Overview of Biomaterials Characterization

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University of Washington Engineered Biomaterials (UWEB)
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Singapore-University of Washington Alliance

NESAC/Bio (NIBIB)

BEAT
(an NIH Bioengineering Research Partnership)

Workshop

In Vitro Analyses of Cell/Scaffold Products

`Scaffolds are made of biomaterials!`

Porous

Gels

Decellularized tissue

Mechanical/Thermal Properties

stress-strain
strain to failure
flex fatigue testing over time
viscoelastic properties
DSC/TGA

Morphological Characteristics

light microscopy
scanning electron microscopy
atomic force microscopy
permeation of aqueous fluids
BET

Chemical Characteristics

Surface
electron spectroscopy for chemical analysis (ESCA)
static secondary ion mass spectrometry (SIMS)
contact angle
infrared surface studies

Bulk
infrared spectroscopy
NMR

Chemical Stability

in aqueous media
in enzyme solutions
in oxidant solutions

Biological

cell attachment/proliferation
endotoxin

There are many possibilities for characterization of scaffolds

- multi-parameter characterization will be needed
- not all methods will be relevant or possible in every case

Core Issues in physical Scaffold Characterization

1. **Match the mechanical properties to the tissue**
   - flexing environment?
   - relatively static environment?
   - bone, cartilage, skin, heart, liver or brain?
2. **Pore size, pore geometry and pore size distribution**
3. **Interconnectivity and % of void space**
4. **Cell interactions** (surface properties)
5. **Controlled release of active molecules?**
6. **Biodegradation (rate, mechanics vs time, cytocompatibility)**
7. **Contamination issues?**

Mechanical Properties

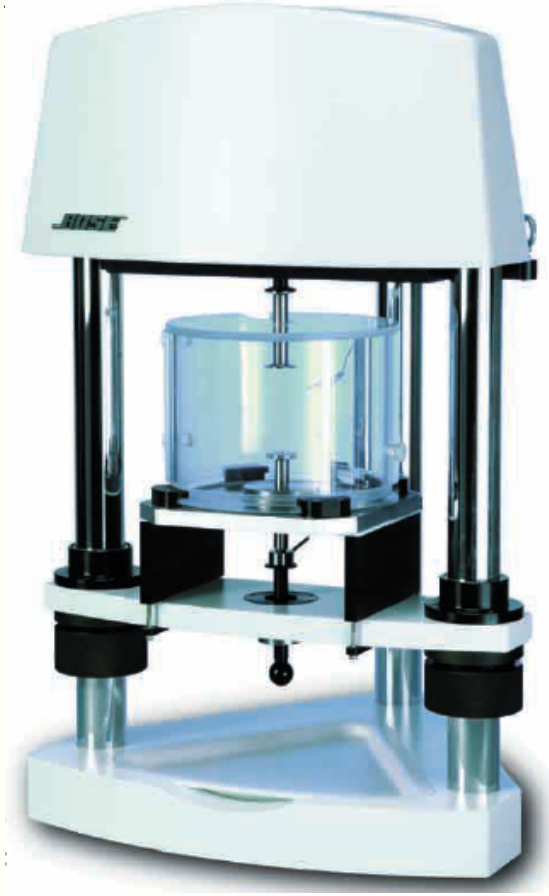
stress-strain
strain to failure
flex fatigue testing over time
viscoelastic (dynamic mechanical) properties

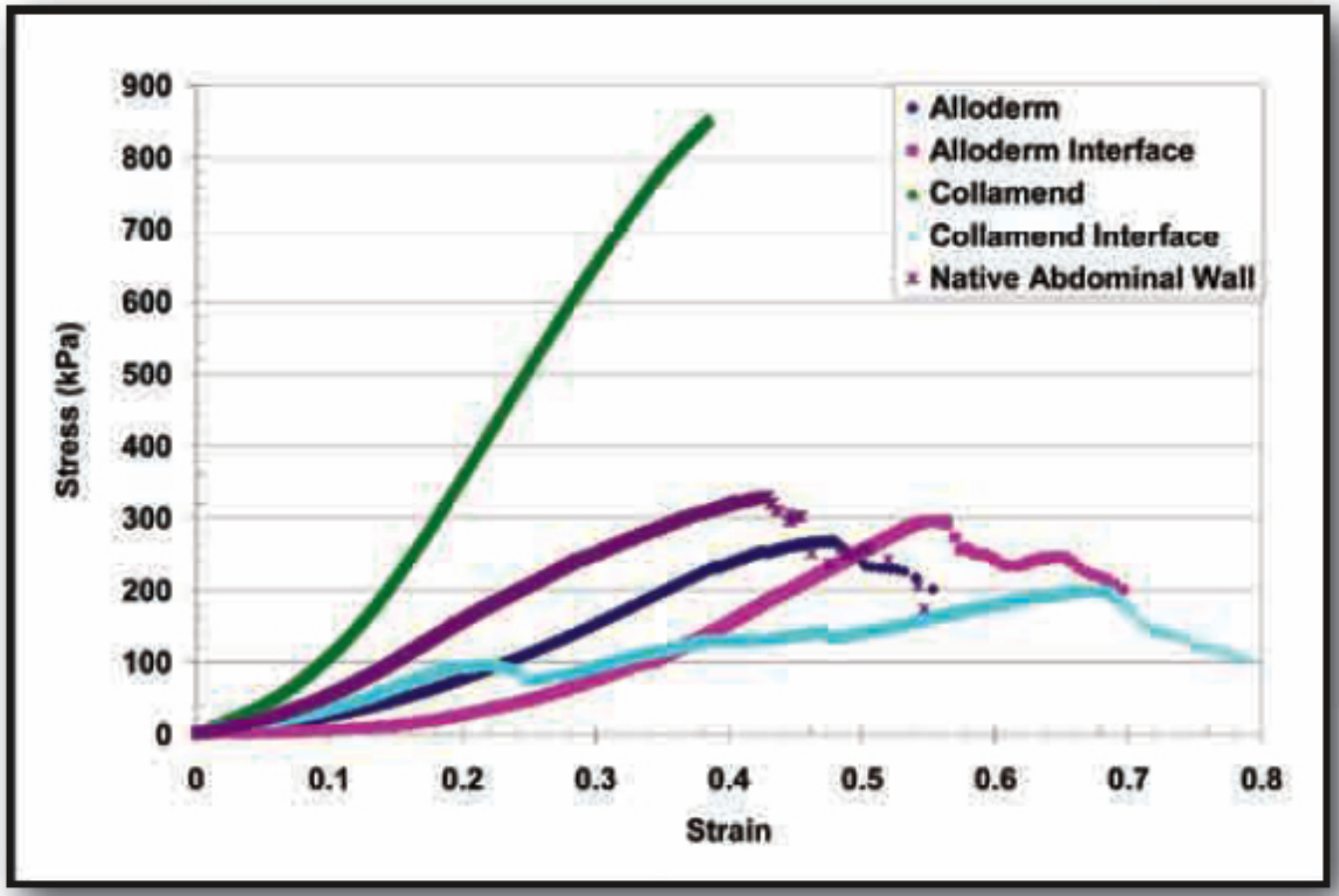
Modulus matching

Decay of properties during degradation

Ultimate strength and toughness

Burst pressure for tubular & hollow structures





The mechanical properties of acellular dermal matrices (ADM) after a 4-week in vivo implantation in a ventral hernia repair model were evaluated under tension in an ElectroForce 3200 biomaterials test instrument with DMA

Bose-Electroforce

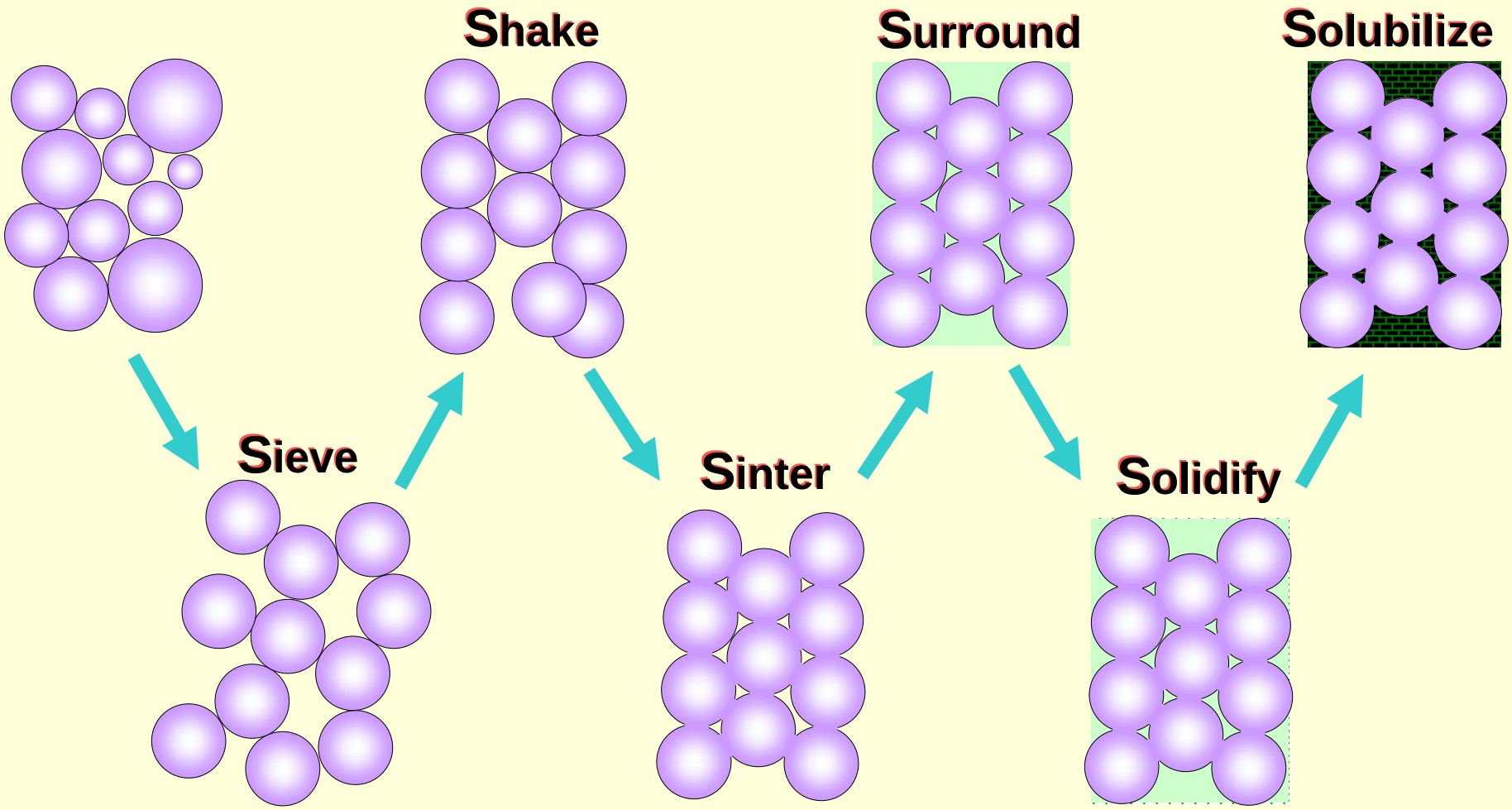
Two scaffold examples wil be used throughout this talk:

Decellularized natural tissue

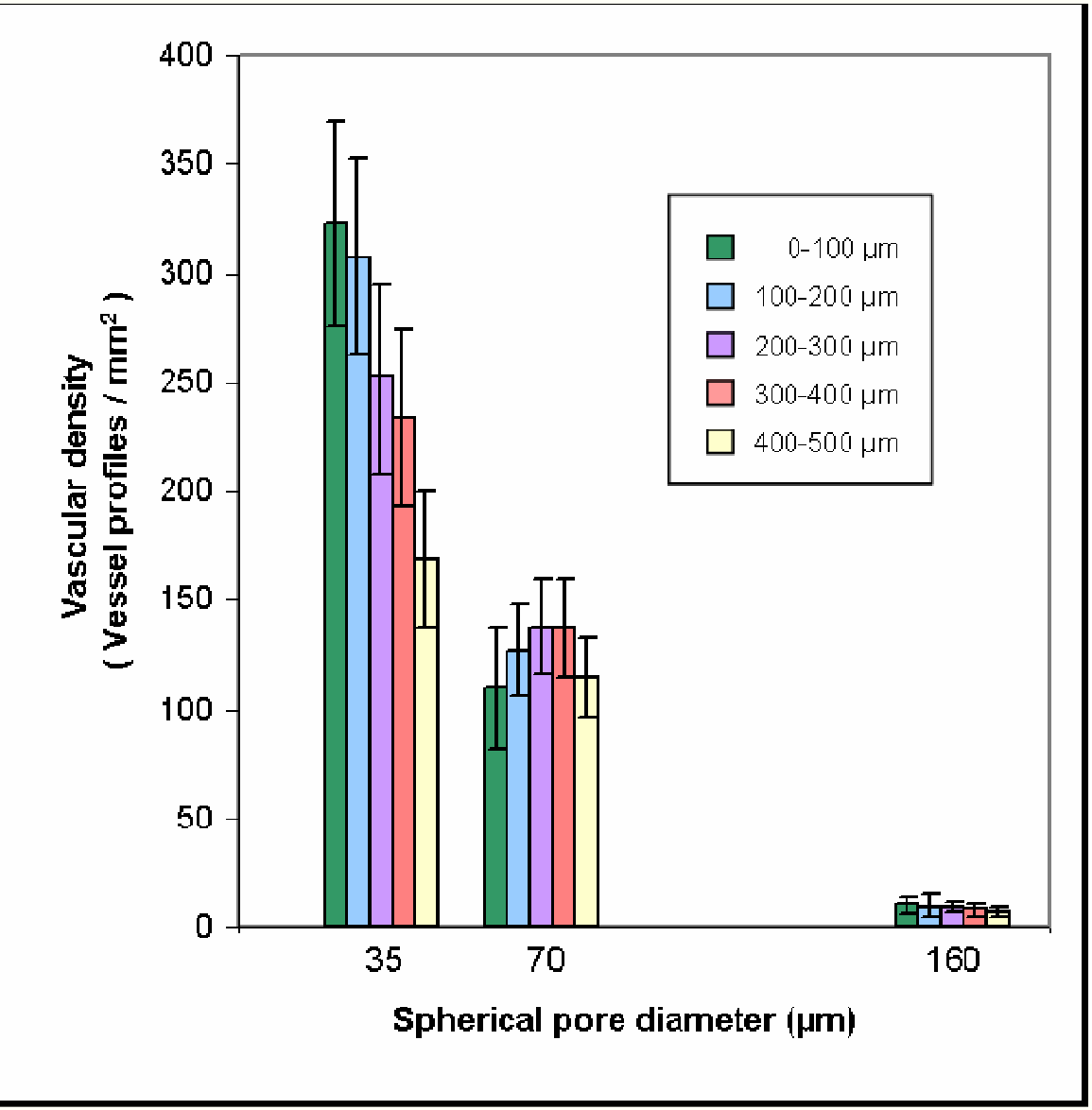
Sphere templated scaffolds

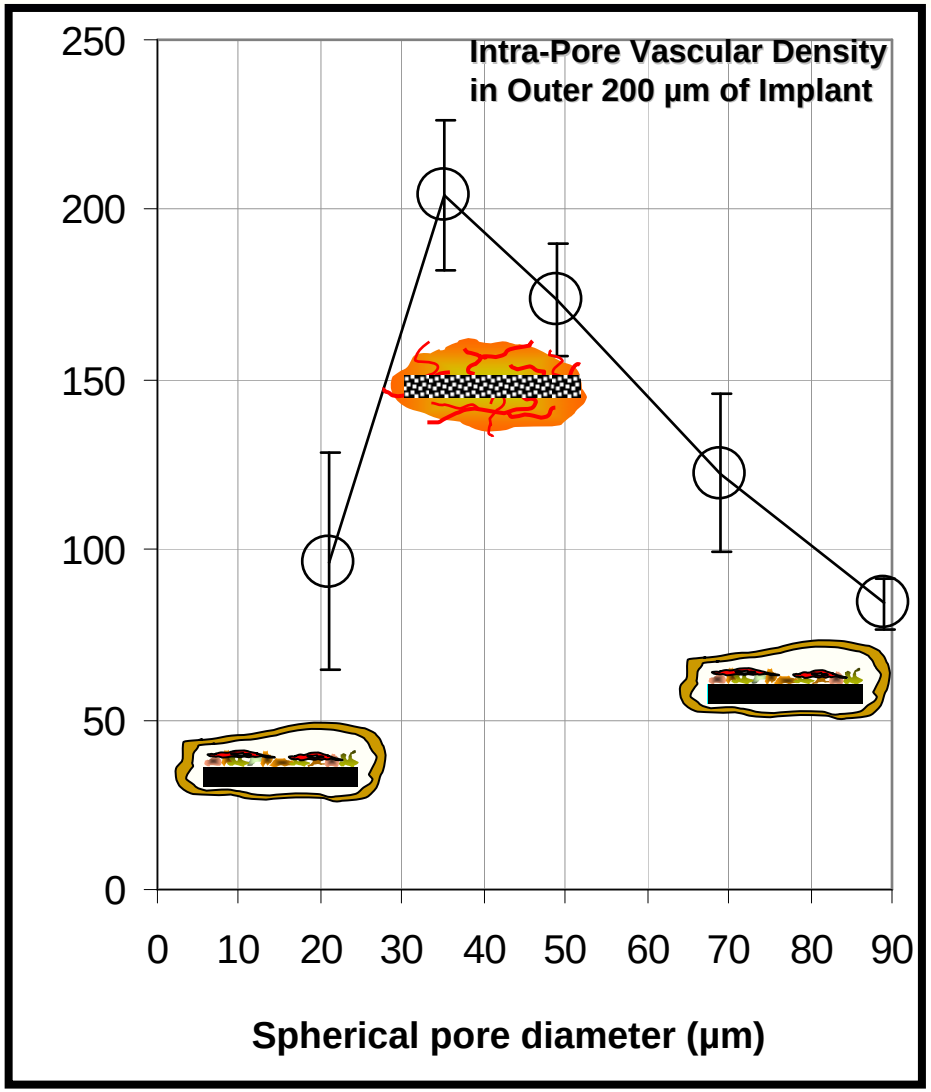
Sphere-Templated Porous Hydrogels:

