Cellular and molecular mechanisms that can be exploited for therapeutic commercialization

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Introduction & Motivation

We have put together an interdisciplinary team of motivated scientists that seek to develop innovative solutions to urgent medical needs. The combination of our eclectic skill set will allow us to examine multifaceted problems from our various perspectives. By combining independent projects with a common goal of biofabrication for medical technology, we are well suited to investigate therapeutic challenges and propose innovative solutions that converge at the intersection of basic research and commercialization.

Our aim is to:
- Address important medical needs with an entrepreneurial mindset
- Alleviate the bottleneck within the transition from research to medicine
- Combine our interdisciplinary skill set to develop innovations with commercialization potential

Our team's expertise:
- *Todd Alexander* – Chemical Engineer, focus in antimicrobial peptides
- *Heather Cirka* – Biomedical Engineer, focus in cell mechanics
  - Patent: WO2012135165
- *Karen Levi* – Biomedical Engineer, focus in tissue construction
- *Sarah Runge* – Biologist, focus in molecular biology

Questions & Methods

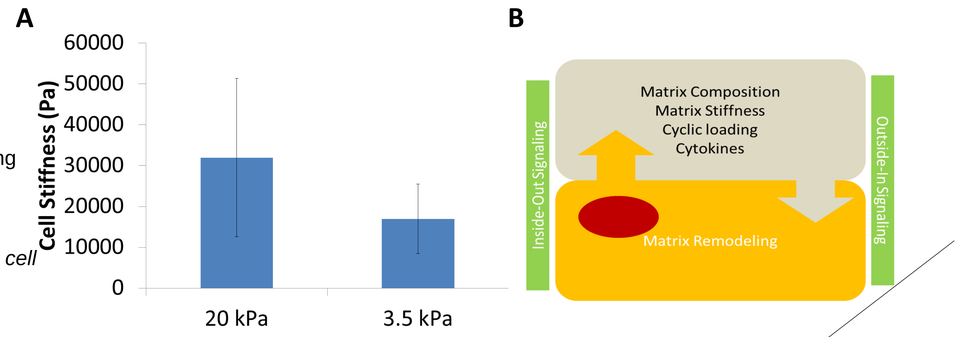
- **Cell mechanics**
  - Can several different types stimuli (substrate stiffness, protein coating) modulate cell stiffness to similar levels?
  - Is there a relationship between cell traction force generation and cell stiffness?
    - Indentation via atomic force microscopy
    - Traction force microscopy
- **Tissue generation**
  - Cell sourcing
    - How much is senescence delayed and population doubling increased in low oxygen culture conditions?
      - Population doubling study
    - Does extended culture in low oxygen affect cell differentiation?
      - Western blotting; qPCR; FACS
  - Molecular mechanism:
    - What molecular mechanism allows for increased lifespan of our experimental cells?
      - PCR; Western blotting
    - What genes are necessary for increased lifespan of our experimental cells?
      - Knockdown; overexpression; population doubling study
- **Surface / antimicrobial peptides**
  - Antimicrobial Efficacy
    - How does the length of the spacer (polyethylene glycol tether) effect the efficacy of the bound peptide system?
      - Use Quarts Crystal Microbalance (QCMD) monitoring and Live/Dead staining
    - How does the peptide density effect the efficacy of the bound peptide system?
      - Flourosectroscopy and calibration curves

Clinical Relevance

- Understanding more about mechanical cell properties will allow for intentional manipulation of substrates to develop and commercialize therapeutic solutions that more successfully relieve pathologies.
- Increasing cellular proliferation solves problems of cell sourcing and allows for cellular manipulation to a more plastic state. One commercial potential of this approach is the development of small-diameter vascular grafts.
- Optimizing antimicrobial peptides for therapeutic implantations has commercialization potential to treat any synthetic surface used in hospitals today for the prevention of biofilms.

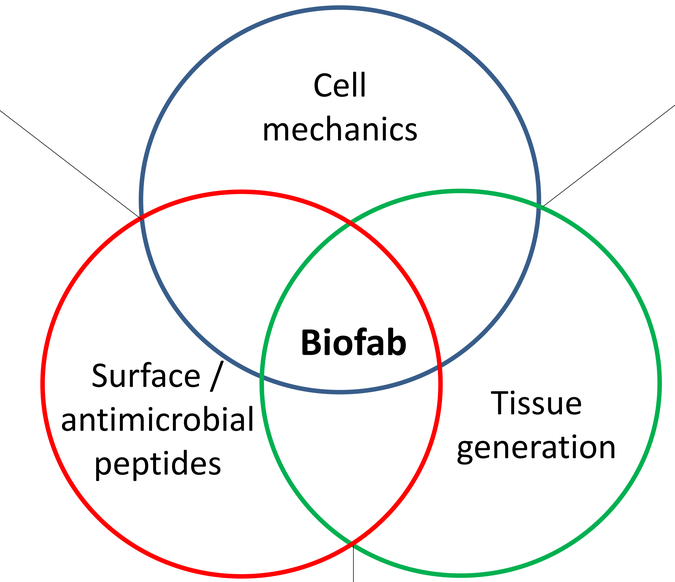
Cells are constantly sensing (outside-in signaling) and remolding (inside-out signaling) their environment[2]

Cell Mechano-response can be quantified using
- Traction Force Microscopy- *measures the amount of tension in the whole cell*
- Atomic Force Microscopy- *measures cytoskeletal stiffness at discrete points in a cell*



2.Quinlan et. al (2011) *PlosOne*, **6**(8): e23272
3. Byfield et. al (2009) *Biophys J*, **96**(12): p. 5095-5102.

