NIH NIGMS-Funded Research in the CSU Part 1

Moderated by:
Dr. Frank A. Gomez
Executive Director, STEM-NET
Office of the Chancellor

https://www2.calstate.edu/impact-of-the-csu/research/stem-net

Speakers

Edgardo Falcon-Morales, National Institute of General Medical Sciences
NIGMS Research Funding Opportunities

Perla Ayala, Cal State Long Beach
Bioengineered Scaffolds for Muscle Repair

Junjun Liu, Cal Poly Pomona
Inhibit Breast Cancer Cell Migration and Invasion by Targeting TWIST1

Maria Soledad Ramirez, Cal State Fullerton
Acinetobacter Baumannii, a Dangerous Threat: The Better We Know it, The Better We Fight it

Patrick Jurney, San Jose State University
Understanding the Vascular Adhesome to Improve Cardiovascular Biomaterials

Erin McCauley, Cal State Dominguez Hills
Probing for Bioactive Natural Products from Marine Derived Fungi

Edgardo Falcon-Morales, PhD.
Program Director

National Institute of General Medical Sciences (NIGMS)
National Institutes of Health (NIH)
Division of Training, Workforce Development, and Diversity (TWD)

STEM-NET CSU Webcast
February 24, 2023

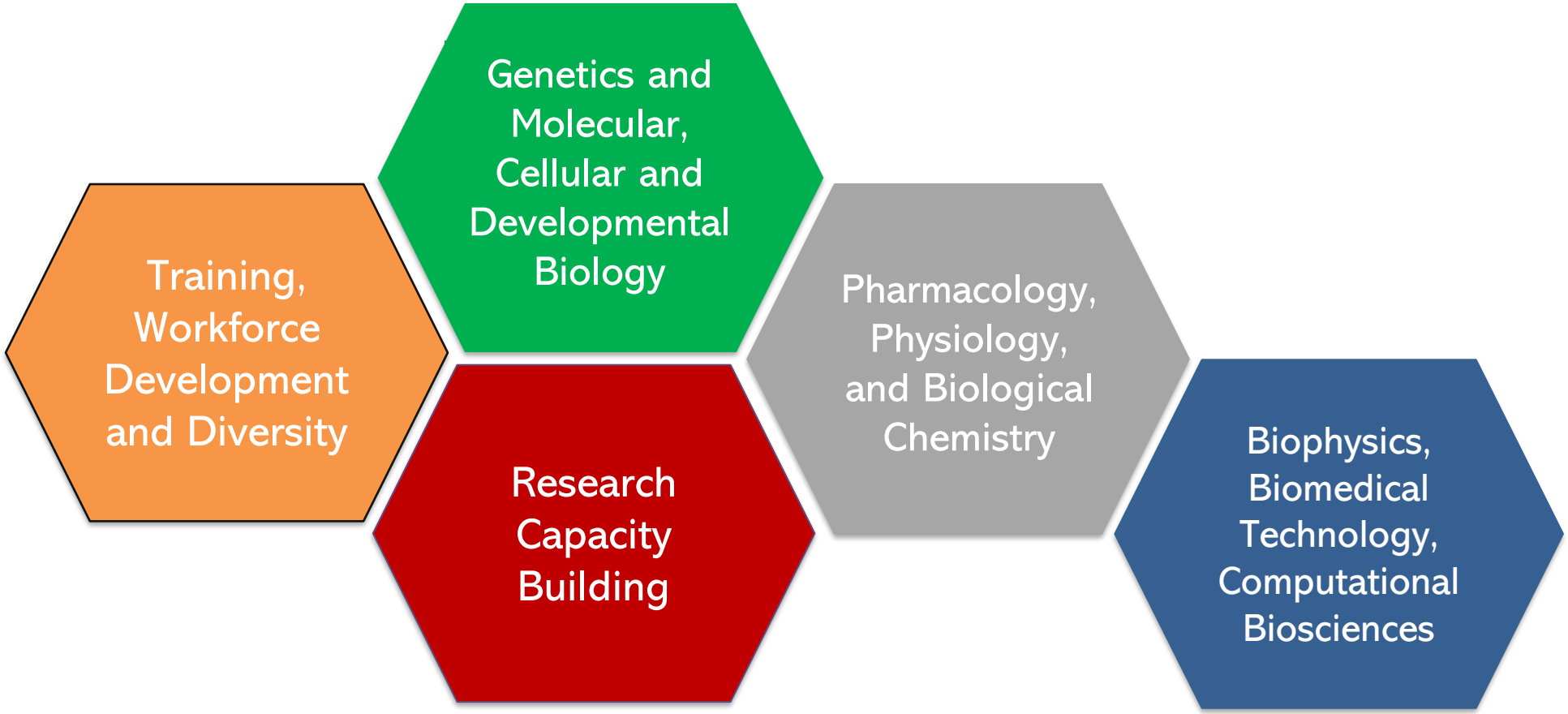
NIGMS Research Funding Opportunities

National Institute of General Medical Sciences (NIGMS)

Our Mission

- One of the **27 institutes/centers** of the National Institutes of Health (NIH)
- Supports **basic research** that increases our understanding of biological processes and lays the foundation for advances in disease diagnosis, treatment, & prevention
- Funds scientists to investigate **how living systems work** at a range of levels, from molecules and cells to tissues and organs, in research organisms, humans, and populations
- Provides leadership in **training** the next generation of scientists, in enhancing the **diversity** of the scientific work-force, and in developing **research capacities** throughout the country

NIGMS Scientific Divisions



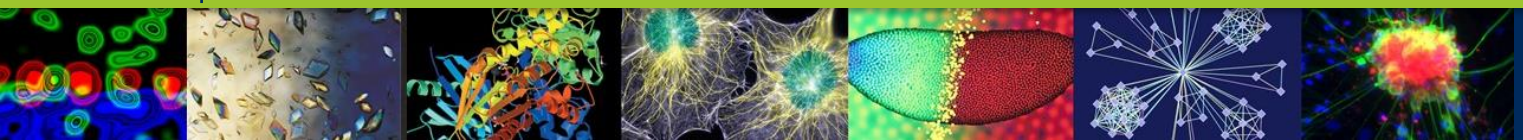
https://www.nigms.nih.gov/about/pages/contactbyarea.aspx

Academic Research Enhancement Awards (AREA) R15

PAR-21-155: Academic Research Enhancement Award for Undergraduate-Focused Institutions

- **Goal:** To support small scale research grants at institutions that do not receive substantial funding from the NIH, with an emphasis on providing biomedical research experiences primarily for undergraduate students and enhancing the research environment at applicant institutions (<$6 M in NIH research support in 4 of past 7 years).
- **Eligibility**: 1) faculty appointment at *AREA-eligible institution*, 2) cannot be PI of an active NIH RPG at time of award
- Provides up to $300,000 in direct costs total for up to 3 years.
- Emphasize providing research experiences for undergrads.
- **Contact:** Varies by IC (See FOA). For NIGMS, contacts are Anne Gershenson and Charles Ansong.

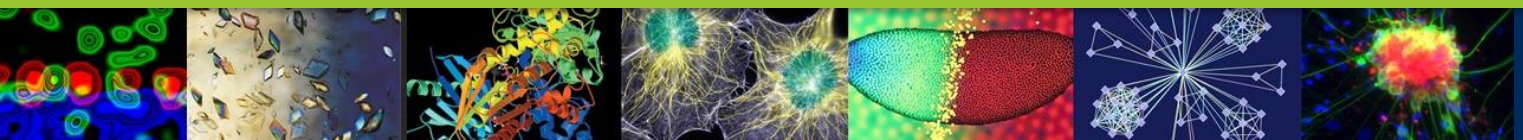
https://www.nigms.nih.gov/Research/mechanisms/Pages/AREA.aspx



AREA R15 Eligibility Criteria

- **Institutions:**
  - Must award baccalaureate degree **in biomedical sciences**
  - Total NIH support **less than $6 million per year in 4 of the last 7 years**
  - Undergraduate student enrollment is greater than the graduate student enrollment

- **Principal Investigators (PIs):**
  - Must have a primary appointment at eligible institution
  - May not have an active NIH research grant at time of award
  - May not hold multiple AREA awards at the same time
  - **All PIs** on a multi-PI application must be from eligible institutions

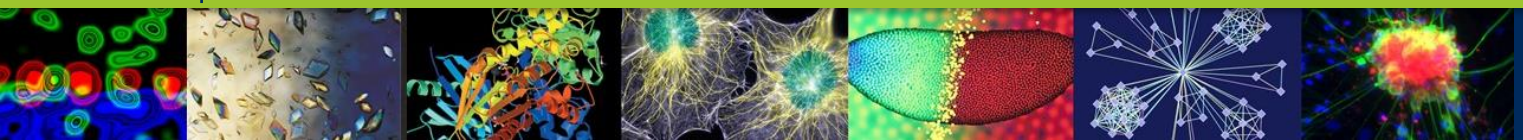


Support for Research Excellence (SuRE) R16

PAR-21-169: Established faculty (SuRE) : $100,000 DC/yr up to 4 yrs.; renewable

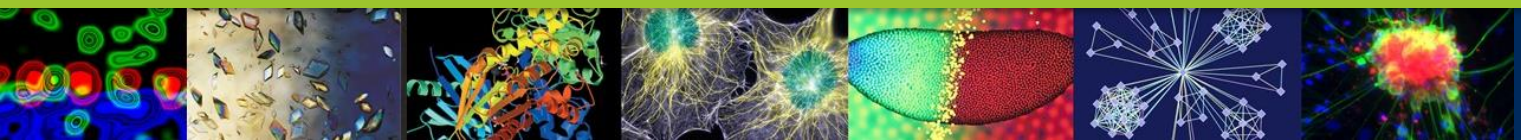
PAR-21-173: First-time awardees (SuRE-First) : $125,000 DC/yr up to 4 yrs.; non-renewable

- **Goal**: To develop and sustain research excellence of faculty, provide students with research opportunities, catalyze institutional research and enrich the research environment.
- Supports **research capacity building** at institutions that:
  - Enroll significant numbers of students from backgrounds nationally underrepresented in biomedical research (see NOT-OD-20-031).
  - Award baccalaureate and/or graduate degrees in the biomedical sciences.
  - Receive limited NIH Research Project Grant funding.
- Research activities require participation by students.
- **Contact:** Varies by IC (see FOA). For NIGMS, contact Irina Krasnova



Institutional Eligibility for R16s

- Award BA/BS and/or graduate degrees **in biomedical sciences**
- Have **< $6 M/year (total costs) from NIH Research Project Grants (RPG) in past 2 years** calculated using NIH RePORTER
- **Enroll ≥ 25% undergraduate students supported by Pell grants** using the IPEDS database as a reference; or medical/health professional school founded to educate students from underrepresented groups
- Institutions with no more than 20 total active SuRE, SC1, and SC3 awards for SuRE applications (not applicable for SuRE-First applications)
- PI cannot have an active NIH RPG as a PI (e.g., R01, R35, U01, P01, R21, R03, R00, R15)

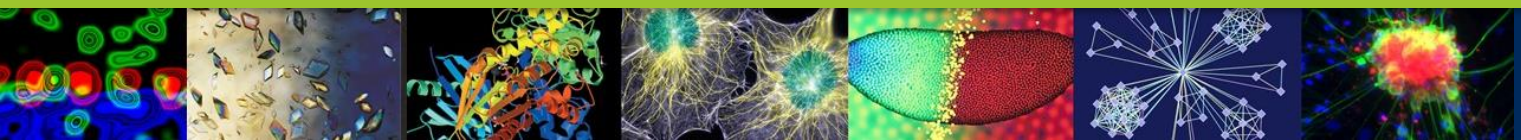


NIH R01 Grant

The NIH Research Project Grant (R01) at NIGMS:

PA-20-185: NIH Research Project Grant (Parent R01 Clinical Trial Not Allowed)

- **Goal**: Support for investigator-initiated research relevant to the mission of the NIGMS.
- Support for a **discrete, specified, circumscribed project** representing the investigator's specific interest and competencies.
- Awarded for **up to 4 years** with a budget justified by the proposed work.



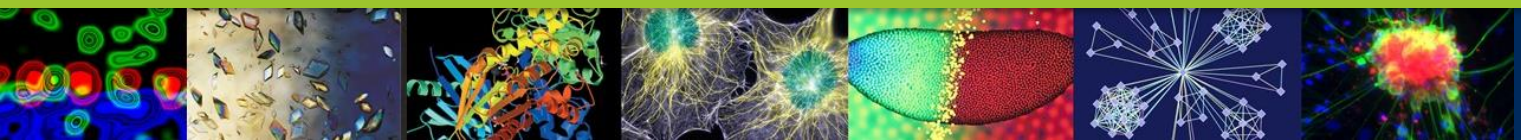
Early-Stage Investigator R01

What is an Early-Stage Investigator (ESI)?

A PD/PI who has completed their **terminal research degree** or **end of post-graduate clinical training** (whichever date is later) **within the past 10 years** and who has not previously been a PD/PI on a substantial NIH independent research award.

- At Study Section, ESI R01 applications are “clustered” during review to enable evaluation as a group distinguished from Established Investigators.
  - All PIs on Multi-PI projects must have ESI status to qualify as an ESI R01 application.
- NIGMS support for ESI R01 applications is a high priority.
- ESI R01s receive five years of support at NIGMS compared to four for established investigators.

https://grants.nih.gov/policy/early-investigators/index.htm



Maximizing Investigators' Research Award (MIRA) (R35) Program

PAR-20-117: Early-Stage Investigators (ESI; to be re-issued)

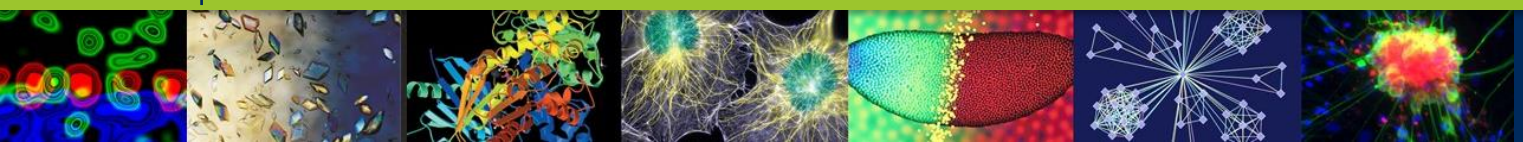
PAR-22-180: Established and New Investigators

Established Investigators are those with existing GM support (R01, R35, SC1, DP1, DP2, R37, or NRMN U01).

A **New-Investigator (NI)** is beyond 10 years post-PhD but has not been PD/PI of a substantial NIH grant.

- Must be in the mission of NIGMS
- No preliminary data is required for ESI MIRA
- Impact of proposed work while deemphasizing details of approach
- Applications **focus on the investigator and the overall research program**
- Significance of past and recent contributions to science and to the scientific community
- Requires 51% of total research effort
- Improved success rates

https://www.nigms.nih.gov/Research/mechanisms/MIRA/Pages/default.aspx



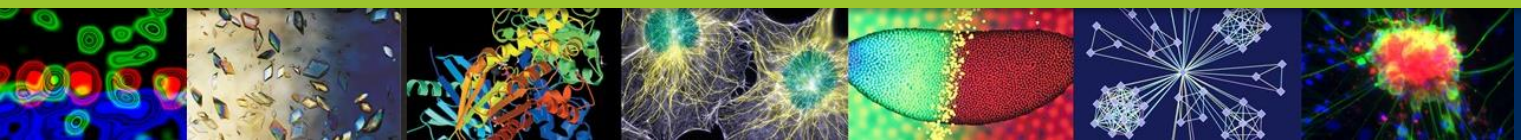
Technology Development Programs

- PAR-22-126: R21 Exploratory Technology Development:
  - Up to 2-year project periods
  - Maximum budget $275,000 DC for 2 years; no more than $200,000 in a single year
  - Unpublished data not allowed; must be novel, high risk is acceptable
  - No untested biomedical hypotheses
  - Project outcome is a proof-of-concept study

- PAR-22-127: R01 Focused Technology Development:
  - 4-year maximum project periods (ESIs eligible for 5 years), renewable one time
  - Budget requests are not limited but need to reflect the actual needs of the proposed project.
  - Preliminary data to support feasibility of the approach is allowed.
  - Validation studies against known standards are allowed but no untested biomedical hypotheses
  - Project outcome is a working prototype of the technology

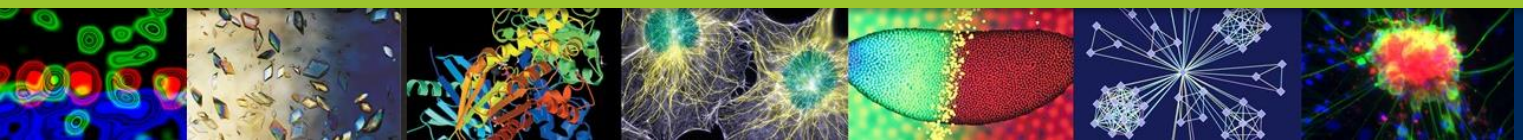
- Investigators are strongly encouraged to contact program staff (NIGMS_TechDev@nigms.nih.gov) prior to and in preparation for submitting an application to these programs.

https://www.nigms.nih.gov/grants/R21-R01/Pages/NIGMS-Technology-Development-Programs-R21-and-R01.aspx



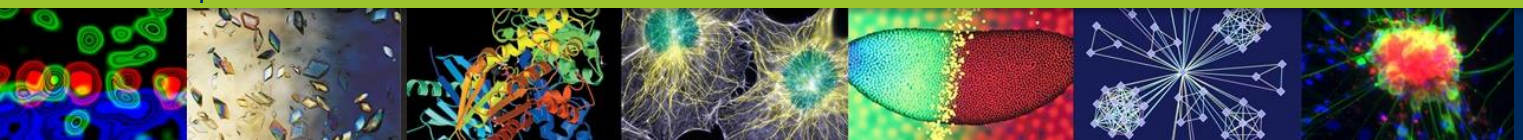
Other research funding opportunities of interest

- Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR)
  - **Phase 1 (R41/R43)** establishes the scientific and technical merit and feasibility as well as the potential for commercialization of the proposed research.
  - **The Phase 2 (R42/R44)** grant continues research or research and development (R&D) efforts initiated in Phase 1.
  - The goal of NIGMS is to support innovative SBIR projects that could benefit the research communities related to its mission. SBIR/STTR grant applications are accepted in most of the scientific areas for which the Institute provides support.
  - For additional information on NIGMS SBIR/STTR programs, contact Eddie Billingslea, Ph.D.
- PAR-20-103: Collaborative Program Grant for Multidisciplinary Teams (RM1)
  - Supports applications from a highly integrated team of investigators addressing a single-focused, ambitious, and challenging project that cannot be addressed by individual R01 applications.
  - The team of researchers can be located at a single institution or multiple institutions throughout the United States.
  - For additional information on the NIGMS Collaborative Program Grant for Multidisciplinary Teams (RM1), please contact Alexandra Ainsztein, Ph.D. at RM1mailbox@nigms.nih.gov.



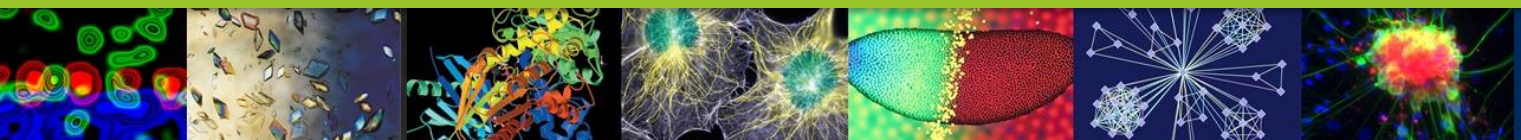
Research on Interventions that Promote the Careers of Individuals in the Biomedical Research Enterprise

- R01: PAR-21-269
- **Hypothesis-driven research** to test interventions for efficacy and replicability across career stages and at a range of institution types and to provide empirical evidence of the factors contributing to success, including the social and behavioral factors.
- This grant will support research designed to **test interventions to enhance research-oriented individuals' interest, motivation, persistence and preparedness for careers in the biomedical research workforce**.
- Not designed to support evaluation of an existing or planned program(s), nor is it intended to support a training program, curriculum development, or other activity disguised as an experiment.
- Examples of areas of study:
  - Training, Mentoring, and Networking
  - Navigation of critical transition points
  - Harassment
  - Institutional factors that influence persistence



Other Types of Support: Administrative Supplements

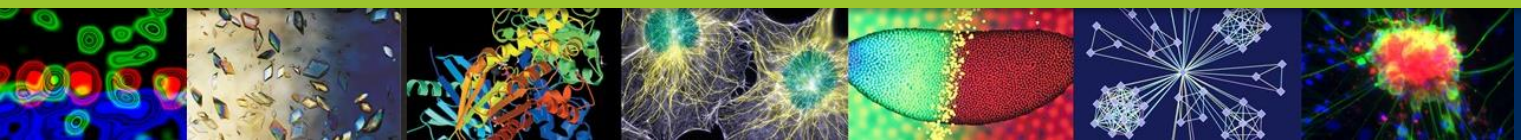
- NIH (and NIGMS) offer:
  - Administrative supplement FOAs for specific programs (such as the Research Supplements to Promote Diversity)
  - A parent administrative supplement FOA for requests that do not fall under a specific program
  - Notices of Special Interest (NOSI's) that identify an administrative supplement FOA for application submission
- A noncompeting award that provides additional funding to a currently funded grant to meet increased costs that are within the scope of the approved project, but that were unforeseen when the new or competing renewal application was awarded.
- Applicants are strongly encouraged to contact the Program Officer assigned to their grant with questions related to developing a supplement application



Research Supplements to Promote Diversity in Health-Related Research

PA-21-071

- **Goal:** To improve the diversity of the research workforce by recruiting and supporting high school and undergraduate students, postbacs, masters, predocs, postdocs, and early-career investigators developing independent projects from groups that have been shown to be underrepresented to participate in grant supported research.
- Also available to PI's of eligible research grants who are or become disabled and need support and accommodations.
- Several participating ICs: https://grants.nih.gov/grants/guide/contacts/Diversity-Supp_contacts.html
  - Depending upon the IC, there are different rules for eligibility, submission, etc.
  - **Be sure to reach out to the IC contact to discuss before applying.**



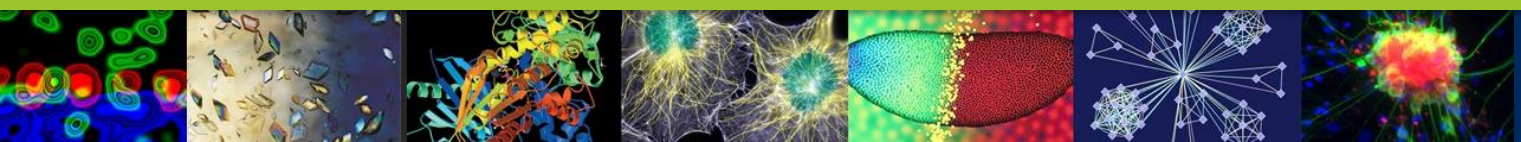
Research Supplements to Promote Re-Entry and Re-Integration into Biomedical Research Careers

NOT-OD-21-134: Notice of Special Interest

PA-18-592

- **Goal:** To support individuals with high potential to re-enter an active research career after an interruption for family responsibilities, or re-integration for graduate students or postdocs affected by unsafe or discriminatory environments to transition into a new and safer environment.
- **Eligibility**:
  - For re-entry: Doctoral degree or equivalent; some ICs allow for predoctoral students.
  - For re-integration: predoctoral and postdoctoral trainees.
  - Planning for a career in biomedical, behavioral, clinical, translational, or social science research.
  - Citizens or non-citizen nationals of the United States or to individuals who have been lawfully admitted for permanent residence (i.e., in possession of a Permanent Resident Card, Form I-551) at the time of the award.

https://www.nigms.nih.gov/Research/Mechanisms/Pages/PromoteReentry.aspx



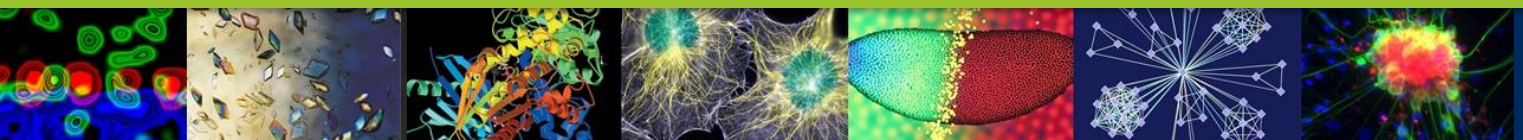
Administrative Supplements for Continuity of Research During Critical Life Events

NOT-OD-20-054

NOT-OD-20-055

- **Goal:** To support career development (K) or first-time research project grant (R) awardees whose progress is likely to be hindered by a critical life event (e.g., childbirth, adoption, or primary caregiving responsibilities). To help awardees sustain research and remain competitive by minimizing impact of departure from the workforce.
- **Eligibility**:
  - PD/PIs of the following activity codes are eligible for the award: K01, K07, K08, K22, K23, K25, K38, K43, K76, and K99/R00 **OR** the following activity codes: DP1, DP2, DP5, R01, R00, R15, R21, R35, RF1, and U01 and who have a qualifying critical life event.
  - PD/PIs with more than one independent research project grant award are ineligible for this supplement
  - Individual(s) must hold an active grant, and the research proposed in the supplement must be accomplished within the competitive segment of the active award.

https://www.nigms.nih.gov/training/Pages/Administrative-Supplements-for-Continuity-of-Research-During-Critical-Life-Events.aspx

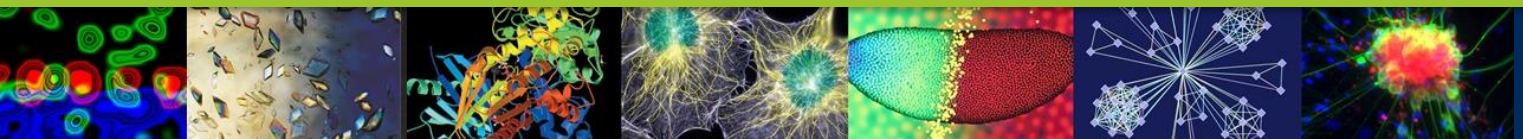


Grantsmanship

- NIGMS TWD "Training Resources" webpage
- Grant Writing Webinar Series for Institutions Building Research and Research Training Capacity
- NIH "How to Apply" training videos
- NINDS's "Building up the Nerve" podcast
- Sample grant applications from NCI, NIAID, NHGRI, NIA K99/R00, NIA SBIR/STTR, NIDCD on a variety of mechanisms

Remember

- Understand the mechanism you're applying for – research grants; training or career development awards; technology development; research capacity building
  - Always **read and study the entire Funding Opportunity Announcement (FOA)** – purpose, guide notices, review criteria, etc., and follow all instructions.
- Program Officers and Scientific Review Officers are a resource to applicants
  - Contact PO **early** in the process - send **biosketch** and **specific aims** page.
    - Helpful to determine **eligibility** and **responsiveness** of proposal to the Institute or Center's (IC's) mission and priorities
    - Reach out **after summary statement** is released to discuss next steps
  - Contact SRO for compliance questions
- Go for it – don't self-eliminate (and resubmit if needed)



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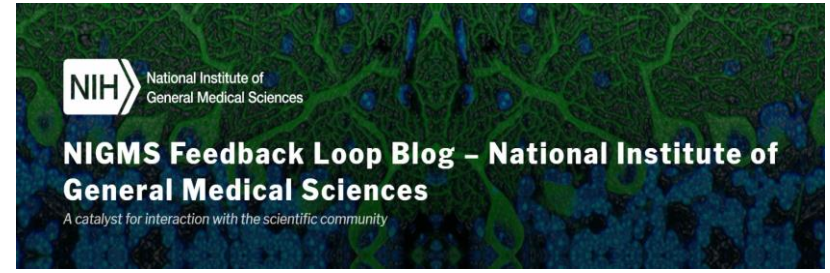
- Consult our website: https://www.nigms.nih.gov
- Read Feedback Loop blog: https://loop.nigms.nih.gov
- Follow us on Twitter:



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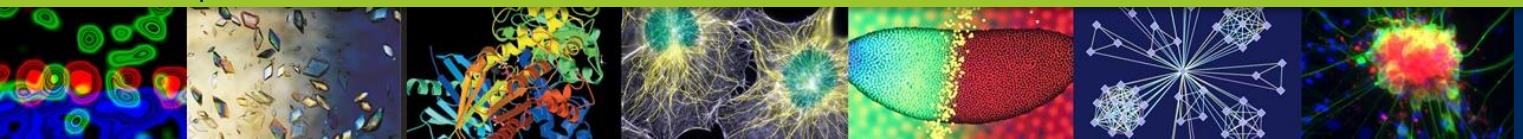


National Institute of General Medical Sciences

CALIFORNIA STATE UNIVERSITY LONG BEACH

Bioengineered Scaffolds for Muscle Repair

California State University Long Beach

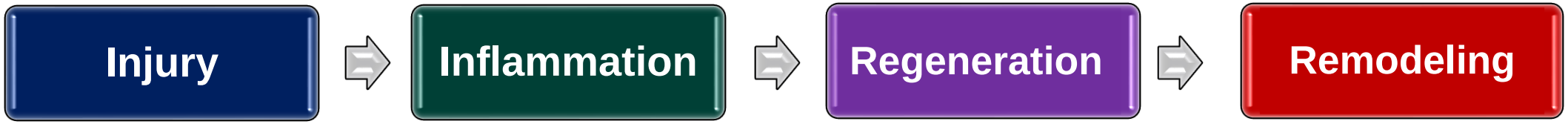
Perla Ayala, Associate Professor

Long Beach, Department of Biomedical Engineering

Perla.Ayala@csulb.edu

Project Overview

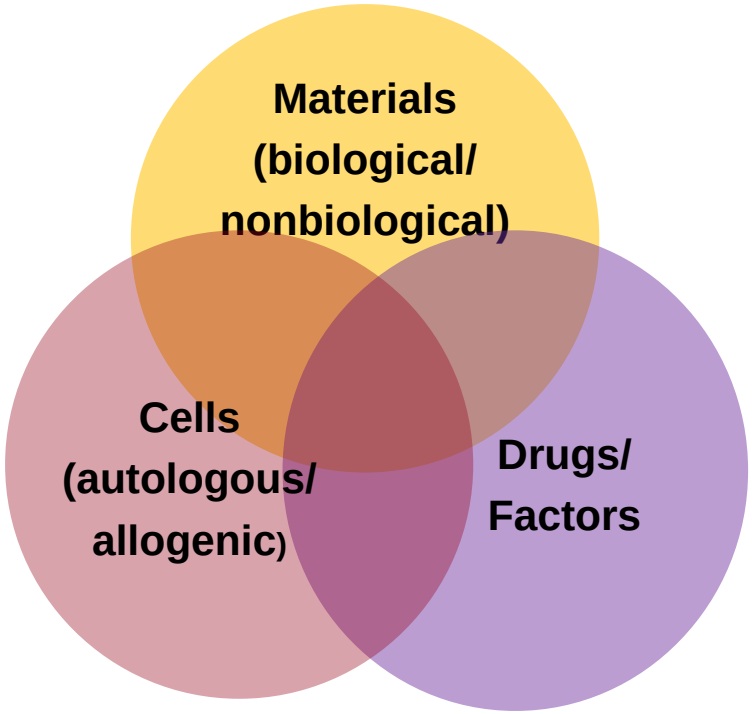
Tissue Repair Process



Pathological fibrosis: overproduction of extracellular matrix as a response to tissue damage.