The Steps to “6 S”



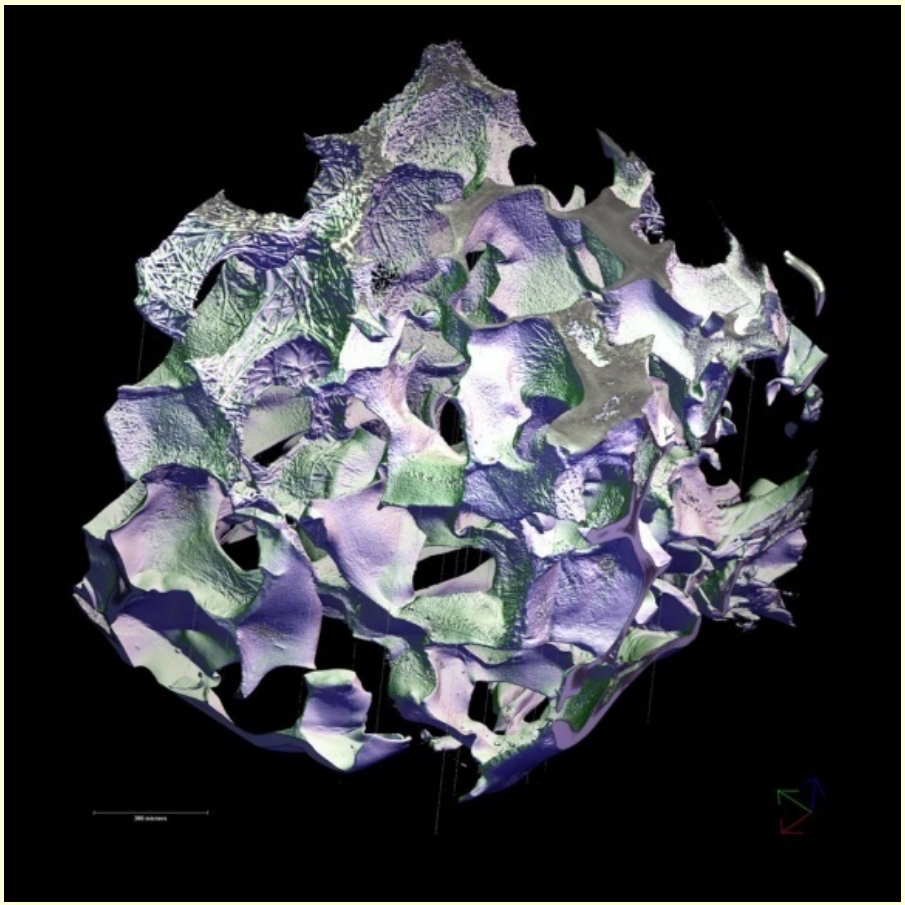
6S Vascular Density vs. Pore Diameter



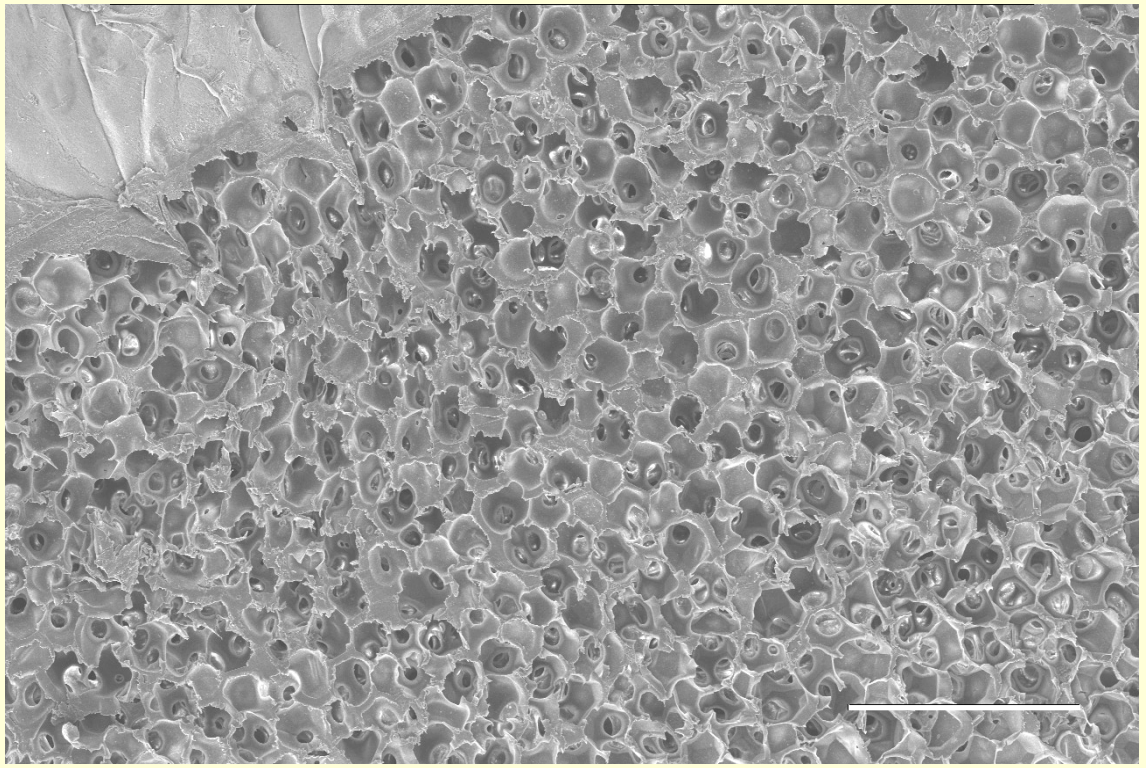


Andrew Marshall, et al

6S fabrication of fibrin scaffolds

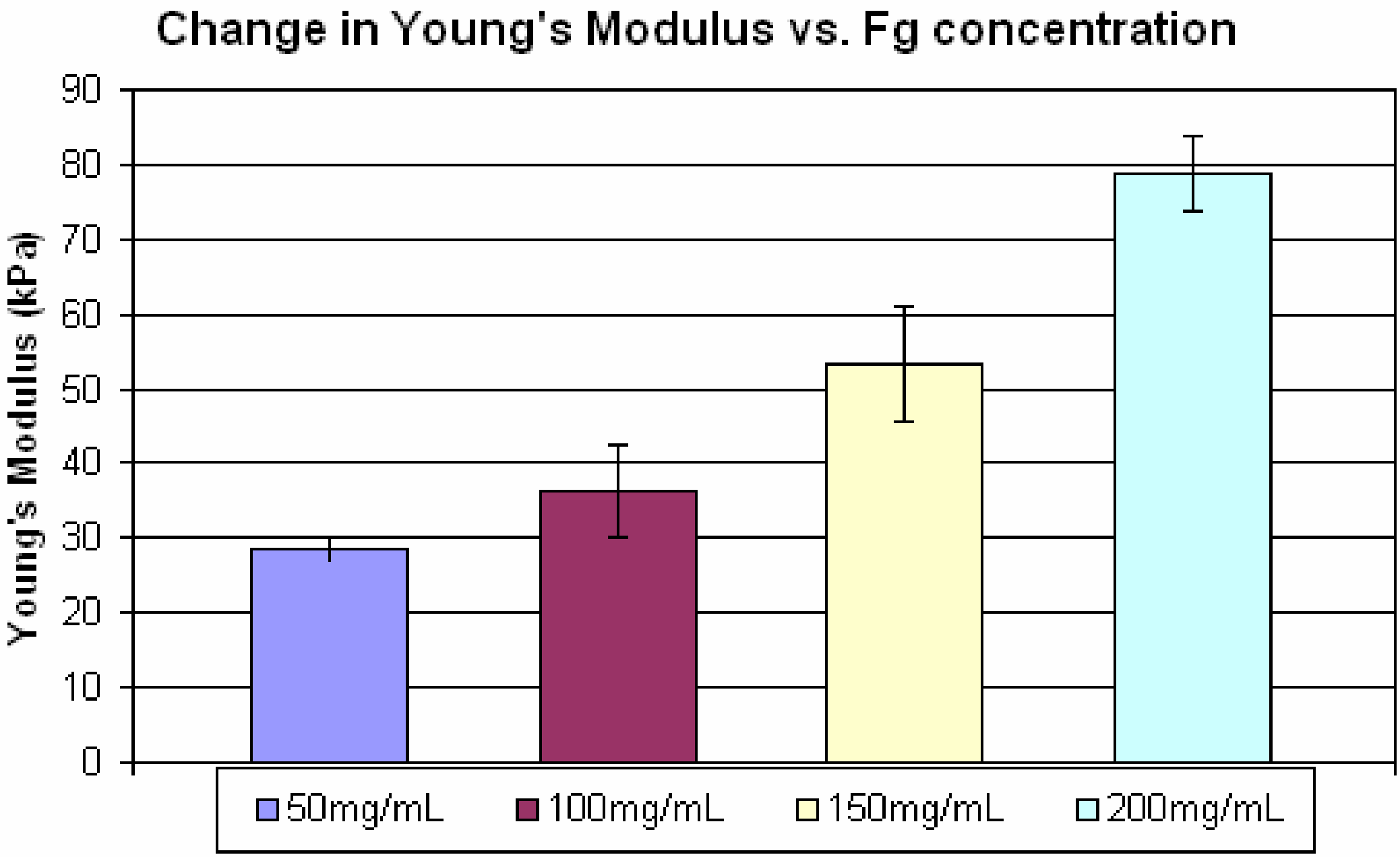


Digital Volumetric Imaging



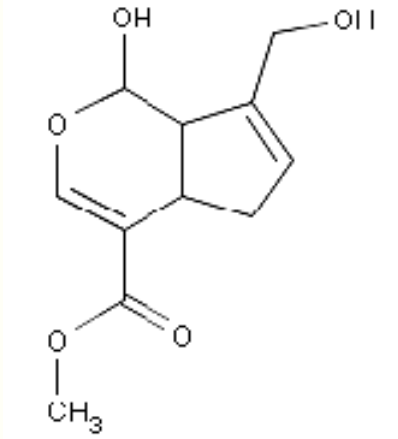
Scanning Electron Microscopy

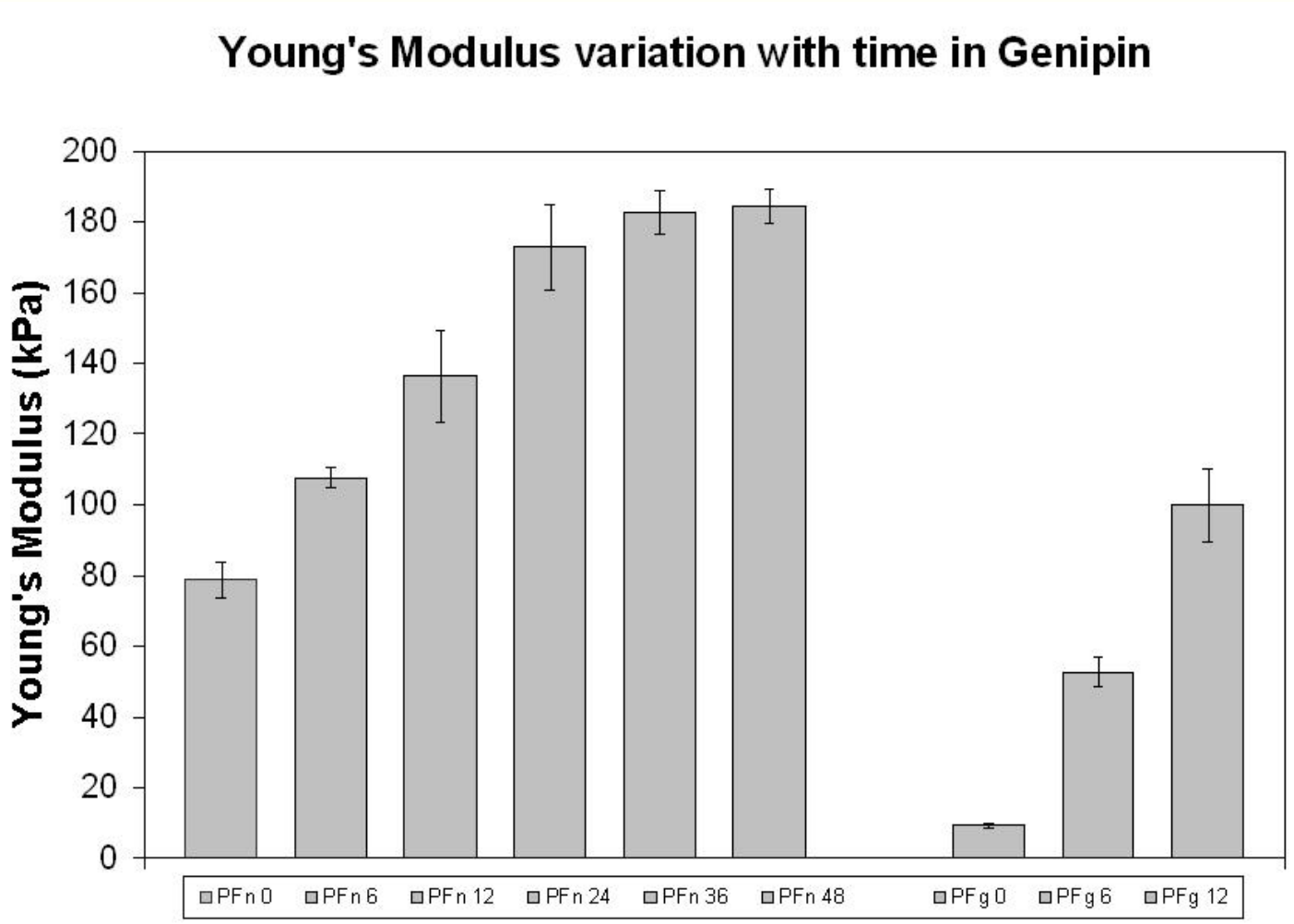
Michael Linnes, Ceci Giachelli, Buddy Ratner



Michael Linnes

Genipin Crosslinking



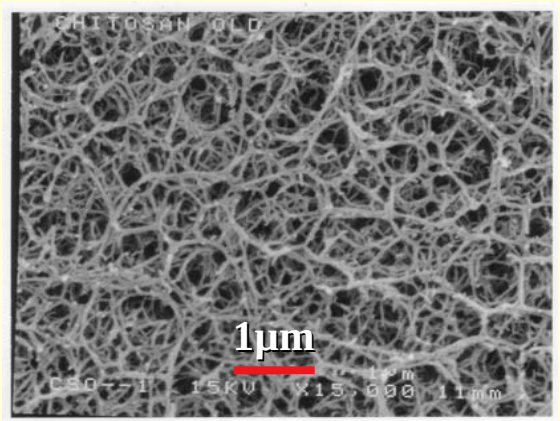


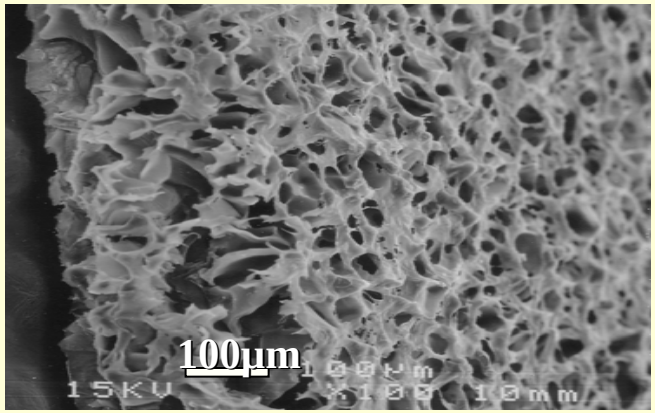
Gardenia jasminoides Ellis

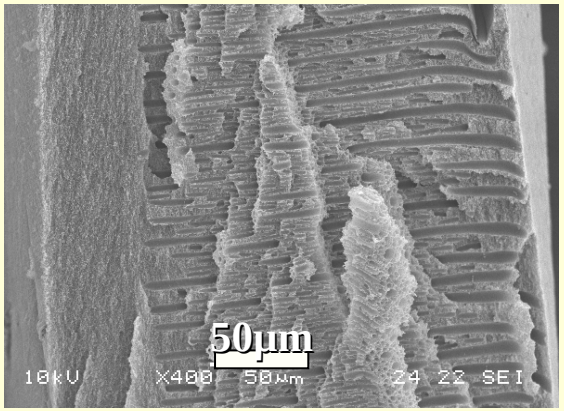
http://www.wou.edu/las/physci/ch350/Projects_2006/Vaandering/Genipin.htm

