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The Microgravity Research Platform:
Novel Insights into the Mechanisms and Treatment of Infectious Disease

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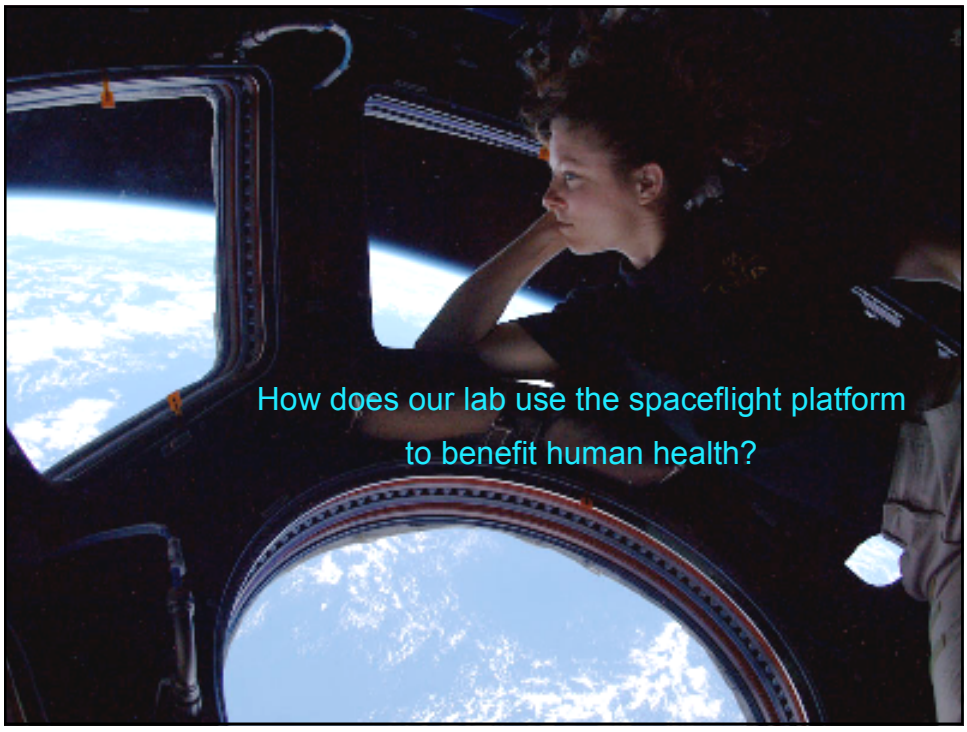

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New Insights into Disease Mechanisms Not Observed using Traditional Culture

The pathogen

SPACE ACT AGREEMENT

In-flight infections
Risk assessment/
Countermeasures

The host

Fluidic environment relevant to conditions encountered by pathogens in human body

Physiologically relevant

Biomedical phenotypes ***not*** observed during conventional culture

Vaccines/Therapeutics/ Tissue Engineering
Treatment and prevention

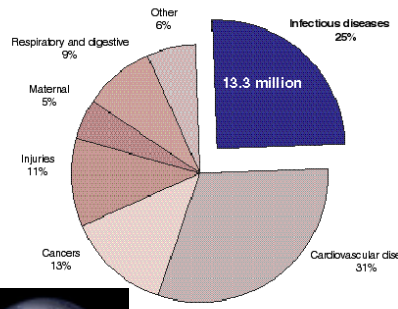
Why Infectious Disease?

Outpacing Infectious Disease!
Better understanding of the mechanisms of microbial pathogenesis leads to new strategies to combat infectious disease

- **Infectious disease - leading cause of death world-wide**
- **National and global social, economic, political, and security impact**
- **Total cost in US exceeds $120 billion annually - direct medical and lost productivity costs. Globally staggering costs.**
- **New and re-emerging infectious disease, antibiotic resistance, bioterrorism threat**

Leading causes of death

53.9 million from all causes, worldwide, 1998



Note: Cancers, cardiovascular and respiratory/digestive deaths can also be caused by infections and raise the percentage of deaths due to infectious diseases even more.

