Characterization of Sterilization Techniques on a Microfluidic Oxygen Delivery Device

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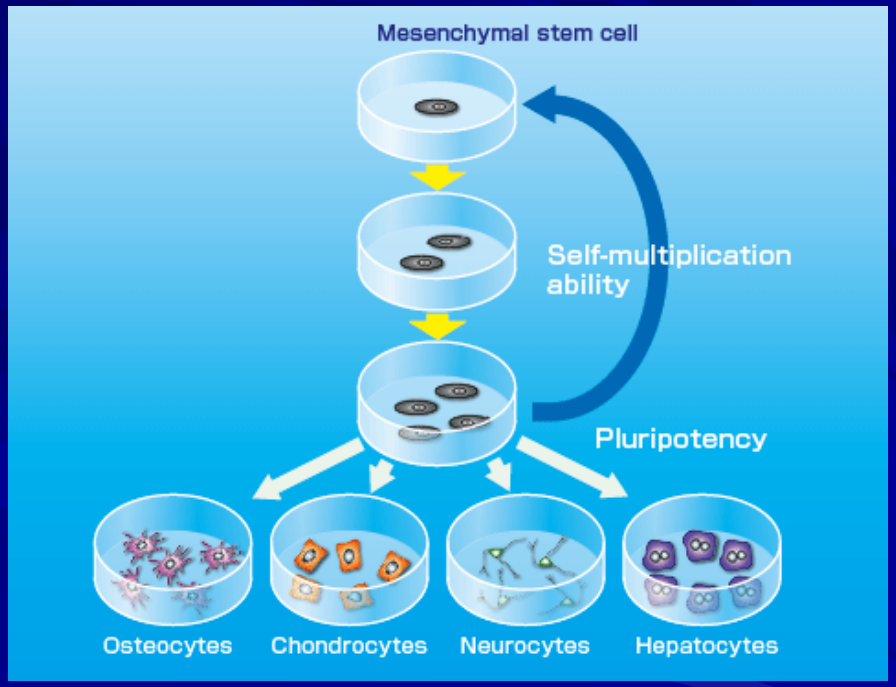
Objectives

- General: Validate utility of microfluidic device fabricated in lab
  - Designed to carefully and efficiently control oxygen exposure to cell cultures
- Specific: Characterize effects of sterilization methods on effectiveness of the device

Motivation: Why control oxygen content in cell culture?

- Simulate hypoxia and hyperoxia *in vitro*
- Some experimental applications:
  - Response of cardiac cells to changing oxygen levels (eg. heart attack)
  - Regulation of stem cell differentiation using oxygen signals



www.aist.go.jp/aist_e/aist_today/2006_22/feature/feature_06.html

Problem: Limitations of existing exposure system

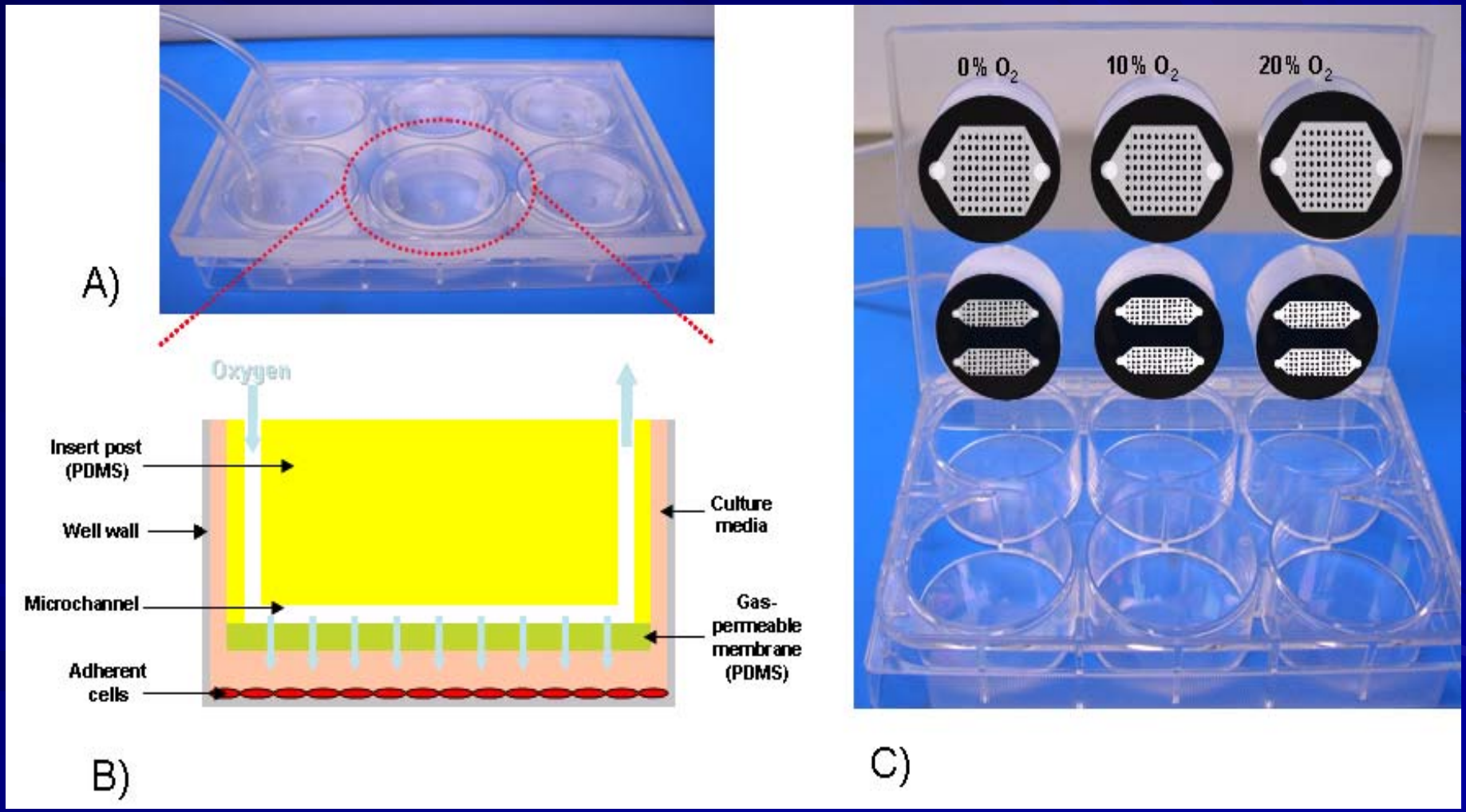
- Modular hypoxic chamber
  - Floods entire chamber with gas
  - All cells exposed to same oxygen concentration
  - Takes >3 h to equilibrate with media