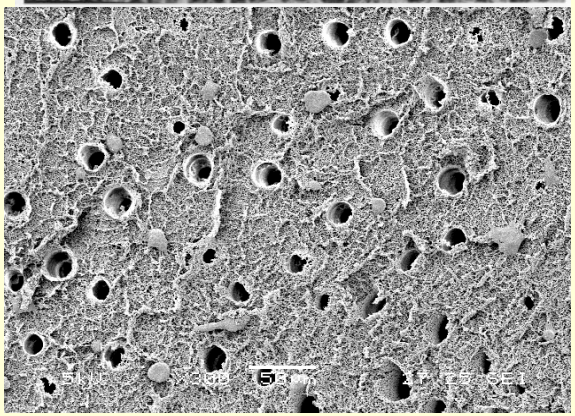
Morphological Characteristics

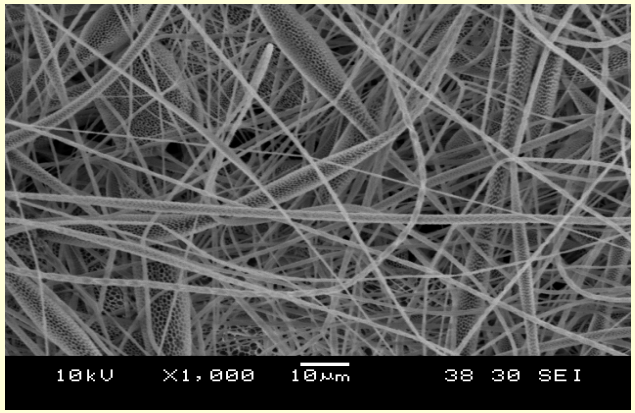
light microscopy
scanning electron microscopy
atomic force microscopy
permeation of aqueous fluids
BET

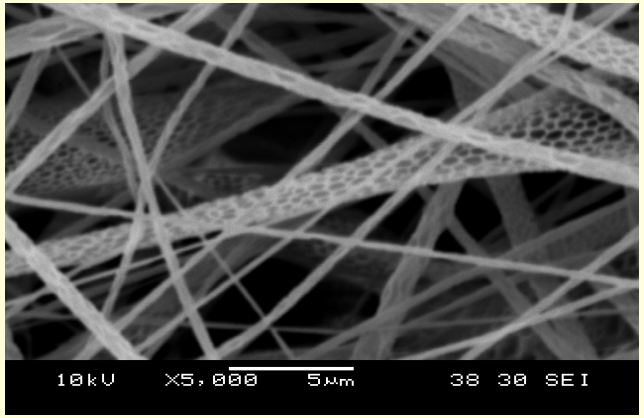












Darcy's Law

Henry Philibert Gaspard Darcy, (1803-1858)

The rate of flow of liquids through porous media

$$Q = kS \frac{H + e}{e}$$

where

Q = volume of liquid/unit time,

S = porous bed area,

e = porous bed thickness,

H = height of the liquid on the bed

k = coefficient (nature of the bed, etc.)

http://biosystems.okstate.edu/darcy/

To characterize interconnectivity

- **We can use a correlation to determine the critical throat radius from measurable properties.***

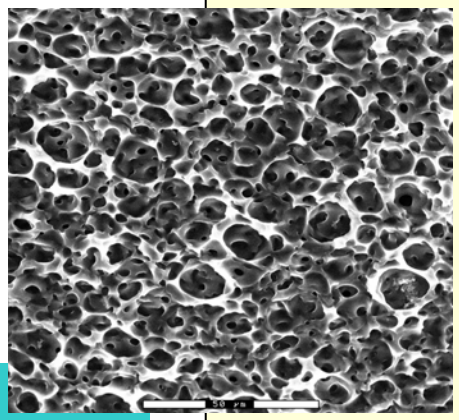
r_c = critical throat radius (~1.4 μm)

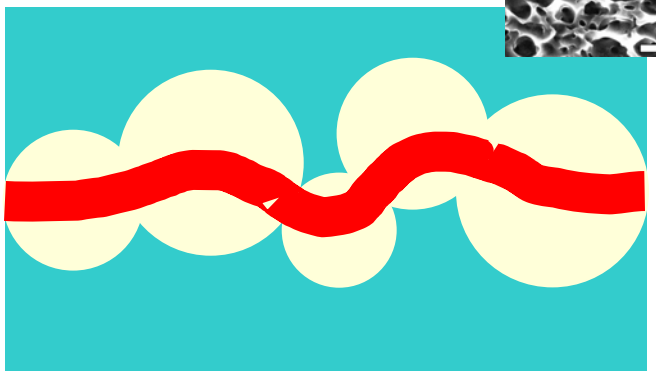
k = hydraulic permeability (~1.3×10^{-11} cm^2)

α = tortuosity (~1.2)

ϕ = porosity (%68)

$$r_c = \sqrt{\frac{226 k \alpha}{\phi}}$$





*Katz, A.J. and Thompson, A.H.,*Phys. Rev. B*, **34**, 8179 (1986)

"Quantitative Characterization of Sphere-templated Porous Biomaterials," A.J. Marshall and B.D. Ratner, AIChE Journal, Vol. 51, No. 4, 1221-1232, 2005.

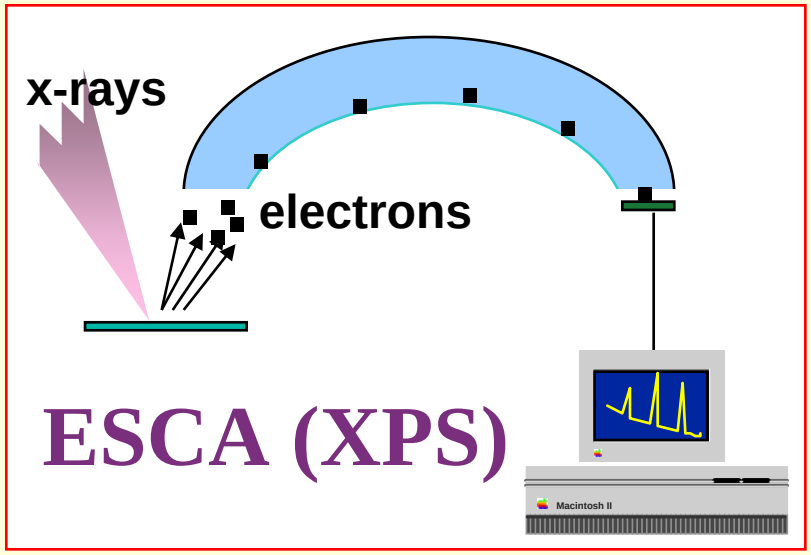
Chemical Characteristics

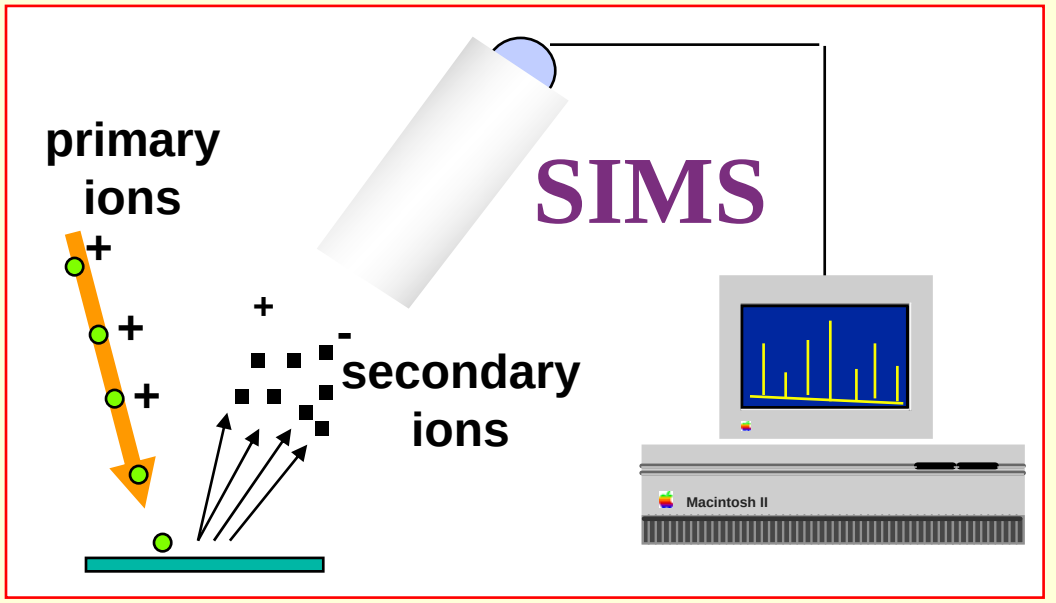
Surface	electron spectroscopy for chemical analysis (ESCA) static secondary ion mass spectrometry (SIMS) contact angle infrared surface studies
Bulk	infrared spectroscopy NMR Size exclusion chromatography Thermal analysis

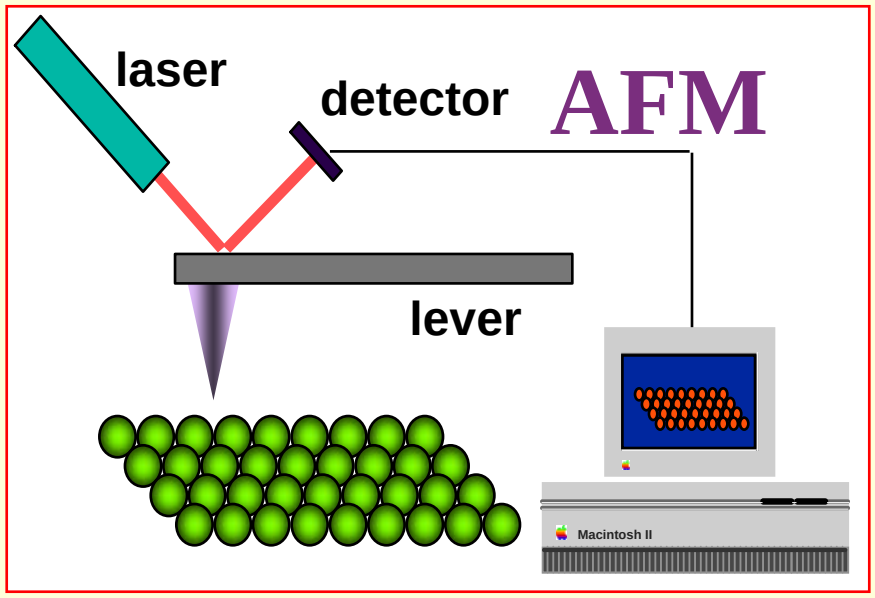
Surface methods provide information relevant to biological interactions and to contamination issues

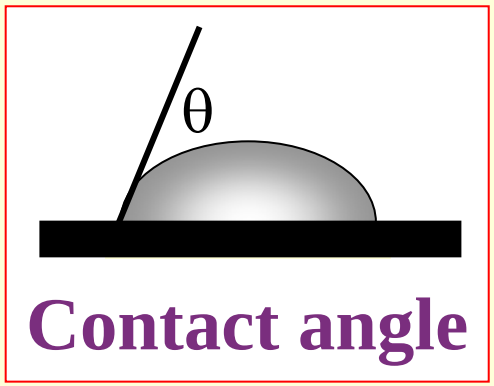
Bulk methods are critical for complete characterization, but are of a more routine nature

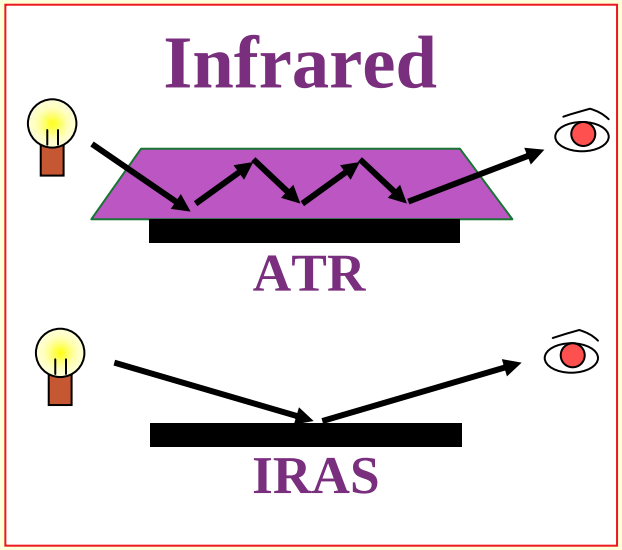
Basic Repertoire of Surface Analysis Tools



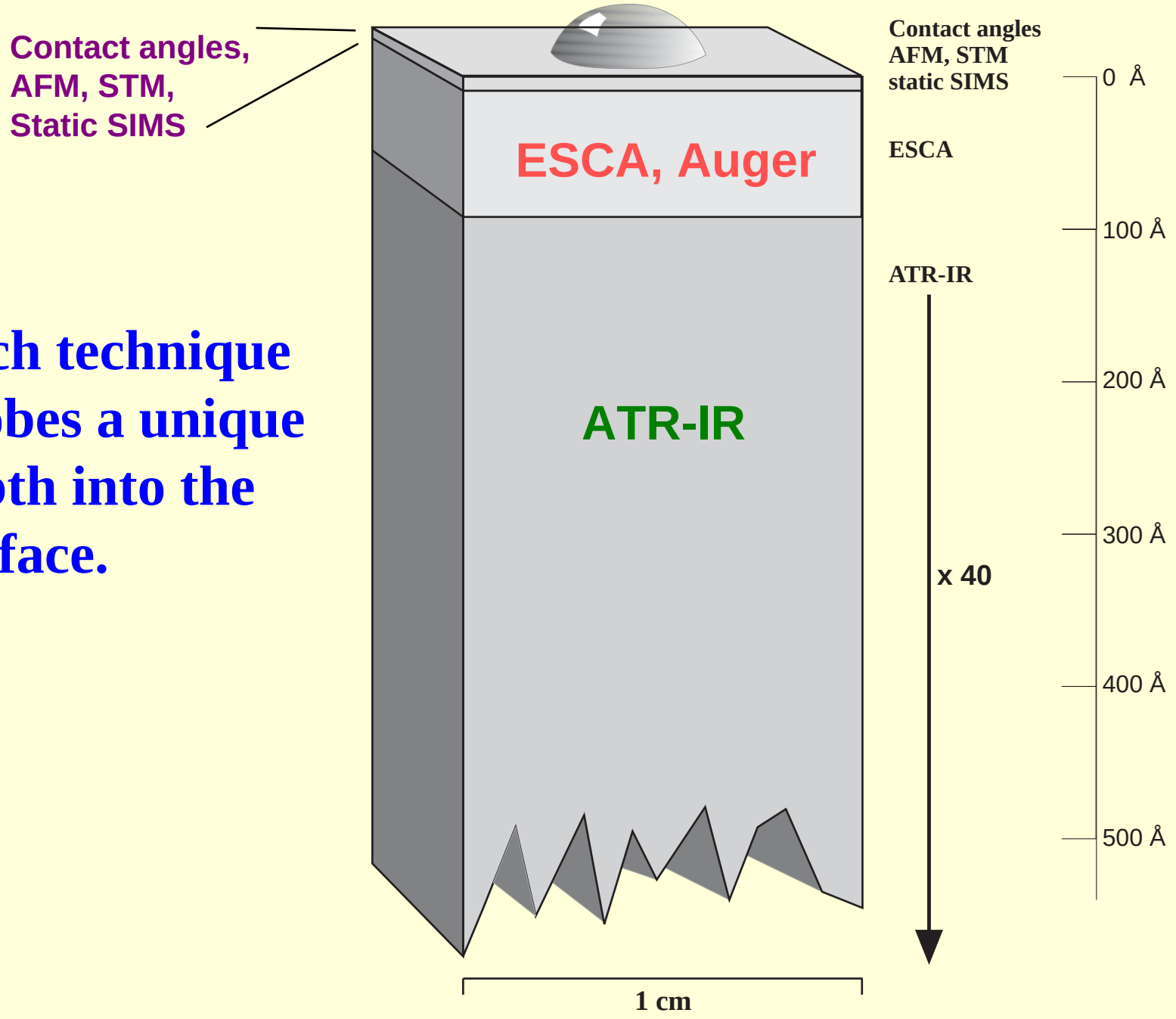








Each technique probes a unique depth into the surface.

