types of biothreats and radiation.

*"This concept establishes a disease model in laboratory grown tissues and translates well for use in DoD applications"*

# Armed Forces Institute of Regenerative Medicine

Since 2007, the Armed Forces Institute of Regenerative Medicine (AFIRM) has promoted R&D for treating severely wounded military servicemen and women. Regenerative medicine therapies have come from matching funds and collaborations with US Army-Medical Research and Materiel Command, US Navy-Office of Naval Research, US Air Force-Office of the Surgeon General, the National Institutes of Health, Veterans Administration, Department of Defense, and Health Affairs.

In continuation of the tissue engineering movement, AFIRM has made great strides in promoting the science of regeneration for damaged tissues and organs. While development of engineered tissues for skin and wound healing in DoD applications supported by AFIRM are becoming more available, the regenerative application of engineered tissues for the cardiovascular system needs further attention. As described further in the following section, damage to the heart may not be immediately apparent even though biothreats and radiation are well-documented to be life-threatening in the long run. Thus, regenerative application of tissue engineering targeted to the cardiovascular system, including the heart, is a critically underserved area.


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Jaklenec, A., et al., Progress in the tissue engineering and stem cell industry "are we there yet?". Tissue Eng Part B Rev, 2012. 18(3): p. 155-66.

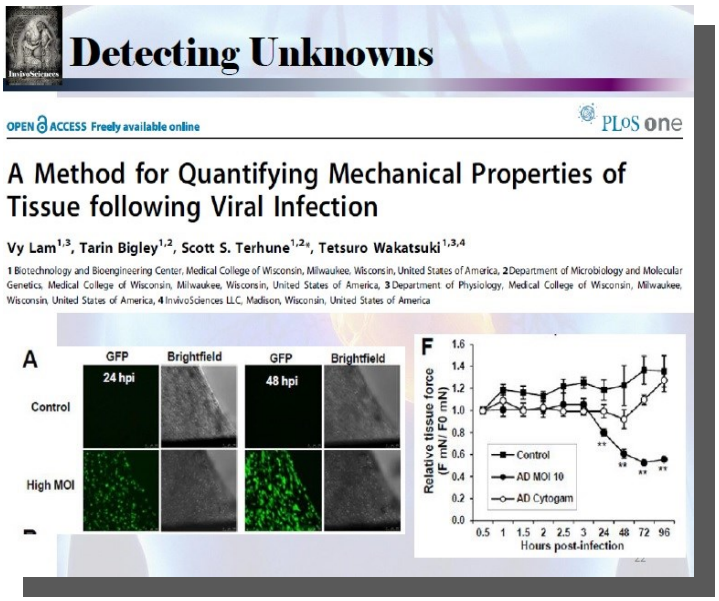
Takahashi, K. and S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 2006. 126(4): p. 663-76.

Wakatsuki, T., J.A. Fee, and E.L. Elson, Phenotypic screening for pharmaceuticals using tissue constructs. Curr Pharm Biotechnol, 2004. 5(2): p. 181-9, Polini, A., et al., Organs-on-a-chip: a new tool for drug discovery. Expert Opinion on Drug Discovery, 2014. 9(4): p. 335-352.

# Detecting Unknown Biothreats

DNA and protein analyses are used to identify known biothreat agents and, in general, are sensitive and effective approaches. It is difficult, however, to respond efficiently if biological weapons are unknown, rare, or even of known but of different strains (i.e., anthrax). When conventional analytical approaches fail to understand the extent of toxicity, laboratory animals or culture cells are used as test subjects and exposed to the agents. Yet, the use of laboratory animals is difficult and expensive, and accuracy and sensitivities of the cell-based approach are questionable. So, what can be done?

In the heart injury treatment strategy proposed by InvivoSciences, more than one hundred thousand cells reconstitute engineered tissues. Therefore, biological effects of agents, including infection, radiation, or chemicals, on each cell are integrated over many cells in the tissues. The agent-induced tiny changes on cells can be amplified 10s to 100s of thousand times in engineered tissues. An early sign of biothreat will be detected through functional changes of vital organs such as the heart. Hence, the human engineered tissue-based detection system is a viable alternate approach to analyze biothreat agents that affect physiological properties of organs and tissues.




In a noted test case, (engineered tissue-based detection of a viral infection), InvivoSciences studied effects of cytomegalovirus (CMV) infecting engineered tissues and fabricated human fibroblasts isolated from human skin biopsy for testing. This collaborative study with a CMV expert, Dr. Scott Terhune at the Medical College of Wisconsin, showed that CMV infection induced softening of connective engineered tissues in an ultra-sensitive manner. While CMV does not cause life-threatening damage, the detection method used for CMV infection demonstrates potential applications of engineered tissue-based viral detection in many other life-threatening viruses.