Source: WHO 1998

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Investing in Infectious Disease Research

- Current estimates of bringing a new drug to market ~ $1 billion and requires extended development times of over a decade before it reaches patients
- Even incremental decreases in this cost and time are of tremendous importance
- *Spaceflight holds tremendous promise to benefit infectious disease research*

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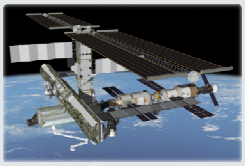
Commercial Development through Spaceflight

A unique environment for innovative discoveries to advance human health

Spaceflight culture induces novel changes in both human and microbial cells directly relevant to infectious disease development and its treatment

Virulence, Immune system function, Antibiotic resistance, Tissue engineering



Identify target mechanisms in space

Investigate target mechanisms on Earth

Discovery of previously unknown molecular targets and mechanisms

Commercial product development

Incremental decreases in cost of drugs/vaccines and time to clinical bedside

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The Reality of Spaceflight Research

Advances U.S. efforts to maintain technological leadership against international competition with a lasting impact on our scientific capability, economy, and quality of life

- ✓ **Does** provide novel vaccine and therapeutic targets for future research investigations on Earth
  - Potential acceleration and cost savings in the discovery process
- ✓ **Does** provide a platform to modify existing vaccines and therapeutics
- ✗ **Does not** create a manufacturing platform for vaccine production
- ✗ **Does not** bypass current federal regulations, including clinical trial timelines
  - Accelerated downstream vaccine approval should *not* be expected
  - FDA clinical trials ~8-10 years

We Must Communicate Realistic and Scientifically Accurate Goals and Expectations*!*

This platform has *tremendous potential*

We must reject unsubstantiated claims!

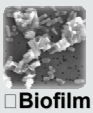
Microgravity Culture Uniquely Alters Gene Expression, Stress Resistance and Virulence in Major Human Pathogens

Virulence

Salmonella typhimurium

Pseudomonas aeruginosa

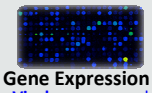
Stress resistance



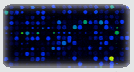
Biofilm

Spaceflight

Virulence gene expression
Quorum sensing, biofilm, adherence, cytotoxicity

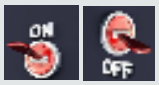


Gene Expression
- **Virulence genes** down-regulated
- **Hfq** Master regulator



Gene Expression
- **Hfq** Master regulator

Modulating ion levels turns off virulence



Hfq – ***Evolutionarily conserved master regulator of microgravity response in Salmonella and Pseudomonas***

Nickerson et. al., 2000, Infect. Immun; Wilson, et al., 2002, Proc. Natl. Acad. Sci. USA; Wilson, et al., 2002, Appl. Environ. Microbiol.; Wilson et al., 2007, Proc. Natl. Acad. Sci. USA: Wilson et al, 2008, PLoS ONE; Crabbe et al, 2011, Environ Microbiol

ASU BIODESIGN INSTITUTE — Our Recent Spaceflight Experiments

STS-131
STL-IMMUNE

First study to profile the infection process in human cells during spaceflight

Characterization of the host-pathogen interaction when *both* host and pathogen are *simultaneously* exposed to the microgravity environment of spaceflight.

STS-135
RASV

Spaceflight experiment to enhance (Recombinant Attenuated Salmonella Vaccine) strain against pneumococcal disease in human clinical trials

Flown under ASU's Space Act Agreement with NASA

Our discoveries led us to hypothesize the spaceflight environment could be used to accelerate genetic engineering of RASV strains as immunizing vectors against infectious disease by maximizing their ability to induce a protective immune response.