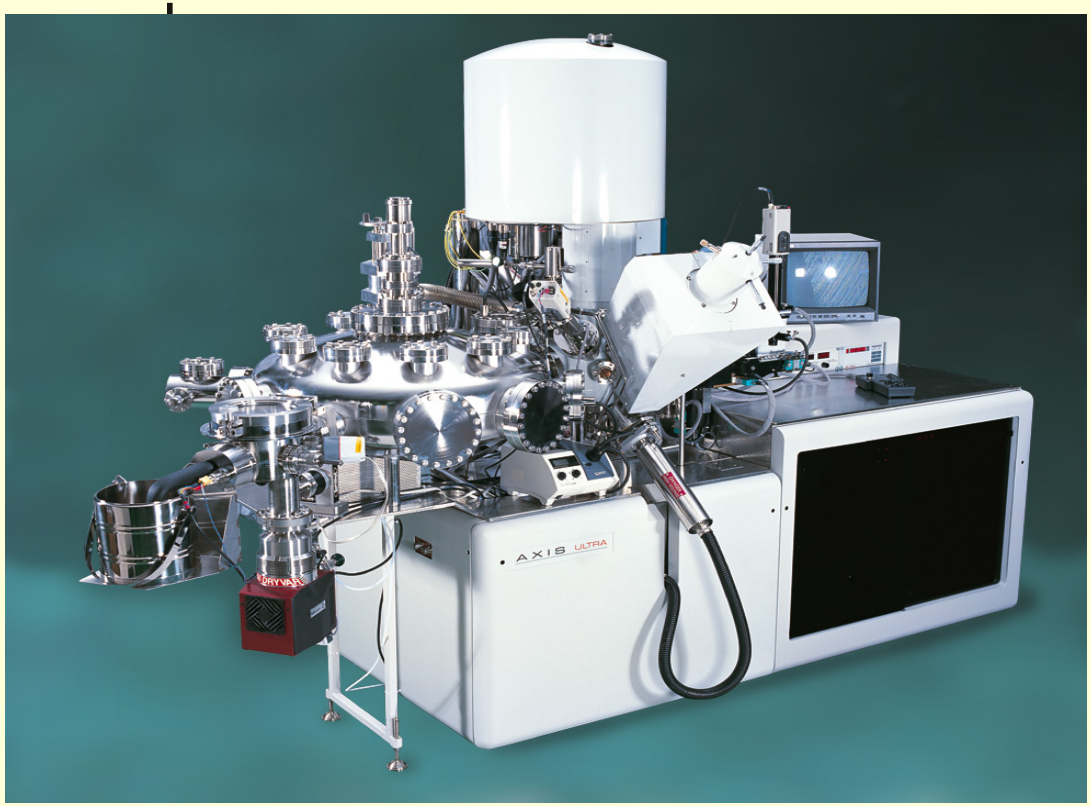


two names for the same technique

Electron Spectroscopy for Chemical Analysis (ESCA)

X-ray Photoelectron Spectroscopy (XPS)

Of all the techniques used in contemporary surface science and surface analysis, ESCA is probably the most widely



What Information Can We Obtain With ESCA?

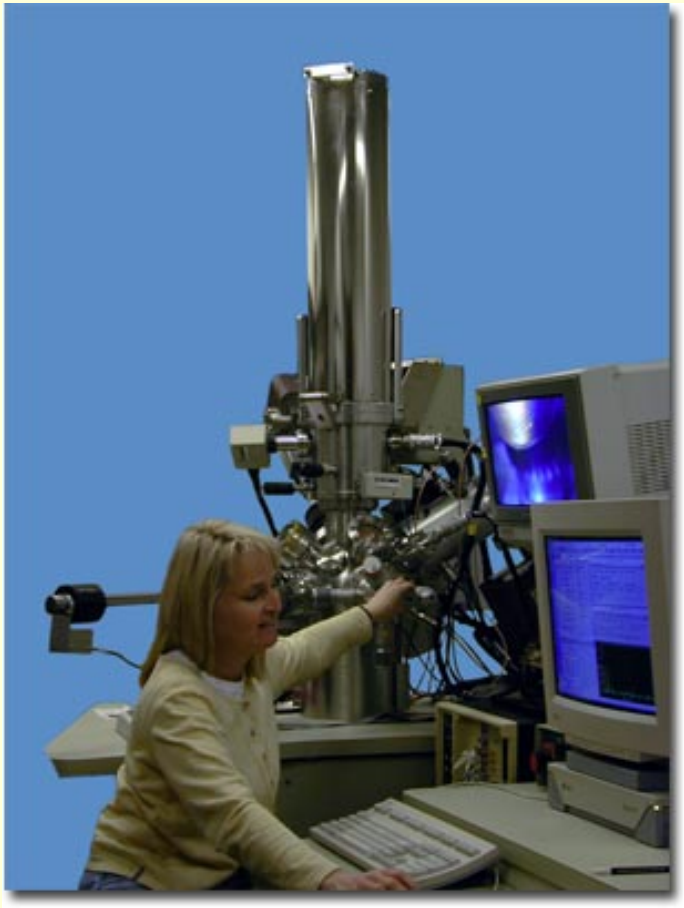
in the uppermost 50-100Å:

1. all elements present except H and He
2. amount of each element (±10%, under good conditions, ±1%)
3. molecular environment or oxidation state

   [e.g., $\underline{C}$, $(\underline{C}H_2)_n$, -$\underline{C}$-OH, -$\underline{C}$H=O, -$\underline{C}F_3$]
4. non-destructive depth profile
5. shake-up and shake-off information
6. inelastic scattering and background information (information on films and overlayers)
7. Elemental imaging (10μm spatial resolution)

Secondary Ion Mass Spectrometry (SIMS)
Time-of-flight (ToF) SIMS; Static SIMS

Probably the most information-rich of the modern surface analysis methods



Special Advantages of Static SIMS

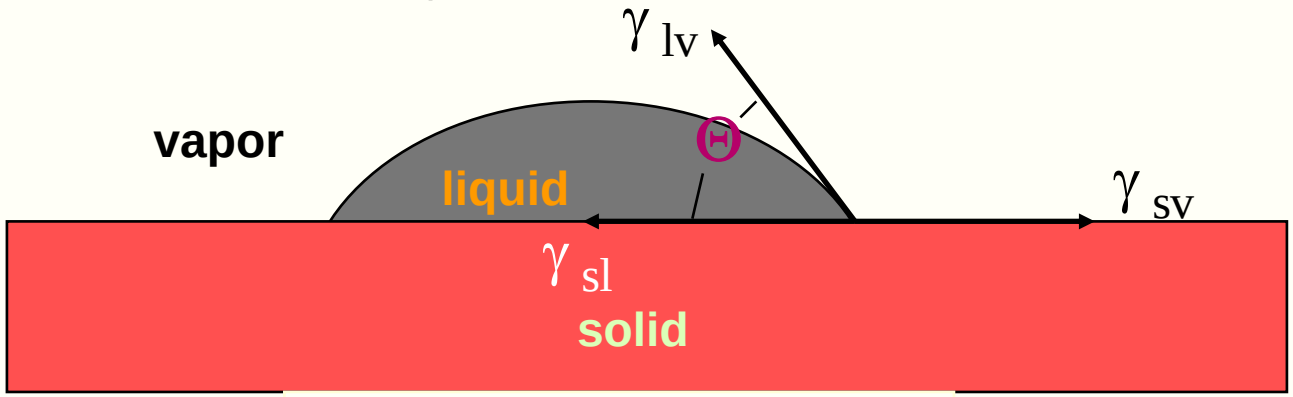
- High mass resolution (precise identification)
- Very high analytical sensitivity
- High spatial resolution (0.1μm x,y resolution)
- Shallow sampling depth (10-15Å)
- Depth profiling in uppermost layers (recent)

Contact Angle (θ)

The $5 surface analysis method

γ_{lv} for liquid in equilibrium with its own vapor

γ_{sv} for solid in equilibrium with vapor

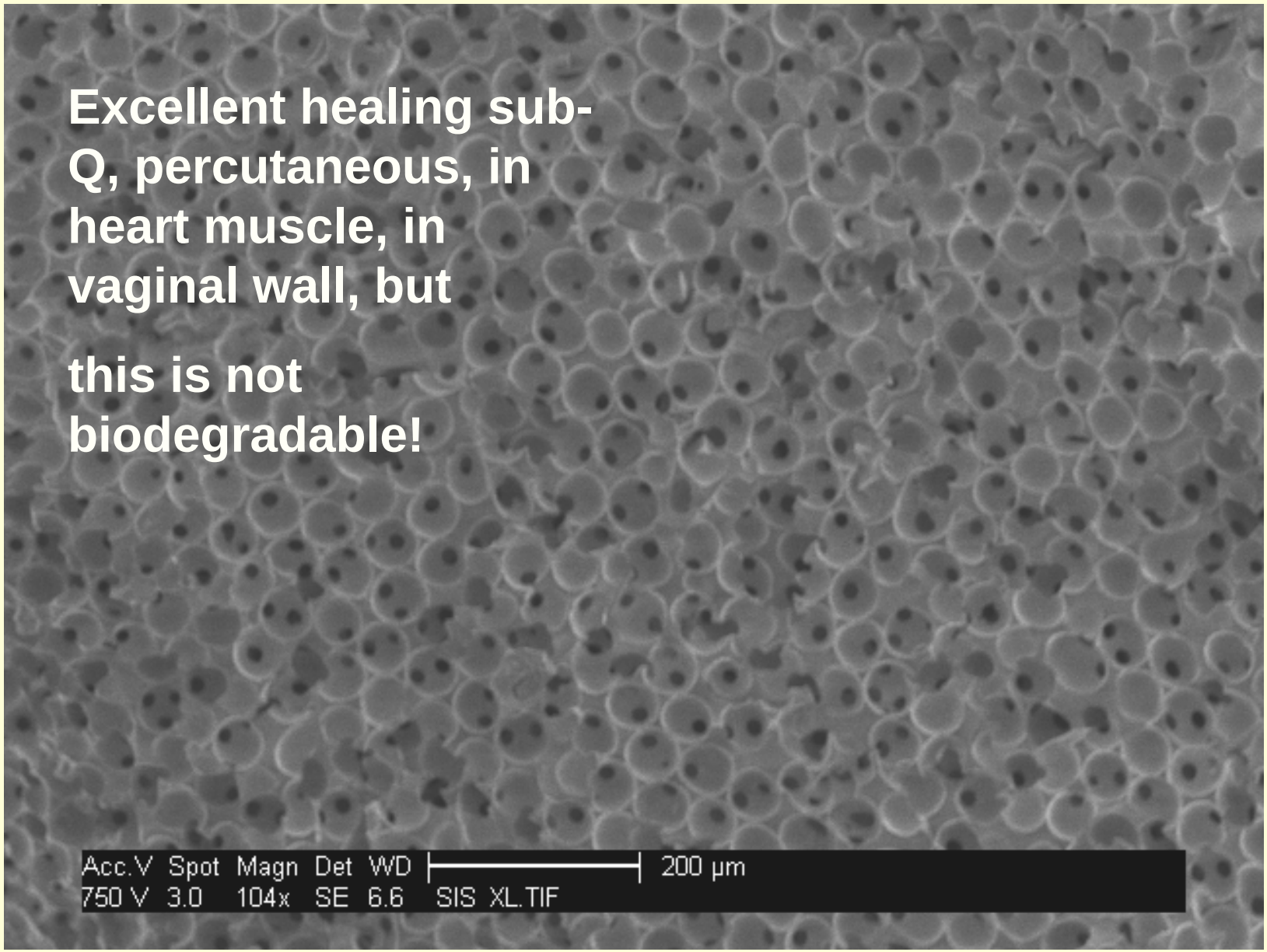


γ_{sl} for solid in equilibrium with liquid

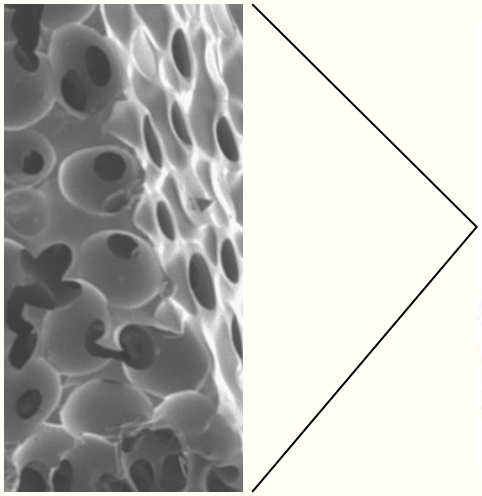
- Can be performed in any lab
- Very surface sensitive
- Many artifacts
- Hard to interpret
- Minimally useful for scaffolds

The application of these surface methods to scaffolds

Porous pHEMA hydrogel templated with 40µm beads

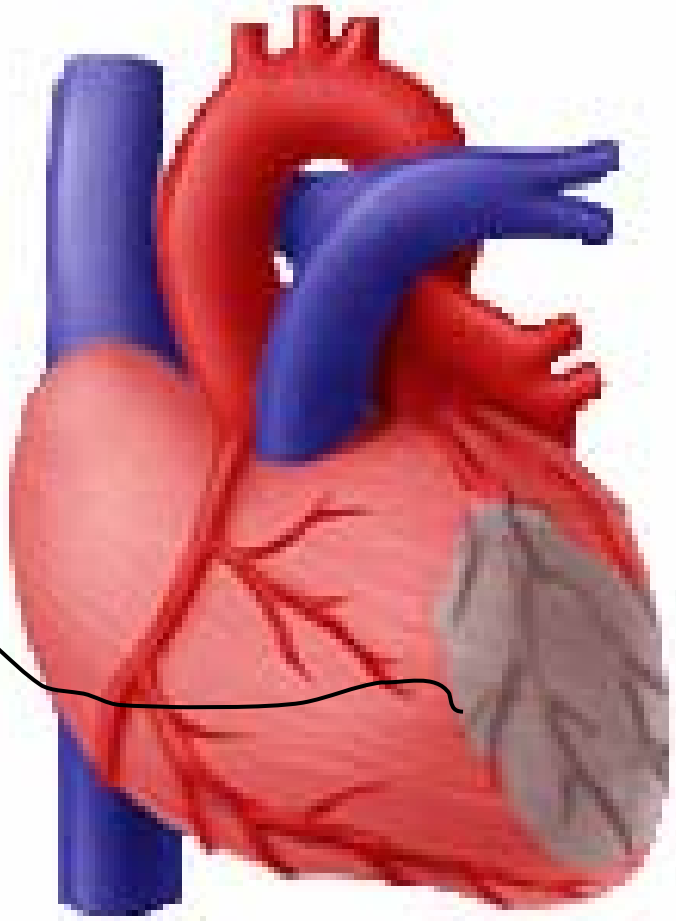


Tissue Engineered Cardiac Muscle



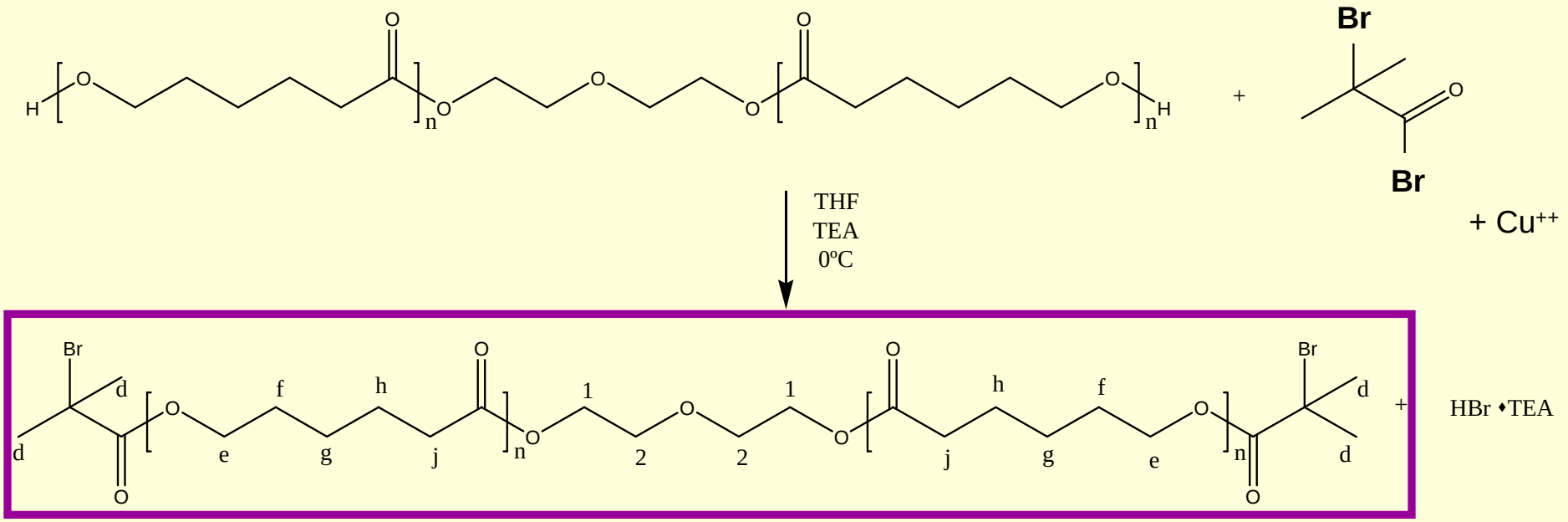
Porous structure seeded with cells

Culture in vitro



Funded through the NHLBI BRP (BEAT)

