Assessing Technology Best Suited for Regenerative Medicine Biomanufacturing Biomonitoring

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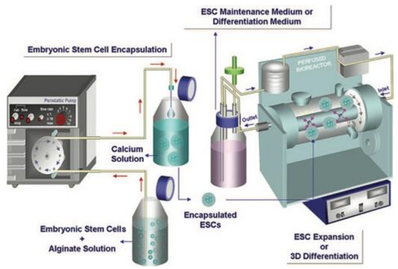
Introduction

- Regenerative medicine (RM) is an emerging and game-changing field of medicine that uses living cells as therapeutics in replaces or treating diseased tissue.
- Production of cells for the purpose of RM use is a complicated process that requires careful control of cell environments' pH, temperature, nutrient supply, waste disposal, sterility, and more.
- Special focus on stem cells, which are able to turn into many different kinds of cells.

Biomanufacturing:

- Bioreactors are the technological heart of biomanufacturing, providing controlled environment required for cell survival, proliferation and directed development of personalized RM treatments.



Schematic representation of a bioreactor

- Monitoring is a critical aspect in ensuring cells remain healthy and ideally characterized.
- FDA encourages use of process analysis technology (PAT)
  - A quality assurance system integrated into manufacturing process
  - Goal of ensuring final product quality through real time monitoring of critical quality factors, such as cell health, nutrient and waste levels, and other possible secretions[1].

Biomonitoring Techniques Considered

Dyes:

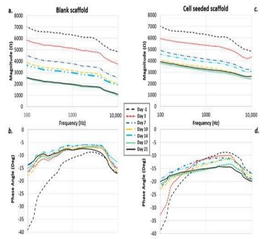
- Permanent and cytotoxic[2], even popular GFP

Imaging:

- Lack of ways to characterize cell other than count and vitality.
- Limited to 2D imaging

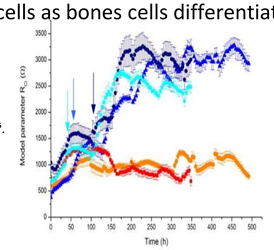
Scaffold Impedance Sensing:

- Incorporates cellular scaffold as sensor
  - Ohm's law: Voltage = Current * Impedance,
  - Capacitance Impedance = Capacitance * Rate of change of Voltage
- Impedance and capacitance change under different current frequencies and different cellular conditions
- Create an "Impedance or Capacitance Spectrum" to deduce cellular conditions[4].



Impedance magnitude and phase over current frequency with and without cells and compared with different growth times[4].

- Alternatively: track changes in impedance/capacitance over time to monitor changes in cell characteristics, such as the morphological transition from fibroblastic- to cuobidal-shaped cells as bones cells differentiate[5].

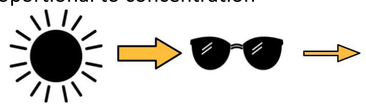


Impedance magnitude over culture time with cultures of differentiating osteocytes in blues and control cells in reds[5].

- Relies on database of cellular characteristics correlated to their impedance/ capacitance spectrum, which is still under development.

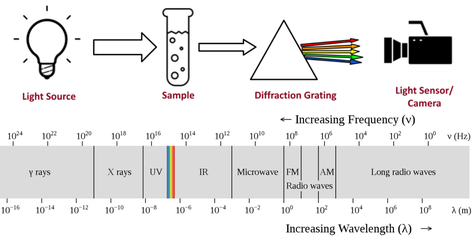
Infrared Spectrophotometry:

- Beer–Lambert law: $A=\varepsilon \ell c$
  - A = absorbance of light (at specific wavelength); ε = absorptivity, a property specific to material; ℓ = pathlength of light beam through sample; c = concentration of substance
  - Absorption is proportional to concentration



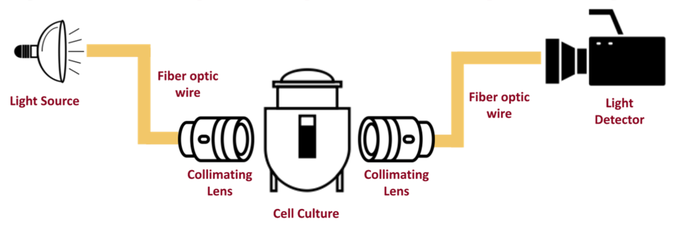
Absorbance measures attenuation, the ability of a material to reduce intensity of light as it passes through.

- Measure absorption of light passing through cell culture with **spectrophotometry**



- Light beams,comprised of photon particles of various energy and momentum, can be differentiated by their wavelength
- Spectrophotometers, analytical instruments that use diffraction gratings to separate light beams into specific wavelengths, can be used to interrogate samples
- Measuring light absorbance at different wavelengths, allows multiple analytes to be measured at once in real time.
- Light near visible and infrared (IR) wavelengths especially promising for monitoring analytes of interest in biomanufacturing RM products:
  - Partial Least Square modeling of 1000nm-2500nm light proved accurate in predicting cell viability, glucose, lactate, and glutamate concentration *in vitro*[6].
- At-line, real time monitoring of multiple parameters at once is possible, but requires advanced signal processing techniques.

Proposed IR Spectrophotometry Test System



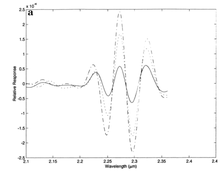
Key Biomanufacturing Biomarkers

Glucose:

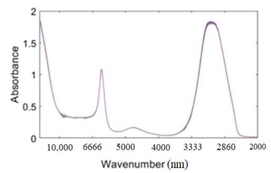
- During somatic reprogramming (turning a normal cell into a stem cell) there is a burst of glycolysis and glucose uptake[7]
- Naïve stem (not ready to differentiate) cells also consume more glucose than primed stem (ready to differentiate) cells.

Lactate:

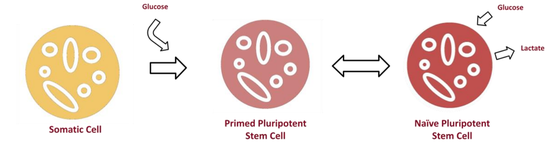
- In stem cell cultures, lactate production differentiates between pluripotent stem stages: naive cells produce more than primed cells[9]



Glucose Absorbance Spectrum[8]



Lactate Absorbance Spectrum[10]



Design of Biomonitoring Test System

Next steps:

- Arquire spectrometer test system components
- Assemble and validate custom spectrometer system
- Test accuracy, reliability, rapidness, and feasibility of measuring glucose and lactate concentrations over IR range initially performed on mock solutions followed by testing in a bioreactor-like setting.
- Further development would be to test these same parameters across different scales of production and monitoring development of stem cells between naïve and primed states.

References

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