Our Future Spaceflight Experiments

- **NASA funded SpaceX Experiment MICRO-5/PHOENIX**

Goal: Investigate susceptibility and associated cellular, molecular and innate immune responses in the human surrogate host, *Caenorhabditis elegans*, to infection when *both* host and pathogen are simultaneously exposed to spaceflight culture

-Utilization of ASU's Space Act Agreement with NASA to use the ISS National Lab platform

Goal: Identification of cellular and molecular responses to the microgravity environment with innovative biomedical and biotechnological applications to solve major human health challenges

An Additional Research Area for our Future Spaceflight Investigations:

GOAL: *In vitro* models of human tissues which better approximate *in vivo* to study host-microbe interactions, drugs and therapeutics

Explore

Better models = more relevant research outcomes

Validate

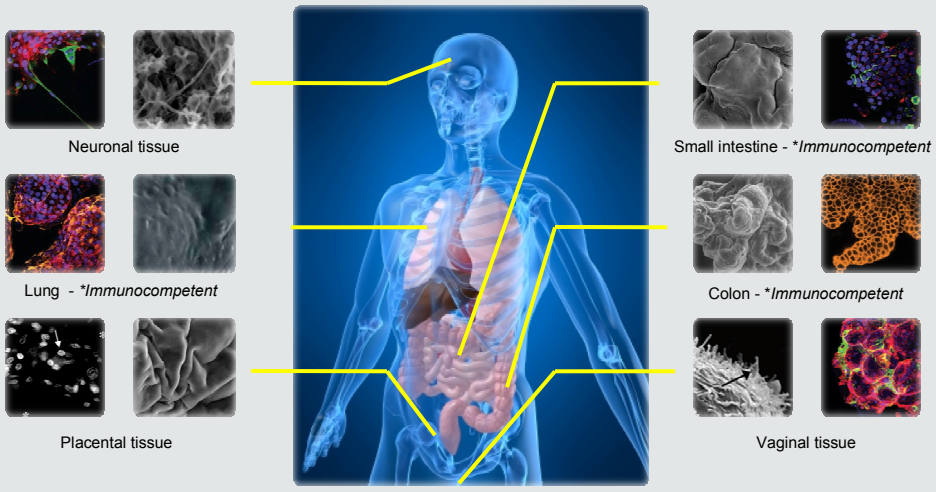
Reduce, Replace, Refine

Translate

Disease mechanisms, drug discovery, environmental health, organ generation

Benefit human health

OUR 3-D CELL CULTURE MODELS DEVELOPED FOR INFECTION STUDIES



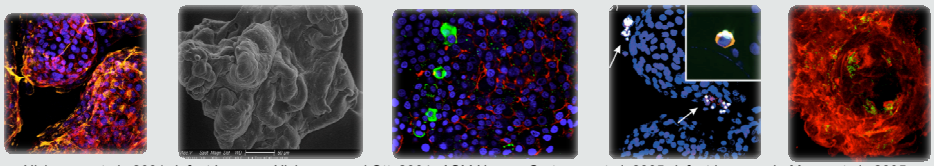
Engineering state-of-the-art 3-D tissue models under physiological low fluid shear: Mimicking immune response and underlying tissue microenvironment

Barrila *et al.* 2010. Nat Rev Microbiol; Radtke et al. 2010. PLoS ONE; Crabbé *et al.* 2011. Cellular Microbiology

Pathogens Establish Infection of 3-D Cells in Ways that Model Important Aspects of an *in vivo* Infection

*In vivo-like validation of infectious disease mechanisms **not** mimicked by conventional cell culture models*

- **Tissue Pathology**
- **Adherence, invasion, apoptosis**
- **Innate immune responses**
- **Host biosignatures – transcriptomics, proteomics, metabolomics**
- **Microbial virulence mechanisms**
- **Growth of pathogens not previously culturable**
- **Mimic human responses to antimicrobial therapeutics**