PCL Macroinitiator

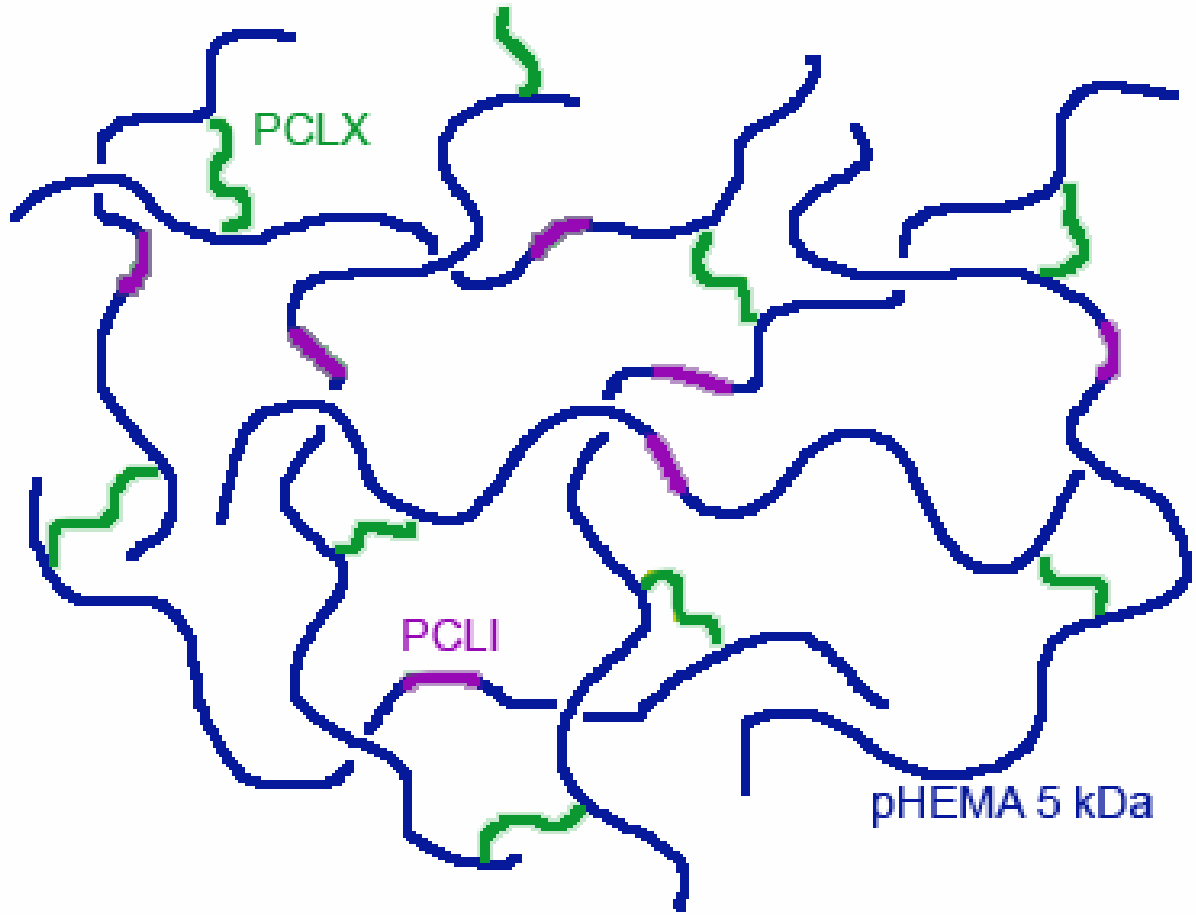


- PCL diol functionalized using α bromoisobutyrl bromide
- Characterized with ^{1}H NMR found to be ~80% functionalized