Figure 1. Cell stiffness dependent upon substrate stiffness
(A) Valvular Interstitial Cells (Passage 4) were seeded onto collagen coated polyacrylamide gels overnight at low density (2,000 cells/cm^2). 20 um force maps were taken on n=10 cells per gel. (B) A custom MATLAB script was then used to extract the Young's modulus from each curve by fitting the first 200 nm of indentation data to the Hertz model for a conical indenter .[3]



By modifying a surface with a tethered antimicrobial peptide (AMP) we can:
- Chemically immobilize crysopsin-1 to a flexible linker molecule which enables lateral motion and proper orientation of the AMP without decreasing the bactericidal activity
- Increased bactericidal activity of the tethered antimicrobial peptide as compared to the physically absorbed peptide against *E.coli HB101*

Which leads to therapeutic solutions for biomaterial implant infections

Simply by altering culture conditions, we have developed an *in vitro* model system which:

- increases telomerase reverse transcriptase (TERT) levels[1]
- increases proliferative potential of the cells[1]
- increases time to senescence[1]
- remains non-tumorigenic when injected into SCID mice[1]

[1]Page et al. (2011) *Tissue Engineering 17*, 2629-2640

Our novel model system allows for:

- cell sourcing for tissue regeneration and biofabrication
- molecular study of factors that control the balance between replicative senescence and cancerous self-renewal for therapeutic purposes

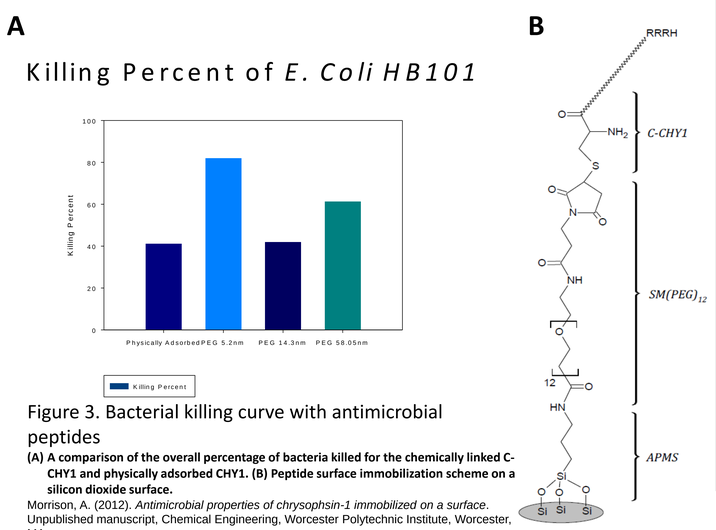


Figure 3. Bacterial killing curve with antimicrobial peptides
(A) A comparison of the overall percentage of bacteria killed for the chemically linked C-CHY1 and physically adsorbed CHY1. (B) Peptide surface immobilization scheme on a silicon dioxide surface.
Morrison, A. (2012). *Antimicrobial properties of chrysophsin-1 immobilized on a surface.* Unpublished manuscript, Chemical Engineering, Worcester Polytechnic Institute, Worcester, MA, .

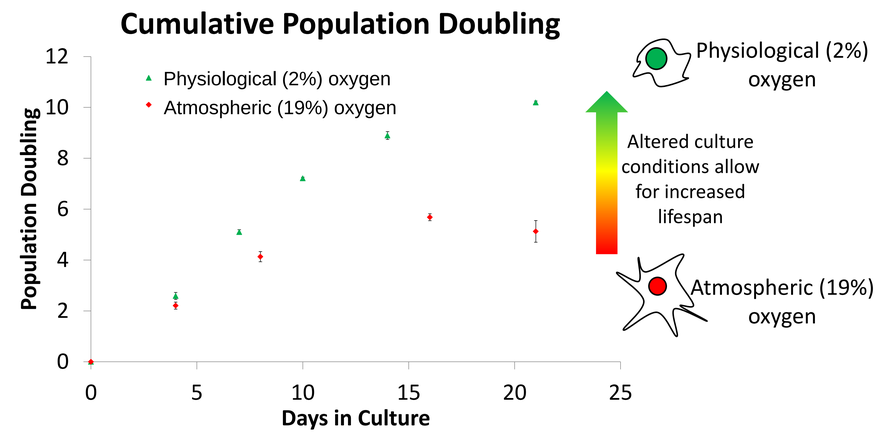


Figure 2. Cumulative Population Doublings
(A) Low oxygen culture increased the cumulative population doublings and decreased the doubling time of the primary human cells over three weeks of culture **(B)** By simply altering culture conditions, primary human cells have displayed molecular changes that allow for the adoption of a transitional phenotype of increased lifespan and increased time to senescence.