A Metabolomic Approach to Diagnosing Prosthetic Joint Infection

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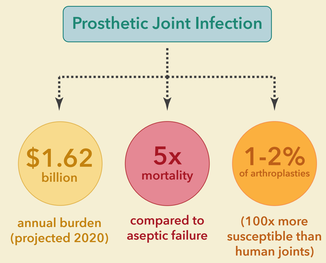
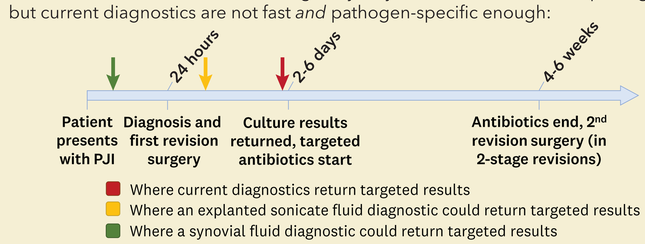
Introduction

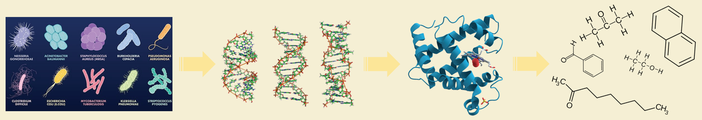
Background:
- Total joint replacements provide some of the greatest improvement in quality of life per dollar spent of any treatment [1]
- Knee and hip replacements together rep resent well over 90% of all joint replacements in the United States [2]
- Americans are leading longer lives [3], so the number of hip and knee replacements will continue to rise.
- Knee and hip replacements significantly increase the susceptibility of these joints to infection, known as prosthetic joint infection (PJI).



Need: PJI treatment and antibiotics greatly vary based on the causative pathogen [4], but current diagnostics are not fast *and* pathogen-specific enough:

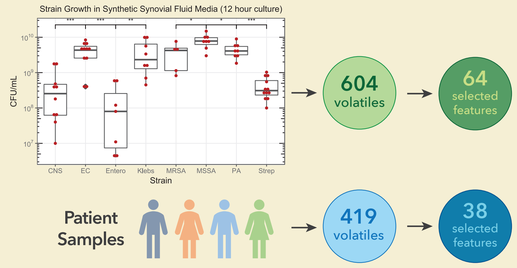


Hypothesis: Volatilomic (volatile metabolomic) analysis can deliver a rapid, pathogen-specific diagnostic test for PJI. We believe such a diagnostic is feasible because of a fundamental multi-omics approach (below). First, I hypothesize that analysis of explanted joint sonicate fluid can determine if a joint has been infected within the current workflow of surgeries. I then hypothesize that we can design a synovial-fluid based diagnostic for PJI that can allow PJI diagnosis before surgery.



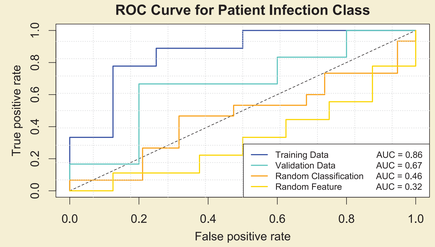
Results

1. We identified the volatilome and discriminatory VOCs of explanted prosthesis sonicate fluid and of cultures of PJI-causing strains grown in SSF.



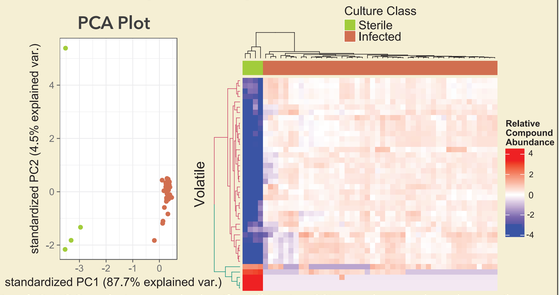
Result 1: In our final SSF recipe, all strains grew to at least 10^6 CFU/mL. VOC analysis returned 604 constituent VOCs after contaminant removal, of which 64 were discriminatory. Analysis of patient samples yielded 419 constituent VOCs, 38 discriminatory.