Sarah Atzet

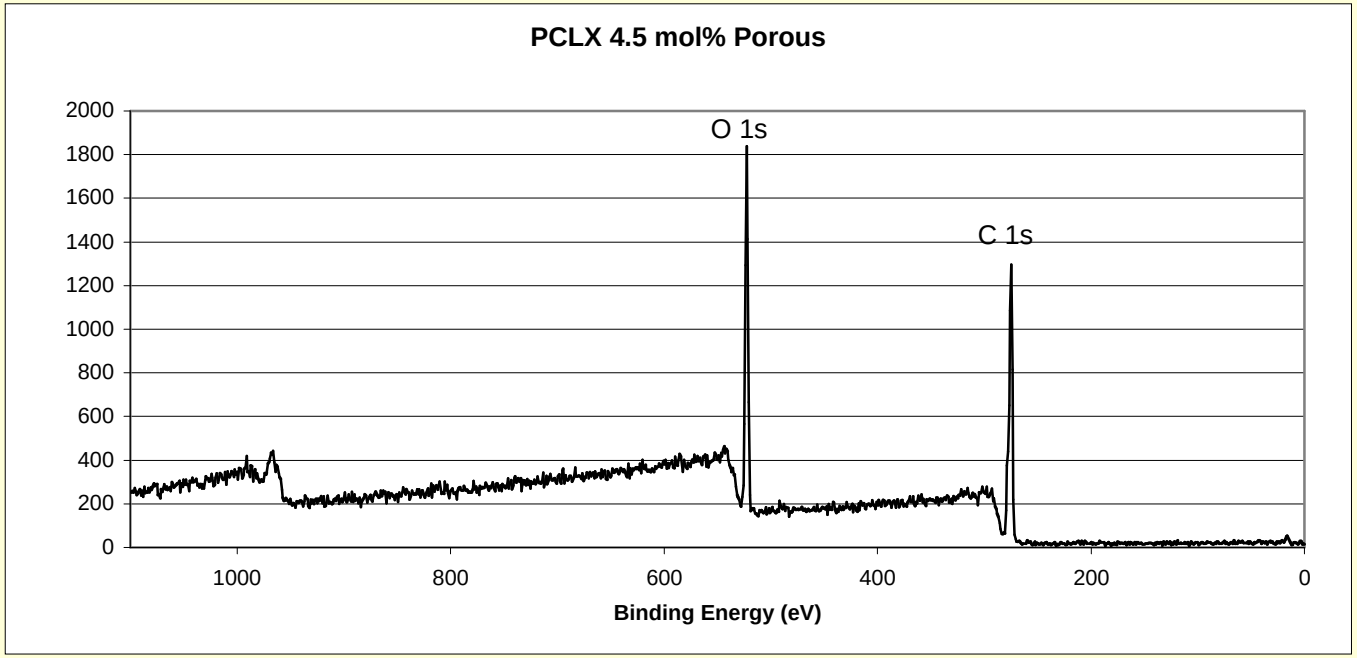
Degradable pHEMA-b-PCL Hydrogels

Precise block lengths by ATRP



- 5 kDa pHEMA is water soluble
- 2 crosslinks per chain

Sarah Atzet

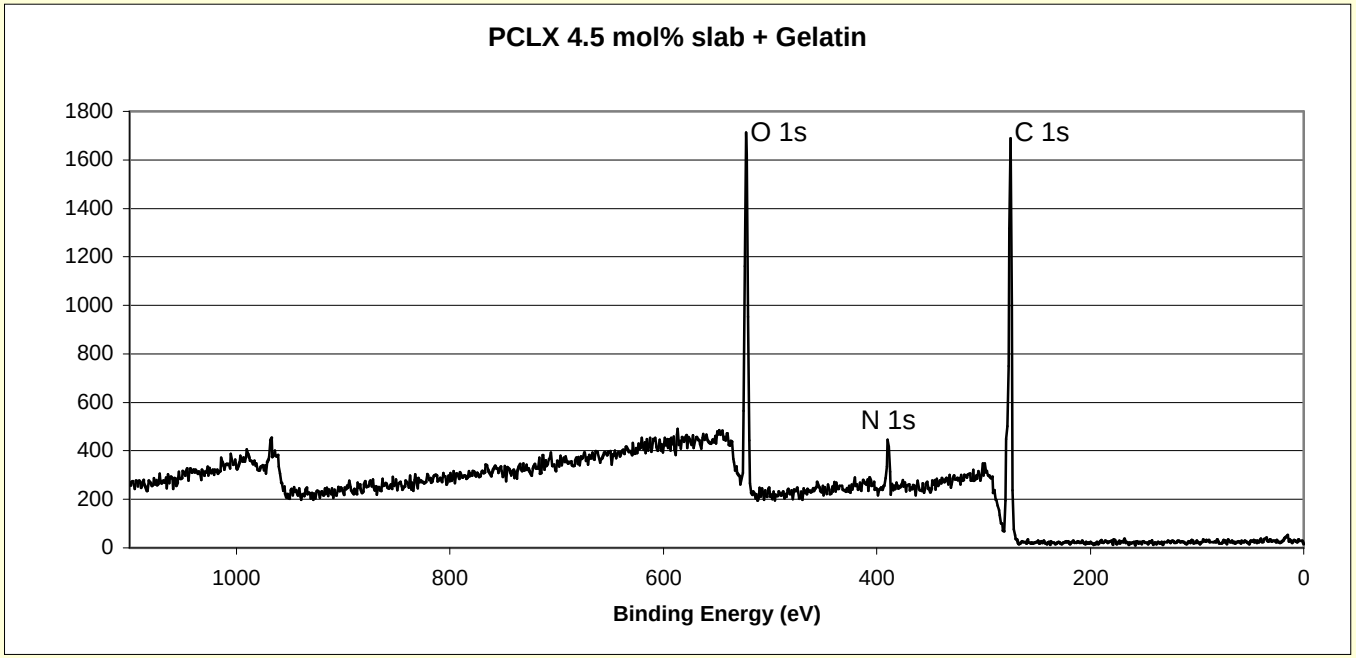


ESCA wide scan

4.5% PCL x-linker

No Br

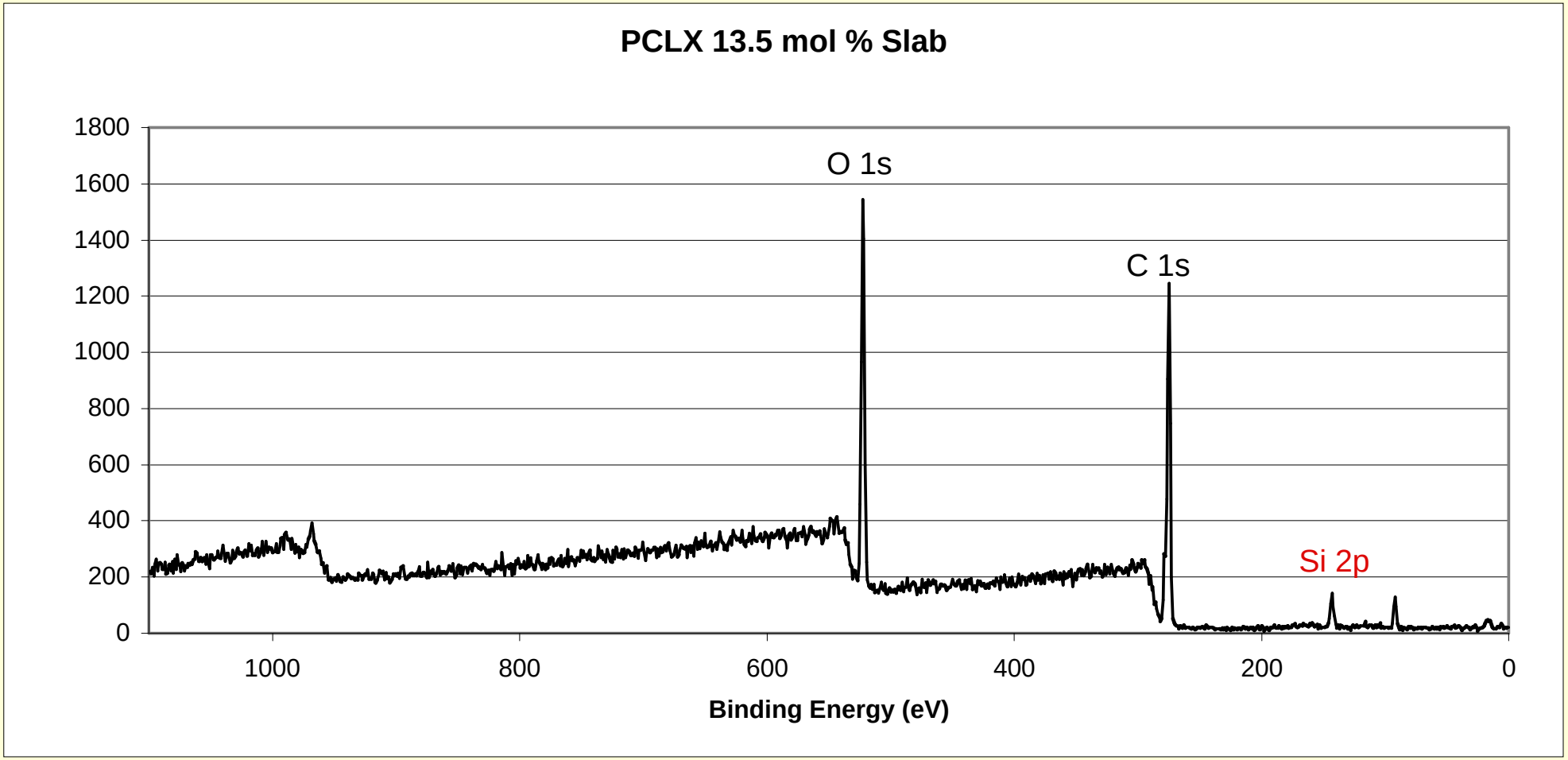
No Cu



Adsorb gelatin as an attachment factor and an N signal appears

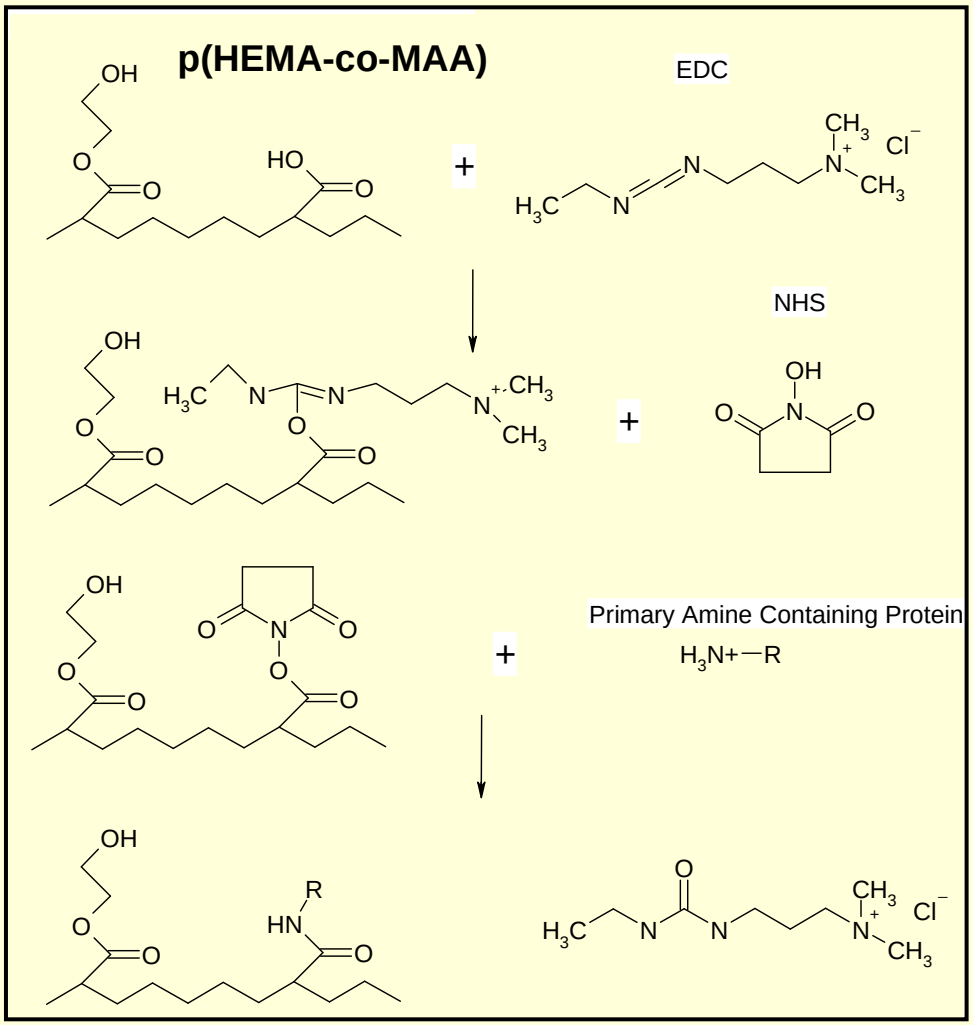
ESCA wide scan

13.5% PCL x-linker



Si contamination is noted

A p(HEMA-co-MAA) copolymer for EDC/NHS mediated protein immobilization

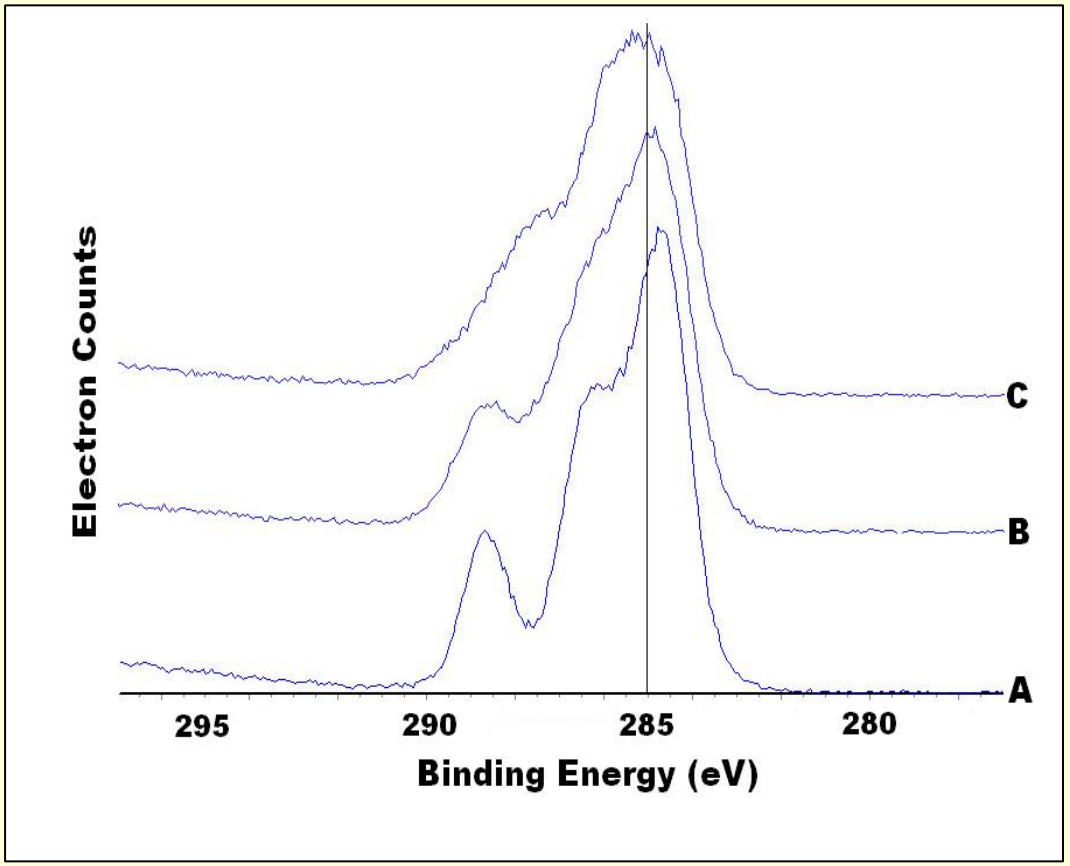


N-(3 dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)

N-hydroxy succinimide (NHS)

S. Curtin, D. Mortisen

XPS (C1s) confirms covalent bond formation

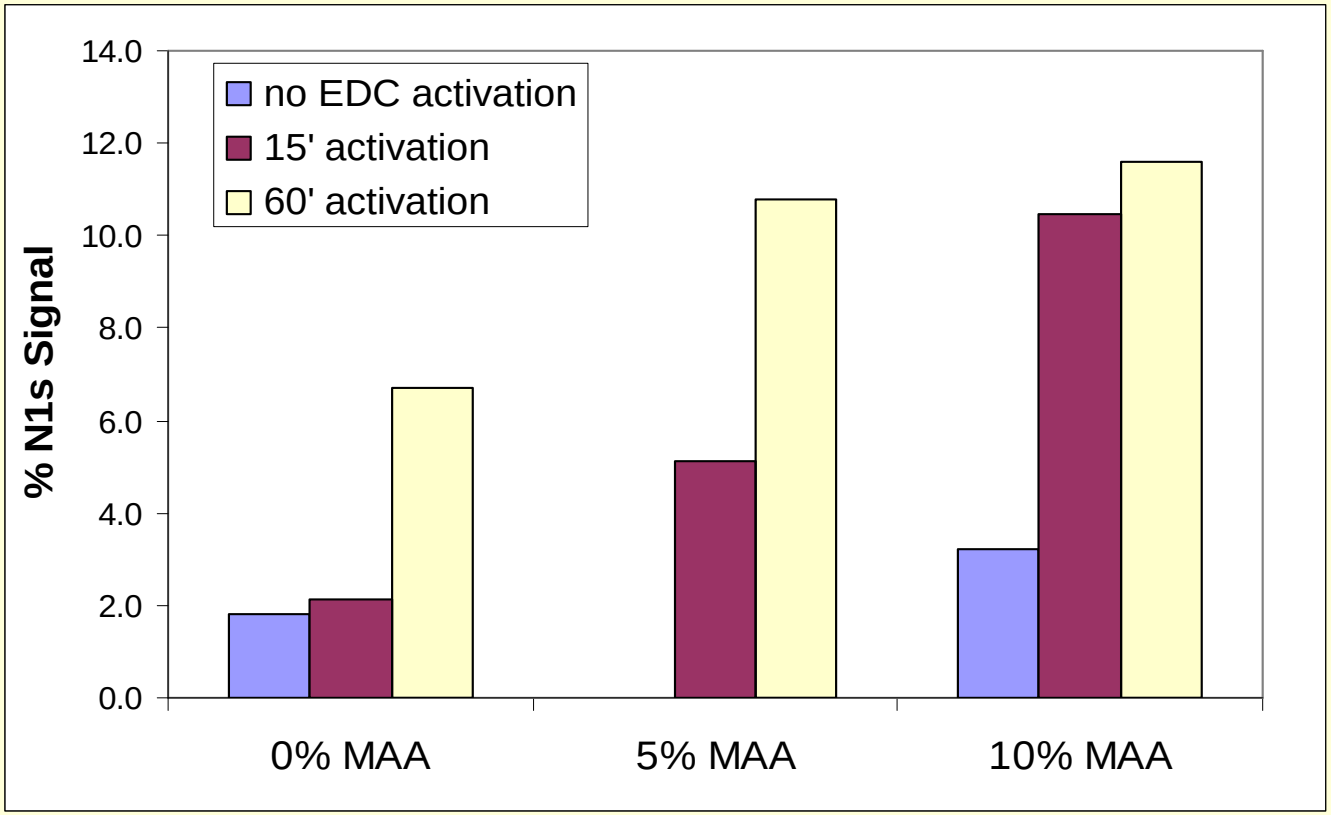


Amide bond formation appears at 288.2 eV as reaction time is increased

Disappearance of carboxyl peak (O=C-O) at 289 eV indicates reaction at methacrylic acid

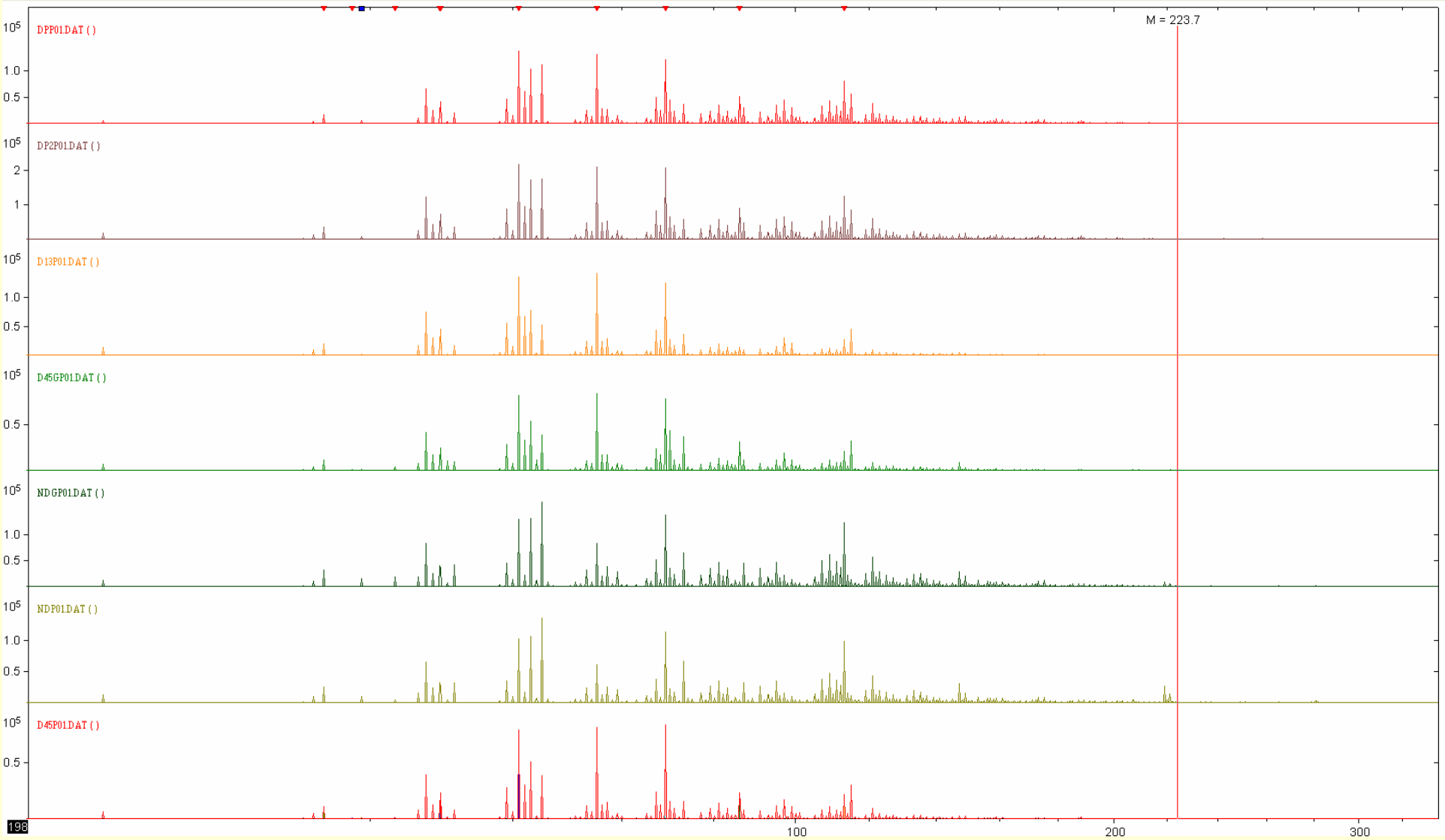
S. Curtin, D. Mortisen

N1s signal increases with EDC/NHS activation time and MAA content



SIMS Spectra Encode for a Huge Amount of Information

ToF-SIMS spectra of PCL scaffolds, positive mode



We can generate huge amounts of data!

How can we convert data into useful information?

Multivariate analysis methods, sometimes called “chemometrics”

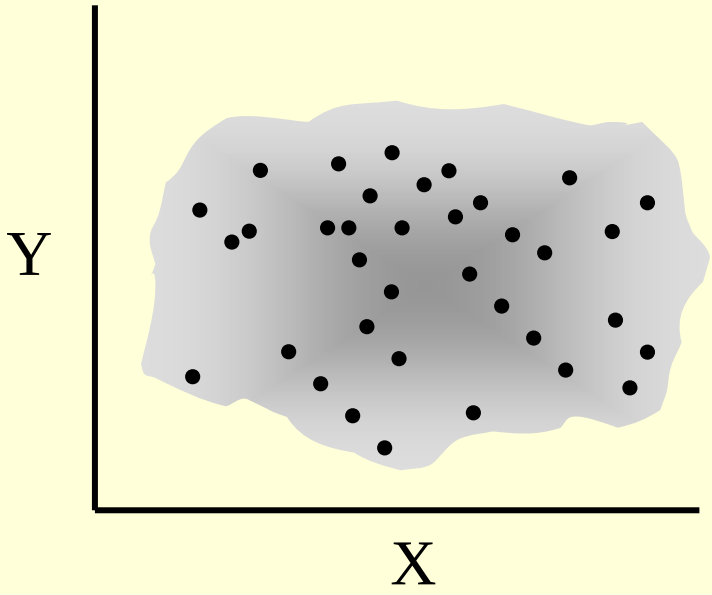
Allows us to identify trends that might be hidden in the data

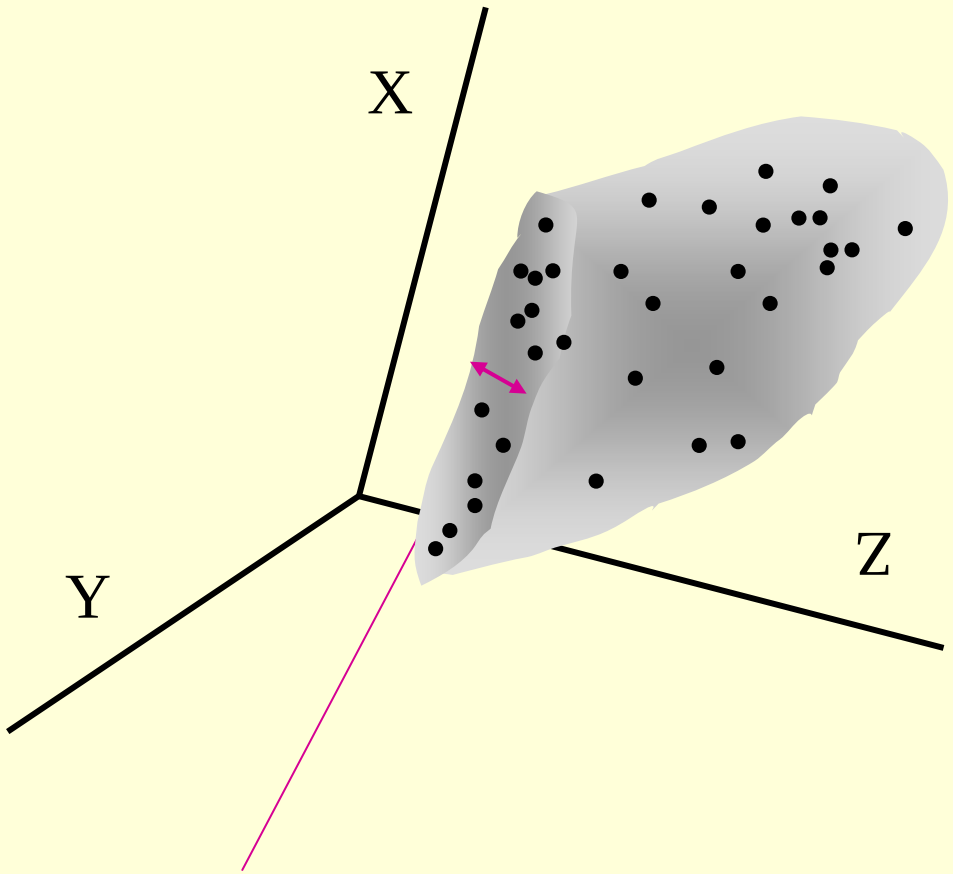
Makes use of large amounts of data

Uses all the data, not just that which we think is important

A hypothesis generator!