Nickerson et al., 2001. *Infect Immun;* Nickerson and Ott, 2004, *ASM News*; Carterson et al, 2005, *Infect Immun*; LaMarca et al., 2005, *Placenta*; Honer zu Bentrup, et al., 2006, *Microbes and Infect*:; Straub et al., 2007, *Emerging Infect Diseases*; Myers et al., 2008, *J Neurosci Methods*; Barrila et al,, 2010, *Nature Reviews Microbiology*; Crabbe et al, 2010, *Cellular Microbiology*; Radtke et al, 2010, *PLoS ONE;* Mellata et al, 2012, *PLoS ONE;* De Weirdt, et al, 2012, *PLoS ONE*

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What Does the ISS Microgravity Research Platform Offer?

A unique environment for innovative discoveries to advance human health

- **Novel environment offers insight into fundamental biological response parameters from *both* the host and pathogen perspective that are directly relevant to infectious disease - *and which cannot be observed using traditional experimental approaches***
- **Scientific advances and commercial potential for innovative solutions toward treatment and control of infectious disease.**

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STL - IMMUNE

STS-131

- **Objective**

  To characterize the host-pathogen interaction when *both* host and pathogen are *simultaneously* exposed to the microgravity environment of spaceflight.

  Implications for understanding how human cells function normally - how physical forces and mechanical stress influence balance between normal tissue homeostasis and disease progression

– Profiling cellular responses of human intestinal cells before and after infection with *S. typhimurium* during spaceflight

  - Changes in immune function and cellular stress responses
  - Changes in cellular differentiation
  - Targeted gene expression profiling

First study to profile the infection process in human cells during spaceflight

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Discovery Timeline Investigating Effect of Spaceflight on Microbial Virulence

First Basic Science Finding - Spaceflight analogue culture (RWV) alters *Salmonella* virulence, stress resistance, and protein expression (Nickerson et al, Infect Immun, 2000).

↓

Wilson, JW, et al. 2002. *Proc Natl Acad Sci USA* 99(21):13807-13812.
Wilson, JW, **et al.** 2002. *Appl Environ Microbiol* 68:5408-5416.
Nickerson, CA. 2003. *J Microbiol Meth* 54:1-11.
Nauman, EA, **et al.** 2007. *Appl Environ Microbiol* 73:699-705.

↓ **STS-115**

True spaceflight alters *Salmonella* virulence and gene expression and identifies role for global regulator Hfq (Wilson et al, Proc. Natl. Acad. Sci. USA, 2007)

↓ **STS-123**

Ion concentration controls regulatory and virulence response of *Salmonella* in true spaceflight culture (Wilson et al, PLoS ONE, 2008)

↓ **STS-115**

True spaceflight alters *Pseudomonas* virulence characteristics and gene expression, with Hfq regulation (Crabbé, et al. Appl Environ Microbiol 2011)

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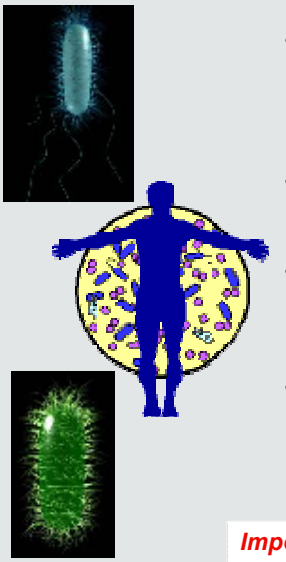
Summary Part 1

