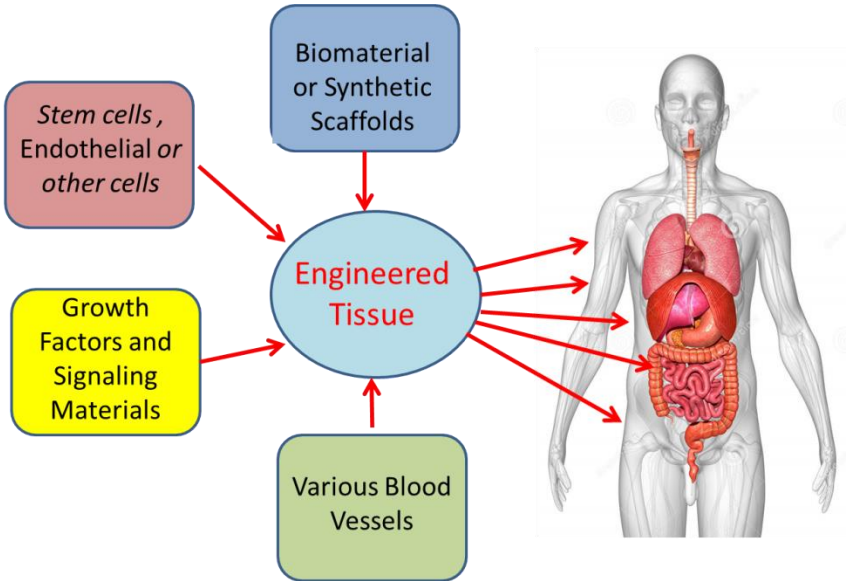
MDX LIFE SCIENCES INC

Breakthrough Technologies in Tissue Vitality

Monitoring of Tissue Oxygenation during Tissue Engineering and Regenerative Medicine Processes

1. Background



The promise of regenerative medicine is the complete, functional repair of human tissue damaged by disease or injury. This vision includes directing the integration of transplanted cells into damaged tissue, replacing damaged structures with new tissues and organs generated ex vivo using cells and materials, and regenerating damaged structures by recruiting endogenous repair mechanisms in vivo. Advances in stem cell biology are central to achieving this vision, but certain therapeutic approaches require additional breakthroughs in tissue engineering.

The combination of human stem cells, engineered products, novel drug and protein therapies, and breakthrough surgical techniques may all be required to realize this regenerative potential. Tissue engineering thus encompasses a variety of tools and approaches, ranging from developing synthetic or bioengineered scaffolds that recruit or enhance the body's natural repair processes, to optimizing transplantation with products that direct the migration and integration of cells into damaged tissue, to creating three-dimensional tissues seeded with cells and built in vitro.

Once a tissue engineered product has been conceptualized and developed, the product must be tested for safety and efficacy before it can be advanced to the clinic. This requires animal models, tools for tracking engraftment and survival, and consideration of the immunological profile of the product. Participants in CIRM's[1] workshop on tissue engineering discussed the approaches and tools needed to evaluate the scientific merit of a TE product before therapeutic development could be warranted.

Many of the standard preclinical assessment tests used in other fields might be applicable to cell therapy, including assessment of the cardiac, neural, liver, and reproductive toxicity profiles of each product. However, there are evaluations that are more specific to tissue engineering products, and some of the tools to conduct these evaluations remain to be developed. For instance, there are currently no good methods for real-time, non-destructive, high-content assessment of the health and stability of an engineered tissue either in vitro or in vivo. A need in the field and an active area of research is the development of new analytical techniques to monitor successful engraftment and function of engineered tissues. Techniques are also needed

to evaluate host responses to the implant including inflammation, apoptosis, cell trafficking and gene expression. These tools might incorporate intelligent nano sensors, which can non-invasively sense particular chemical signals indicative of their respective cellular events, into engineered tissues to monitor tissue behavior. More research is required to characterize the optimal approach for implantation of these devices into the joint environment.

The Industrial problem

As of today, no one has described a standard real time technique for the evaluation of oxygen metabolism in the constructed tissues or organs prepared by tissue engineering processes. During the last decade, the three-dimensional printing technology was introduced into the research area of tissue engineering and organ fabrication.

The main severe problem in tissue engineering is the possibility to provide enough oxygen in tissues thicker than 150-200 microns. The development of vascularization within tissues constructs remain the holy grail of tissue engineering processes. It will be necessary to monitor the viability of the constructed tissue or organ. This type of monitoring is necessary in order to comply with the Quality Assurance requirements of the industrial process in production of implantable tissue or organ in patients.

The proposed solution

MDX Life Sciences is proposing to develop and apply unique set of the medical devices based on FDA approved technology. The approved technology is based on intracellular mitochondria NADH/Fp redox state. In addition, the device will enable to monitor microcirculatory blood flow, blood volume and hemoglobin saturation. It will be used as part of the quality assurance protocol of the Tissue Engineering industry in production of tissues and organs to be used in patients.