- **GOAL**: Translate mechanisms of tissue regeneration into feasible therapies that will promote optimal healing.

Tissue Engineering



The field of tissue engineering focuses on the development of methods and technologies to regenerate, repair, or replace tissues.

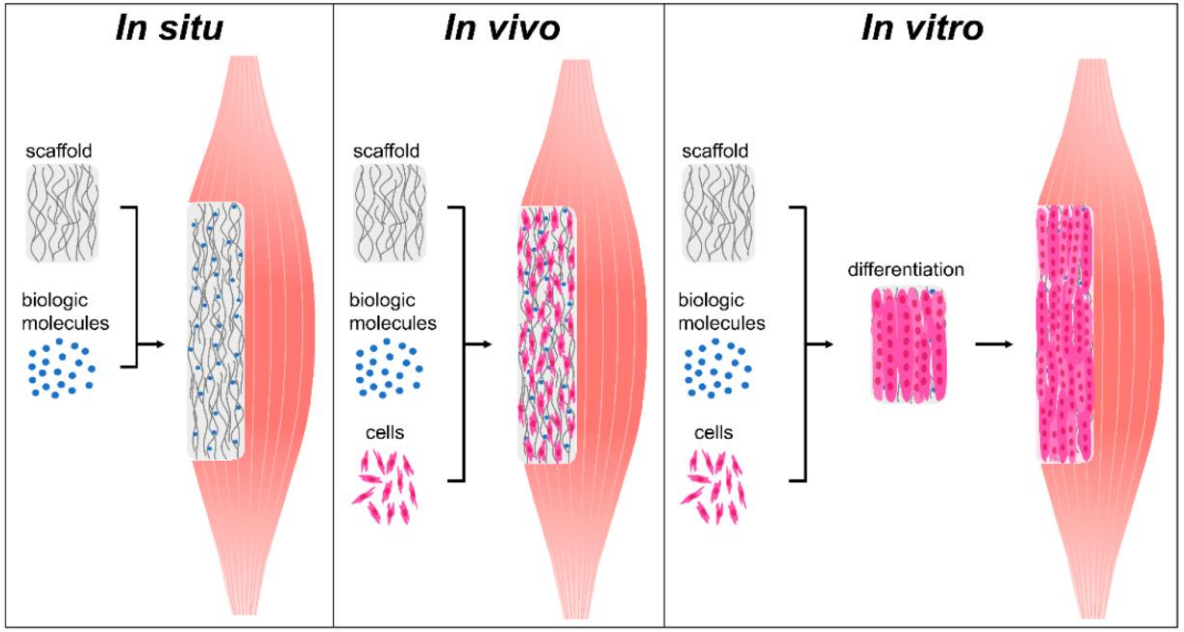
Volumetric Muscle Loss

- Severe muscle tissue damage can result on volumetric muscle loss (VML) which commonly results in significant fibrosis.
- VML can be the result of surgical procedures or major traumatic injuries, including motor vehicle crashes and explosions.
- Inadequate recovery of muscle results in long-term disability and contributes to an economic burden of ~$400 billion in the US annually.

J Orthop Res 2015;33(1):40-46

Physiol Rep 2017;5(7)

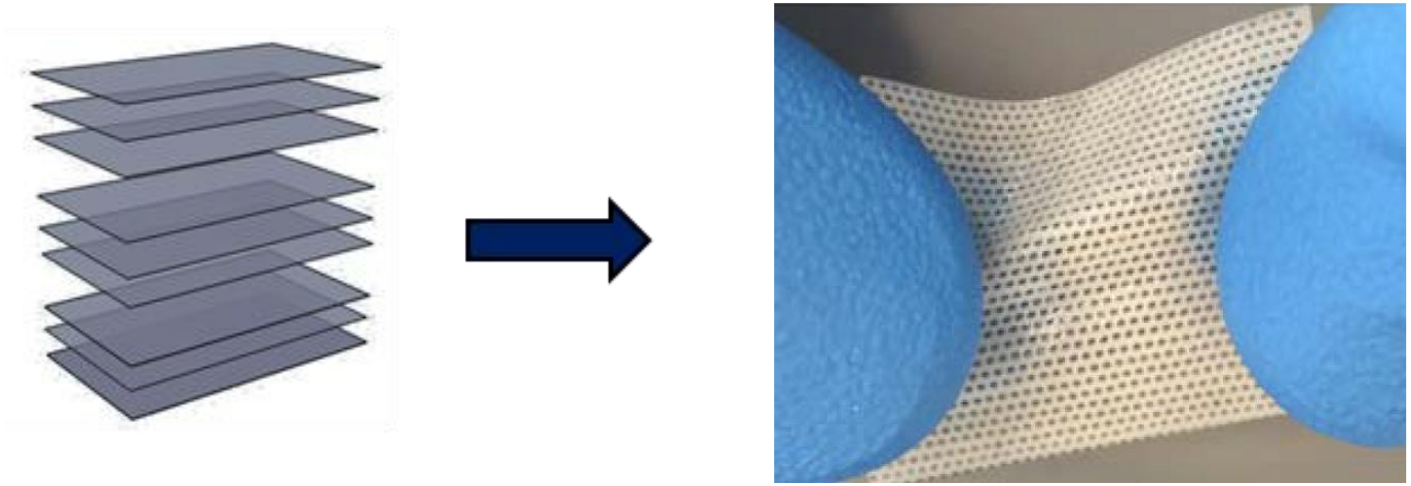
Scaffold mediated repair of VML



Various VML treatment approaches, including in-vitro grafts, which differentiate cells on a scaffold prior to implantation, proven to be the most viable option for more significant tissue damage.

Carnes, M. E., & Pins, G. D. (2020). Skeletal Muscle Tissue Engineering: Biomaterials-Based Strategies for the Treatment of Volumetric Muscle Loss. Bioengineering, 7(3), 85.

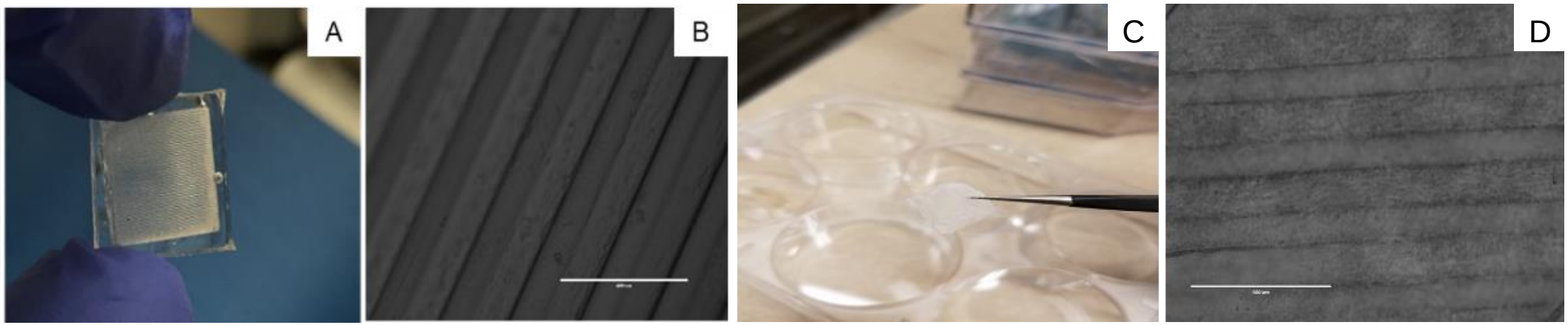
Fabrication of Mechanically Robust Constructs



- Objective: Design biomimetic scalable/implantable scaffold to increase and direct skeletal muscle regeneration.

Ayala, P et. al. Evaluation of a Bioengineered Composite Construct for Tissue Engineering Applications. Journal of Biomedical Materials Research: Part B Applied Biomaterials. 2017.

Collagen Films with Micro-channels

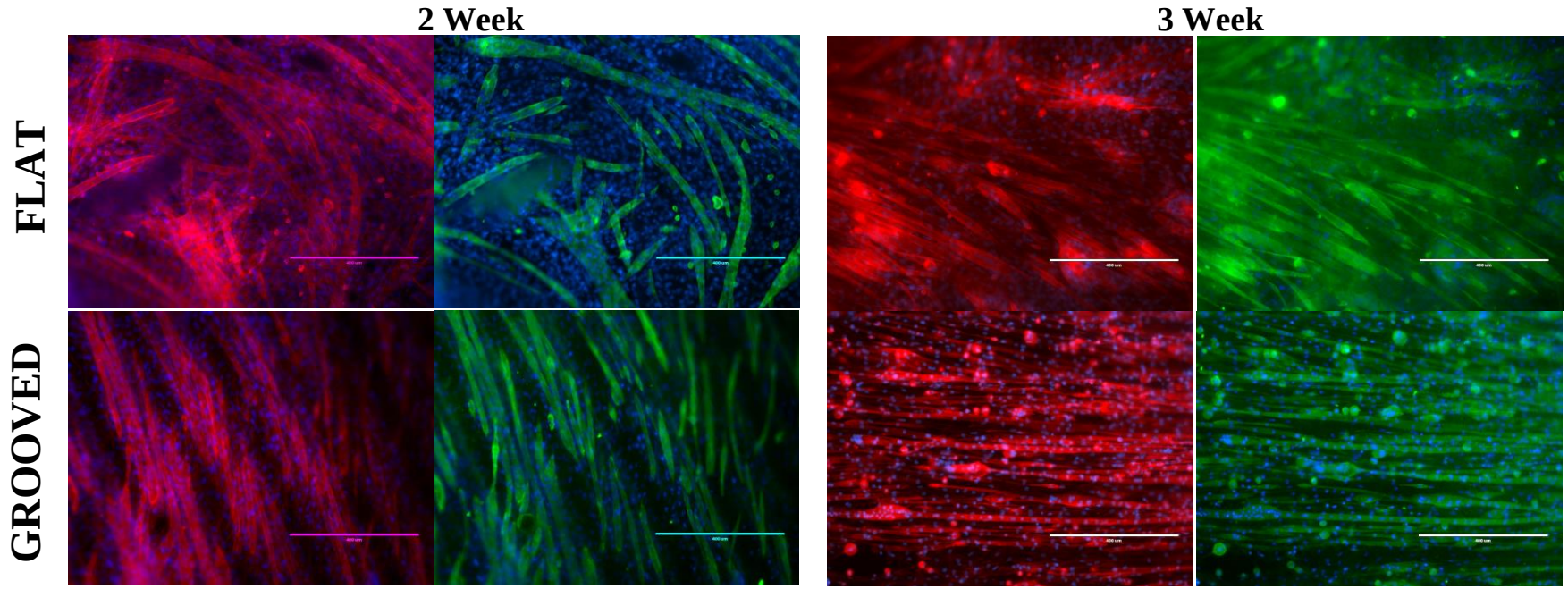


A) PDMS (polydimethylsiloxane) mold with imbedded micro-channels.
B) B) Microscopic image of channels on PDMS mold.
C) C) Collagen sheet extracted from PDMS mold.
D) D) image of collagen sheet with microchannels (right). Scale Bar= 400µm.

CALIFORNIA STATE UNIVERSITY LONG BEACH

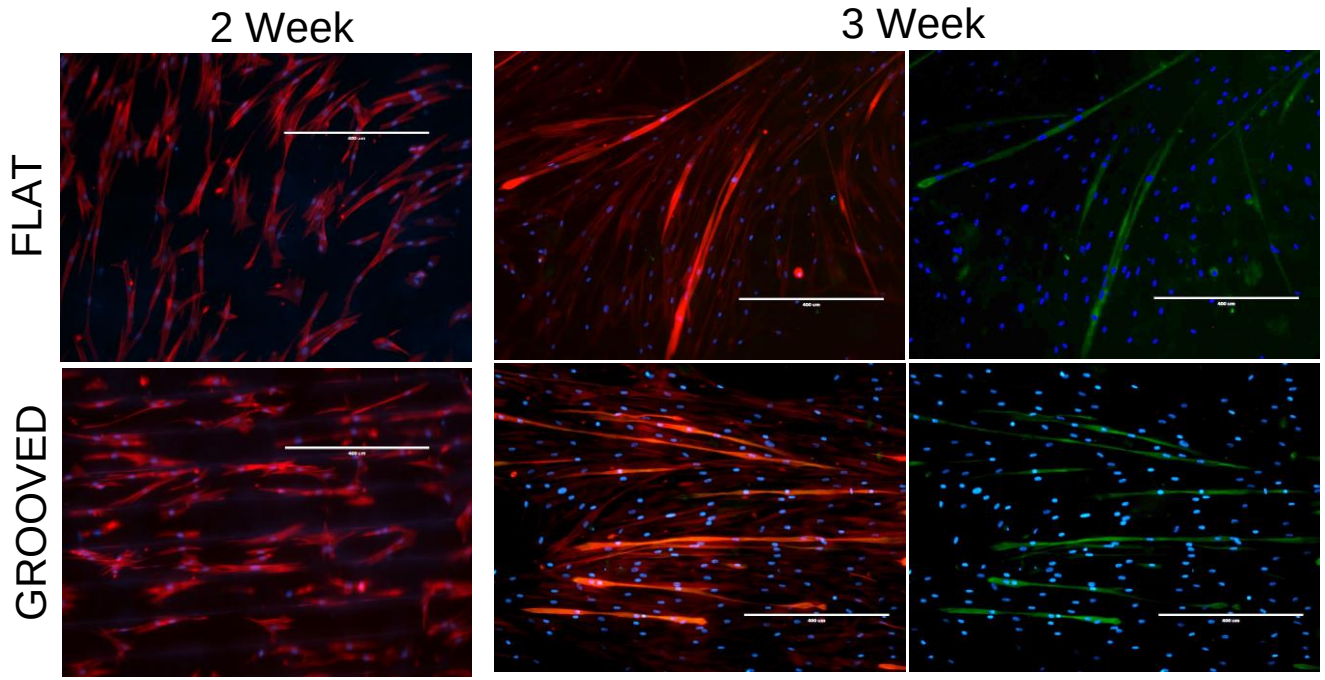
Bioengineered Scaffolds for Muscle Repair

C2C12 Myoblasts on Micro-channeled Collagen Films



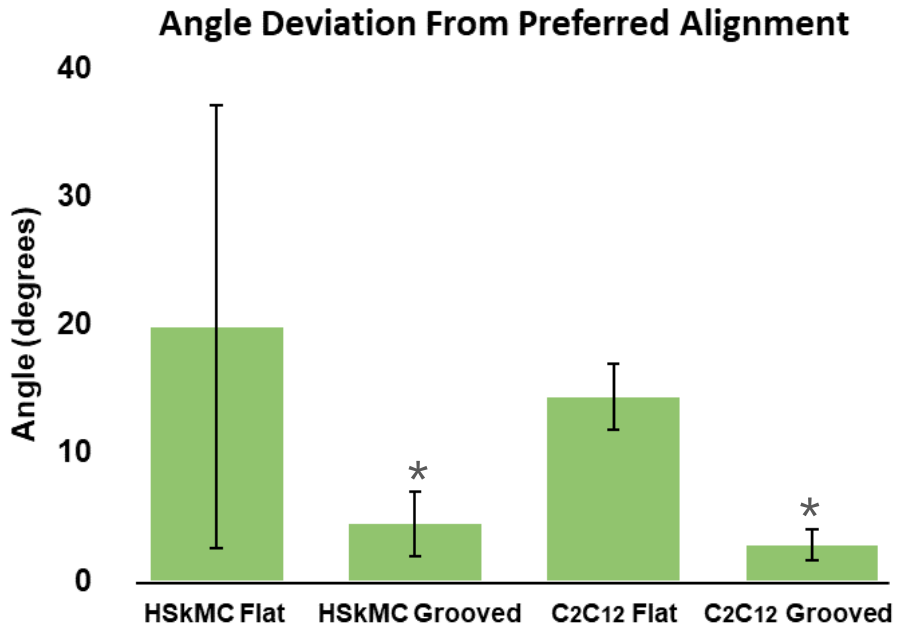
Myoblasts differentiation on bioengineered scaffolds. Fluorescent staining of C2C12 myoblasts demonstrates alignment and myotube formation on flat (top) and grooved (bottom) collagen-based constructs. α-actinin(green), F-actin (Phalloidin, red), Nuclei (DAPI, blue).

Human Myoblasts on Micro-channeled Collagen Films



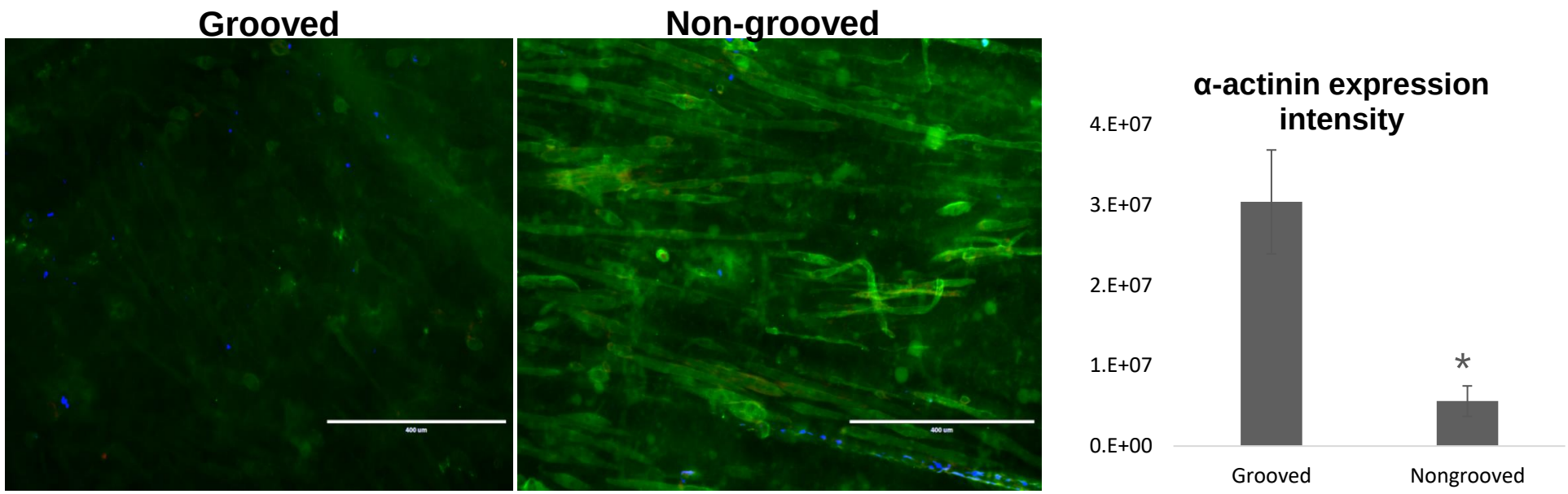
HSkMC myoblasts on bioengineered scaffolds. Fluorescent staining of HSkMC myoblasts demonstrates alignment and myotube formation on flat (top) and grooved (bottom) collagen-based constructs. α-actinin(green), F-actin (Phalloidin, red), Nuclei (DAPI, blue).

Myoblasts alignment on Micro-channeled Collagen Films



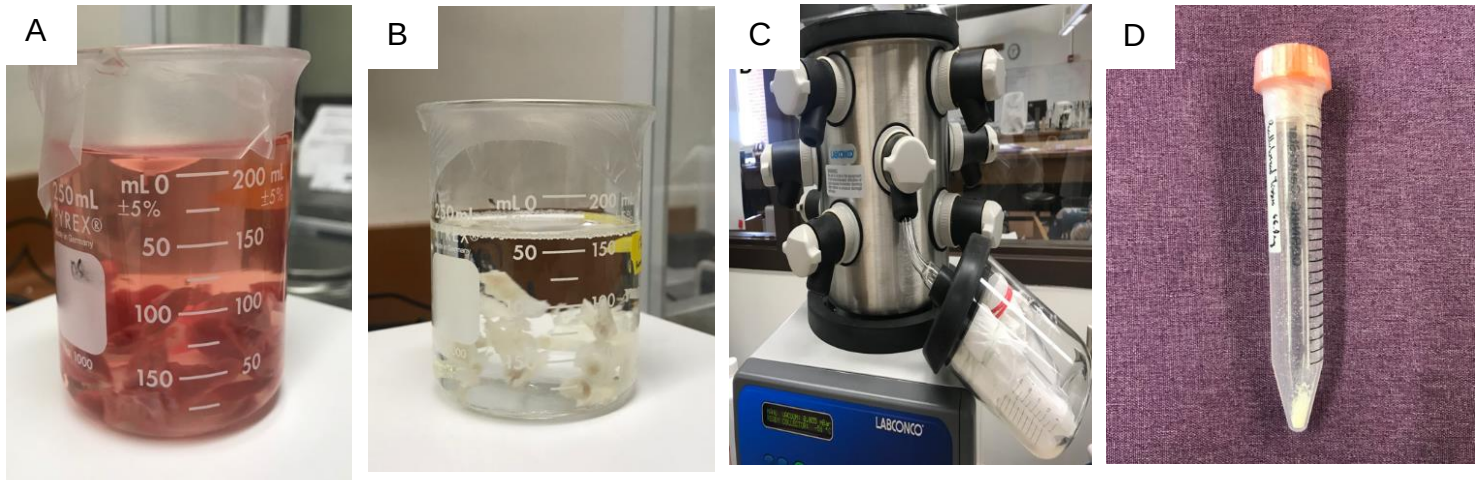
Parallel alignment was enhanced for both HSkMCs and C2C12s on micro-grooved constructs compared to flat constructs. (*$p\text{-value} < 0.05$*)

Myoblasts on Micro-channeled express increased α-actinin



Myoblasts on flat (left) and on micro-channeled (right) collagen films. Immunostained against sarcomeric α-actinin (green). Graph shows relative intensity analysis (*$p < 0.001$, $n=10$). Scale bar =400um.

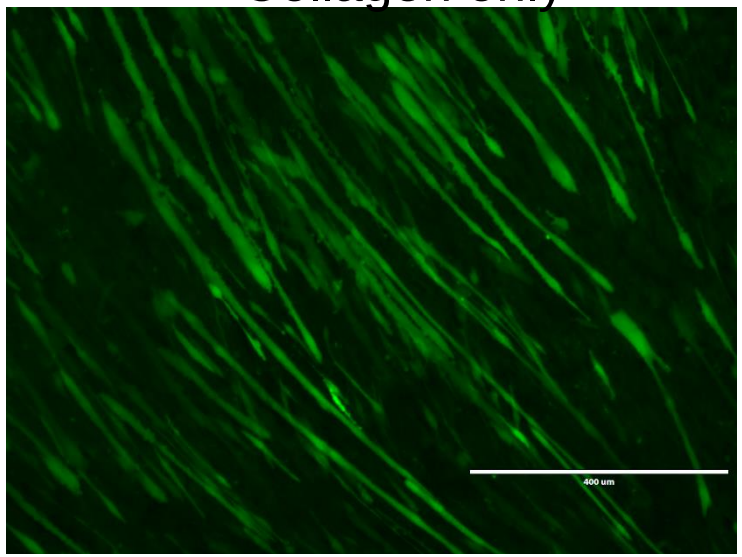
Tissue ECM-decellularized Skeletal Muscle Matrix Scaffolds



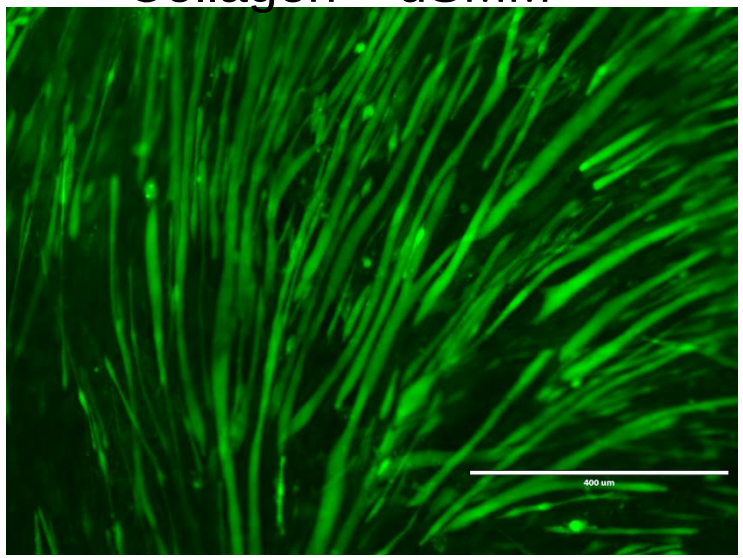
Tissue ECM decellularization. The tissue is placed in 1% (w/v) SDS for 4-5 d. After processing the tissue is dialyzed and then lyophilized. The final tissue is a sterile powder that can incorporated with other materials.