No clear relationship between points





A high correlation between points

Multivariate Calibration Methods for Quantitative Spectral Analysis

CLA - Classical Least-Squares

ILA - Inverse Least-Squares

MLR - multiple linear regression

PCA - Principal Component Analysis

PCR - Principal Component Regression

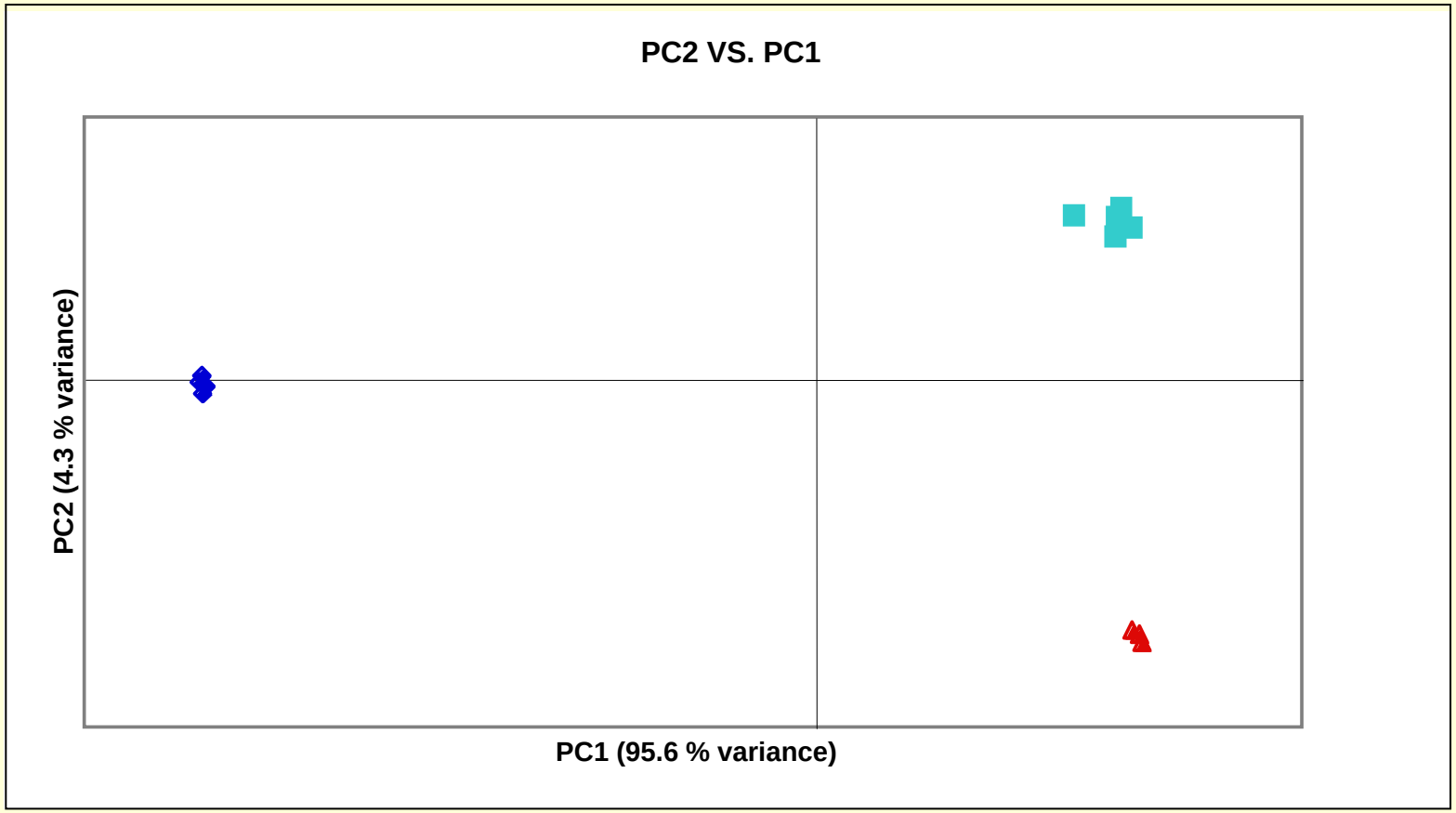
PCA followed by a regression step

PLS - Partial Least-Squares

Maximum Entropy Method

Artificial Neural Networks

TOF-SIMS with PCA easily distinguishes linker chemistries, and protein immobilization



1 - pHEMA + protein
2 - NHS/EDC, no protein
3 - NHS/EDC + protein, pH 9

D. Mortisen, S. Curtin
J. Apte, C. Cezar, NESAC/BIO (UW)

PCA peak assignments

Polycaprolactone (PCL)-containing scaffolds

Polycaprolactone ($C_6H_{10}O_2$):

- $[M+H^+]$ @ m/z=115
- $C_6H_9O^+$ @ m/z=97
- $C_5H_9^+$ @ m/z=69
- $C_6H_{11}O_3^-$ @ m/z=131

PHEMA :

- $C_2H_5O^+$ @ m/z=45
- $C_2H_3O_2^-$ @ m/z=59

Copper :

- $^{63}Cu^+$ @ m/z=63 (69%)
- $^{65}Cu^+$ @ m/z=65 (31%)

Bromine :

- $^{79}Br^-$ @ m/z=79 (51%)
- $^{81}Br^-$ @ m/z=81 (49%)

Remarks:

Positive ionization probability of Br is probably very low…

+ ^{63}CuO and ^{65}CuO @ m/z 79 and 81 in the negative mode !

Isotopic ratios were used to distinguish those signals.

Samples studied

ND - Nondegradable (TEGDMA) slab

NDP- nondegradable porous

NDG- Nondegradable slab + gelatin

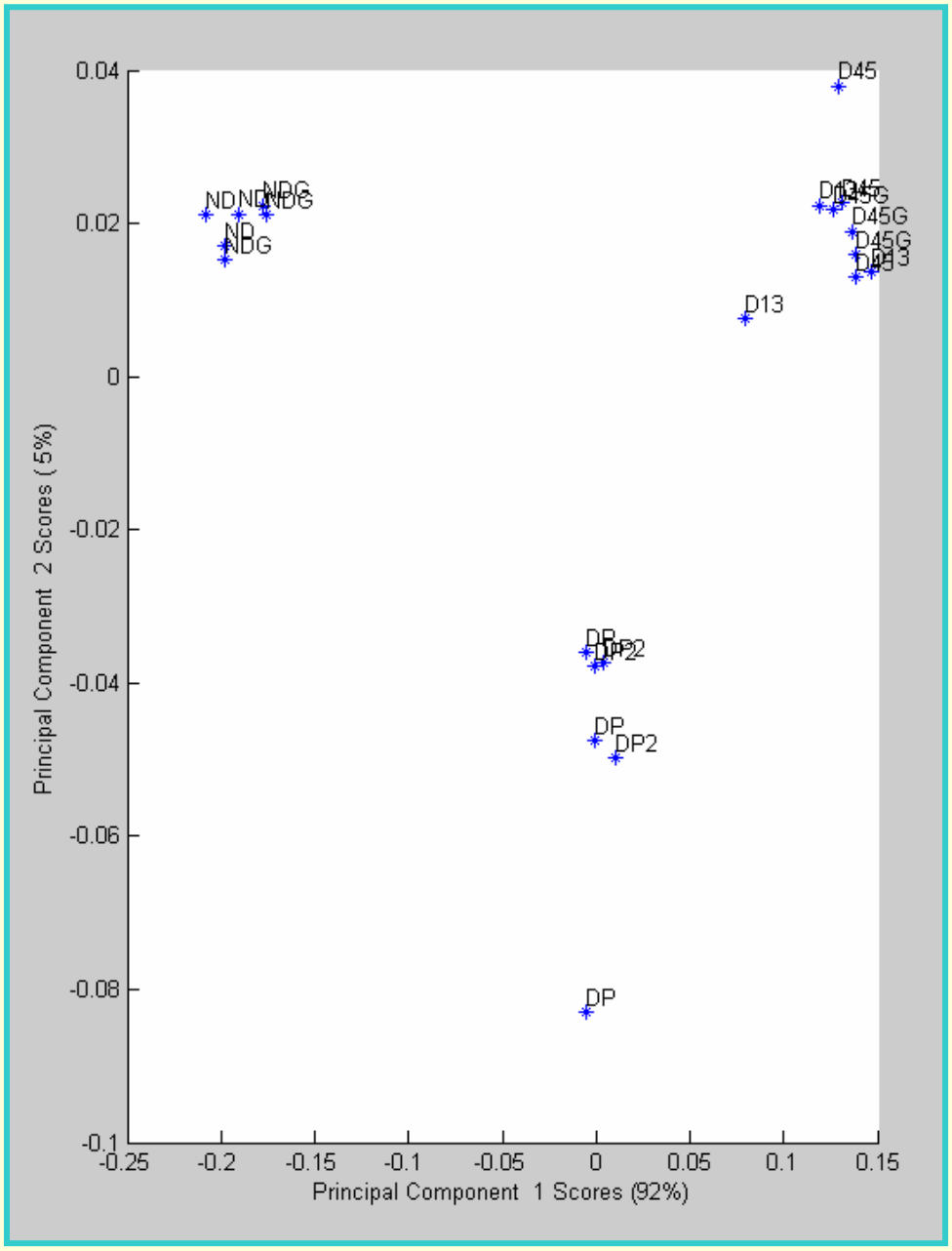
D4.5 - Degradable PCLX 4.5 mol% slab

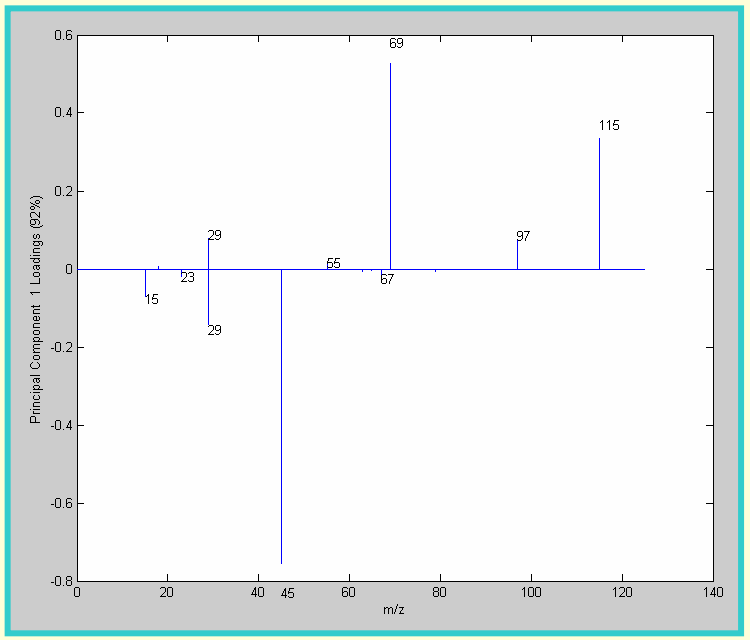
DP - Degradable PCLX 4.5 mol% Porous

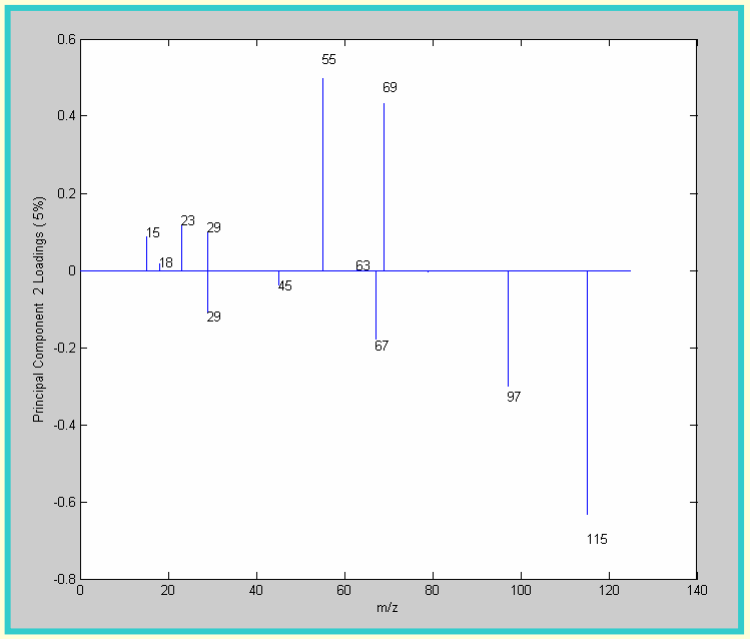
D 4.5 G - Degradable PCLX 4.5 mol% slab + gelatin

D 13.5 - Degradable PCLX 13.5 mol% slab

PCA on PCL specimens – Positive ion mode







Some conclusions about PCl-containing scaffolds:

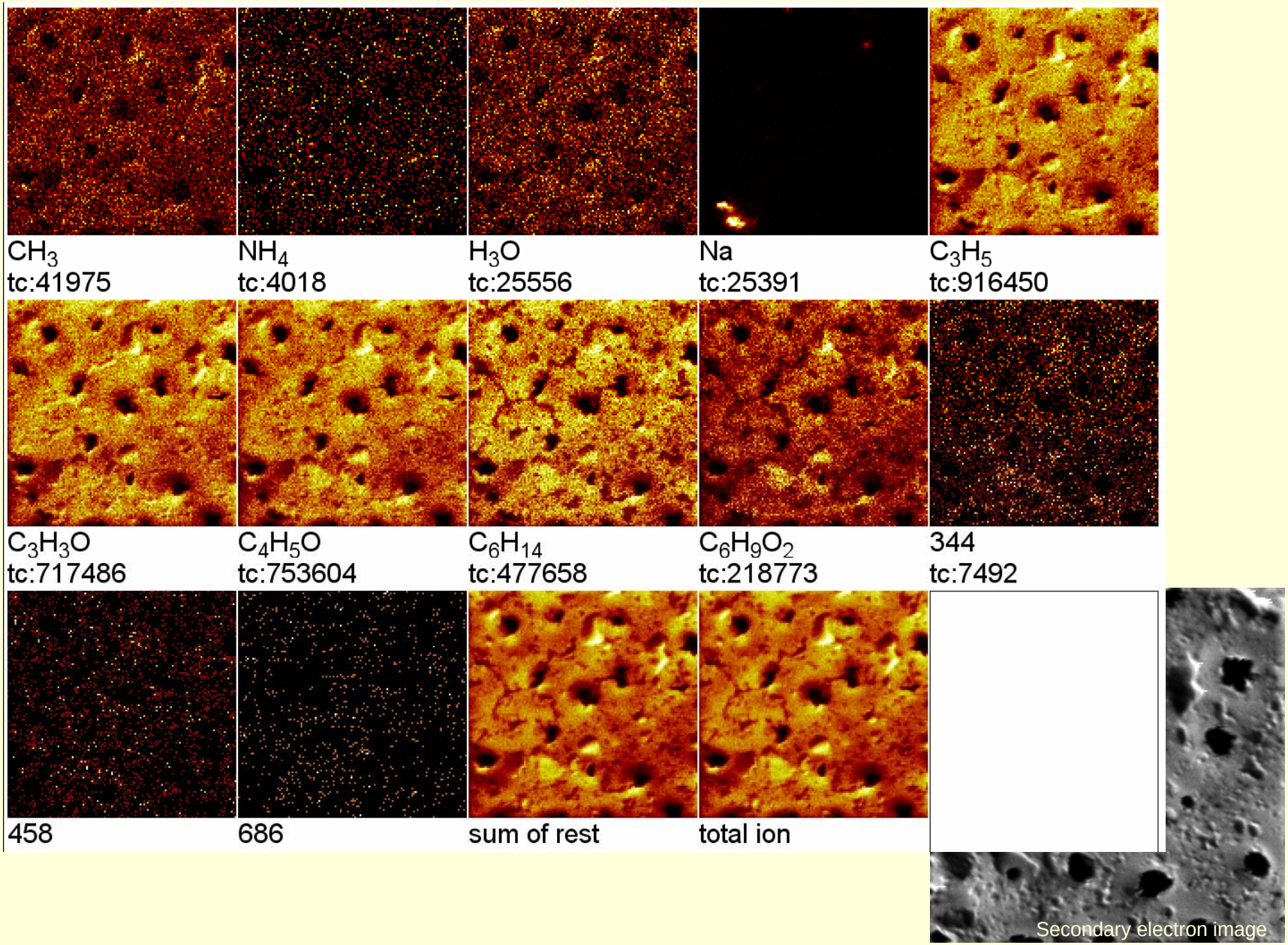
there is residual copper only in the nondegradable samples.

Br was found

There are polycaprolactone groups on the surface of degradable gels.

ToF-SIMS images, **positive** mode, 100x100µm^2

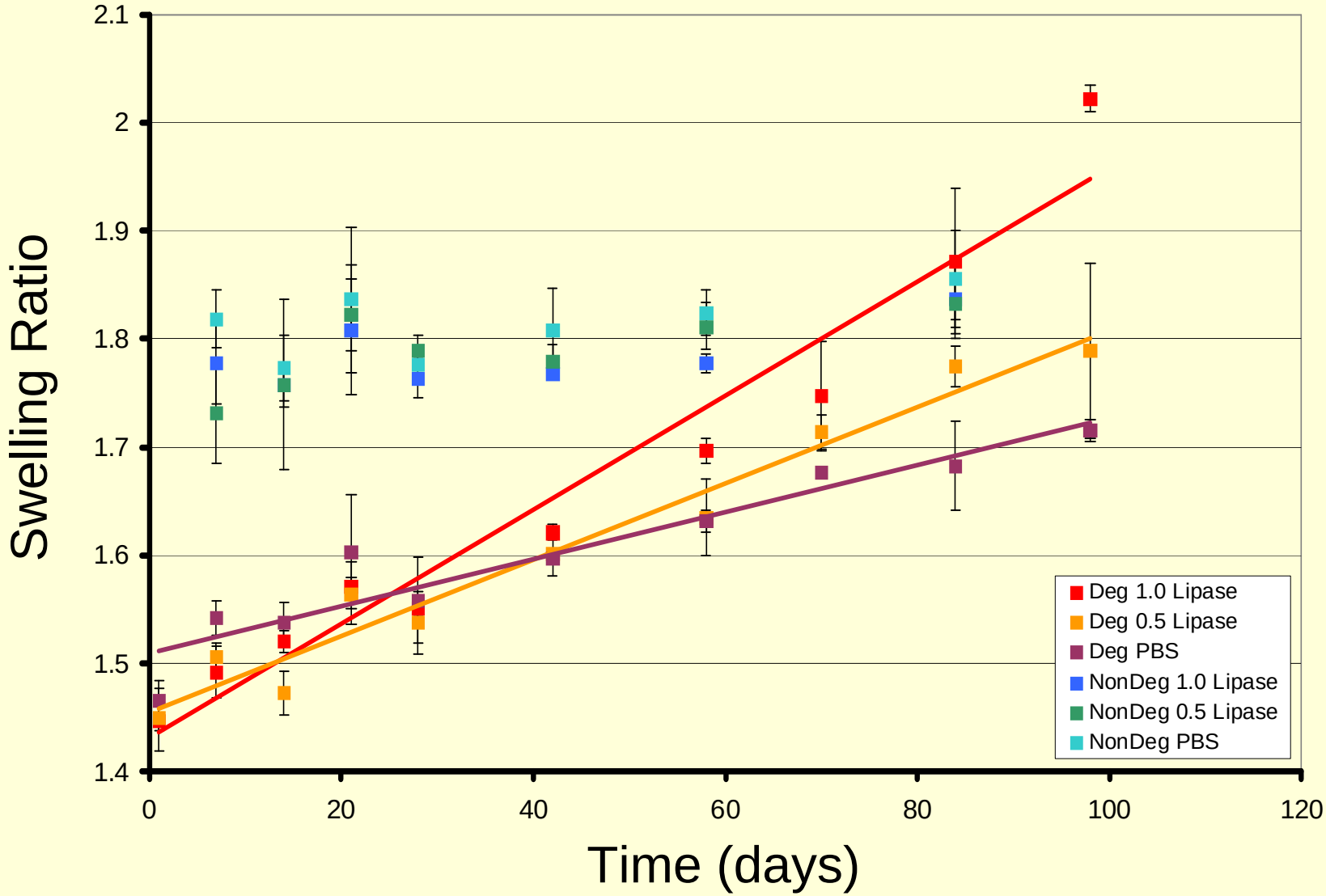
Sample: DP2



Do They Really Degrade?