2. VOC analysis of explanted prosthesis sonicate fluid can identify prosthetic joint infection without a pre-culture step



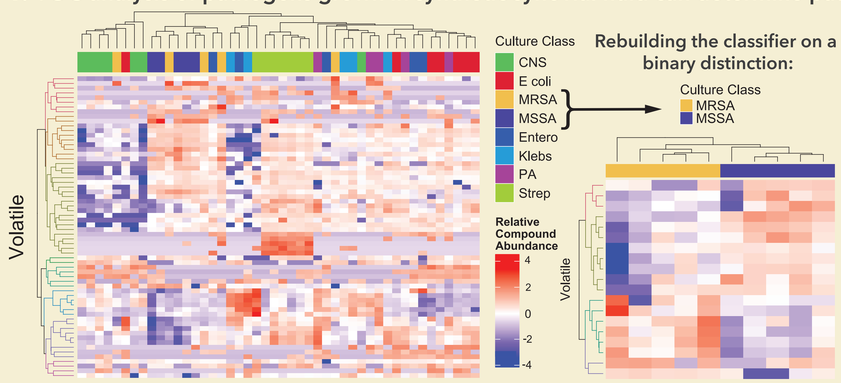
Result 2: A VOC-based test applied to explanted prosthesis sonicate fluid identifies PJI with 72.7% accuracy, and an area under the receiver operator characteristic curve (AUROC) of 0.67 on the validation set. This classification outperforms random sample classification or selecting a random set of 38 features.

3. VOC analysis can discriminate between sterile and cultured synthetic synovial fluid



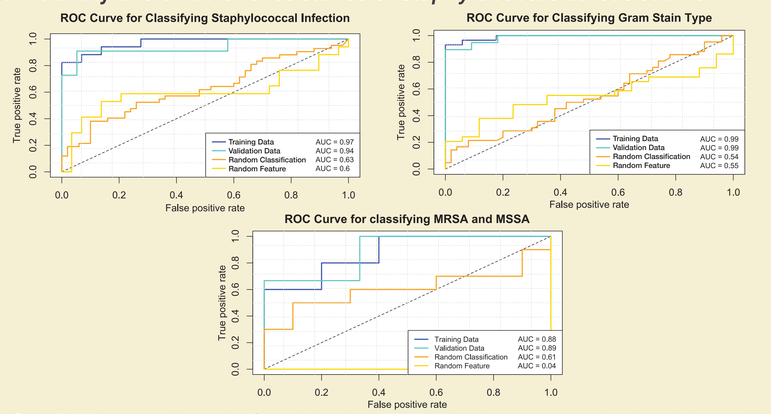
Result 3: VOC analysis correctly classifies all in-vitro SSF samples as cultured or sterile, with clear differences in their volatilome showing up both directly on the heatmap and in the principal components analysis (PCA) plot as strong inter-class separation.

4. VOC analysis of pathogens grown in synthetic synovial fluid can determine pathogen identity and antibiotic resistance of *Staphylococcus aureus* strains



Result 4a: VOC analysis of SSF cultures correctly classifies samples 76.7% of time time, with notable patterns in their volatilomes causing strains to cluster together. Heavily related strains (such as MRSA and MSSA) also appear to cluster together, indicative of their genetic similarity.

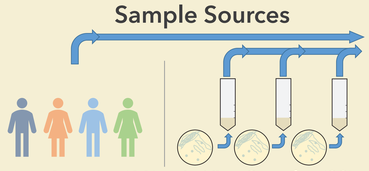
Result 4b: We can re-build the RF classifier to target a binary distinction and generate a new set of discriminatory compounds for more targeted purposes, such as classifying MRSA from MSSA.