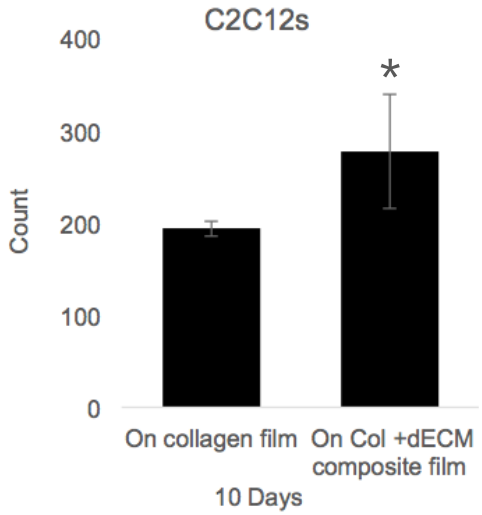
Myoblast Proliferation on Collagen-dSMM Scaffolds

Collagen only



Collagen + dSMM

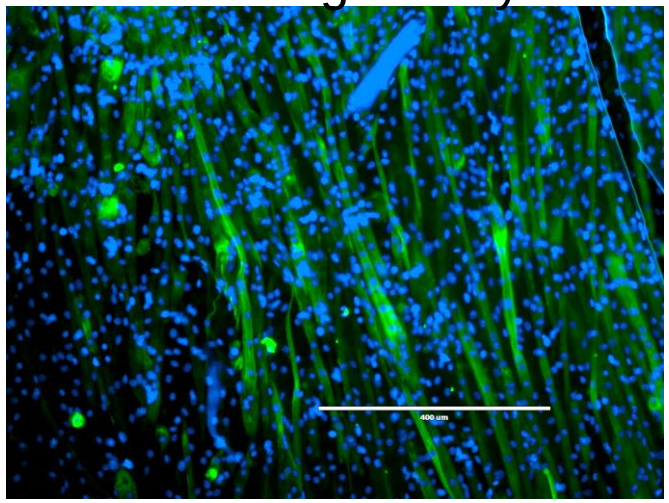




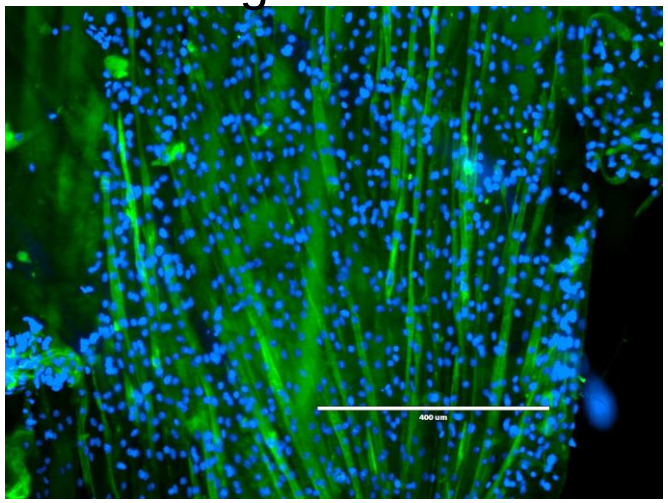
Myoblasts growing on collagen only (left) and collagen with dSMM (right) for 10 days ($p < 0.05$, n= 4). Staining of live cells (Calcein AM). Scale bar =400µm.

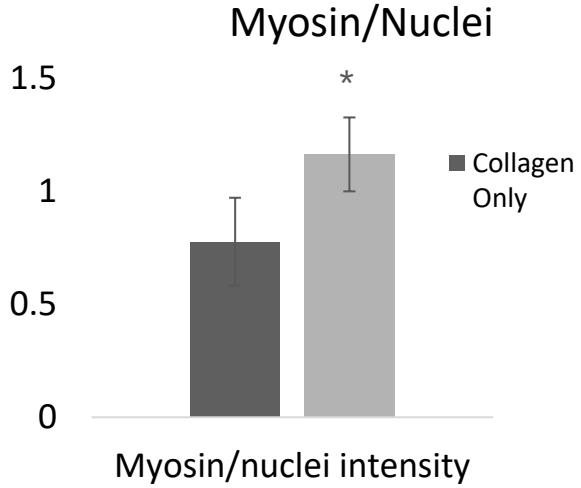
Myosin Expression on Collagen-dSMM Scaffolds

Collagen only



Collagen + dSMM

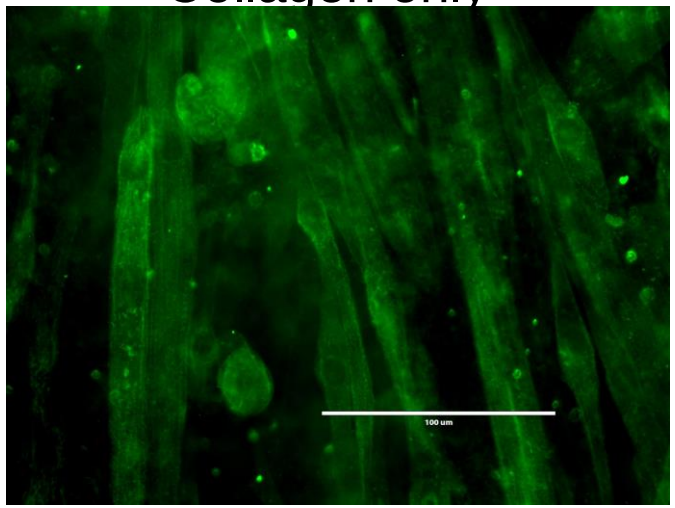




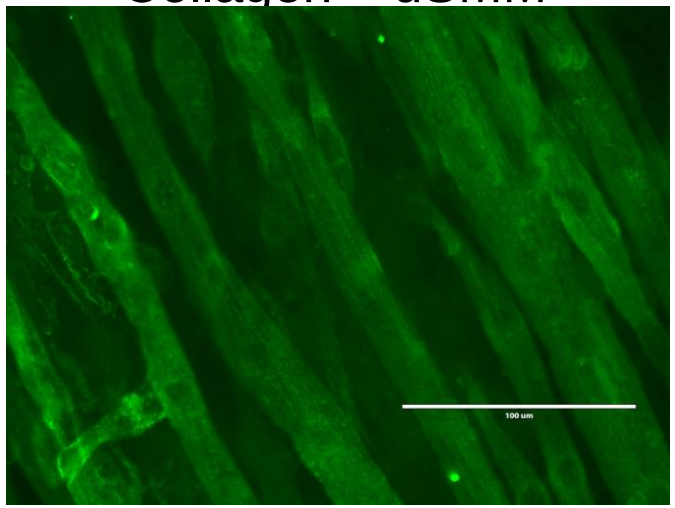
Increased myosin expression. Immunofluorescence showed detection of myosin expression in C2C12s cultured for 14 days on collagen only and collagen + dSMM constructs (F-actin=red, Myosin=green, Nuclei=blue). ImageJ analysis (*p *value* = *0.02*).

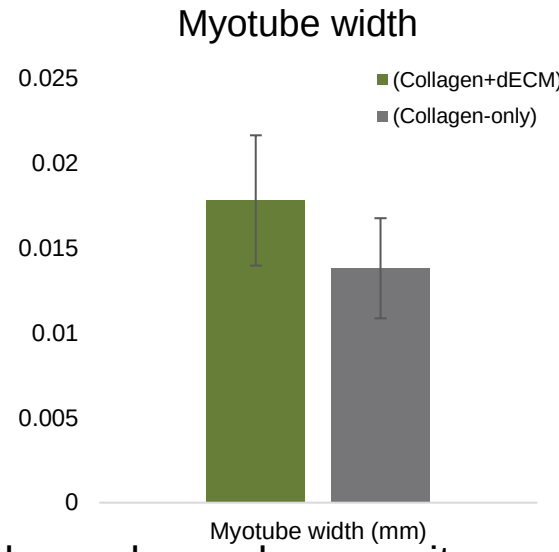
Myotube formation on Collagen-dSMM Scaffolds

Collagen only



Collagen + dSMM





Striations on α-actinin stained scaffolds. Several myotubes in the control samples and composite samples were observed to have ordered striation patterns. Scale Bar 100 μm. (*p value=0.10).*

Summary/Next Steps

- Micro-grooved collagen-based constructs promote myoblast differentiation and myotube formation with the expression of α-actinin, the contractile unit of a myofiber.
- Preliminary results indicate that dSMM samples display an earlier differentiation and formation myotubes compared to the scaffolds that only contained collagen.
- We are working on completing additional studies with increased dSMM incorporation.
- We also plan on translating this process to a 3D bioprinting approach in the near future.

Acknowledgements and Funding

- Lab members
- CSLB COE
- CSULB BUILD
- CSUPERB
- CSULB ORED
- CSULB UROP
- CSULB RISE
- CSULB LSAMP
- NIH SC2 Grant
- NSF CAREER

Questions?

Contact Information:

Perla Ayala

Cal State Long Beach/Biomedical Engineering

www.ayalalab.com

562.985.5732

perla.ayala@csulb.edu

Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

Junjun Liu- Cal Poly Pomona

Collaborator:
Dr. Carlotta Glackin
City of Hope/ Beckman Research Institute

Junjun Liu, Professor

Cal Poly Pomona, Department of Biological Sciences

Email: junjunliu@cpp.edu

Project Overview—Breast Cancer

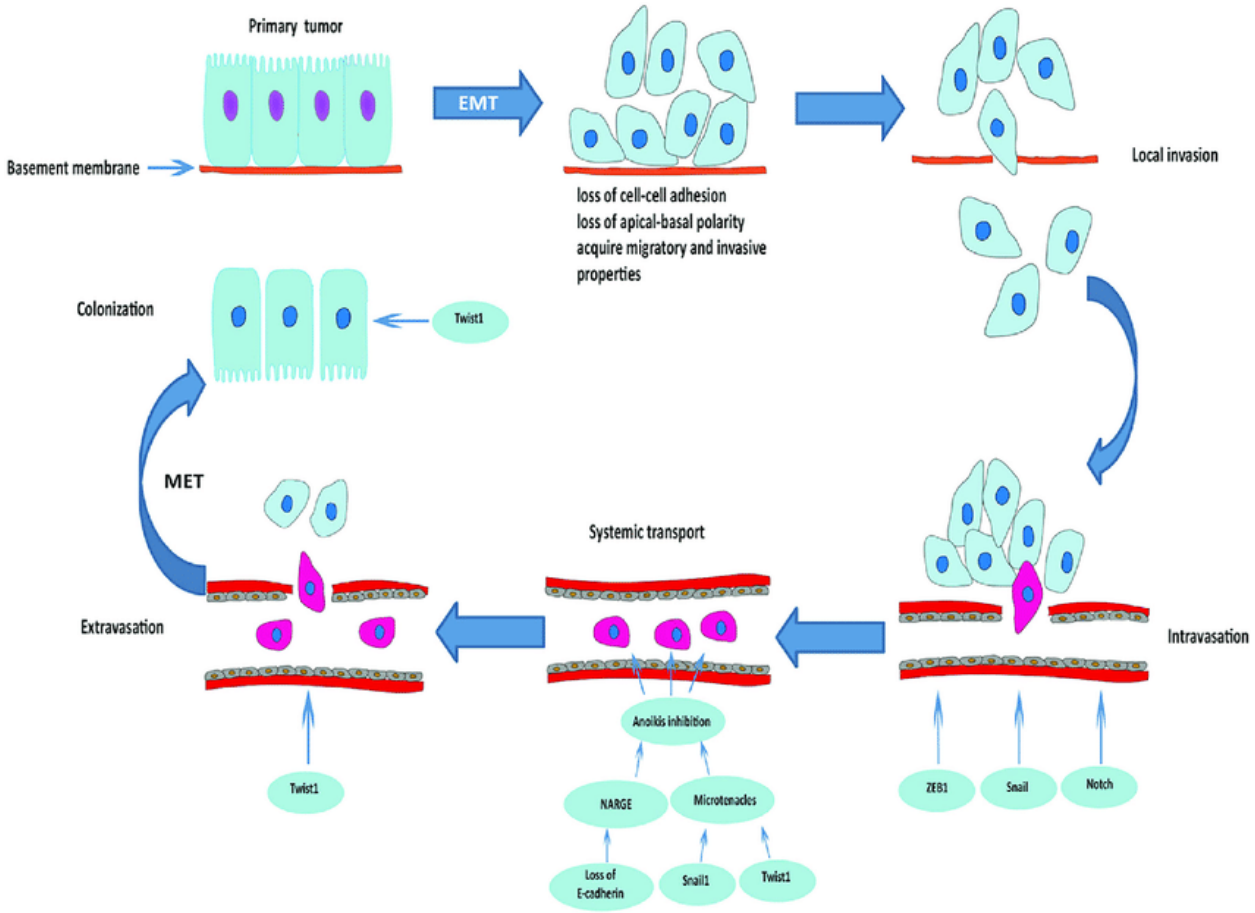
- Breast cancer is a disease in which malignant (cancer) cells form in the tissues of the breast.

- **Ducts** – Ductal Carcinoma
- **Glands** – Lobular Carcinoma
- **Blood and lymph** - Angiosarcoma

Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

Project Overview—Metastasis

- Mortality is usually a result of metastatic breast cancer, not the non-invasive breast cancer.
- Metastasis is a multistep process, and cancer cell migration and invasion are initial steps of the process.



Project Overview--Twist

Cell, Vol. 117, 927–939, June 25, 2004, Copyright ©2004 by Cell Press

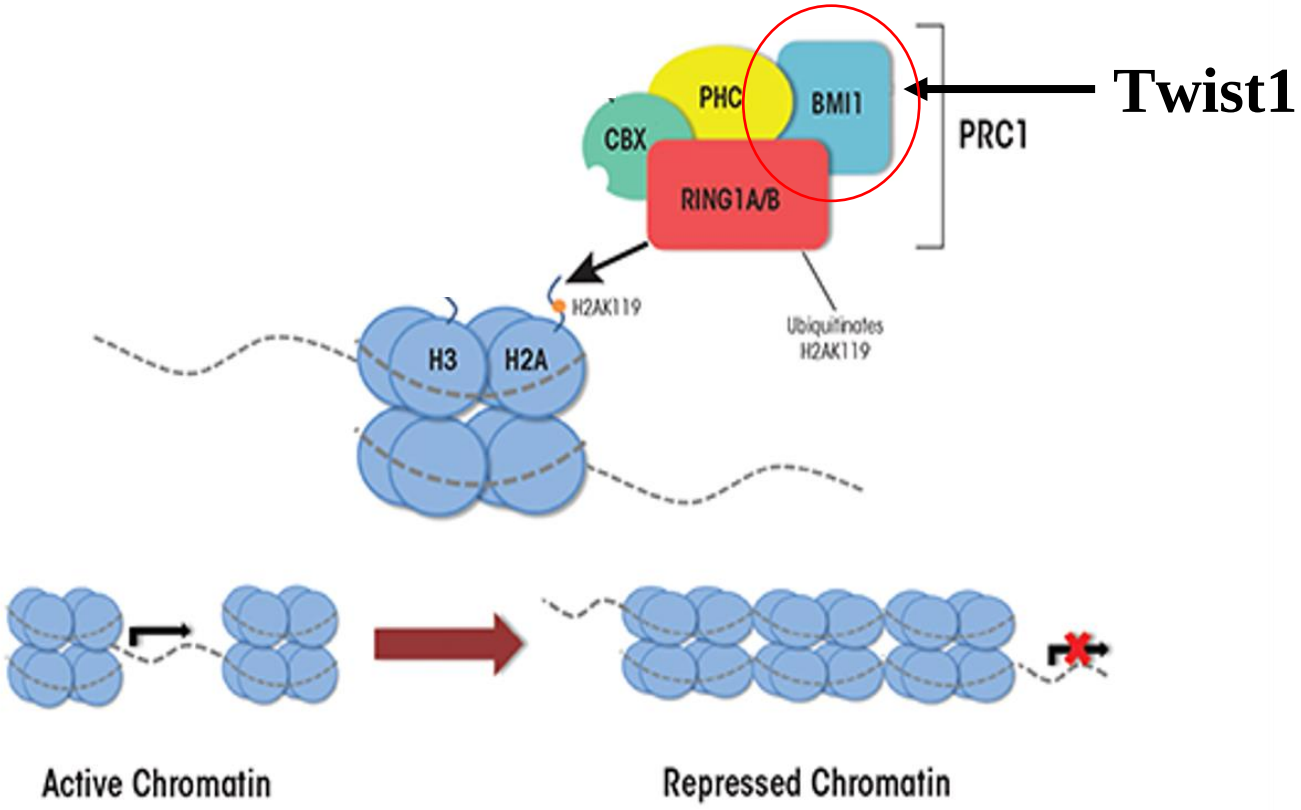
Twist, a Master Regulator of Morphogenesis, Plays an Essential Role in Tumor Metastasis

- Yang *et al.* concluded that "……the transcription factor Twist, a master regulator of embryonic morphogenesis, plays an essential role in metastasis."
- i.e., in addition to its physiological role, Twist also plays an important pathogenic role in tumorigenesis.

Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

Project Overview—Role of Twist

- Twist1 facilitates tumorigenesis, e.g. it promotes the expression of Bmi1, a core unit of PRC1 (*polycomb-group repressive complex 1*), which silences the expression of genes such as *PTEN*, a tumor suppresser gene.
- Twist1-Bmi1 promotes cancer cell migration, invasion leading to metastasis.
- *So, the inhibition of metastasis may be achieved by targeting Twist1.*

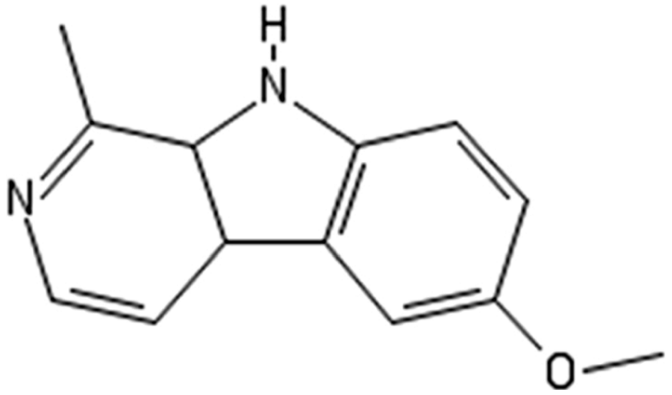


Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

Activities

- **Hypothesis**: We can suppress breast cancer cell migration and invasion by inhibiting Twist1 with harmine.
- Harmine: a beta-carboline alkaloid found in a variety of plants was identified as the first inhibitor of Twist1 (Yochum *et al.*, 2017)

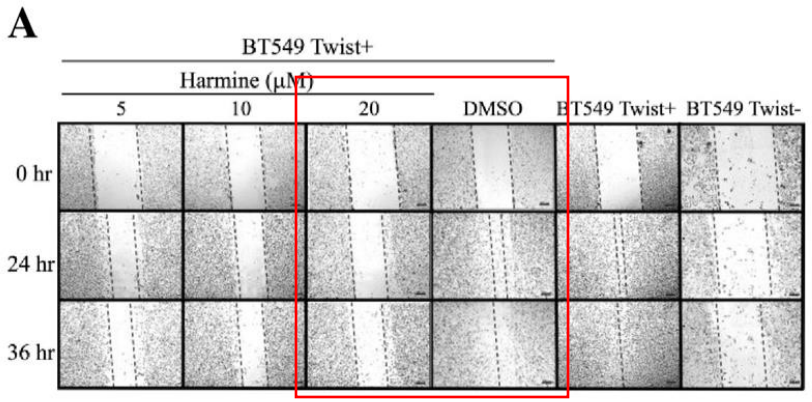
Harmine

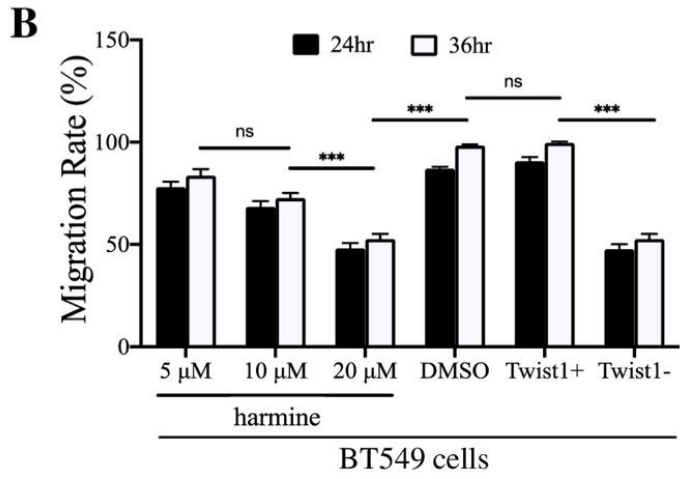


Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

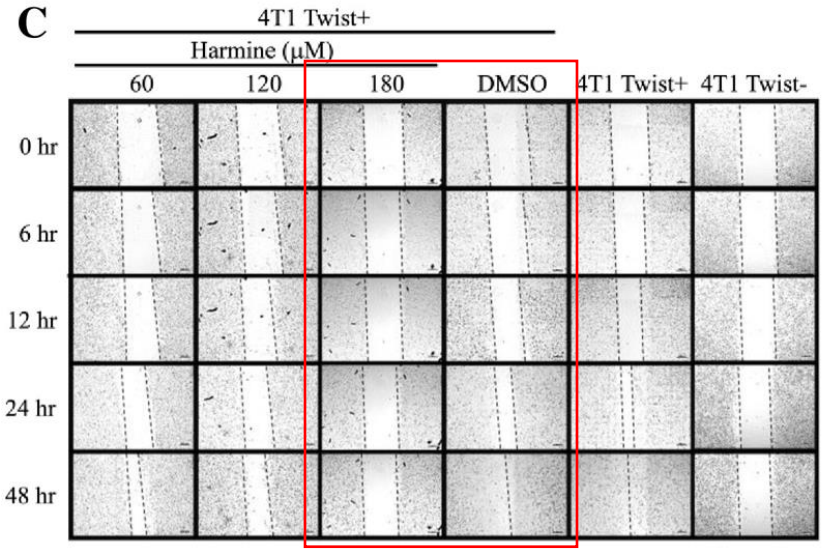
Results–Harmine Inhibits Cancer Cell Migration

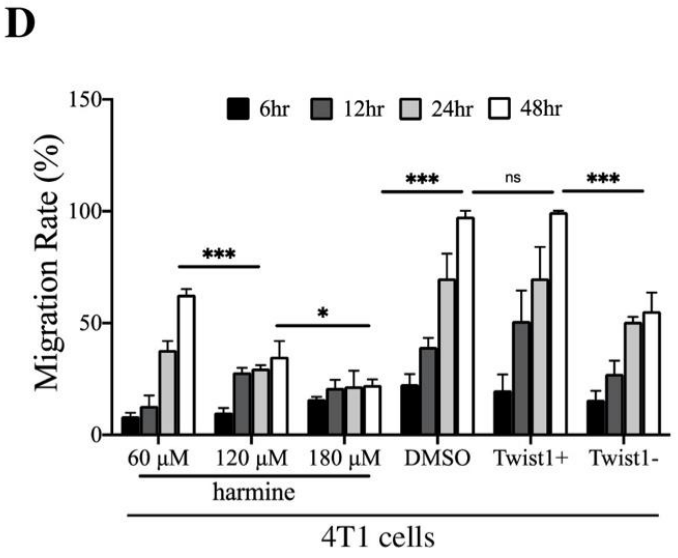
Human breast cancer cell





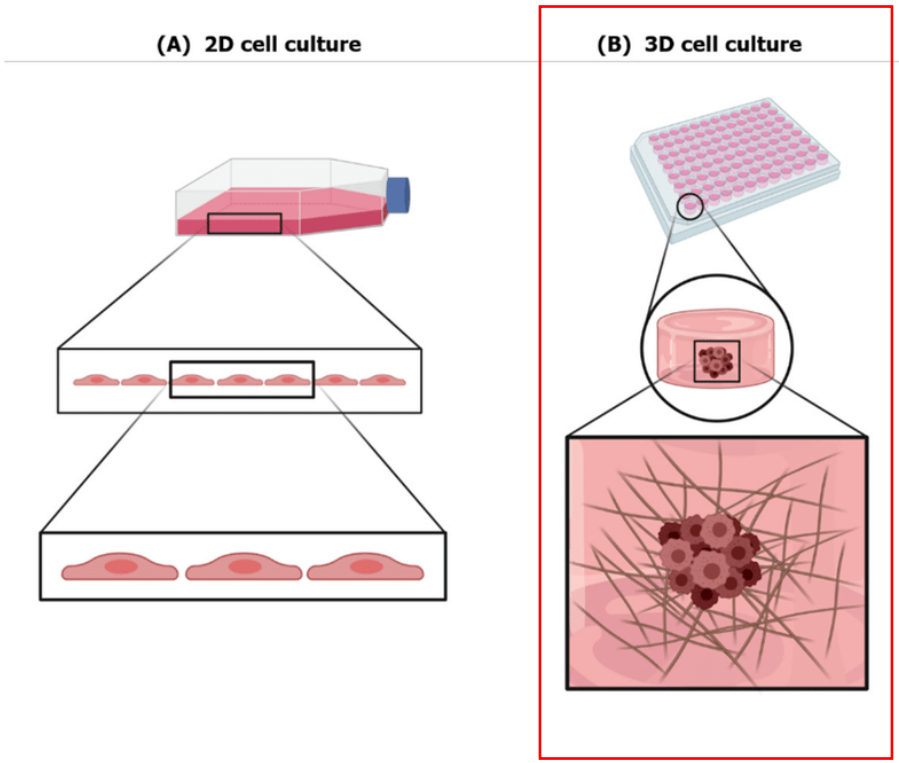
Mouse breast cancer cell

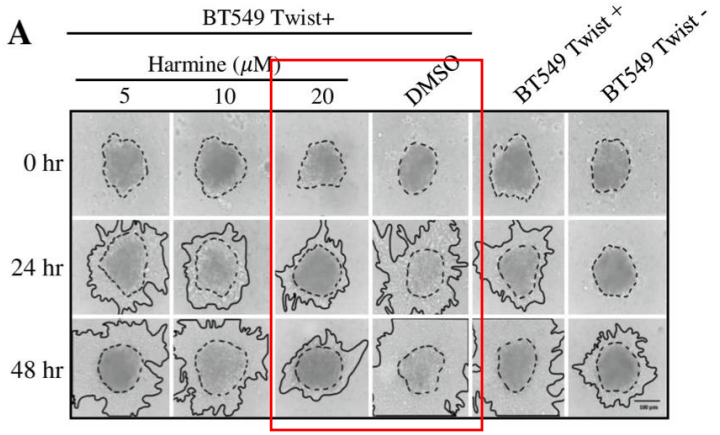


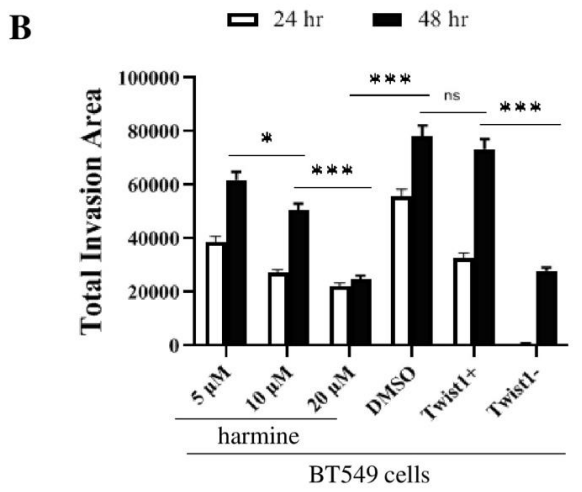


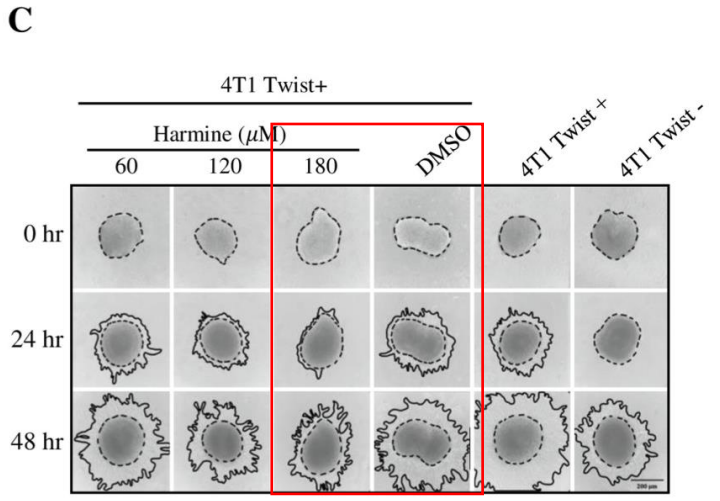
Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

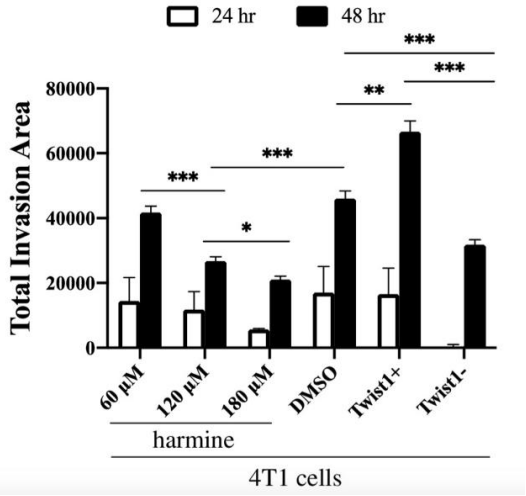
Results–Harmine Inhibits Cancer Cell Invasion