Advantages of microfluidic exposure system

- Microfluidic oxygen delivery device
  - Adapts to multi-well culture plates
  - Can expose cells in different wells or same well to different O_2 concentrations
  - System equilibrates in minutes
  - Made with PDMS: biocompatible, gas-permeable

www.sumibe.co.jp/sumilon/photo/plate1.jpg

Structure of the device



Device sterilization analysis

- Devices must be sterilized before use in cell culture
- Need to investigate:
  - Optimal sterilization technique that withstands repetitive use

- Experimental Plan:
  - Cut up device into 5 separate inserts, one per sterilization technique
  - Repeatedly sterilize and measure oxygen permeability through membranes

Sterilization Techniques

2 hours in culture hood

15 min at 121ºC

15 min soak at 52ºC

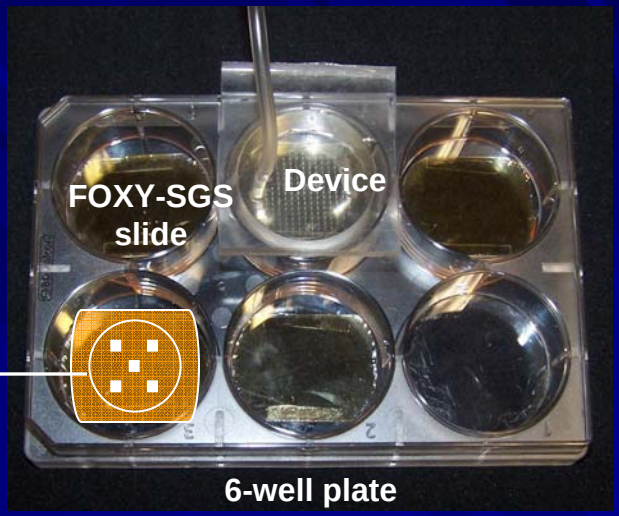
15 min soak

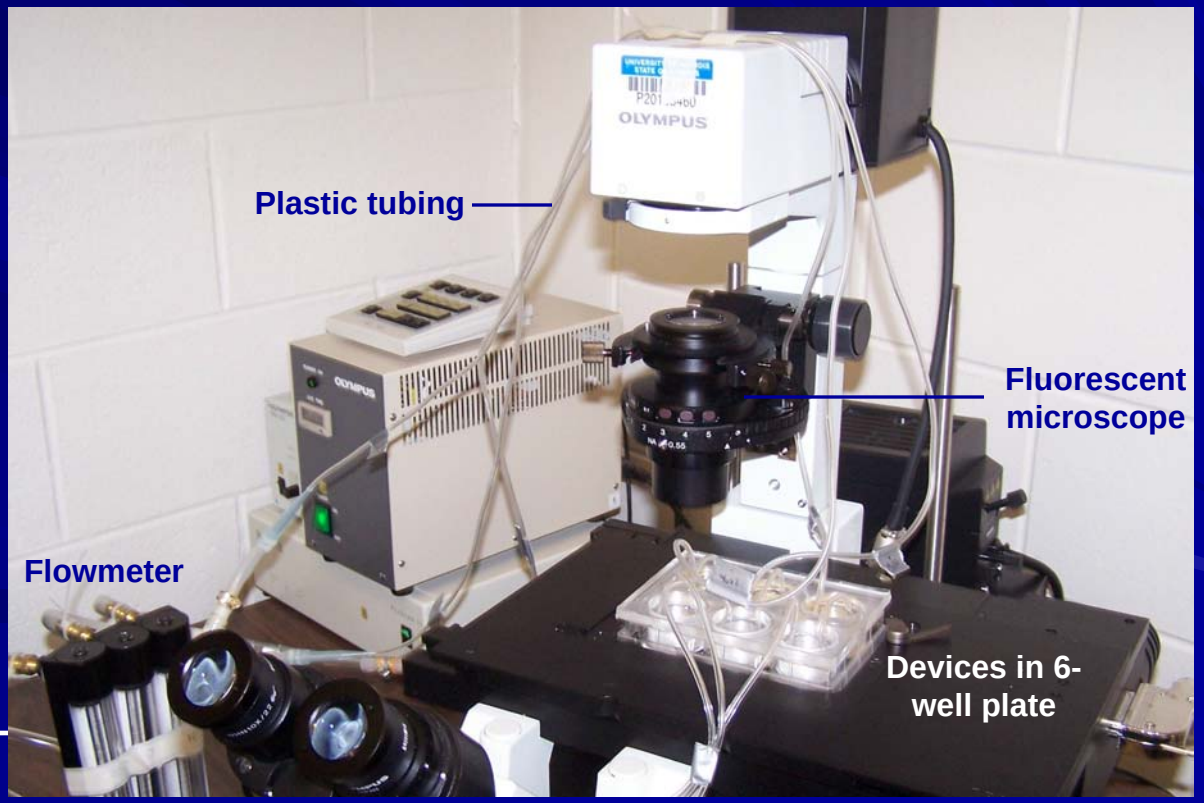
Control - Nothing

Measurement setup

- Generate calibration curve using

  0, 10, 20% O_2 flowing through a device without membrane
- Test experimental devices using 10% O_2