Lessons Learned from Spaceflight Culture of Microbial Pathogens

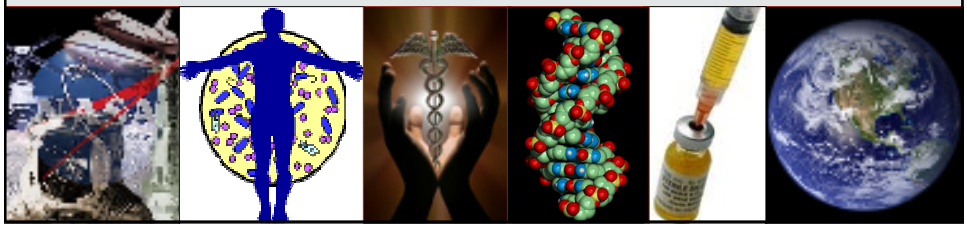


- **Spaceflight culture alters molecular genetic and phenotypic characteristics of pathogens, including virulence *in novel ways*** *not observed using traditional experimental approaches on Earth.*
- **Genes differentially expressed during spaceflight can be targeted for vaccine development**
- **Modulation of different ion concentrations to counteract/inhibit the enhanced virulence of pathogens in spaceflight**
- **Hfq is a conserved global molecular regulator that may be used by other bacterial pathogens (in addition to *Salmonella* and *Pseudomonas*) to respond to microgravity conditions**

Importance for both Earth- and spaceflight-based studies for new strategies to combat infectious disease

Infectious Disease Research and Spaceflight

- Major advances in our knowledge about biological systems - studying their responses to *extreme* environments - (ex. temp, pH, etc) - led to major advances in global human health breakthroughs
- Spaceflight is another extreme environment which offers tremendous potential to provide new insight into biological responses - including *infectious disease*
- Spaceflight produces an environment that is relevant to conditions encountered by the pathogen during infection in the human host – but gravity masks key cellular responses on Earth.



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WHY STUDY BACTERIAL PATHOGEN RESPONSES TO FLUID SHEAR?

- Pathogens experience wide fluctuations in fluid shear *in vivo* during infection.

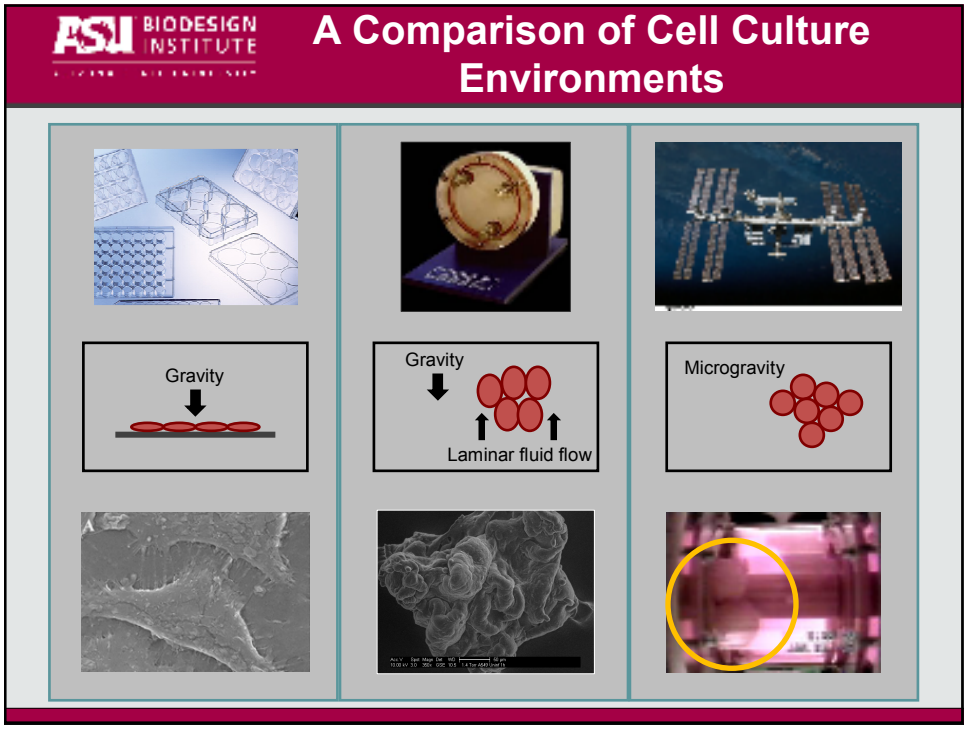
- Most studies have not cultured bacteria under physiological fluid shear conditions encountered during infection.