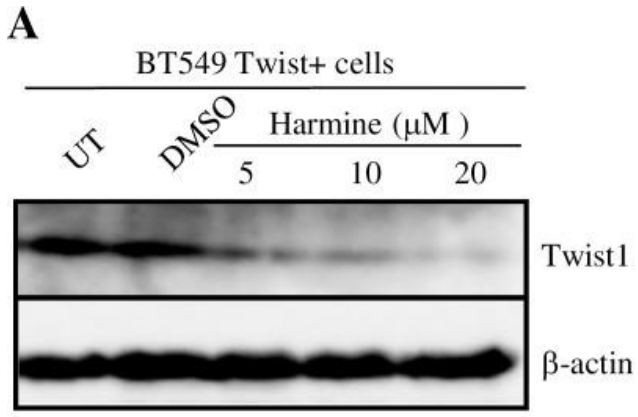


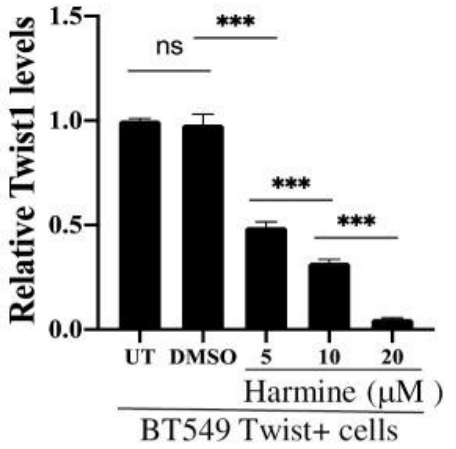


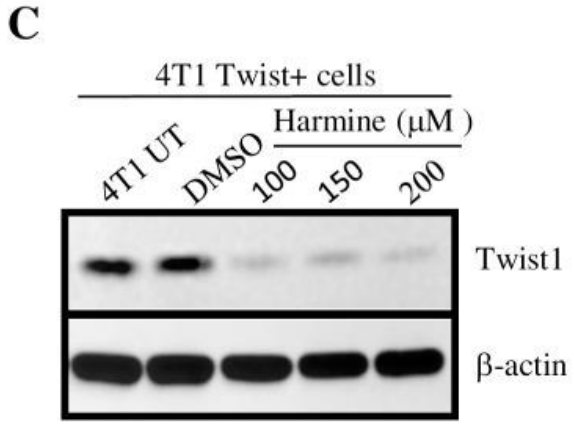


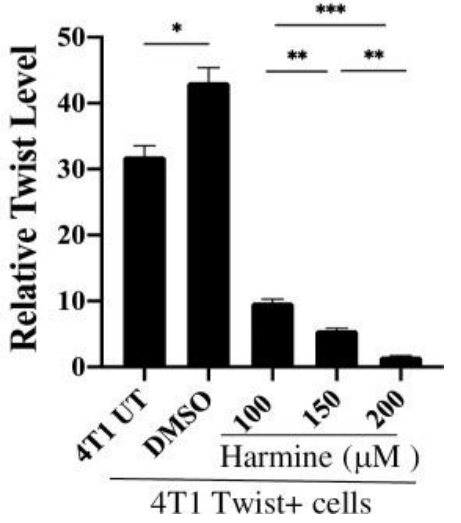
Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

Results–Harmine Induces a Dose-dependent Twist1 Degradation



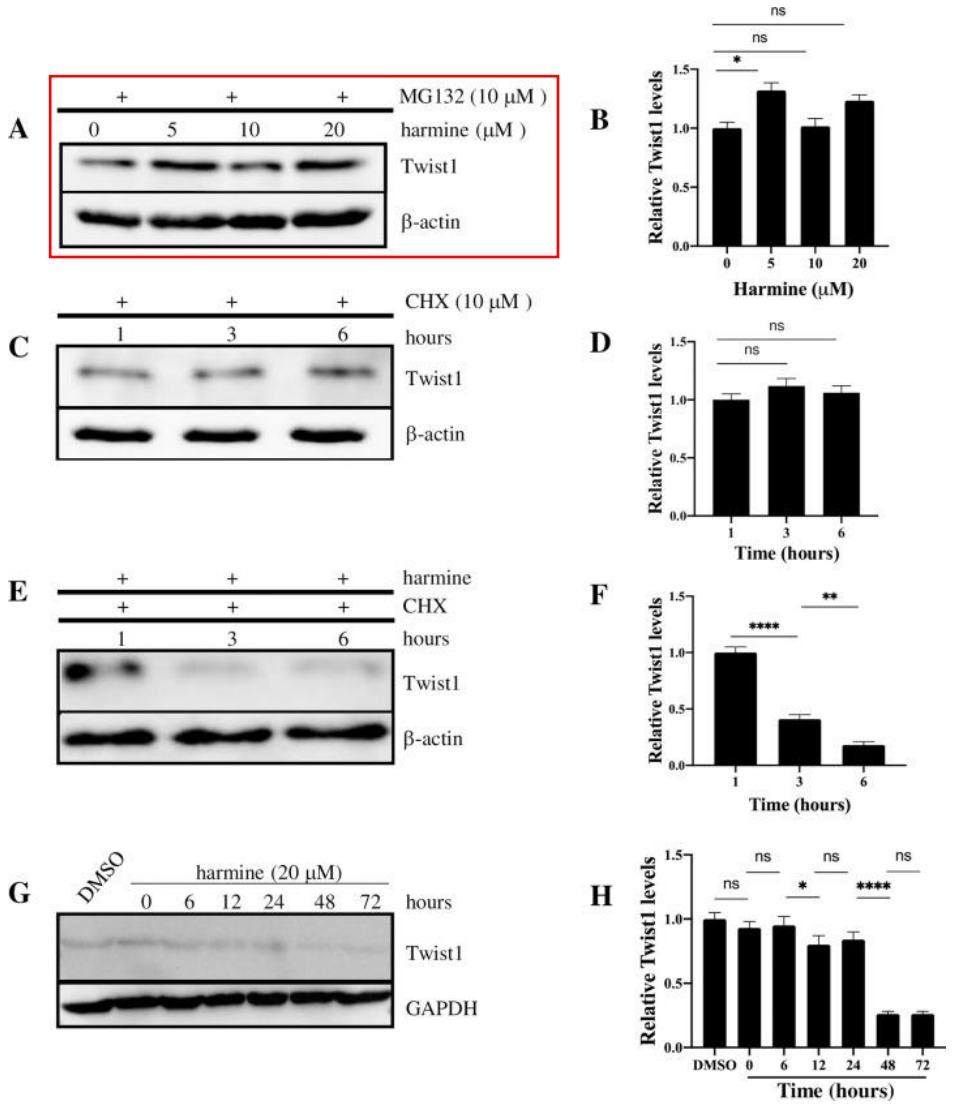






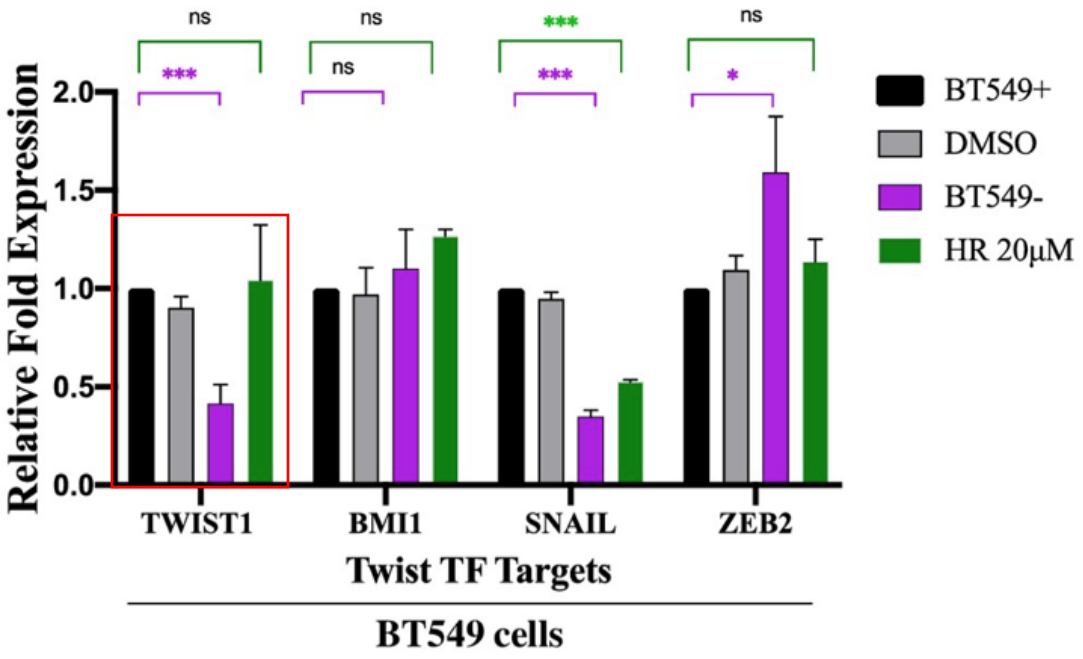
Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

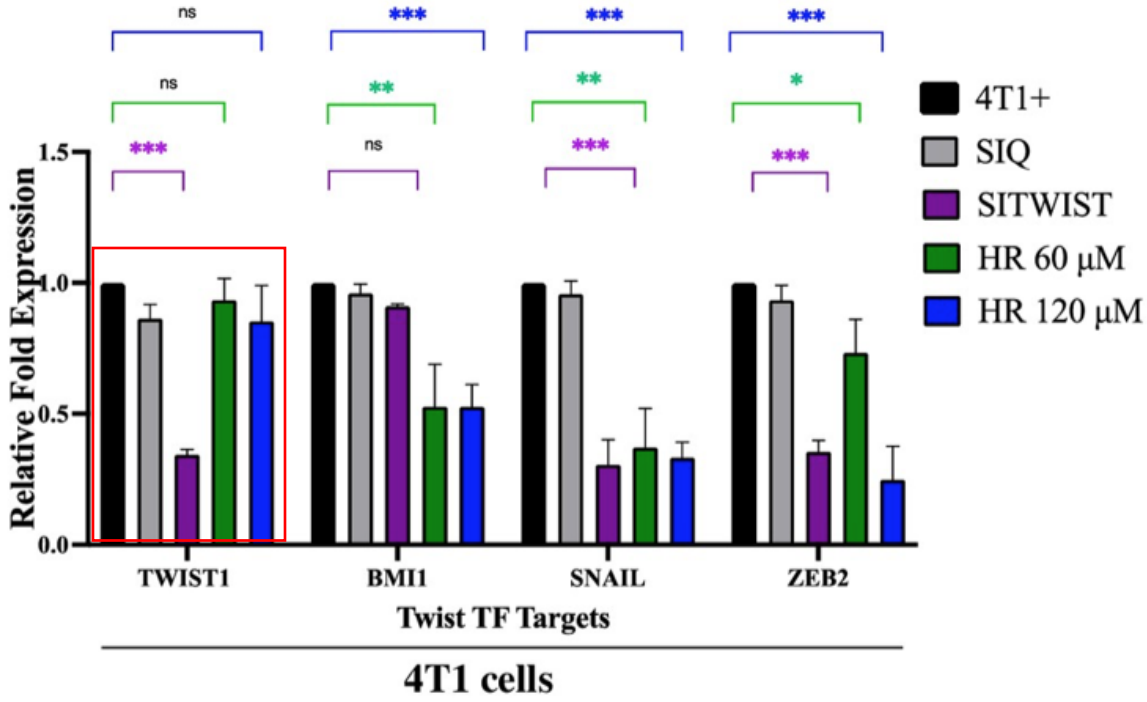
Results–Twist1 Degradation is Proteasome-dependent



Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

Results–Harmine Does not Affect Twist1 mRNA Level





Inhibit Breast Cancer Cell Migration and Invasion by Targeting Twist1

Summary

- In vitro, harmine induces proteasome-dependent degradation of Twist1 and therefore inhibits the migration and invasion of breast cancer cells.

Acknowledgement

Cal Poly Pomona	City of Hope/ Beckman Research Institute
Jade Lolarga	Dr. Carlotta Glackin
Brandon Lam	Dr. Ebtesam Nafie
Jonathan Guo	
Elnaz Abdollahzadeh	

- **Funding**: NIH 5SC3GM132056

Questions?

Contact Information:

Junjun Liu

Cal Poly Pomona/Biological Sciences

909-869-2505

junjunliu@cpp.edu

"Acinetobacter Baumannii, a Dangerous Threat: The Better We Know it, The Better We Fight it"

Maria Soledad Ramirez– California State University Fullerton

Collaborators: Marcelo Tolmasky, Luis A. Actis, Robert A. Bonomo, Fernando Pasteran.

Maria Soledad Ramirez, PhD., Associate Professor

Fullerton, Department of Biological Science

msramirez@fullerton.edu

Project Overview- What we do in the lab?

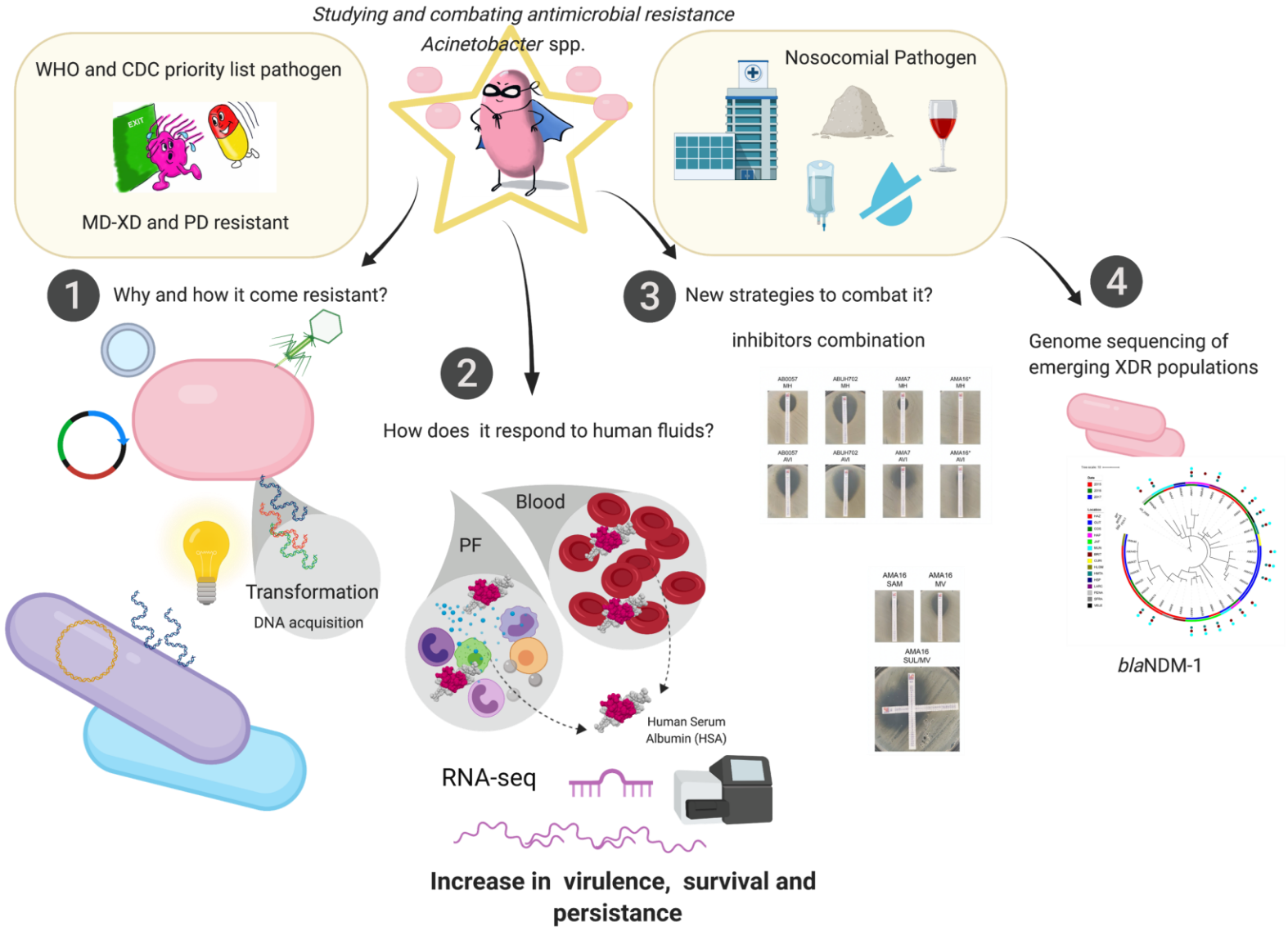
“Acinetobacter Baumannii, a Dangerous Threat: The Better We Know it, The Better We Fight it”

Undergraduate students

Graduate students

Visiting scholars

National and international collaborations

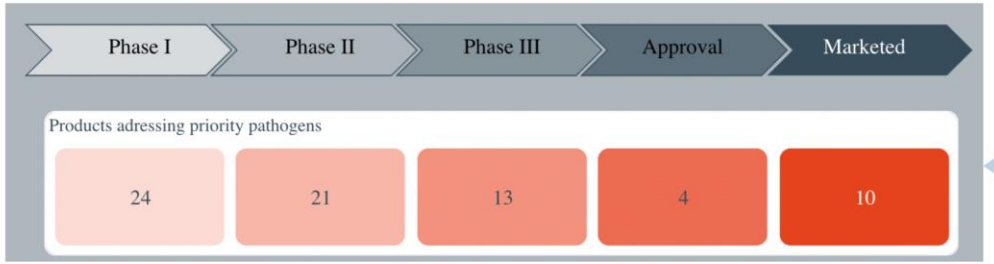


Project Overview- selected topic

"Acinetobacter Baumannii, a Dangerous Threat: The Better We Know it, The Better We Fight it"

What new antibiotics do we have? What options do we have to treat CRAB?

Antibacterial in clinical
(https://dzifhelmholtzdashboard.azurewebsites.net/reports/pipelines/pipelines)



Gram-negatives
1) Meropenem-vaborbactam
2) Imipenem-relebactan
3) Plazomicin
4) Eravacycline
5) Cefiderocol

Additional promising combinations / treatment to inhibit CRAB

*sulbactam — β-lactamase inhibitor of Ambler class A enzymes, exhibited an inherent antibacterial activity against a limited number of bacterial species

β-lactamase inhibitor:
- avibactam
- durlobactam*
- taniborbactam
- QPX7728
- enmetazobactam
- zidebactam
- nacubactam
- ANT431

→

- ceftazidime
- meropenem
- imipenem
- *sulbactam
- cefepime
- aztreonam

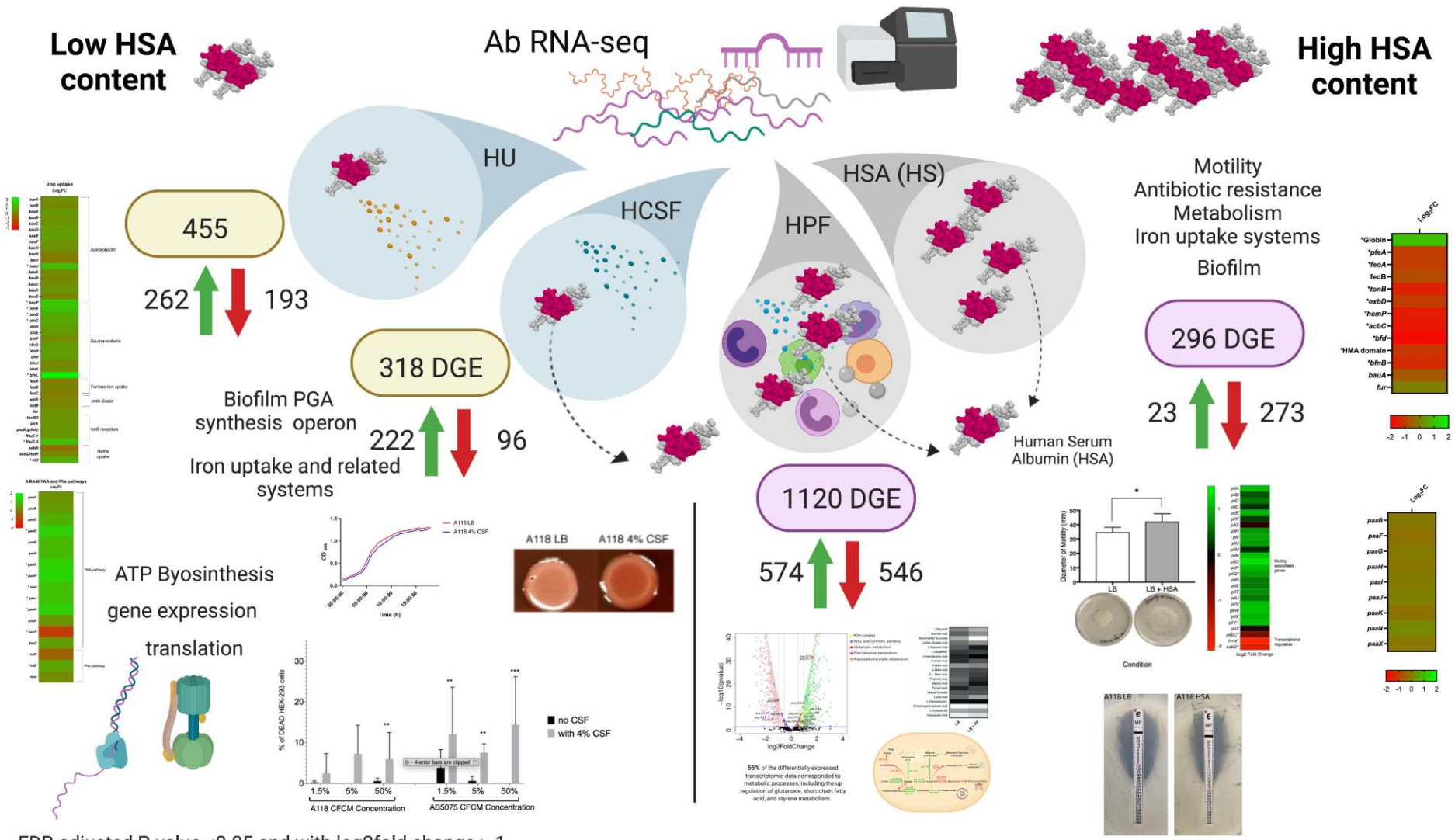
Colistin

Phage theraphy

Bush, K. Antimicrob. Agents Chemother. 2018,.,nel, G.G., Golden, A.R., Zelenitsky, S. et al. Drugs, 2019. Lomovskaya O, et al. Antimicrob Agents and Chemotherapy, 2020. Liu B, Trout REL, Chu GH, et al. J Med, Chem. 2020., Yang Q, Xu Y, Jia P, et al. J Antimicrob Chemother. 2020., Rodriguez et al 2020; Pasteran el al 2020; Papp-Wallace et al 2017; Tooke et al 2019

Project Overview- selected topic

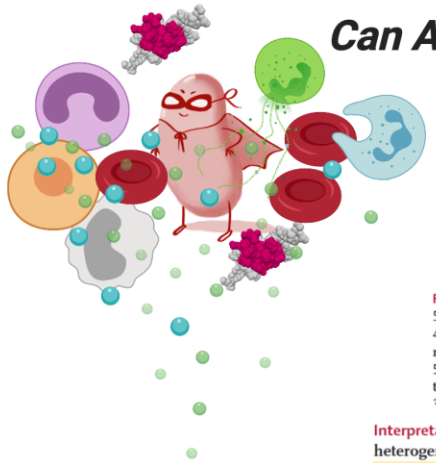
“Acinetobacter Baumannii, a Dangerous Threat: The Better We Know it, The Better We Fight it”



FDR-adjusted P-value <0.05 and with log2fold change > 1

Project Overview- selected topic

“Acinetobacter Baumannii, a Dangerous Threat: The Better We Know it, The Better We Fight it”



Can A. baumannii response to HSA or human fluids affects ATB treatment?

Efficacy and safety of cefiderocol or best available therapy for the treatment of serious infections caused by carbapenem-resistant Gram-negative bacteria (CREDIBLE-CR): a randomised, open-label, multicentre, pathogen-focused, descriptive, phase 3 trial

Matteo Bassetti, Roger Echols, Yuko Matsunaga, Mari Ariyasu, Yohei Doi, Ricard Ferrer, Thomas P Lodise, Thierry Naas, Yoshihito Niki, David L Paterson, Simon Portsmouth, Julian Torre-Cisneros, Kiichiro Toyoizumi, Richard G Wunderink, Tsutae D Nagata

Findings derocol, 51 to best available therapy. 150 patients received treatment: 101 cefiderocol (85 [85%] received monotherapy) and 49 best available therapy (30 [61%] received combination therapy). In 118 patients in the carbapenem-resistant microbiological ITT population, the most frequent carbapenem-resistant pathogens were *Acinetobacter baumannii* (in 54 patients [46%]), *Klebsiella pneumoniae* (in 39 patients [33%]), and *Pseudomonas aeruginosa* (in 22 patients [19%]). In the same population, for patients with nosocomial pneumonia, clinical cure was achieved by 20 (50%, 95% CI 33·8–66·2) of 40 patients in the cefiderocol group and ten (53%, 28·9–75·6) of 19 patients in the best available

Interpretation Cefiderocol had similar clinical and microbiological efficacy to best available therapy in this heterogeneous patient population with infections caused by carbapenem-resistant Gram-negative bacteria. Numerically more deaths occurred in the cefiderocol group, primarily in the patient subset with *Acinetobacter* spp infections. Collectively, the findings from this study support cefiderocol as an option for the treatment of carbapenem-resistant infections in patients with limited treatment options.

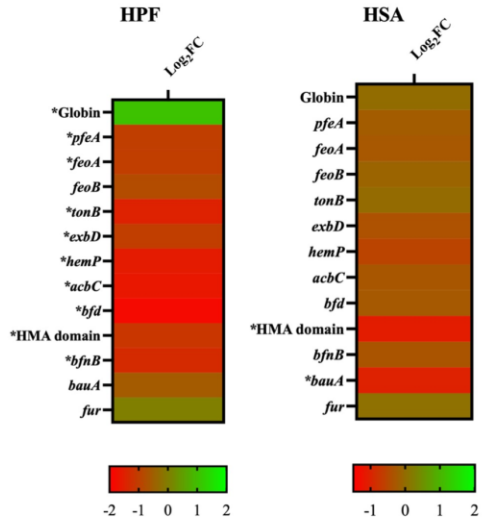
available therapy group) was considered to be related to the study drug.

Interpretation Cefiderocol had similar clinical and microbiological efficacy to best available therapy in this heterogeneous patient population with infections caused by carbapenem-resistant Gram-negative bacteria. Numerically more deaths occurred in the cefiderocol group, primarily in the patient subset with *Acinetobacter* spp infections. Collectively, the findings from this study support cefiderocol as an option for the treatment of carbapenem-resistant infections in patients with limited treatment options.

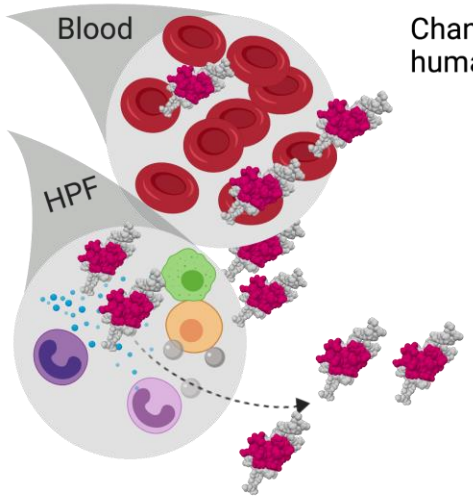
Iron uptake systems
Beta-lactams resistance genes
Porins

?

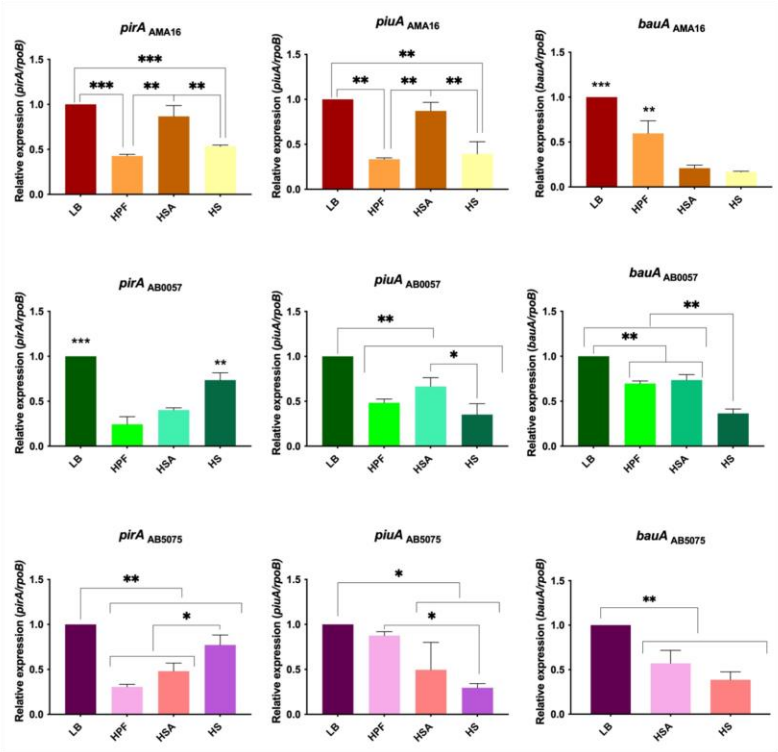
Can this changes affect CFDC activity?