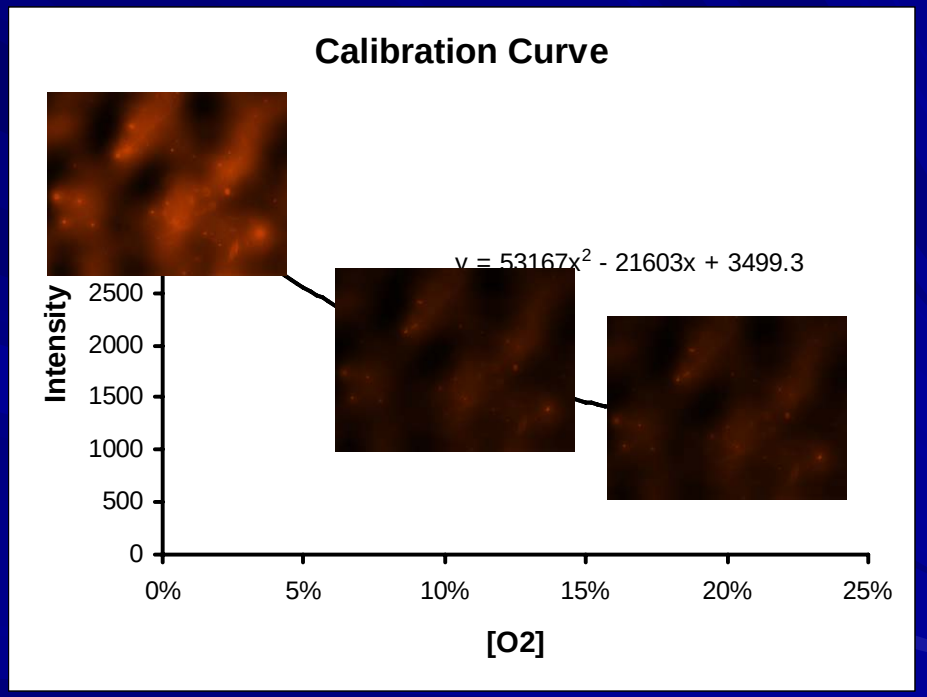




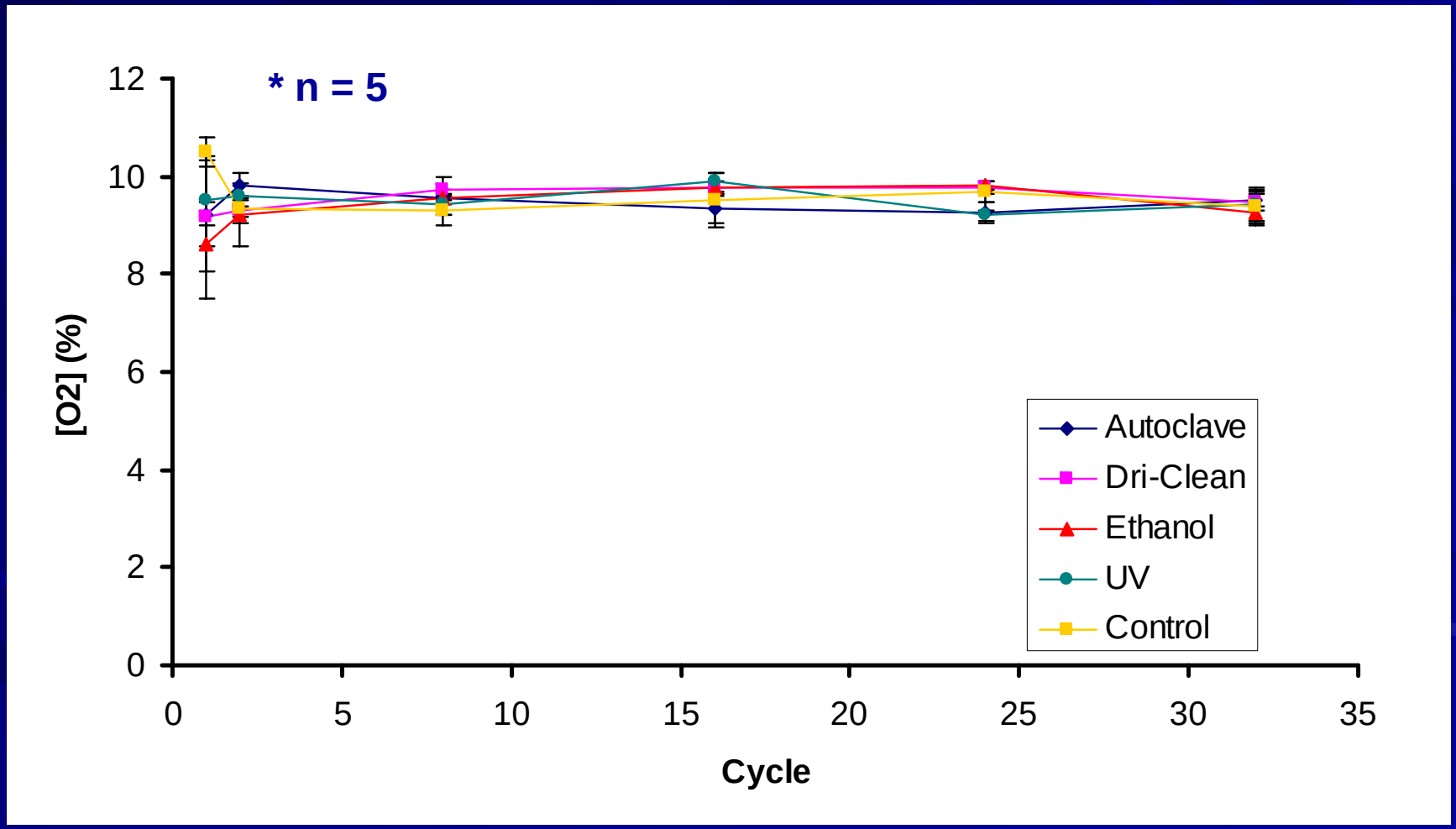

Oxygen tank

Measuring oxygen concentration with fluorescent microscopy

- Imaging software can measure fluorescent intensity
- Intensity and $[O_2]$ are inversely related
- Calibration curve used to solve for $[O_2]$ sensed in test devices



Effects of sterilization on diffusion of 10% oxygen*



Conclusions

- All of sterilized devices performed same as control
  - (2-factor ANOVA, $p > 0.5$)
  - No negative effects due to sterilization techniques
  - User can choose preferred technique
- No significant changes seen due to repetitive sterilization
  - (2-factor ANOVA, $p > 0.4$)
  - Can be used/sterilized many more than 32 times before replacement may be necessary
- Overall: Device very resistant to changes in permeability properties

Ongoing/future goals

- Develop computer models using CFD-ACE+
  - Simulate fluid dynamics and diffusion within device
  - Serves as a form of validation
- Conduct mesenchymal stem cell studies
  - Control differentiation by exposing cells to various oxygen concentrations
- Publish paper, recommend device to potential users

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