- Fluid shear affects bacterial gene expression, physiology, pathogenesis - but mechanism(s) not well understood.

- Entire classes of microbial genes/proteins involved in host interactions not previously identified during growth under conventional culture conditions.

- New targets for vaccine/therapeutic development.

Low fluid-shear regions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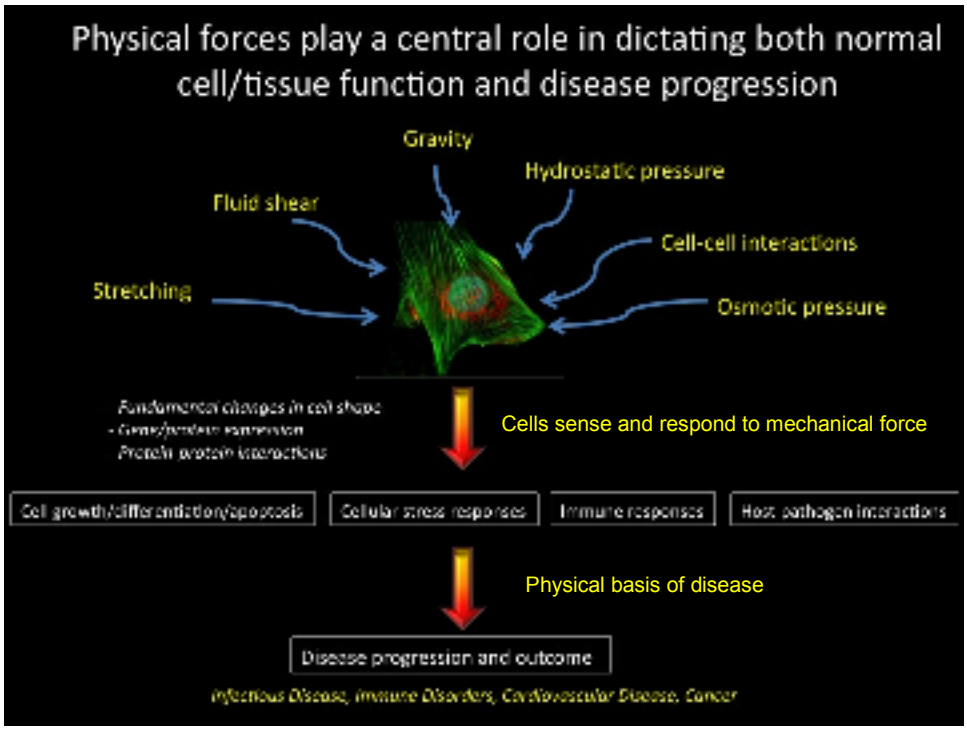
A Comparison of Cell Culture Environments
Gravity
Gravity
Laminar fluid flow
Microgravity


Physical forces play a central role in dictating both normal cell/tissue function and disease progression
Gravity
Hydrostatic pressure
Fluid shear
Cell-cell interactions
Stretching
Osmotic pressure
Cells sense and respond to mechanical force
Cell growth/differentiation/apoptosis
Cellular stress responses
Immune responses
Host-pathogen interactions
Physical basis of disease
Disease progression and outcome
Infectious Disease, Immune Disorders, Cardiovascular Disease, Cancer


VISION FOR COMMERCIALIZATION
FROM DISCOVERY TO THE CLINICAL BEDSIDE
FLIGHT EXPERIMENTS
INTELLECTUAL PROPERTY
INNOVATIVE SOLUTIONS TOWARD TREATMENT AND CONTROL OF INFECTIOUS DISEASE
PATENTABLE VACCINES, THERAPEUTICS, AND DIAGNOSTICS