Results Part 1



Changes in the levels expression of iron uptake genes in the presence of human fluids



The expression of most of the iron uptake related genes was reduced in the tested strains under most of the conditions evaluated

	CFDC MICs (mg/L)		
Strain	LB	HPF	3.5% HSA
AB5075	0.5	1	2
ABUH702	0.38	1.5	3
AMA16	>4.5*	>256	32*
AB0057	1	8	1.5
AMA40	0.5	16*	3
AMA41	0.094	0.5-0.75	2
AMA113	0.5	1.5	1.5
AMA181	0.19	0.19	0.75
AMA3	24	>256	32*
AMA4	16*	48*	64*
AMA5	>256	>256	16*
AMA9	32	48	16
AMA14	8*	16*	12
AMA17	>256	>256	>256
AMA18	64*	16*	16*
AMA19	4	4	2? (48)
AMA28	32*	>256	32*
AMA30	64*	128*	12*
AMA31	>256	>256	96*
AMA33	16*	>256	>256

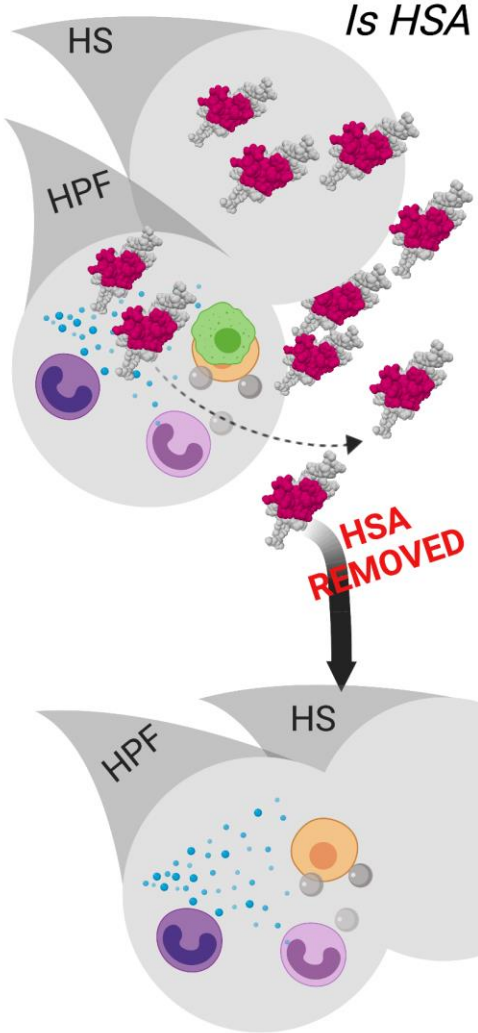
* Intra-colonies are present.

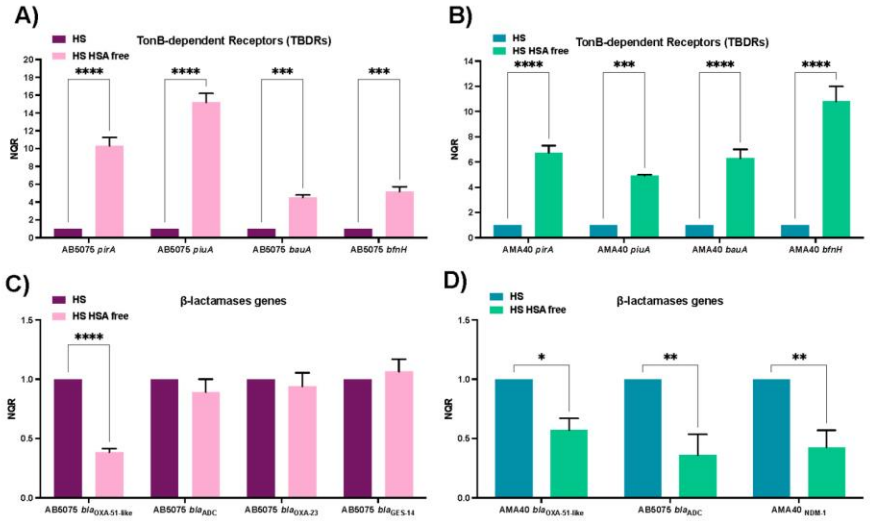
A. baumannii cells were cultured in LB or LB supplemented with 3.5 % HSA or HPF, respectively.

Results Part 2

“Acinetobacter Baumannii, a Dangerous Threat: The Better We Know it, The Better We Fight it”

Is HSA the molecule leading to changes to CFDC sensibility?





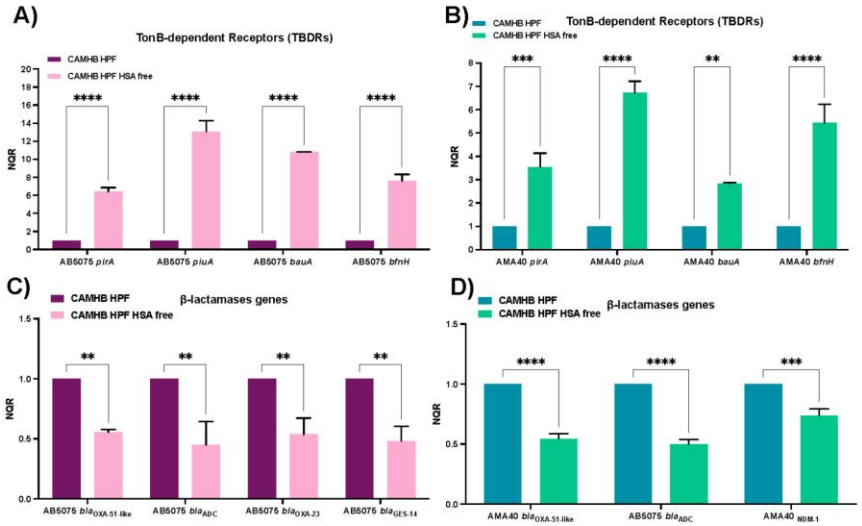


Table 1: Minimal Inhibitory Concentrations (MICs) of cefiderocol (CFDC) for the CRAB AB5075 and AMA40 strains, performed using CFDC MTS strips (Liofilchem S.r.l., Italy) on Iron-depleted CAMHA (Cation Adjusted Mueller Hinton Agar) and the different conditions tested.

Condition	CFDC MIC (*mg*/L)	
	AB5075	AMA40
CAMHB	0.5 (S)	0.5 (S)
4% HPF	1 (S)	16* (R)
4% HPF HSA free **	0.5 (S)	0.25 (S)
100% HS	1 (S)	4* (S)
100% HS HSA free **	0.5 (S)	0.25 (S)

CFDC: cefiderocol, S: Susceptible, R: Resistant
* Intra-colonies are present.
**HSA Removal, Sigma Aldrich

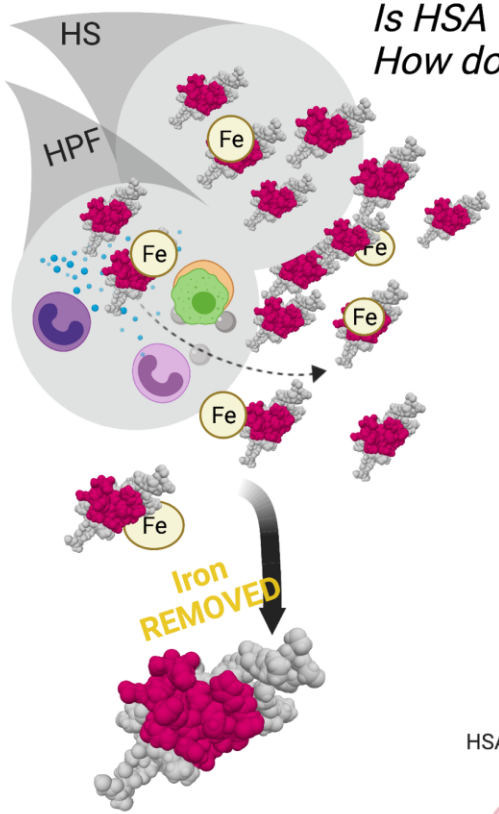
Is HSA the molecule leading to changes to CFDC sensibility? ***YES***

New question.....

What is the role of HSA on the observed effect?
How does HSA trigger A. baumannii’s response?

Results Part 3

“Acinetobacter Baumannii, a Dangerous Threat: The Better We Know it, The Better We Fight it”



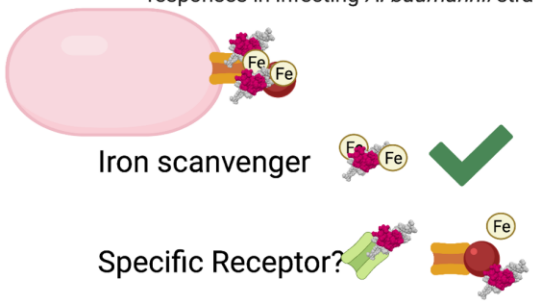
Is HSA the molecule leading to changes to CFDC sensibility? ***YES***
How does HSA trigger A. baumannii’sresponse?

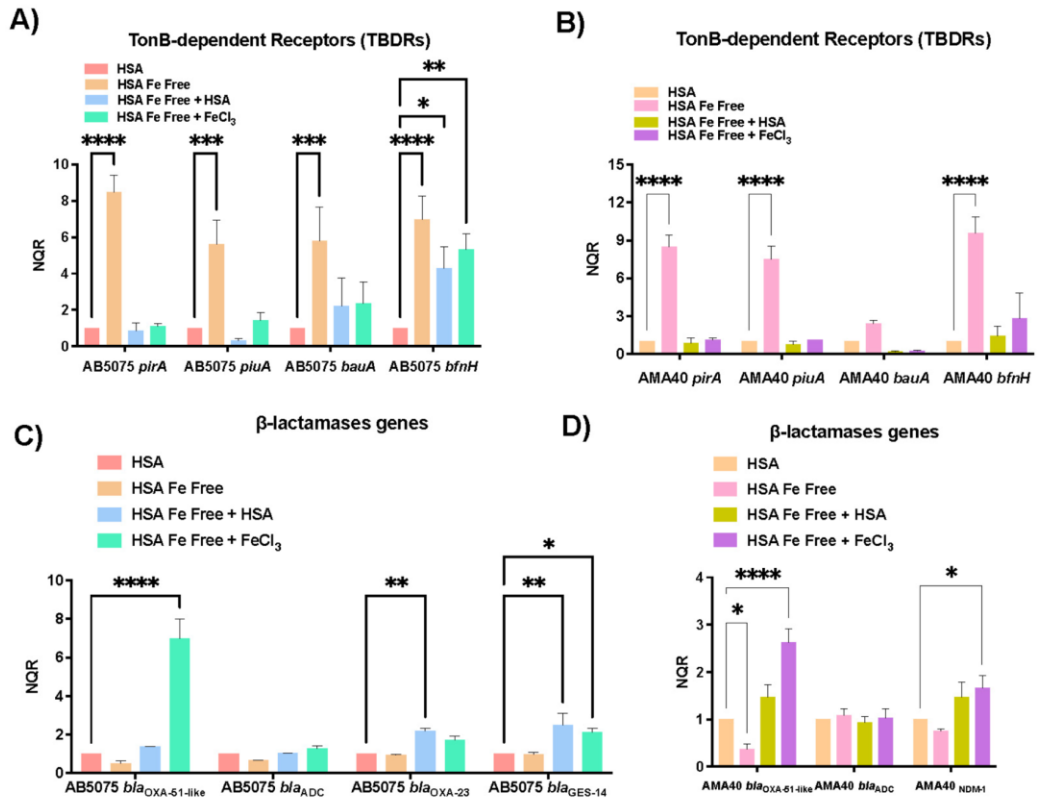
Minimal Inhibitory Concentrations (MICs) and Minimal Bactericidal Concentration (MBCs) of CFDC for the CRAB AB5075 and AMA40 strains, performed by microdilution in iron depleted CAMHB and CAMHB with different experimental conditions.

Strains	CFDC			
	AB5075		AMA40	
	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)
Untreated	0.25 (S)	0.25 (S)	0.5 (S)	32 (R)
HSA pre-Chelex® treatment	8 (I)	32 (R)	2 (S)	64 (R)
HSA Fe-Free (post-Chelex® treatment)	0.125 (S)	8 (I)	1 (S)	16 (R)
HSA Fe-Free + 100µM $FeCl_3$	32 (R)	256 (R)	128 (R)	128 (R)
HSA Fe-Free + 3.5% HSA	8 (I)	64 (R)	4 (S)	64 (R)

CFDC: cefiderocol, S: Susceptible, I: Intermediate, R: Resistant

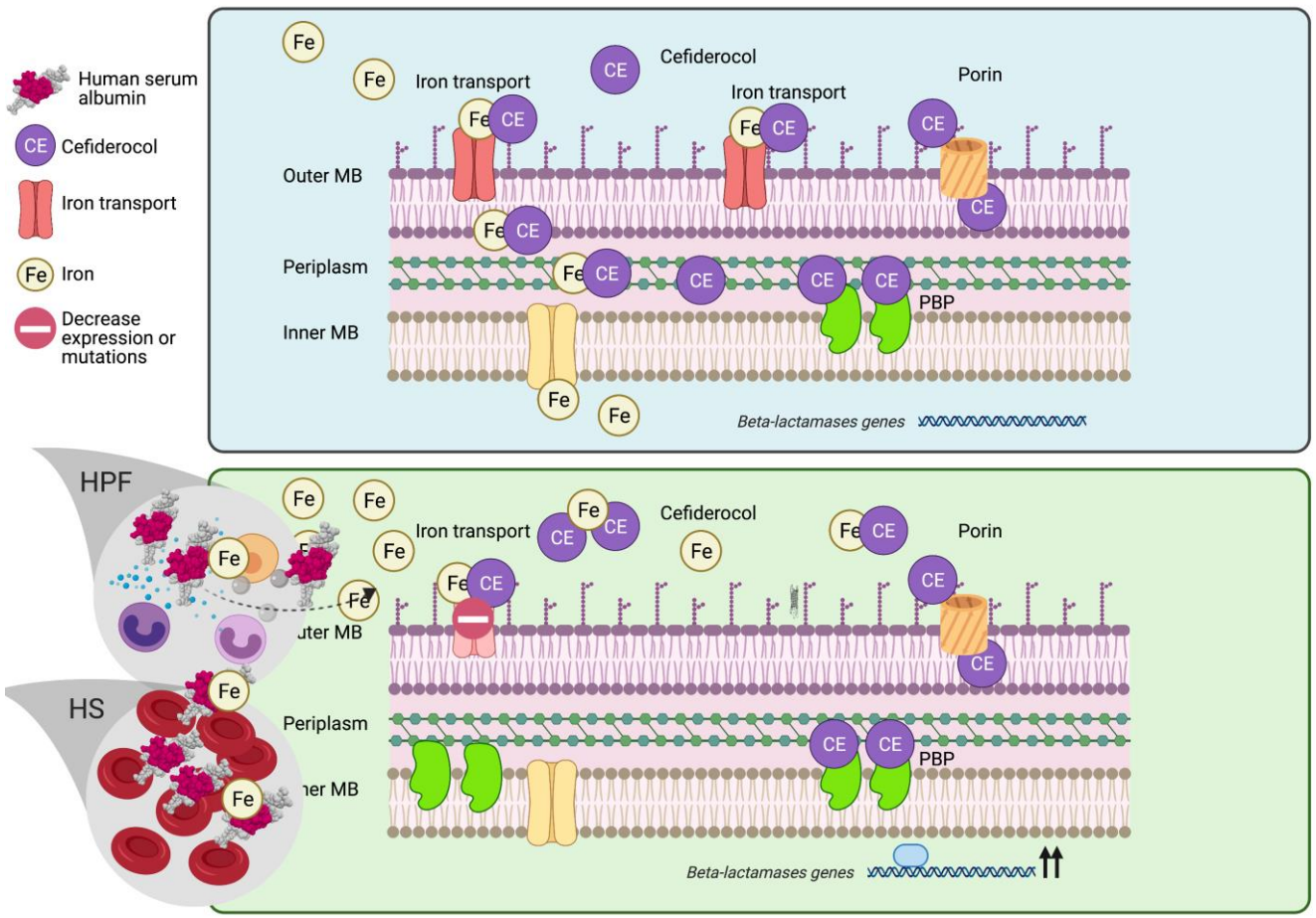
HSA, the main component of human fluids, stimulates a variety of adaptative responses in infecting *A. baumannii* strains





Lessons Learned

“Acinetobacter Baumannii, a Dangerous Threat: The Better We Know it, The Better We Fight it”



Engage students and promote collaborations

Next Steps/ Long- Term Plans

- *Is HSA binding to a specific bacterial receptor?*
- *How is HSA triggering the bacterial response?*
- *Is cefiderocol interacting with HSA? Which molecule is binding more iron?*
- *Is cefiderocol being more degraded in the presence of HSA?*
- *Can our findings help to understand failure in cefiderocol treatment?*

Summary

- *Changes at the transcriptomic and phenotypic level are seen when A. baumannii is exposed to human products*
- *A. baumannii response to human proteins can affect the outcome of antibiotic treatments*
- *HSA, the main component of human fluids, stimulates a variety of adaptative responses in infecting A. baumannii strains*

- *Create a friendly lab environment and encourage your students to get involved in the projects*
- *Search for collaborations, attend scientific meetings, get involved with the scientific community*
- *Share your passion for science with your students*

Questions?

Contact Information:

Maria Soledad Ramirez

CSUF/ Biological Science

657-278-4562

msramirez@Fullerton.edu

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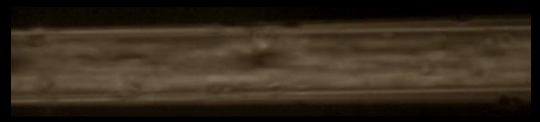
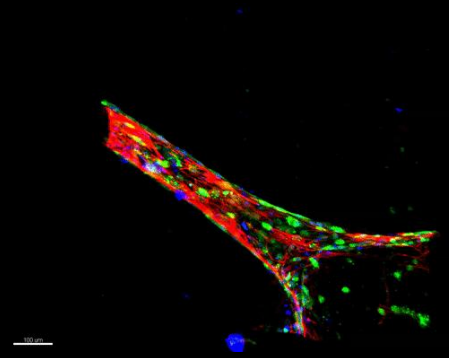
Understanding the Vascular Adhesome to Improve Cardiovascular Biomaterials

Nanoparticle Drug Deliverv



Microfluidics



Thrombosis and Hemostasis

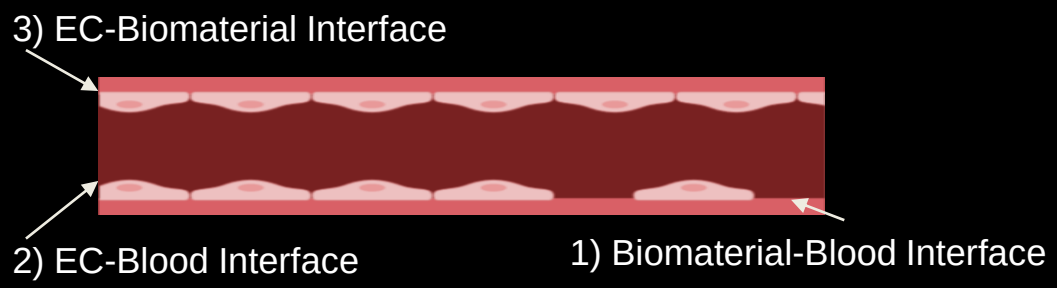
Synthetic Vascular Grafts

Patrick Jurney, Assistant Professor
San Jose State University – Biomedical Engineering
STEM-NET Webcast: NIH NIGMS-Funded Research in the CSU (2/24/2023)

Lab Overview

- *Leverage engineering principles to improve clinical outcomes*

1. Materials characterization
   - Blood-Biomaterial
2. Fluid mechanics
   - Blood-Cell
3. Materials-Bio interactions
   - Cell-Biomaterial
     - Adhesome: the network of structural and signaling proteins involved in regulating cell-matrix adhesion



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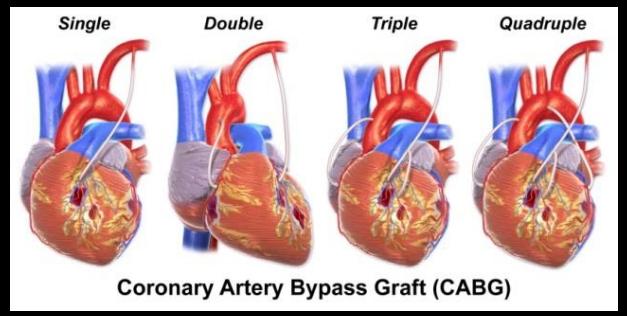
Project Overview

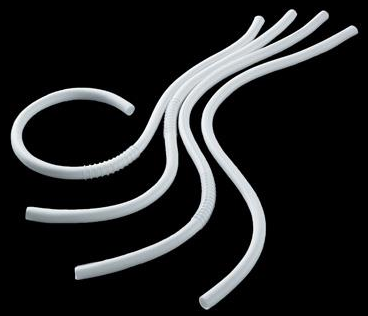
Vascular Graft Applications

- Coronary Artery Disease is #1 cause of death in the US and worldwide
- Coronary artery bypass grafting (CABG)



  - 300,000 to 400,000 Coronary artery bypass surgeries annually in US

Current Vascular Grafts



http://www.goremedical.com/

- Autologous
  - Saphenous vein and internal thoracic artery
- Synthetic
  - 20% of people who require bypass grafting lack suitable autologous targets
  - Clinical Standard: Expanded Polytetrafluoroethylene (ePTFE)

    Small diameter vascular grafts (<6mm) tend to fail at the distal anastomosis

    Neointimal hyperplasia

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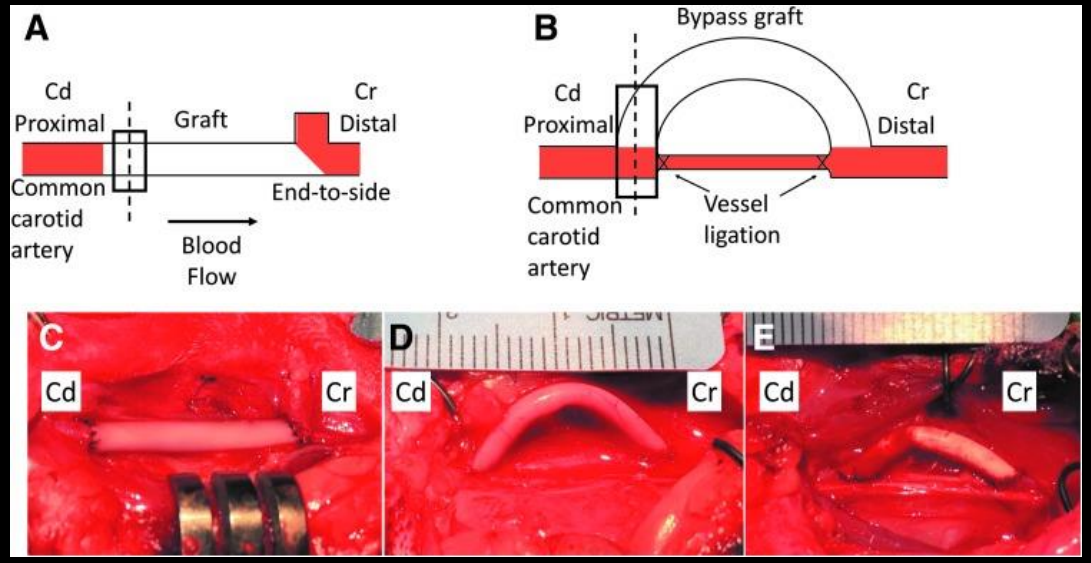
Characteristics of an SDVG

- Withstand cardiac flow conditions
  - Compliance matching
  - Suture retention
- Non-thrombogenic
- Resistant to Neointimal Hyperplasia (IH)



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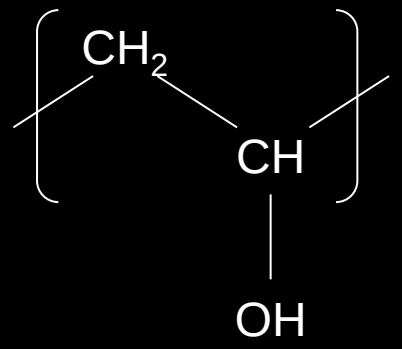
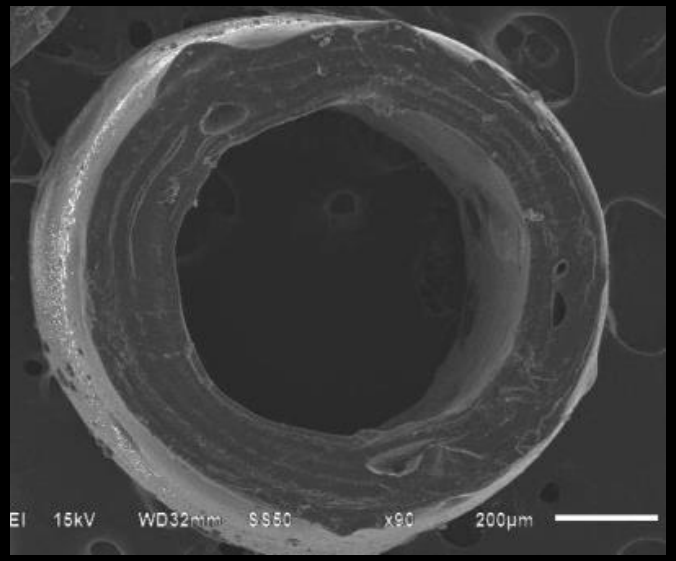
Polyvinyl Alcohol (PVA)

PVA Monomer



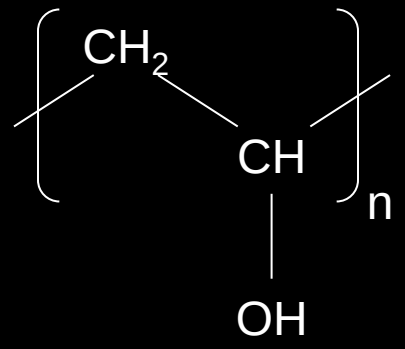


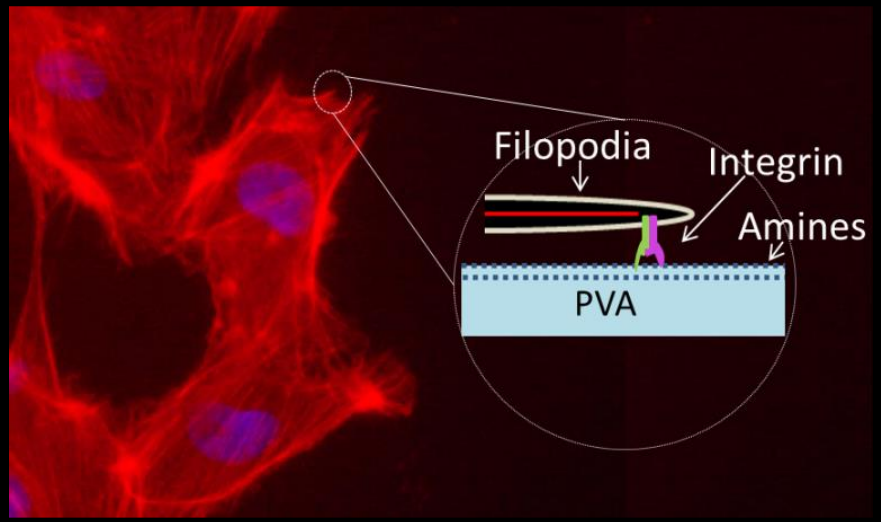
- Advantages:
  - Biocompatible and non-irritating to soft tissues
  - Non-thrombogenic
  - Tunable mechanical properties
  - Amenable to surface modifications
- But it is chemically inert