The company will construct and manufacture three devices that will fit to the various levels of complexity of the manufacturing processes in the tissue engineering industry.

2. High-level Summary of Project Tasks and Deliverables

#	Task	Deliverable	Estimated Timing
1.	Monitoring of NADH/Fp in vitro	MDX-TE1 Device	12 Months
2.	Two dimensional NADH/Fp mapping in vitro.	MDX-TE2 Device	18 Months
3.	In Vivo monitoring of NADH/Fp, Tissue Blood Flow and Hemoglobin Oxygenation.	MDX-TE3 Device	24 Months

3. Technical Approach

The main parameter that represents the energy metabolism of a tissue or organ in vitro and In vivo is the redox state of the mitochondrial oxidative phosphorylation enzymes. In addition, it is necessary for in vivo situation to monitor the function of the microcirculation represented by tissue blood flow, blood volume and hemoglobin oxygenation. All four mentioned parameters will be measured by tissue spectroscopy approaches seen in figure 2.

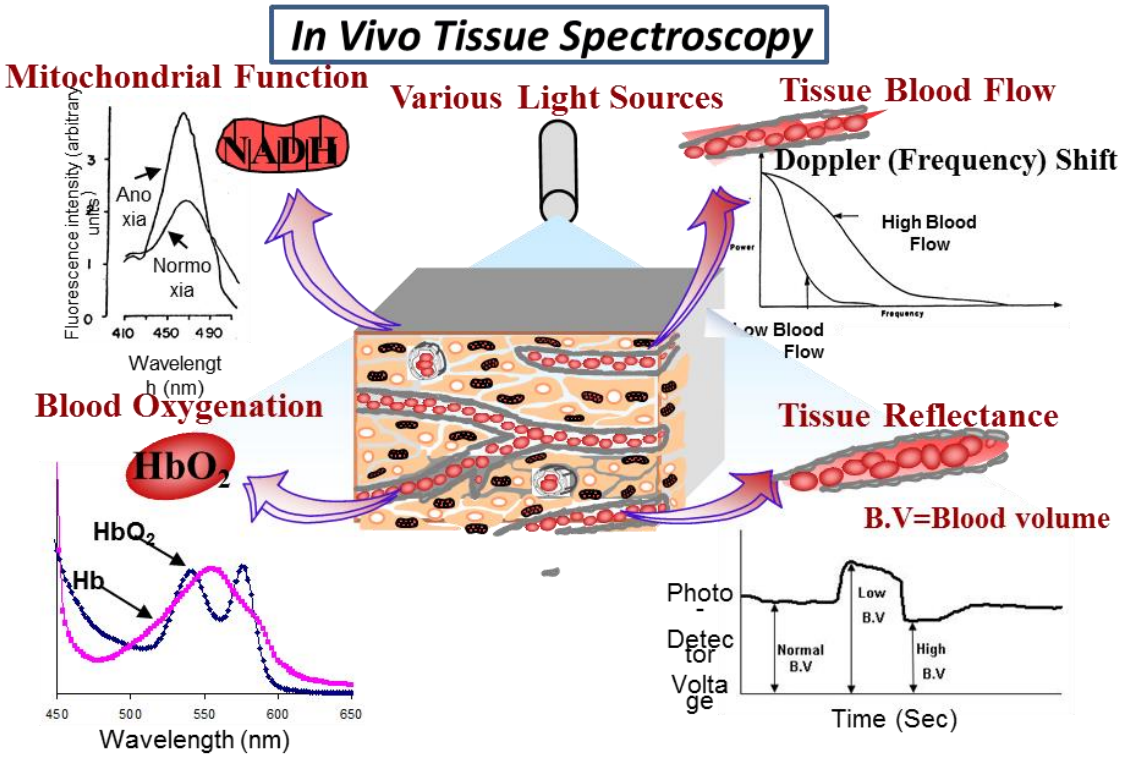


Figure 2: The four parameters to be measured by the three devices to be developed during the two years of activity.