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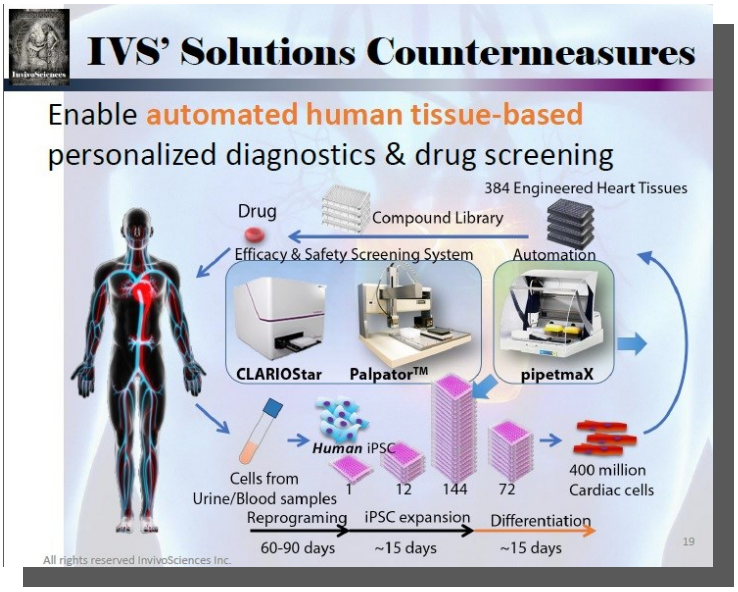
Lam, V., et al., A Method for Quantifying Mechanical Properties of Tissue following Viral Infection. PLoS One, 2012. 7(8): p. e42197.

# Disease Models

Final stages (Phase I, II, and III) of drug discovery employ human subjects to analyze safety and efficacy of drug candidates. Such are tested with healthy patients and those with disease. For countermeasures development, however, it is impossible to recruit patients for clinical trials and expose them to biothreat agents. A draft guideline, "the Animal Rule", outlines a regulatory pathway for approval of drugs and biological products to be used as countermeasures. It was revised in May of 2014. It clearly states, "Approval under the Animal Rule can only be pursued if definitive human efficacy studies cannot be conducted, because it would be unethical and field trials have not been feasible". As an alternative or complementary approach, human engineered tissues treated with biological weapon material can also be considered as a disease model to predict safety and efficacy of novel treatment options. Once the engineered tissues' ability to faithfully mimic the physiological properties of their target tissues and human organs is validated, those tissues can be used to report the safety and efficacy profiles of drug candidates.




InvivoSciences has developed and tested human engineered heart tissues that mimic physiological properties of heart muscle and are ready for use in drug safety and efficacy screening. As mentioned above, using human iPSCs, the company has developed an assay platform for human iPSCs that can test for cardiac safety as well as prove efficacy of new drug candidates based on drugs and/or biological products. This comes from direct induction of changes in physiological properties of engineered heart tissues. Those physiological changes are monitored comprehensively by measuring parameters including cardiac action potential, calcium transient, and contractility. The same technologies make up the disease model that mimics human heart reactions during exposure to biothreat agents. Therefore, the engineered heart tissues not only recapitulate physiological properties of normal heart functions, but also mimic the diversity of patient-specific susceptibility to biothreats.

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www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM399217.pdf

# Summary

Tissue Engineering is an ever evolving field of science & technology seeking to regenerate tissues and organs that have been lost due to injury or disease.

This new field of human tissue engineering is as important as ever to the CBRN consequence community as new technology for drug discovery and safety impact medical countermeasure planning. The Armed Forces Institute of Regenerative Medicine (AFIRM) has promoted regenerative medicine therapies, yet further attention is needed to address the new environment of biothreats.

Recent developments extend the possibility of discovering new drugs or testing toxicity of drug candidates. By applying adult human stem cell technology, engineered tissue can be fabricated to mimic genetic conditions such as inherited diseases and susceptibility to biothreat agents. InvivoSciences offers alternative approaches to detect unknown toxicants affecting cardiac function as well as the development of disease models to predict outcomes of clinical applications of countermeasures.

**About Defense Strategies Institute:**

DSI is a premier non-partisan woman-owned, minority owned small business designed to assist in advancing the mission critical goals of the United States' Military and Government. Through our high level educational and training summits and symposiums , we are able to reach across all offices and departments in a fair and balanced manner. We bring together the mission relevant representatives in our neutral forums in order to foster the necessary discussions and debates to help them achieve efficient and effective mission success. In order to maintain our neutrality, we receive no funding or investment for operating costs from any outside organization, group, or individual.

**About InvvioSciences:**

InvivoSciences (IVS) provides phenotypic compound screening services for first-in-class drug discovery and drug repositioning. Our "2012 Edison Award winning" engineered tissue based high content analysis assay evaluates efficacy and safety of compounds through their effects on physiological properties of engineered tissues. IVS is a leading developer and supplier of proprietary 3D cell/tissue culture models for highly-predictive, multi-parameter phenotypic assays in pharmacology safety and discovery screening. Our functional 3D tissue models recapitulate the physiological properties of cardiac, smooth and skeletal muscle, connective tissue, as well as 3D model diseased states (e.g. hereditary cardiac diseases, cardiac fibrosis, cancer and orphan diseases). Our unique combination of tissue engineering technology with assay automation and sample miniaturization offers a rapid and cost effective platform for unprecedented therapeutic discovery. In support of National Institute of Health, an application of the technology in comprehensive cardiac safety assessment has been in the late phase of validation.