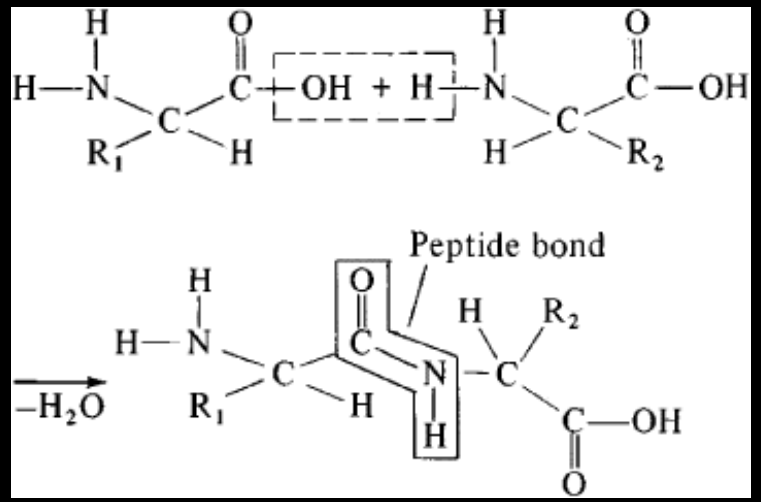
Cell Adhesion (general case)

- Cells bind to surfaces using Integrins
- Peptide bond (linkage)

PVA







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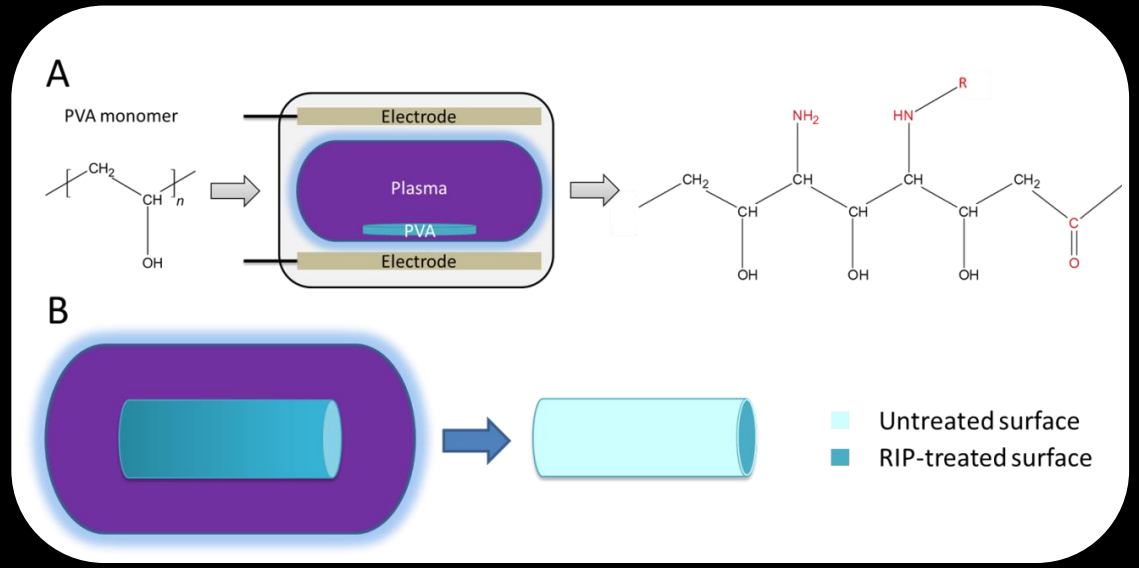
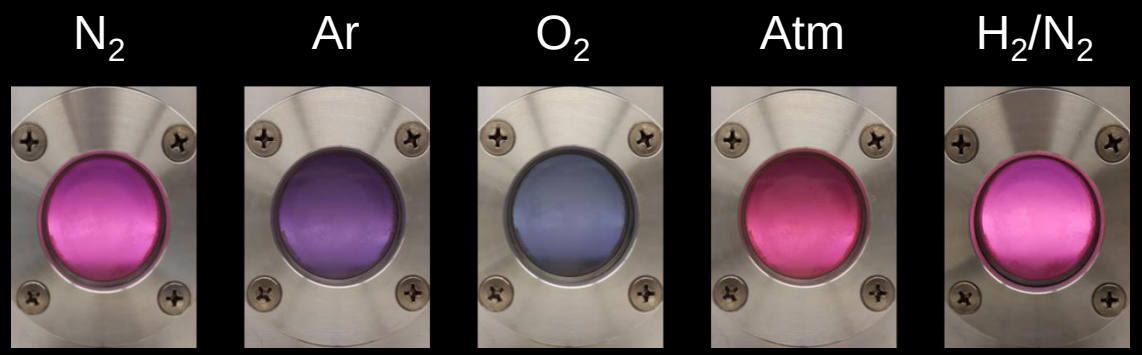
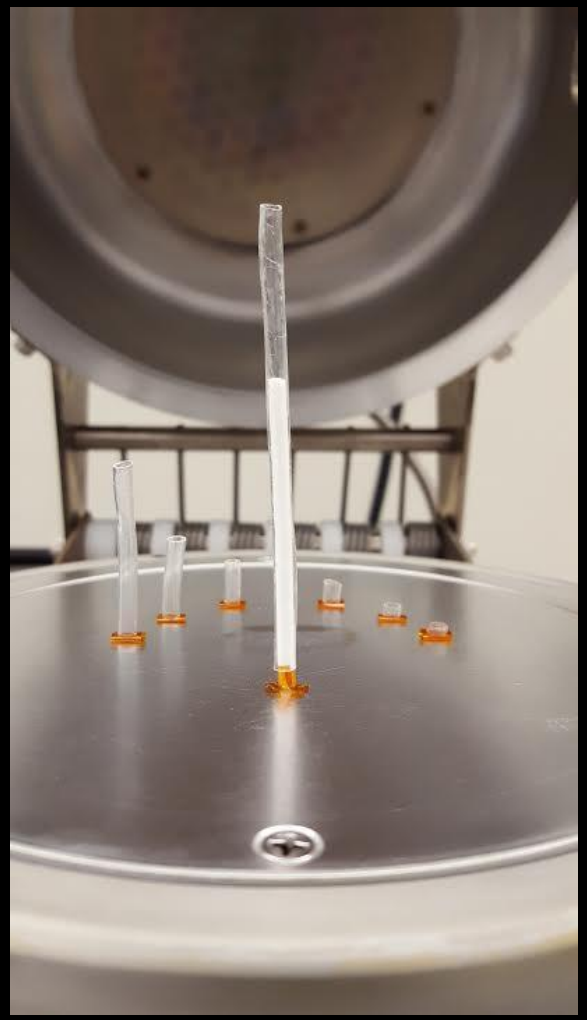
Reactive Ion Plasma (RIP)







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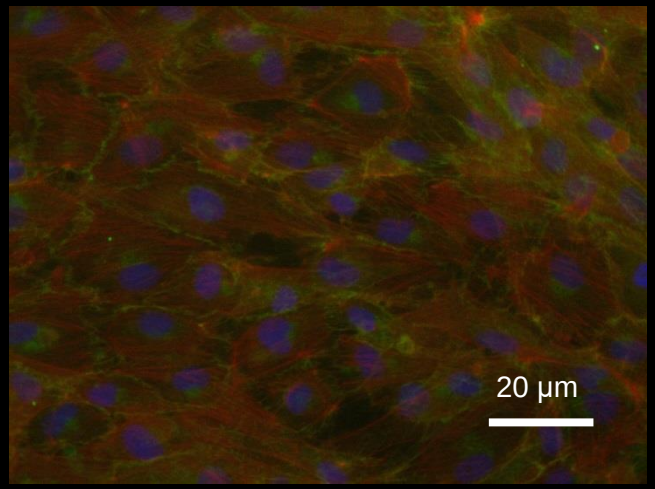
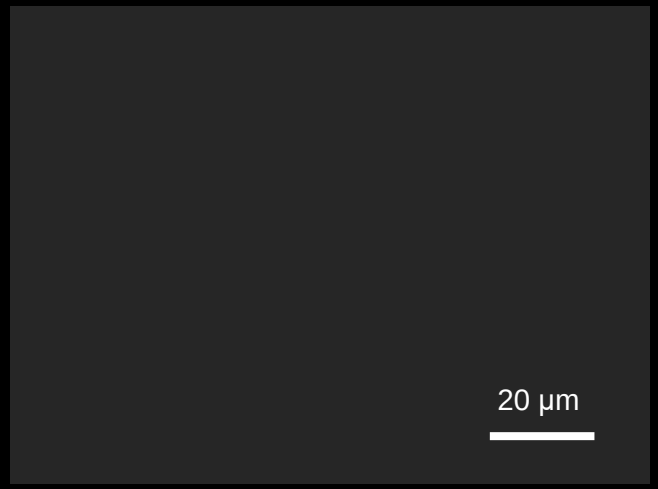
Experimental Observation that ECs Proliferate on Modified Inert Polymers

Endothelial Colony Forming Cells on Modified PVA



Endothelial Colony Forming Cells on Unmodified PVA



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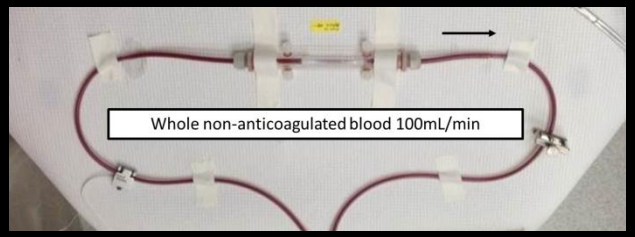
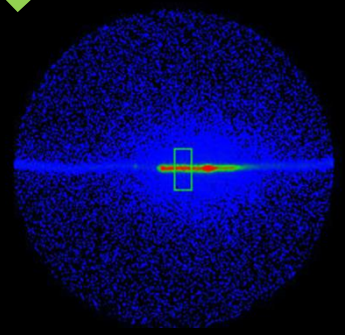
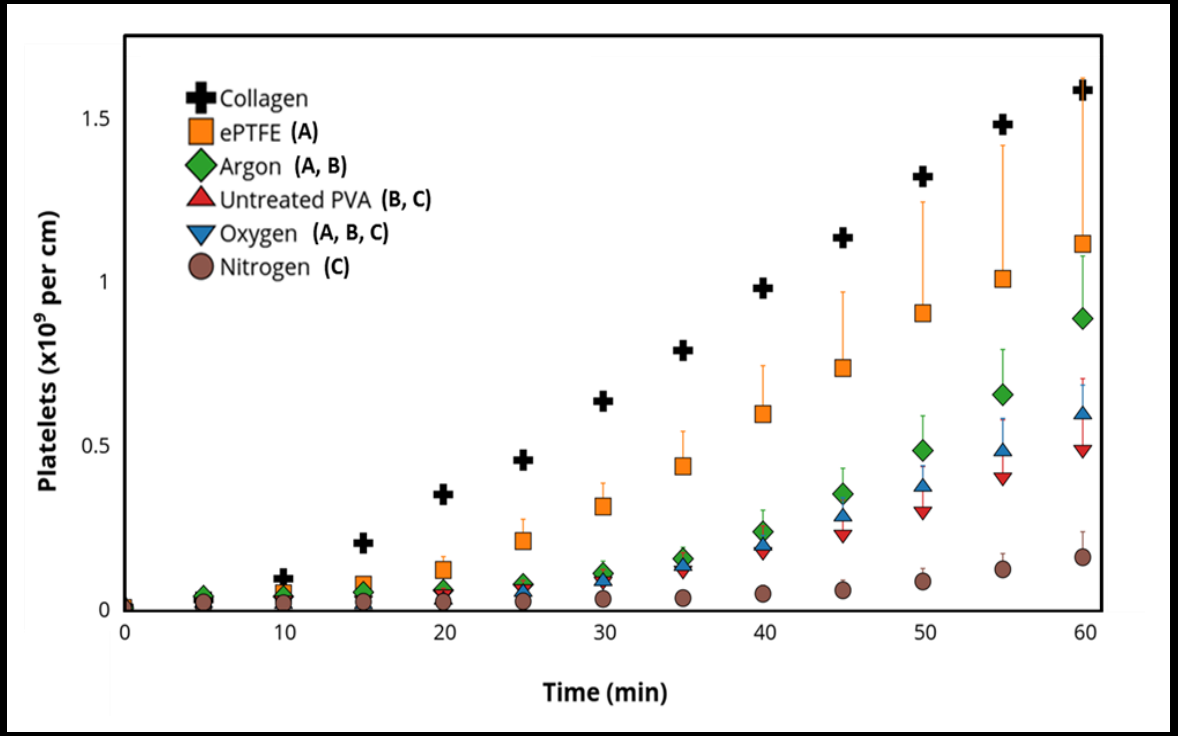
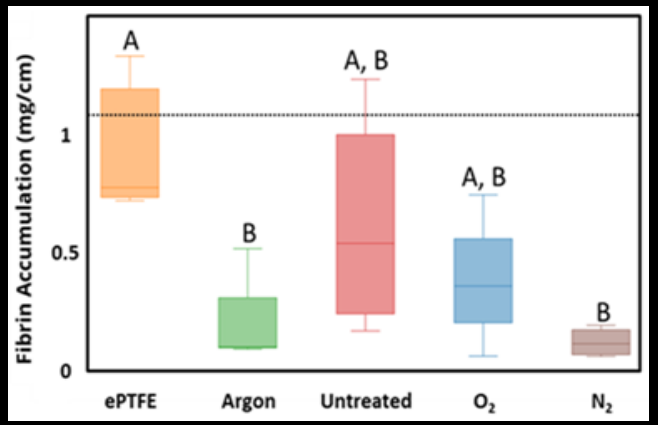
Luminal Surface Properties: Nonthrombogenic ✓









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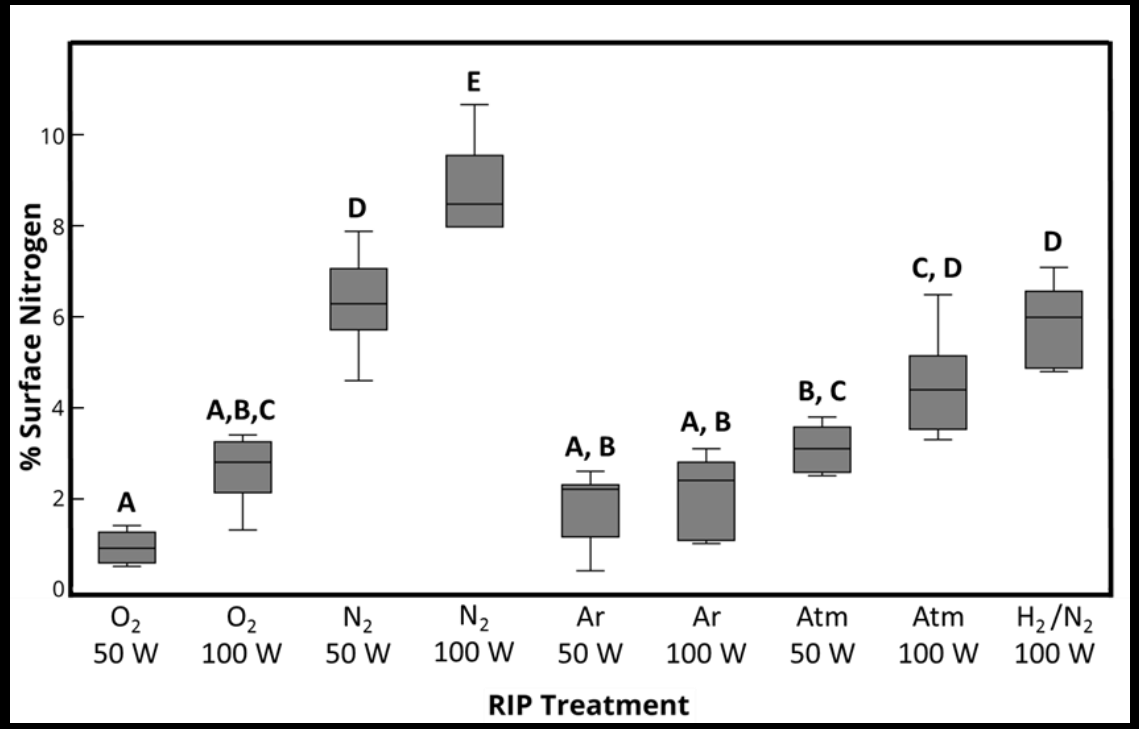
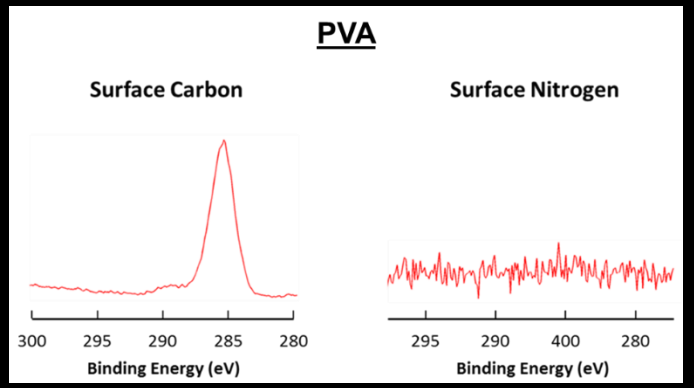
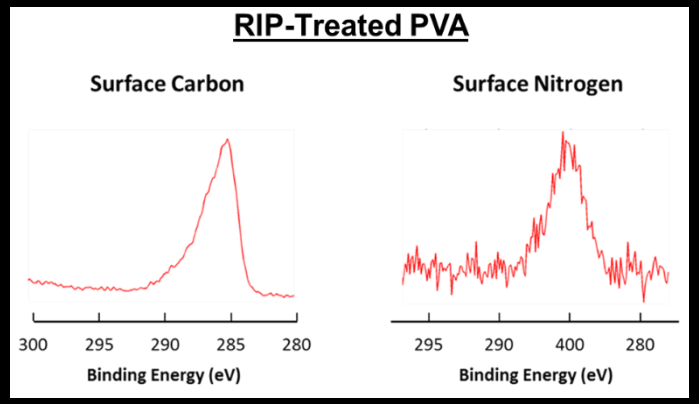
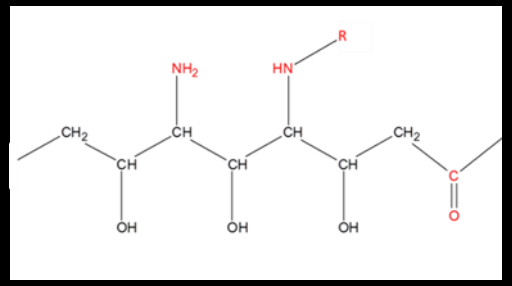
Surface Nitrogen Groups

Nitrogen added to the surface of PVA after RIP-treatment



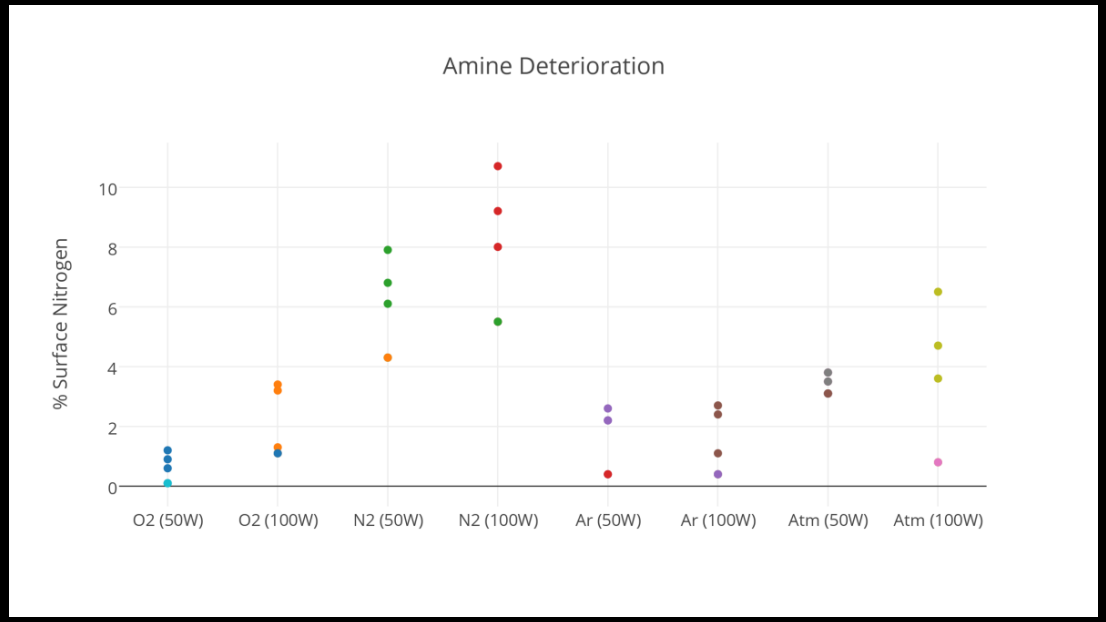






Thermodynamic Relaxation of Surface Effects: Hydrophobic Recovery

Happy Little Accidental Discovery



Project Scope - 1SC2GM140991-01

- Project Title: Reactive Ion Plasma Treatment of Cardiovascular Biomaterials to Understand the Effect of Nanotopography on Endothelialization
- Long-term goal: To manufacture a SBG using RIP which exceeds the patency rates of current PVGs by treating SVG materials to make them rapidly endothelializable.
- We proposed to determine the effect that surface chemistry and nanotexture of SBG materials have on endothelialization using our RIP-treated SVG model.
- Central Hypothesis: through the parameters of RIP, we can promote the rapid endothelialization of SVG materials while maintaining or enhancing their anti-thrombotic properties. We will test our hypothesis through the following three aims:
  - Aim 1: Characterize the surface chemistry and nanotopographic relaxation of RIP-treated SVG materials over time.
  - Aim 2: Determine the relative contributions of surface chemistry and nanotopography on endothelialization of SVG materials.
  - Aim 3: Determine the integrins which are essential for endothelialization on RIP-treated SVG materials.

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Aim 1

- Aim 1: Characterize the surface chemistry and nanotopographic relaxation of RIP-treated SVG materials over time.

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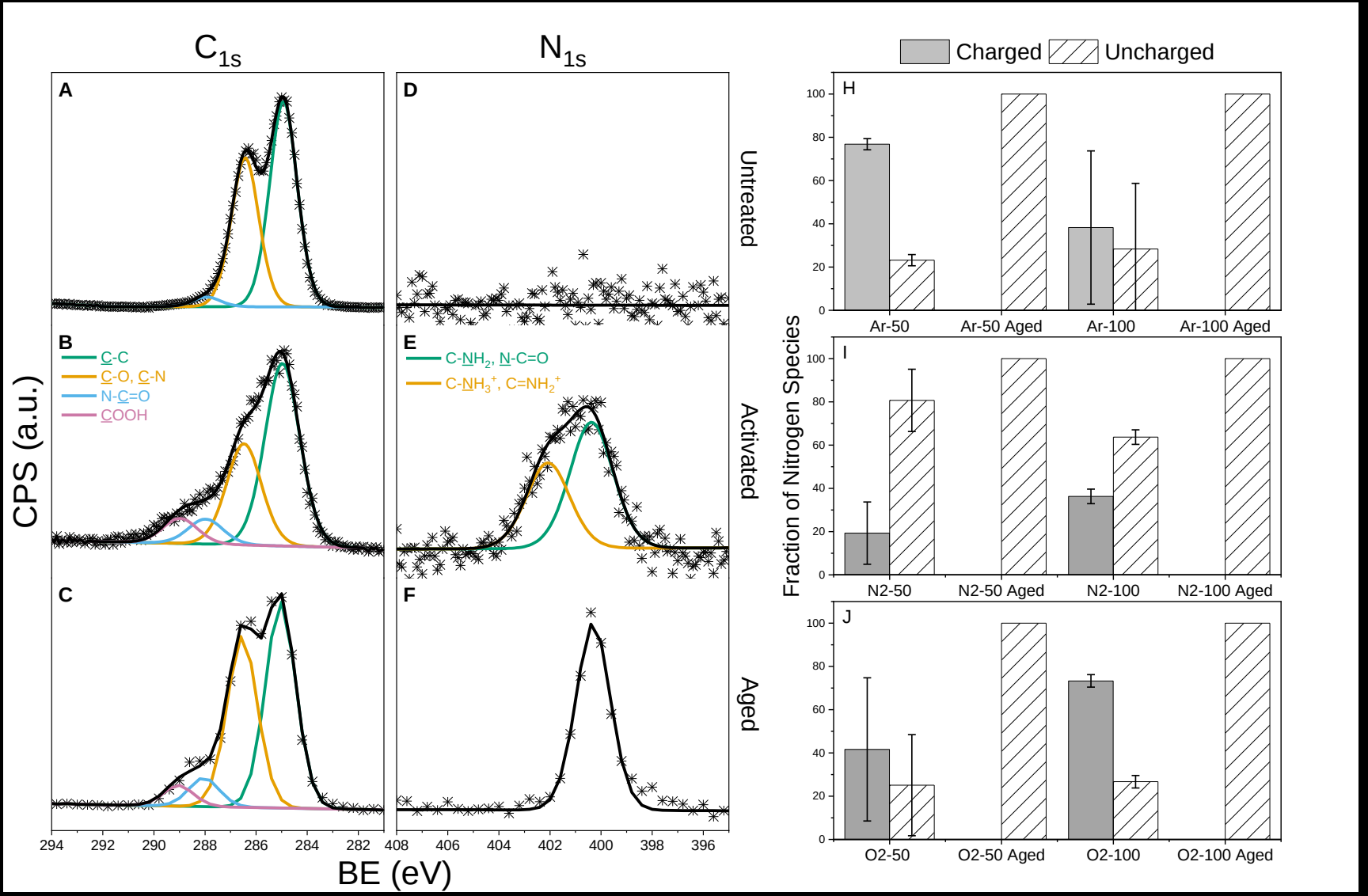
Surface Chemistry - XPS



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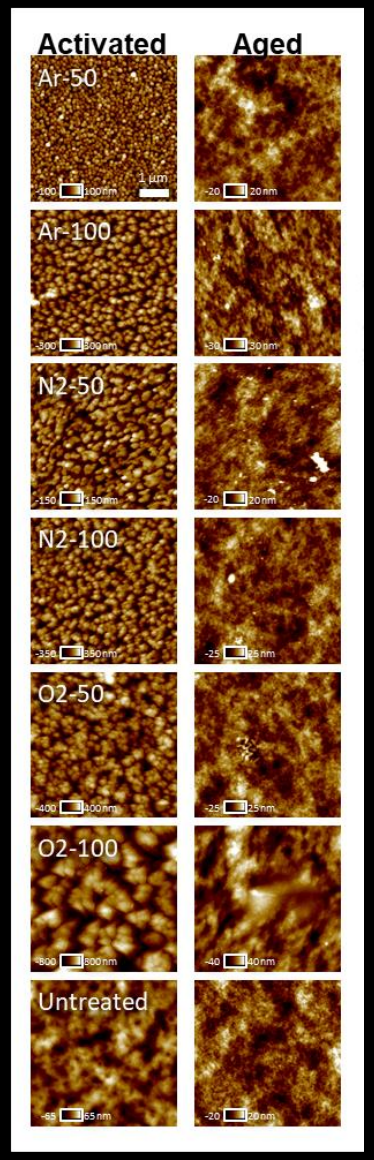
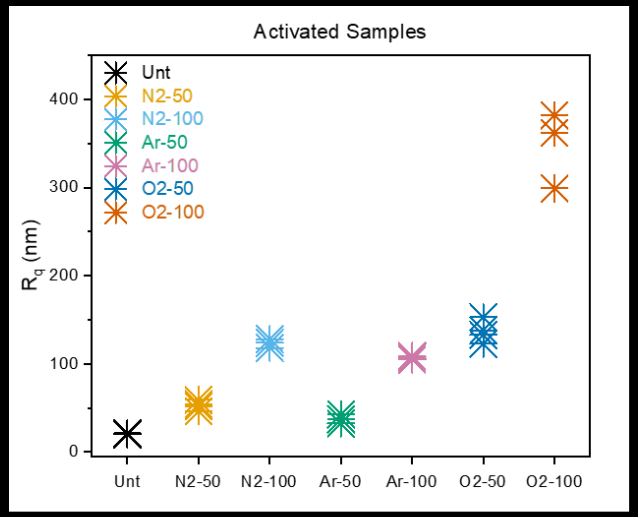
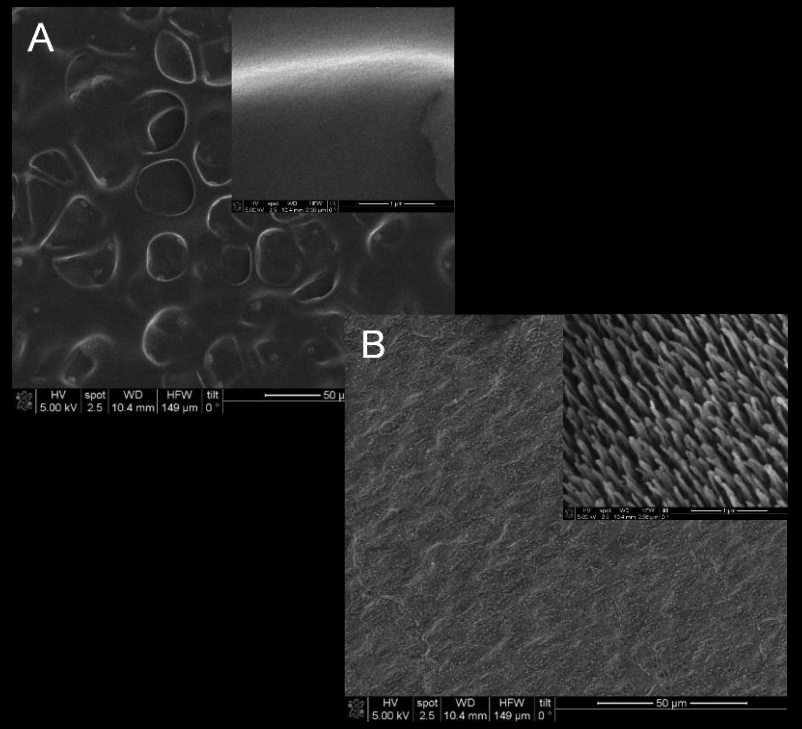
Surface Roughness – AFM/SEM