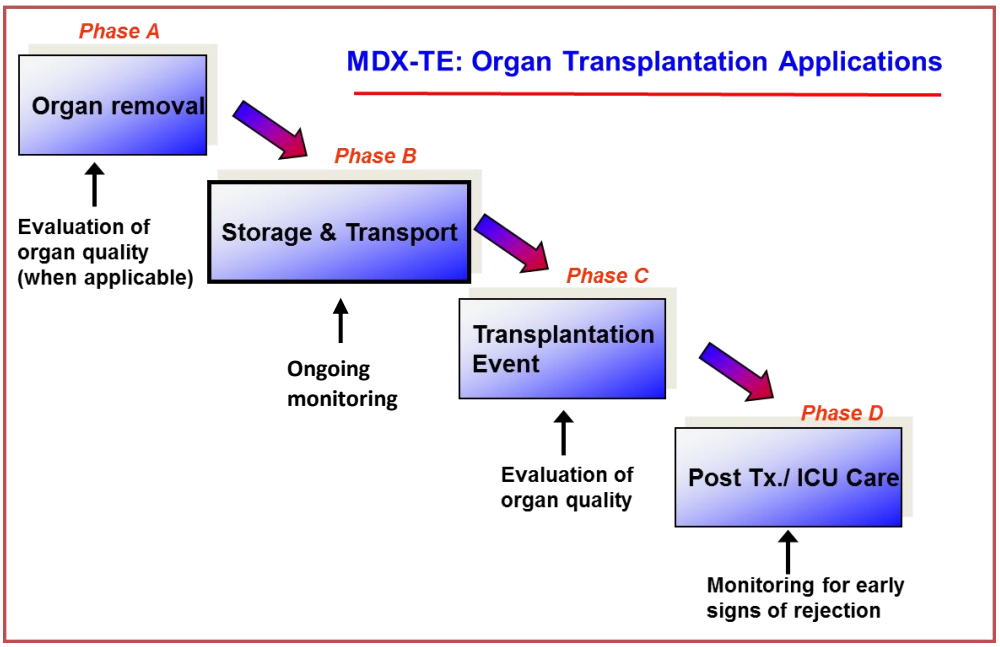


Figure 3: Illustration of the use of the various monitoring devices (MDX-TE1, TE-2 and TE3) during the entire process of organ transplantation procedures. Also, the various developed devices will be used in testing the efficacy of the AOC when used as an oxygen carrier during the Tissue Engineering processes.

The first model to be developed will monitor mitochondrial function by measuring the redox state of NADH alone and afterward together with the fluorescence of the Flavo proteins located

in the inner membrane of the mitochondria. Tissue reflectance will be measured together with the two fluorescence signals in order to obtain the net fluorescence change. This model will be used in testing the quality of organ in the donor (phase A and C in figure 3)

The second model will enable the measurement of the mitochondrial NADH and Flavo proteins in two dimensional configurations. This device will provide a map of the redox state of the tissue. Those two devices will be used only in monitoring tissues that are tested under in vitro conditions and are perfused with a preservative solution. This model will be used in testing the quality of organ in the donor (phase B in figure 3), see also our presentation - Acellular Oxygen Carrier (AOC) for Tissue Preservation and Organ Transplantation.

The third model will be developed for in vivo studies of tissues and organs containing blood. It will include the monitoring of microcirculatory blood flow as well as hemoglobin oxygenation in addition to the monitoring of mitochondrial redox state (NADH/Fp). This model will be used to monitor signs of rejection of transplanted organ in the patient during the post-operative period (phase D in figure 3).

The basic configuration of the set of devices to be developed is shown in the figure 4.

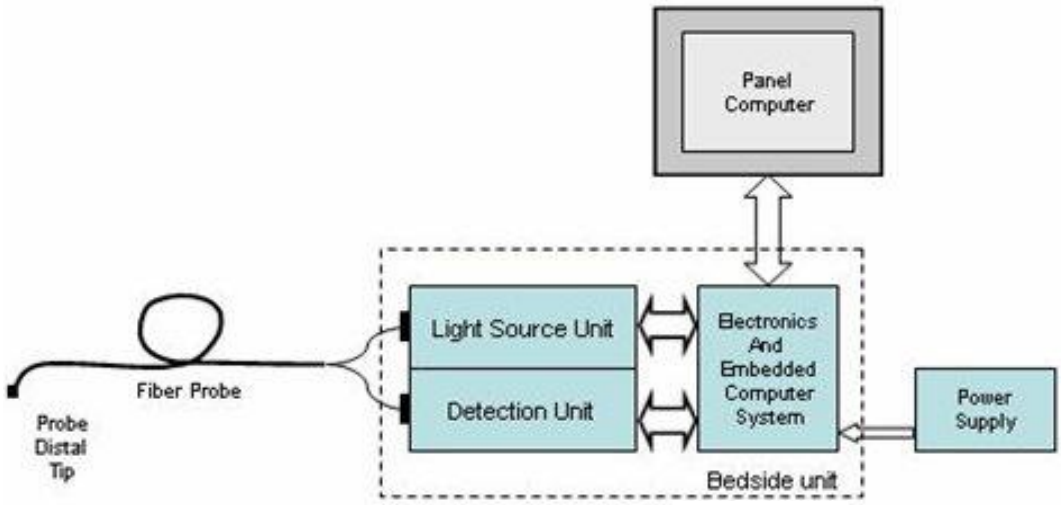


Figure 4: The basic system subunits - The Light Source Unit emits multiple wavelength light into a fiber optic probe that delivers the light to the tissue and collects the returning light. The Detection unit converts light signals into electrical signals. The electronics and embedded computer system that controls the light source and detection unit functions and performs data acquisition and preliminary data processing.

4. Estimated project cost

Total Project Cost	$ 8 million

References

1. California Institute for Regenerative Medicine: *Engineering Strategies, Opportunities, and Challenges for Tissue Repair and Regeneration: CIRM Workshop Summary and Recommendations - San Francisco, CA; January 12-13, 2012*