Result 4c: We built several binary classifiers with clinical importance, each with greater accuracy than the multi-class model. Each classifier outperformed both random sample classification and selecting a random set of features the same size as the model set with area under the ROC curve (AUROC) values of 0.89 or higher on the validation set.

Materials and Methods

Sample Sources



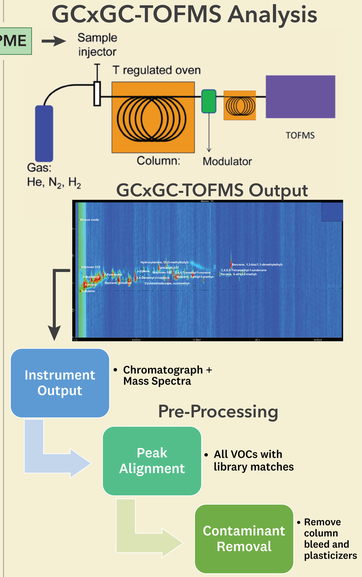
Patient Samples (28)
Explanted joint sonicate fluid, 13 PJI, 15 non-PJI

***In-Vitro* Samples (82)**
In synthetic synovial fluid (SSF) media, 4 bio reps/strain

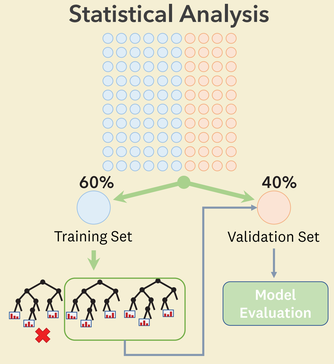
PJI Pathogens and Strains Used

Pathogen	Strain Name	Source	Prevalence	Relative Chance of Leading to Treatment Failure
Coagulase-Negative *Staphylococcus* spp.	BC CNS 4 BC CNS 5 BC CNS 6	Sepsis Isolate Sepsis Isolate Sepsis Isolate	37.00 %	High
Methicillin-Sensitive *Staphylococcus aureus*	ATCC 25923 BC MSSA 5	ATCC Standard Sepsis Isolate	17.50 %	Medium-High
Methicillin-Resistant *Staphylococcus aureus*	ATCC 700698 BC MRSA 6	ATCC Standard Sepsis Isolate	7.30 %	Medium-High
Streptococcus spp.	Group C Group A Group B	Synovial Isolate Sepsis Isolate Sepsis Isolate	8.30 %	No data
Enterococcus spp.	BC VSE 1 BC VSE 6	Sepsis Isolate Sepsis Isolate	5.60 %	Low
Escherichia coli	ATCC 25922 BC EC 13 BC EC 1	ATCC Standard Sepsis Isolate Sepsis Isolate	4.86 %	High
Pseudomonas aeruginosa	PA14 PA 2210	Reference Strain Burn Isolate	4.91 %	Low, but potential co-morbitity
Klebsiella pneumoniae	BC K 11 BC K 2	Sepsis Isolate Sepsis Isolate	1.50 %	No data

GCxGC-TOFMS Analysis



Statistical Analysis



- All sample VOC abundances converted to probabilistic quotient normalization (PQN) scores [5]
- Training, Test, and Validation sets created to optimize model performance without overfitting to the data collected
- Random Forest (RF) classification model classifies a sample into a PJI or non-PJI class based on its constituent VOC

Conclusions and Future Studies

VOC-based PJI diagnostics are:

A metabolomic test for PJI could perform similarly to state-of-the-art diagnostic technologies while providing rapid, pathogen-specific results.

Future Directions:

- Could examine the performance of a patient synovial fluid based diagnostic for PJI
- Could expand the sample size to draw conclusions about pathogen identity from patient ex-vivo samples
- Could investigate the value of an intermediate culture step between obtaining a patient sample and analyzing its volatilome
- Could make a marketable diagnostic device based on these identified discriminatory VOCs

References and Acknowledgements

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