Treatment	Activated		Aged	
	50 W	100 W	50 W	100 W
Oxygen	136.8 (12.5)	347.7 (43.3)	8	13.5
Nitrogen	53 (5.3)	123.3 (5.0)	5.8	7
Argon	37.3 (5.2)	107 (1.8)	5.9	9.3
Untreated	20.4 (0.5)		5.8	

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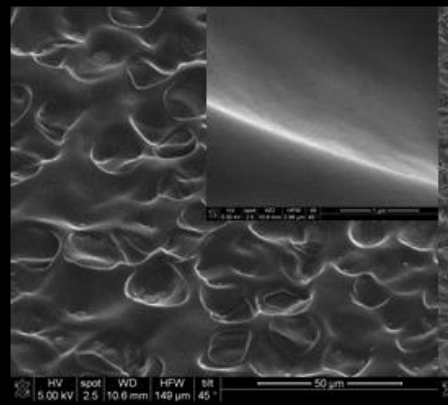
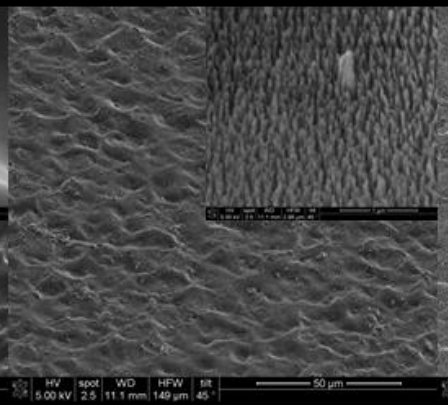
Surface Roughness - SEM

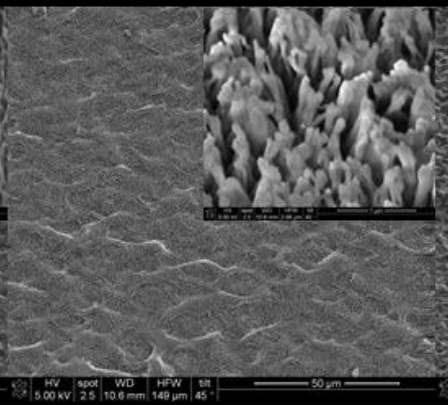
Untreated



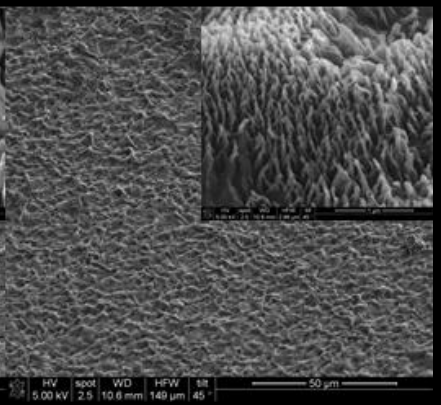
Ar-50



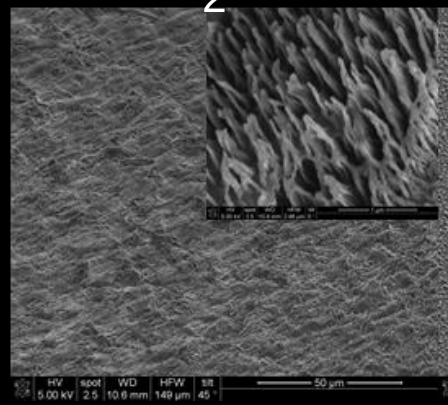
Ar-10



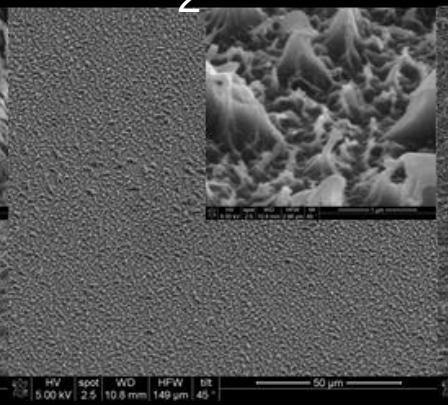
N_2-5



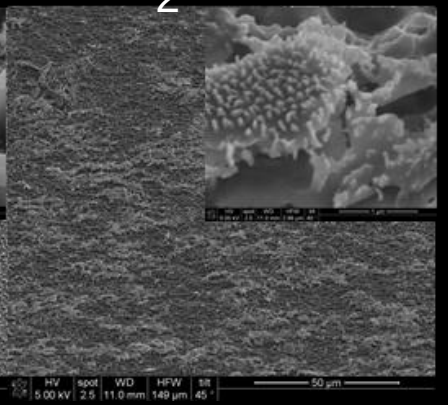
N_2-50



O_2-50



O_2-100



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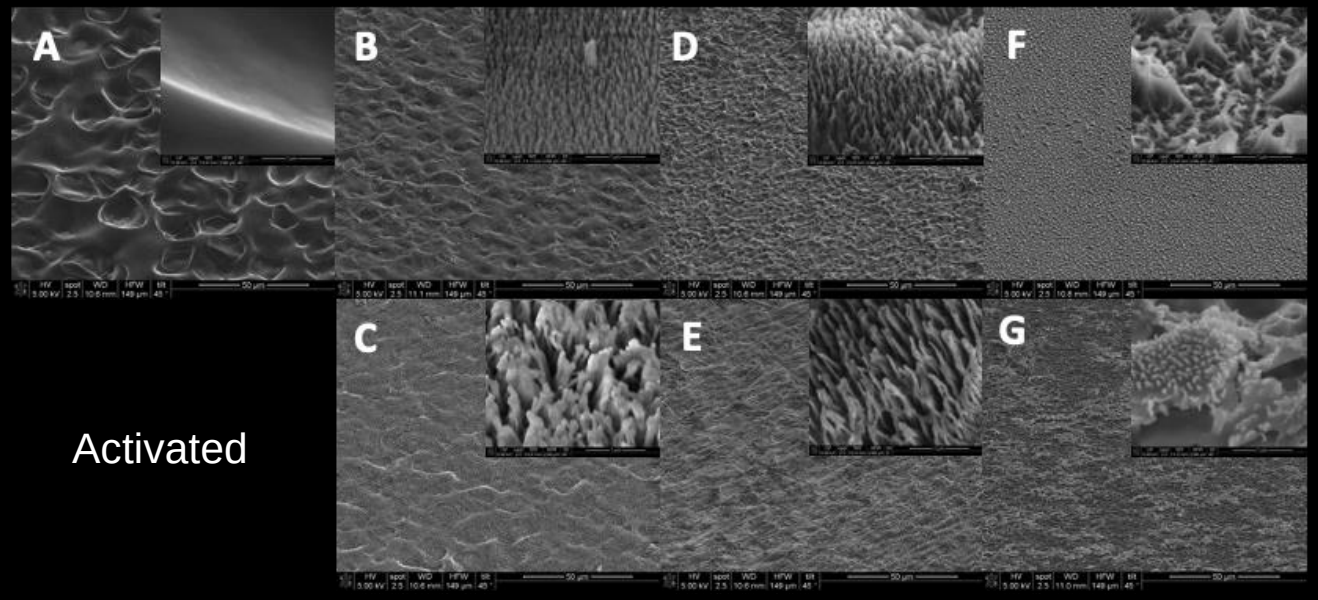
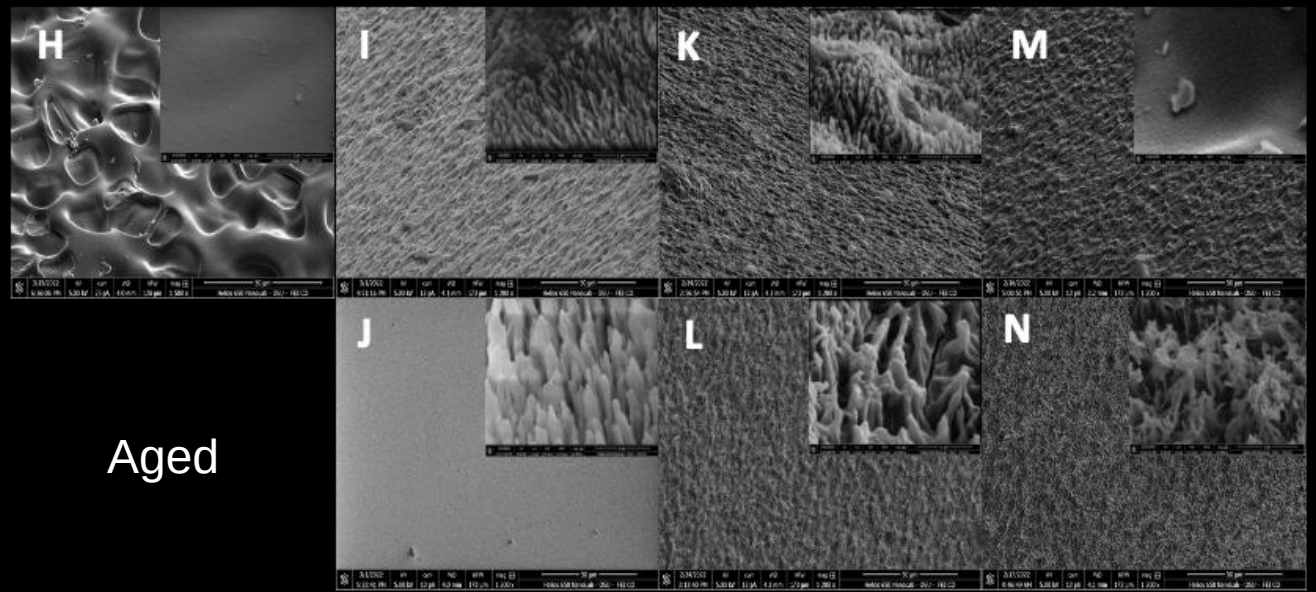
Surface Roughness - SEM



Activated



Aged

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Aim 2

- Quantify EC attachment, proliferation, and migration activated and aged PVA, ePTFE, and Collagen.
- Determine the effect of roughness on EC tip-structure formation, ECM protein deposition, NO, ICAM-1, E-Selectin, and VCAM-1.

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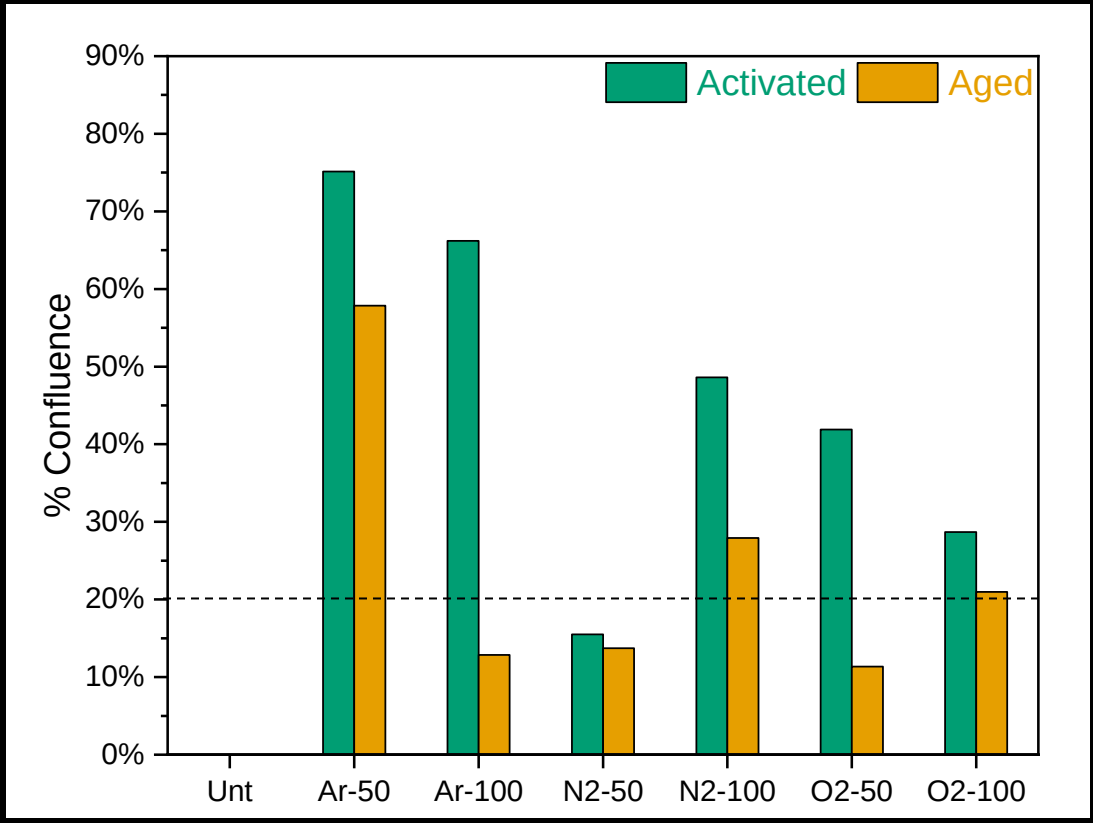
Endothelial Cell Affinity (% confluence at 48 hours)



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Endothelialization

- Quantify EC attachment, proliferation, and migration activated and aged PVA, ePTFE, and CG
- Determine the effect of roughness on EC tip-structure formation, ECM protein deposition, NO, ICAM-1, E-Selectin, and VCAM-1

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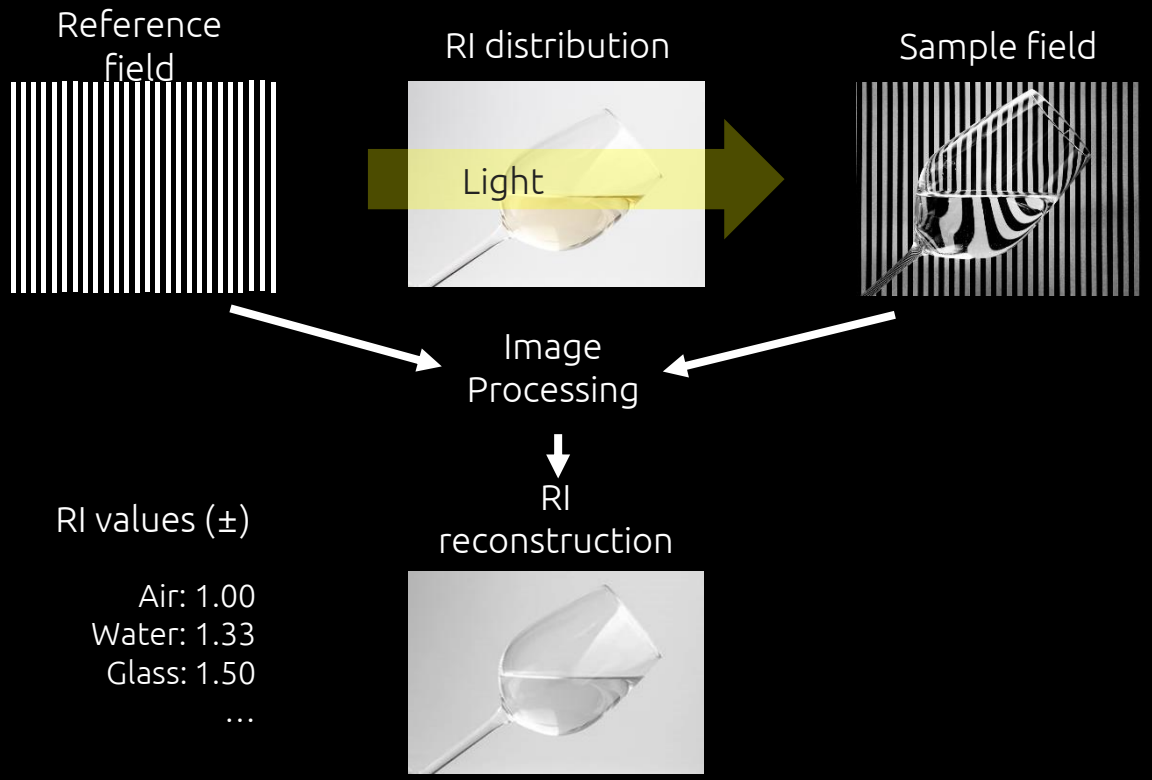
Quantifying Endothelialization Using a 3-Dimensional Holotomography



Information on the Refractive Index (RI) distribution is provided by the difference between the reference field and the sample field

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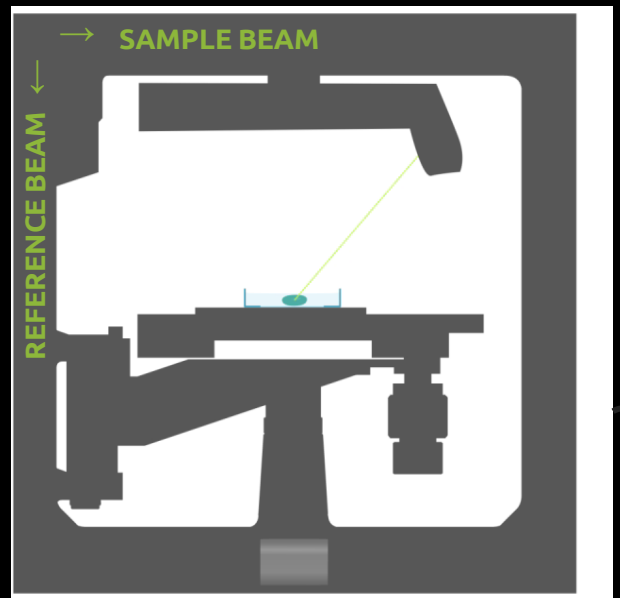
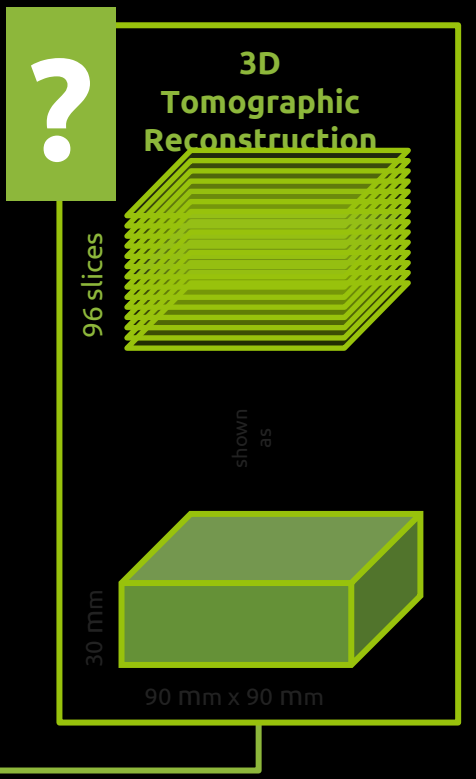
Quantifying Endothelialization Using a 3-Dimensional Holotomography

Laser light
45°angle illumination
λ = 520nm
Light exposure
20mW/cm^2
3-channel fluorescene

Imaging system
Rotating illumination
Resolution (x; y): 200 nm
Resolution (z): 400 nm





* Patent: Holotomographic scanning arm (EU WO 2011/121523)

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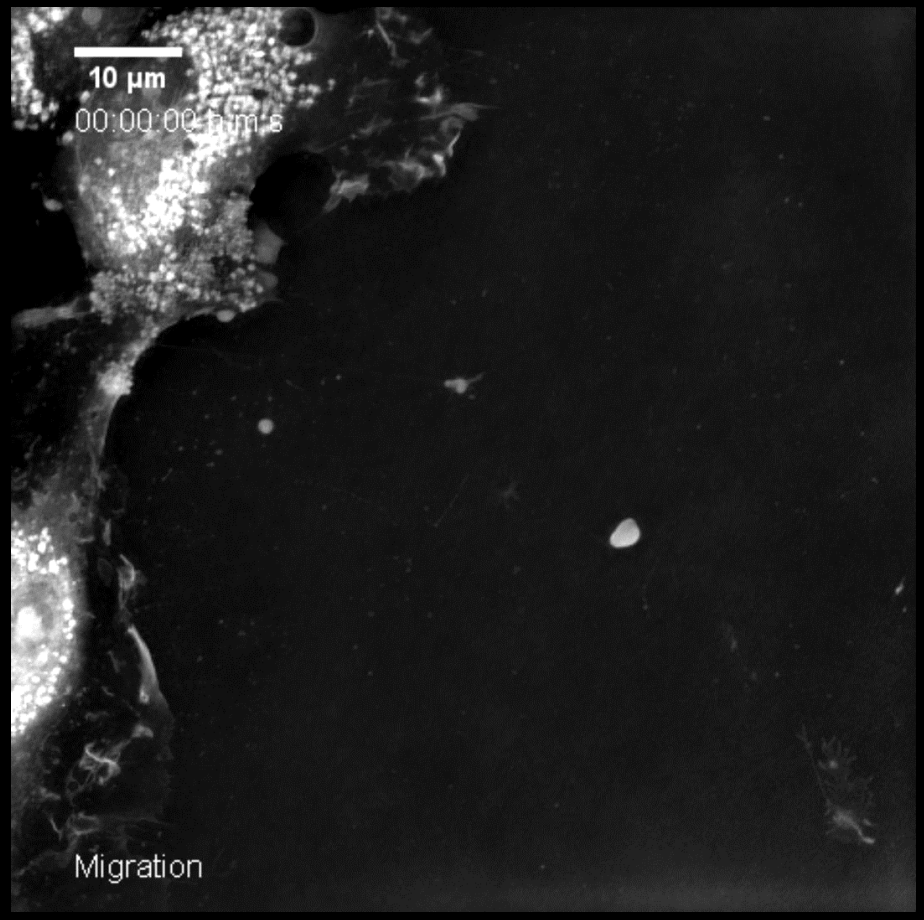
Endothelialization - Migration



- Human Aortic Endothelial Cells Migrating on Collagen-I
  - Imaged using 3-D holotomography

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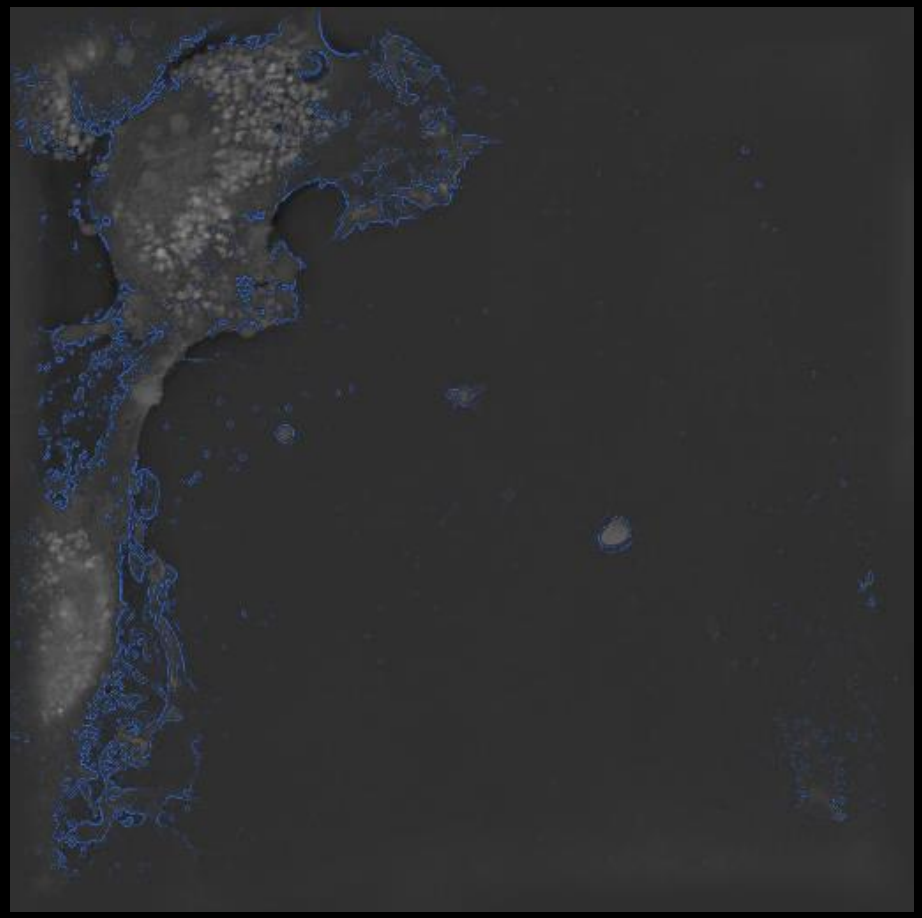
Endothelialization - Migration



- Human Aortic Endothelial Cells Migrating on Collagen-I
  - Imaged using 3-D holotomography

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Aim 3

- Determine the integrins which are essential for endothelial cell attachment, proliferation, and migration on SVG materials

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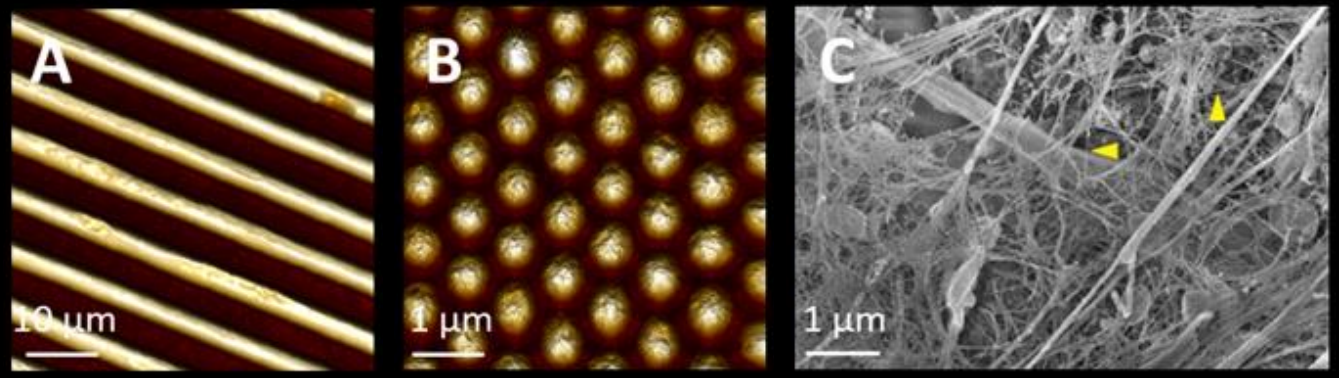
Future Directions

- Geometric mimicry of ECM



	Reactive Surface Chemistry	Surface Roughness	Microtopgraphy Possible?	Hierarchical Structures?
Untreated PVA	NO	NO	YES	NO
Newly RIP-Treated PVA	YES	YES	YES	YES
Aged RIP-Treated PVA	YES	NO	YES	NO
Vascular ECM	YES	YES	YES	YES

Email: Patrick.jurney@sjsu.edu
Lab Website: jurneylab.org
Twitter: @JurneyLab

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Jurney Lab – Vascular Biomaterials Team

Christian Leycam
Gabriela Acevedo Munares
Ammar Babiker
Orion Capuyon
Juliette Noyer
Alex Guerra
Alexis Solorio

Collaborators

OHSU
Dr. Monica Hinds
Dr. Novella Bates

OSU
Dr. Joe Baio (OSU)
Dr. Ryan Fasse

Stanford
Dr. Philip Tsao

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Probing for Bioactive Natural Products from Marine Derived Fungi

Erin McCauley – California State University Dominguez Hills

Erin McCauley, Assistant Professor
CSUDH, Department of Chemistry & Biochemistry
emccauley@csudh.edu

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Probing for Bioactive Natural Products from Marine Derived Fungi

Goals of the McCauley Research Group

Identify microbial natural products with pharmaceutically relevant biological activity

Identify microbial natural products with novel chemical scaffolds

Ensure students are given the opportunity to engage in research experiences that provide them with the skills they need to succeed in the next stage of their career.

- Hands on techniques in bio-, organic, analytical chemistry; microbiology; and molecular biology
- Independent/critical thinking & problem-solving skills
- Communicate scientific finding (writing/presenting) at scholarly level

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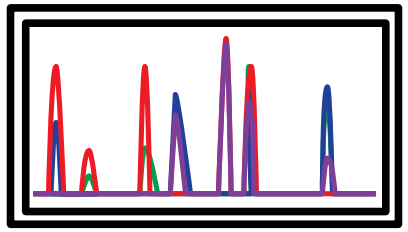
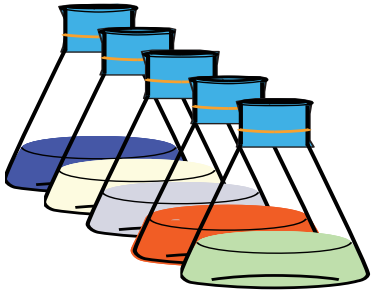
Probing for Bioactive Natural Products from Marine Derived Fungi

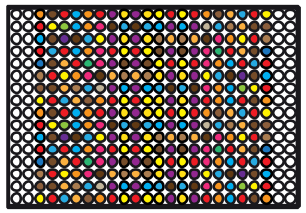
Project Overview



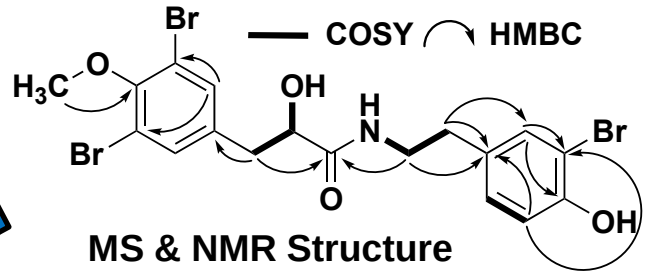
Crude Separation of Metabolites Using Flash Chromatography



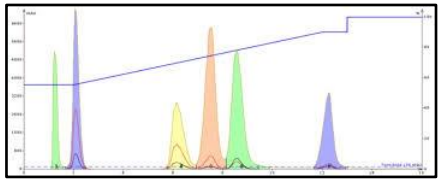
Culturing of Fungal Strain & Extraction of Metabolites



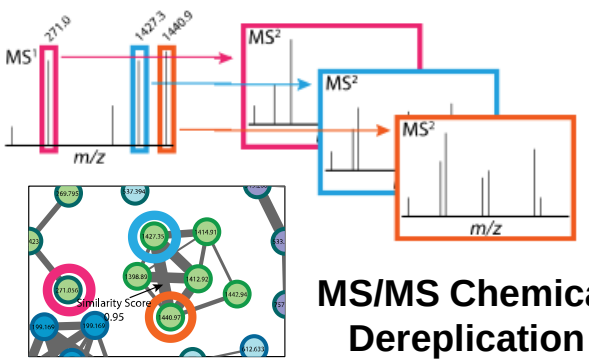
Bioassay Screening



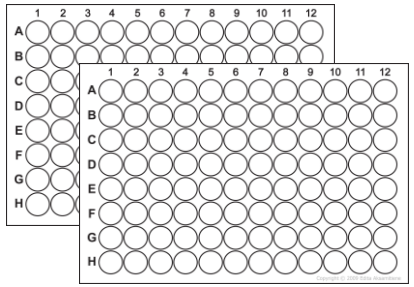
MS & NMR Structure Elucidation



HPLC Purification of Active Compounds



MS/MS Chemical Dereplication



Stock Distinct Testing Unit Screening Plates

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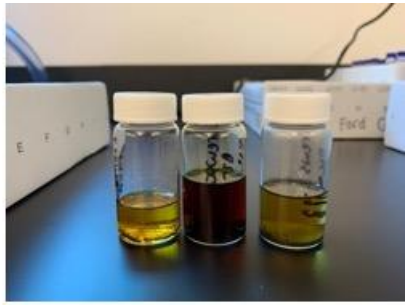
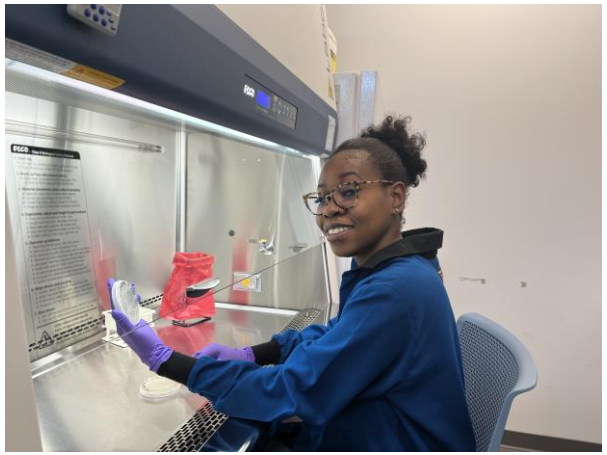
Library of Natural Products

Gifted a Library of +8000 Fungal Strain from Professor Phillip Crews - UC Santa Cruz

Jason Guerrero





Ebonie Bennett

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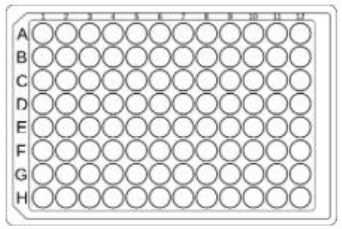
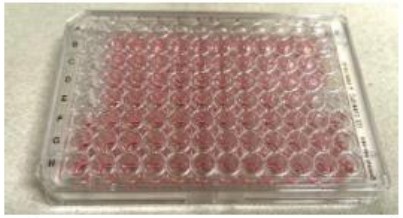
Bioactivity Screening

Cytotoxicity Screening - SRB Assay



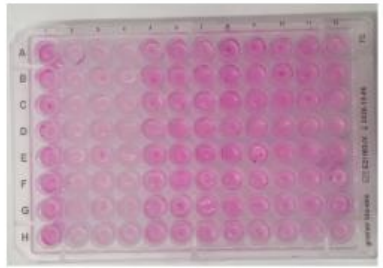
Add 10uL of Extract or Pure Compound in 10% DMSO



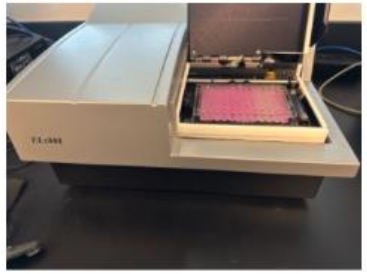
Add 10,000 U87 Cells/well Incubate for 48 hr @ 35°C and 5% CO_2

Fix Cells with 20% Trichloroacetic Acid (TCA)

Stain with 0.057% Sulforhodamine B

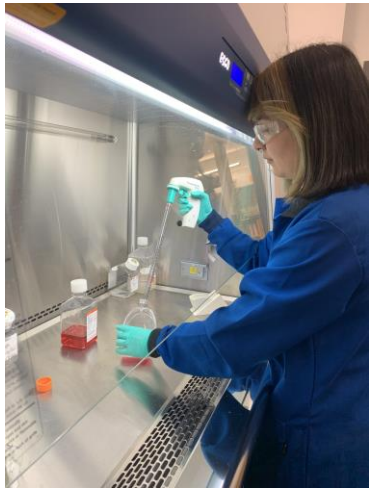


Dissolve SRB in 10mM Tris Buffer



Measure Absorbance at 510 nm

Shaz Sutherland



Melissa Estrada

Vichai, et al, *Nat Protoc.***2006**;1(3):1112-6.

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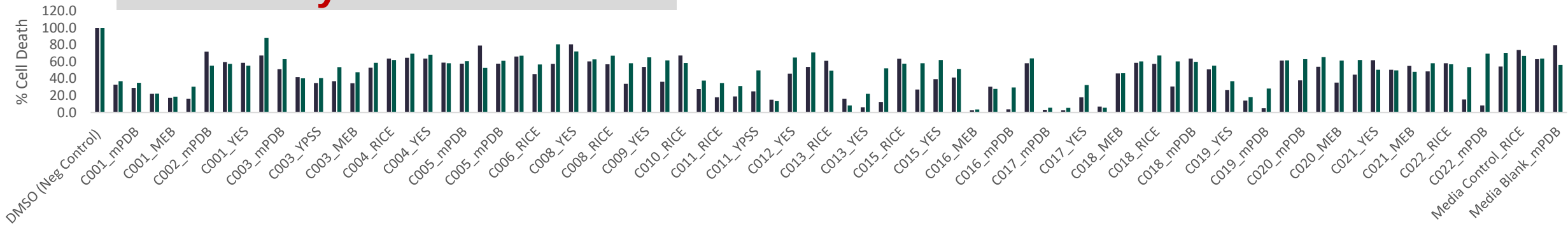
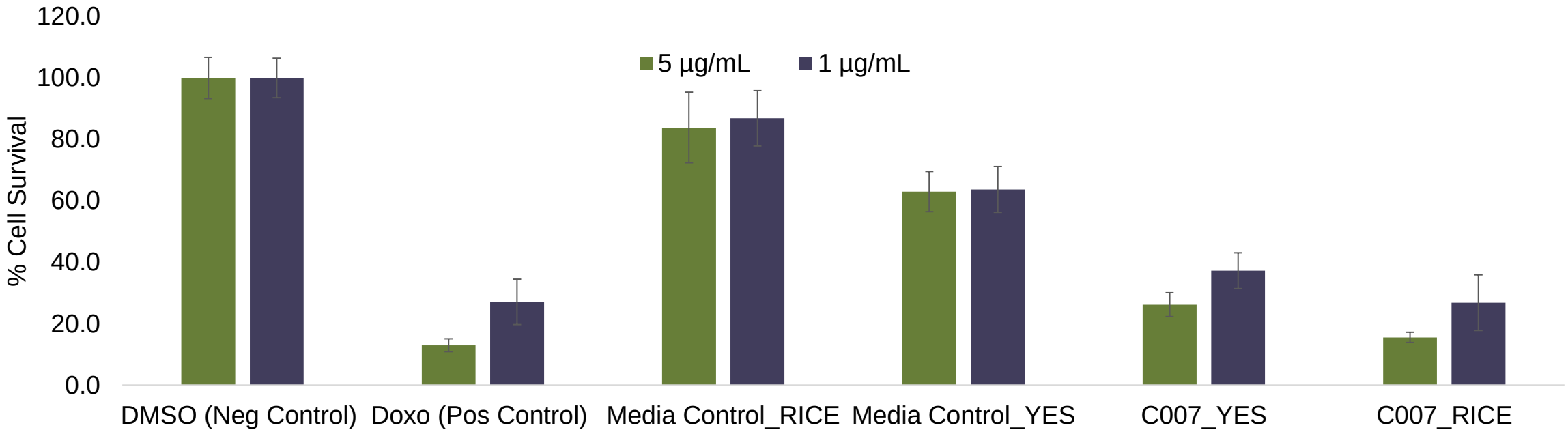
Bioactivity Results





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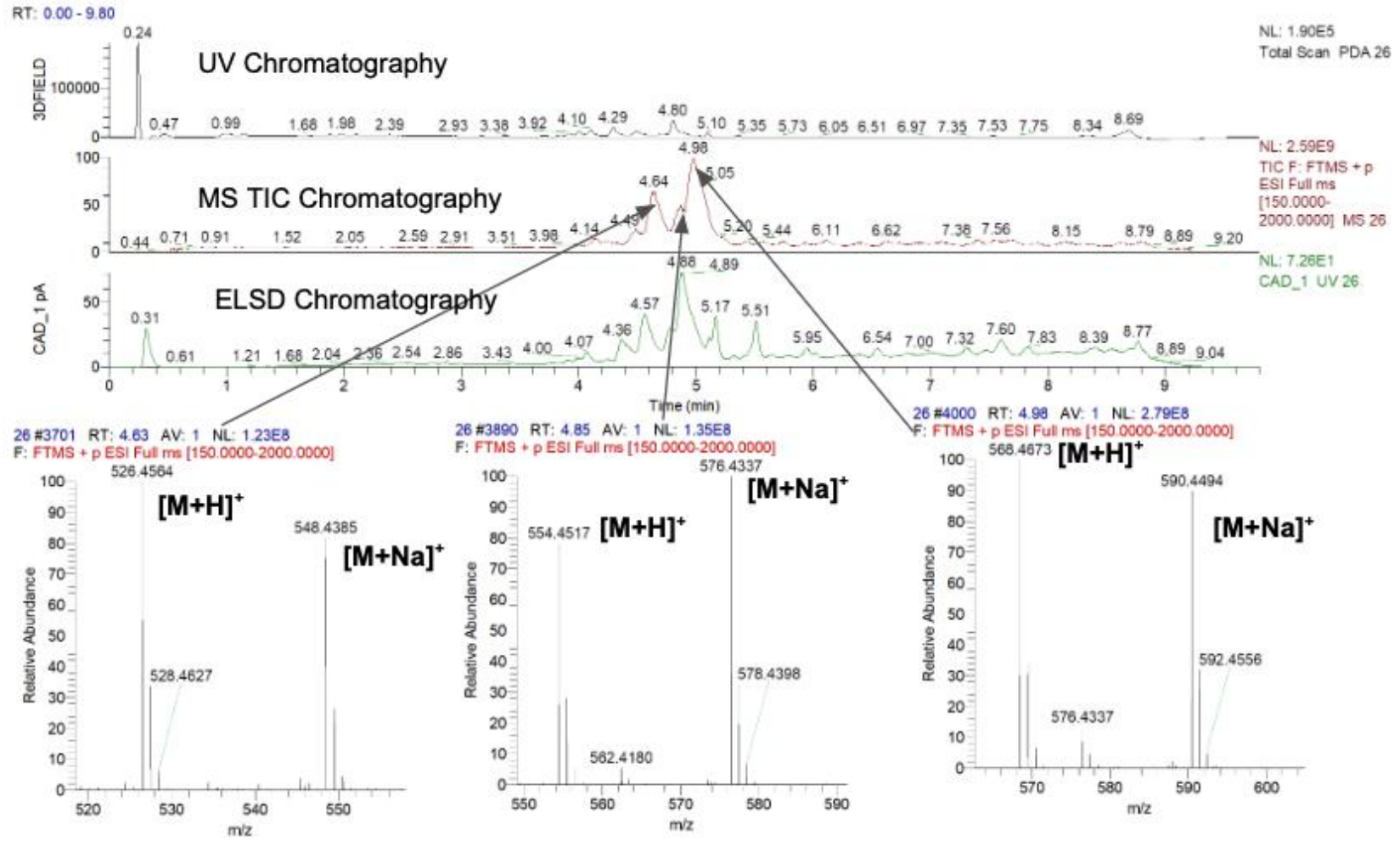
Dereplication



Wang, *et al. Nat Biotechnol.* **2016** Aug 9;34(8):828-837.

van Santen, *et al. ACS Cent Sci.* **2019** 27;5(11):1824-1833.

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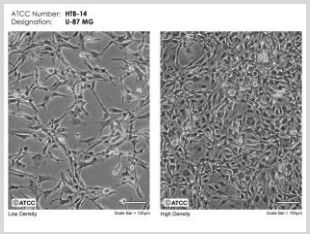
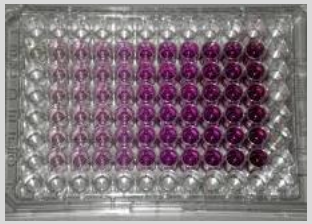
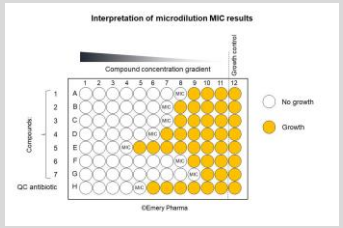
Probing for Bioactive Natural Products from Marine Derived Fungi

Prioritization

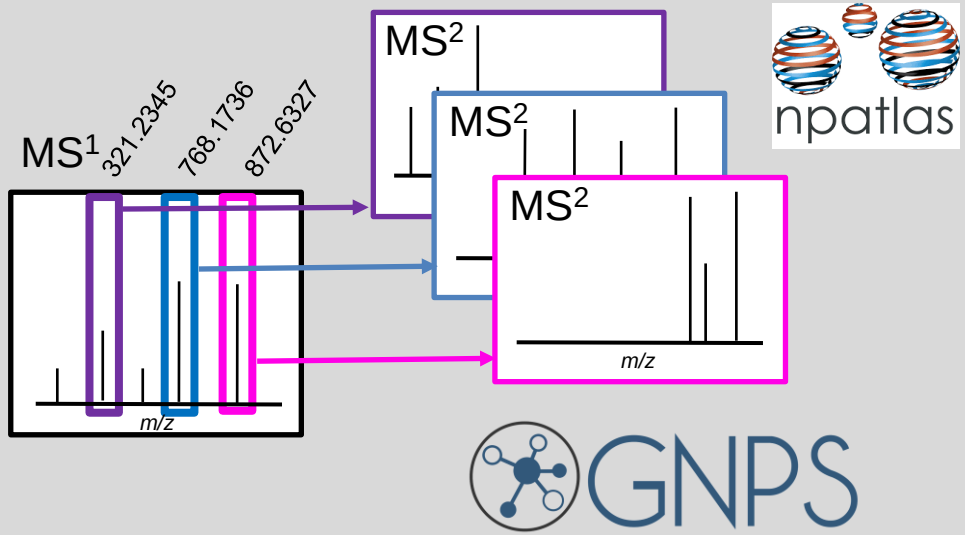
Screen for Biological Activity







Screen for Novel Structures



Vichai, et al, *Nat Protoc.***2006**;1(3):1112-6.
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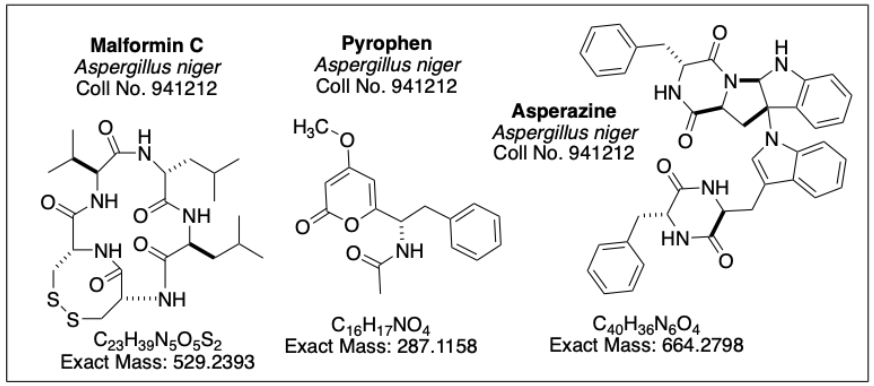
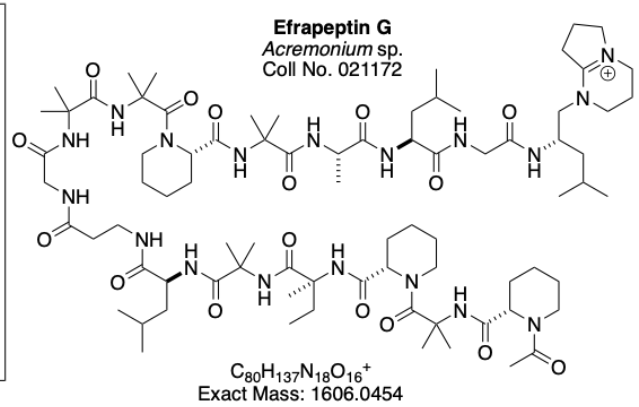
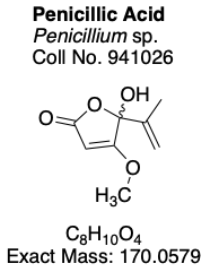
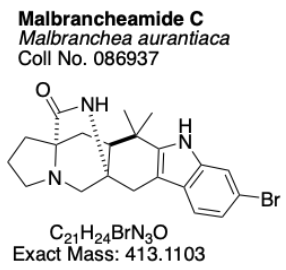
Probing for Bioactive Natural Products from Marine Derived Fungi

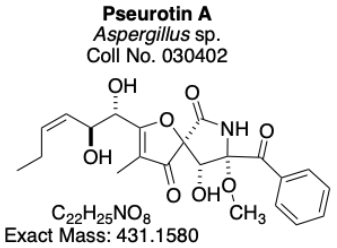
Prioritization/Dereplication

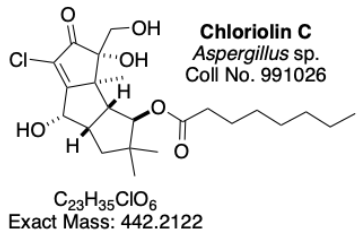


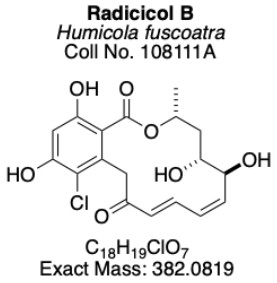


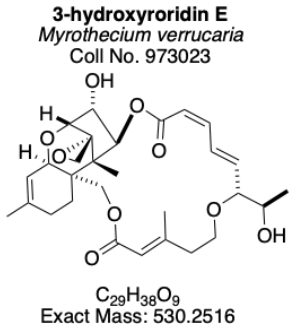


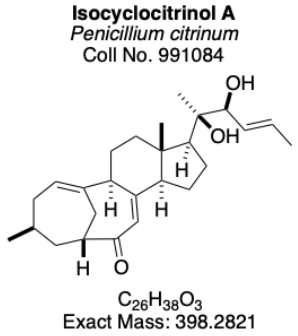


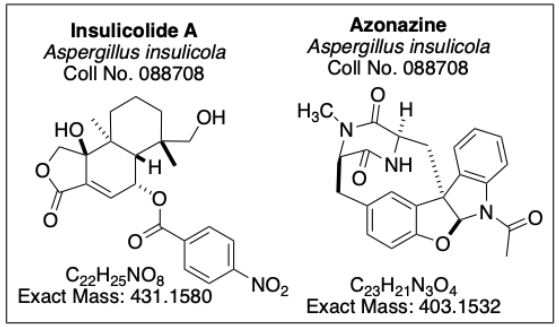












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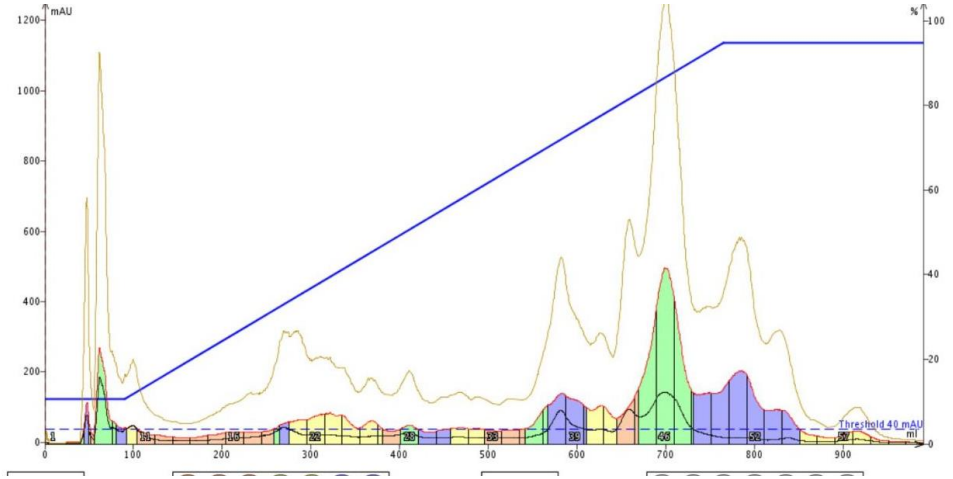
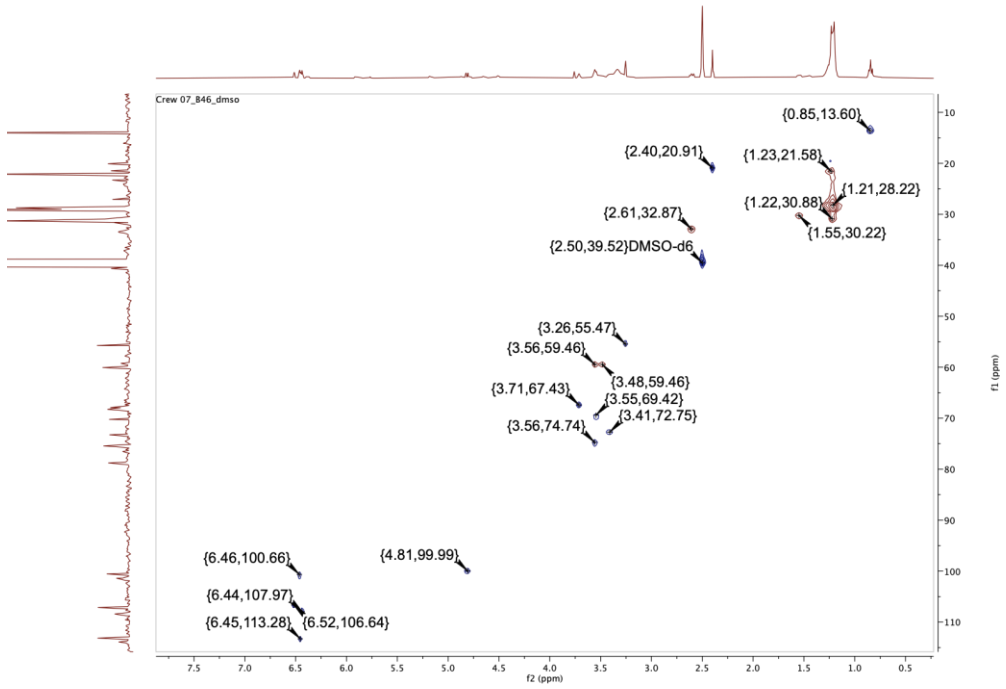
Fusicolla sp. Crews 007 strain



Flash Chromatography Purification of the Putatively Novel Natural Products Present in Crude Extract from the CREWS 007 Fungal Strain.

Lari Smith



HSQC NMR Spectra from Putatively Novel Natural Product Isolated from CREWS 007- Flash Chromatography Fraction B46.

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Probing for Bioactive Natural Products from Marine Derived Fungi

Next Steps/Long-Term Plans

Expand fungal library by culturing fungi from unique high salt environments

Build a mechanism that would provide research opportunities for high school and community college students

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Probing for Bioactive Natural Products from Marine Derived Fungi

Acknowledgements

National Institutes of Health

RISE/Martinez - R25GM62252
McCauley - SC2GM144172

RISE

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Questions?

Contact Information:

Erin McCauley

CSUDH/ Department of Chemistry & Biochemistry

831-435-9174

emccauley@csudh.edu

Speaker Contacts

Edgardo Falcon-Morales, National Institute of General Medical Sciences
edgardo.falcon@nih.gov

Perla Ayala, Cal State Long Beach
perla.ayala@csulb.edu

Junjun Liu, Cal Poly Pomona
junjunliu@cpp.edu

Maria Soledad Ramirez, Cal State Fullerton
msramirez@fullerton.edu

Patrick Jurney, San Jose State University
patrick.jurney@sjsu.edu

Erin McCauley, Cal State Dominguez Hills
emccauley@csudh.edu

Next Steps/Closing Remarks

Dr. Frank A. Gomez
Executive Director, STEM-NET
Office of the Chancellor

https://www2.calstate.edu/impact-of-the-csu/research/stem-net

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STEM-NET Upcoming Events

STEM-NET Virtual Research Café 10.0

Date: Friday, March 10, 2023
Time: 11am-12pm

Register Here

STEM-NET March Webcast

Topic: NIH-Funded Research in the CSU Part II
Date: Friday, March 24, 2023
Time: 10am-12pm

Register Here

Join our CSU STEM-NET Community listserv

csustemnet@lists.calstate.edu

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Frank A. Gomez *CSU Office of the Chancellor* *fgomez@calstate.edu*