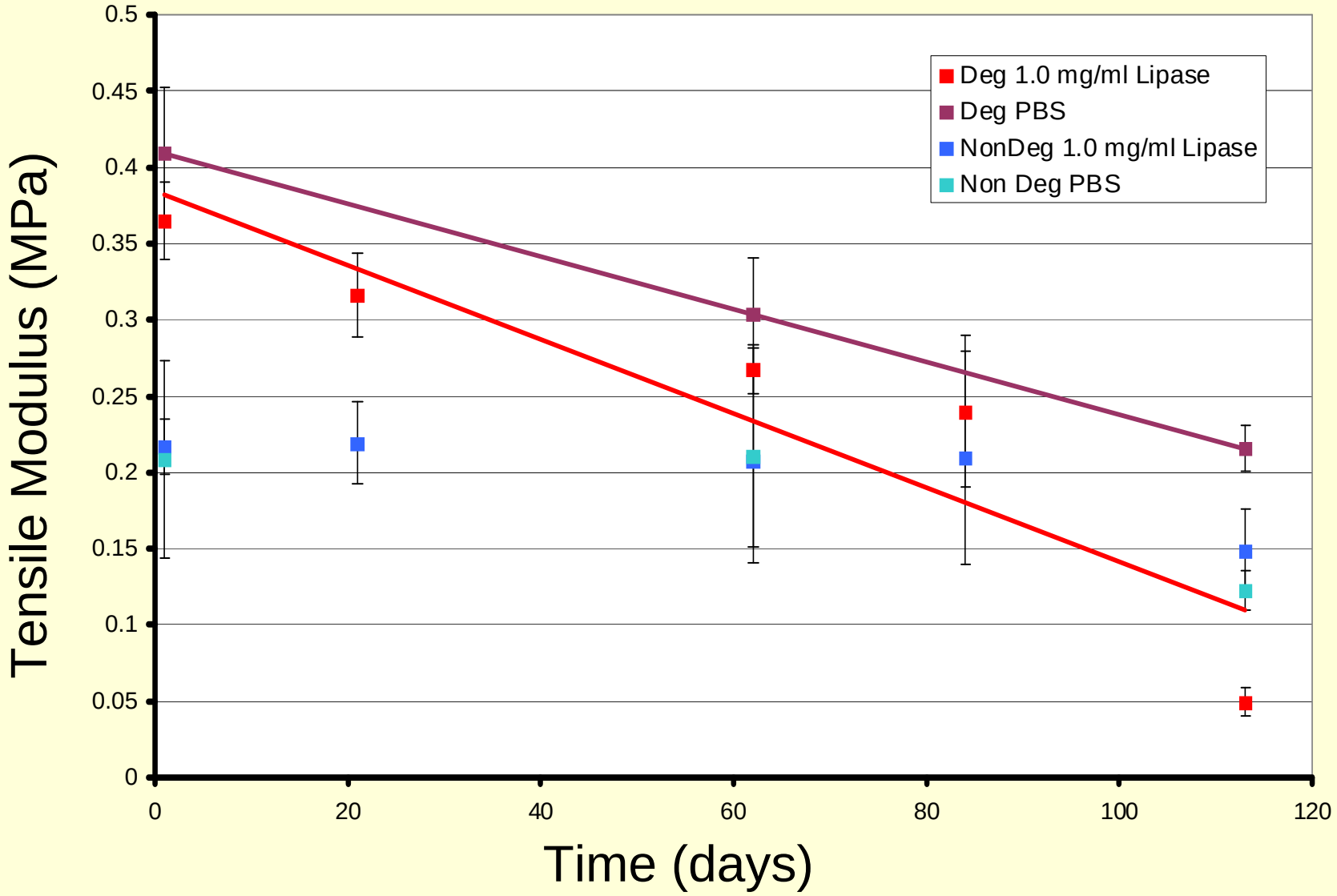
- Measure Degradation 3 Ways
  - Swelling Ratio $$swelling\ ratio = \frac{m_{WF}}{m_F}$$
  - Tensile Modulus
  - Mass Loss $$\%\, mass\, loss = \frac{m_I - m_F}{m_I} \times 100$$

Swelling Ratio ↑ For Degradable Gels



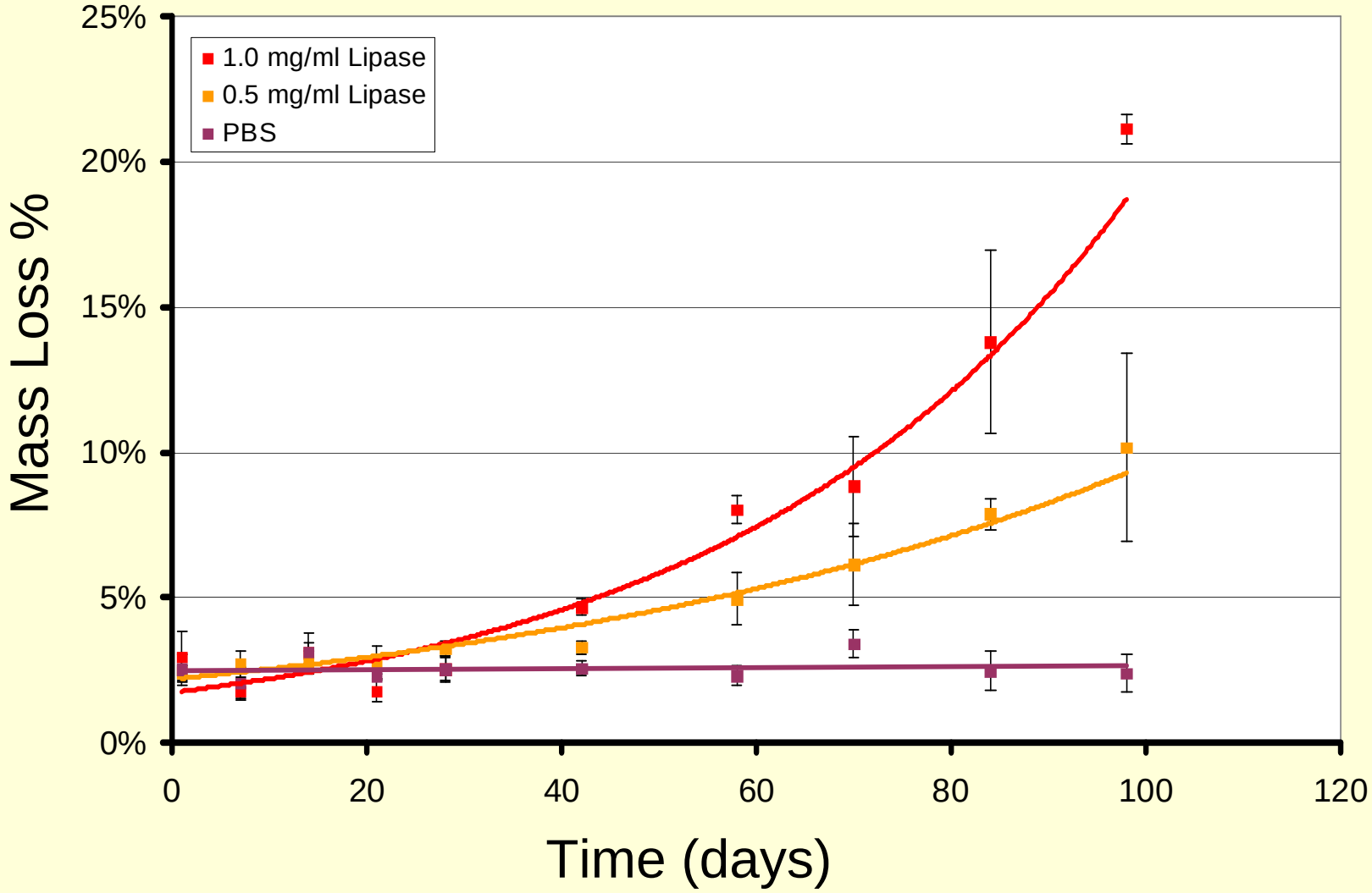
Sarah Atzet

Tensile Modulus ↓ For Degradable Gels



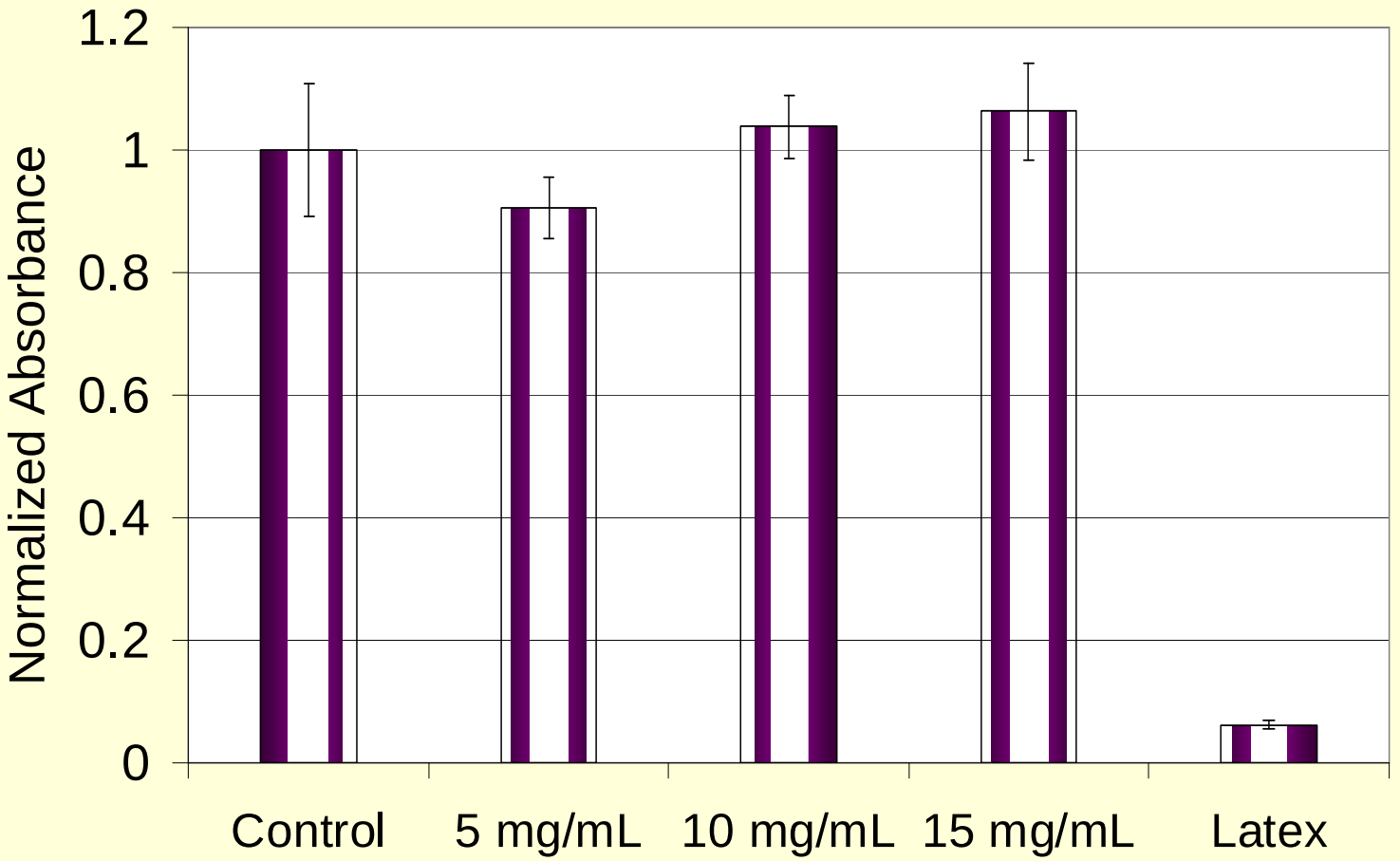
Sarah Atzet

PCL Gels: Mass Loss at Two Enzyme Concentrations



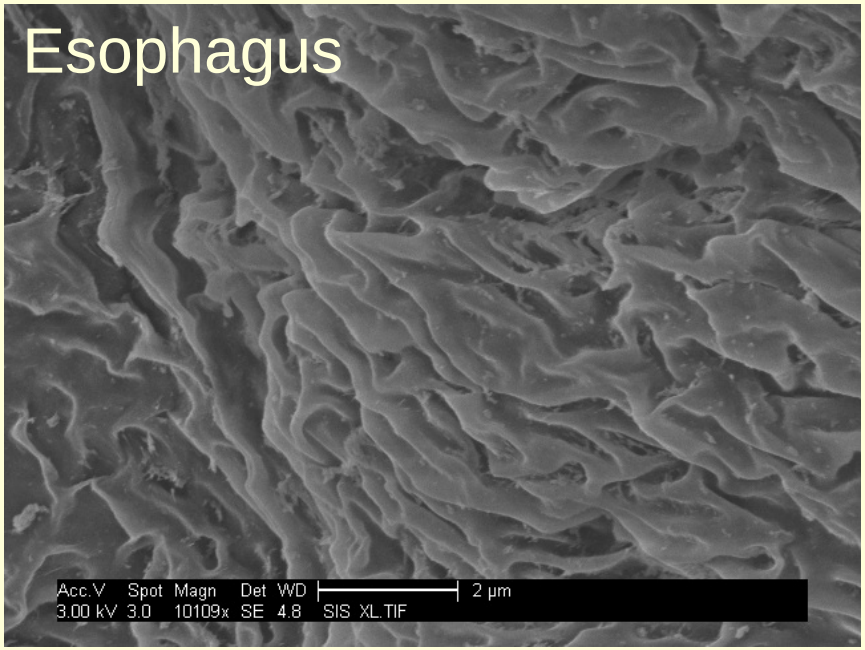
Sarah Atzet

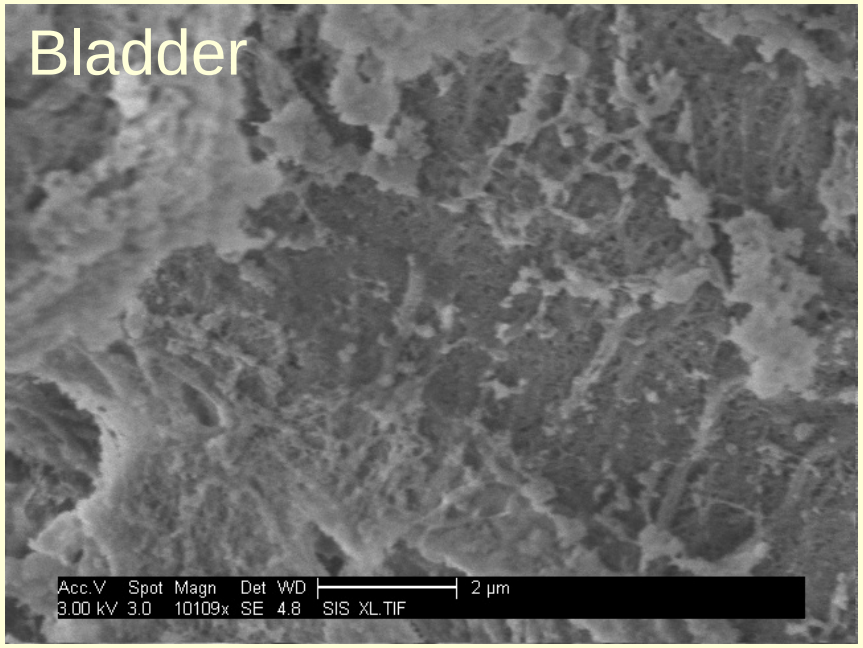
Degradation Products are NOT Cytotoxic

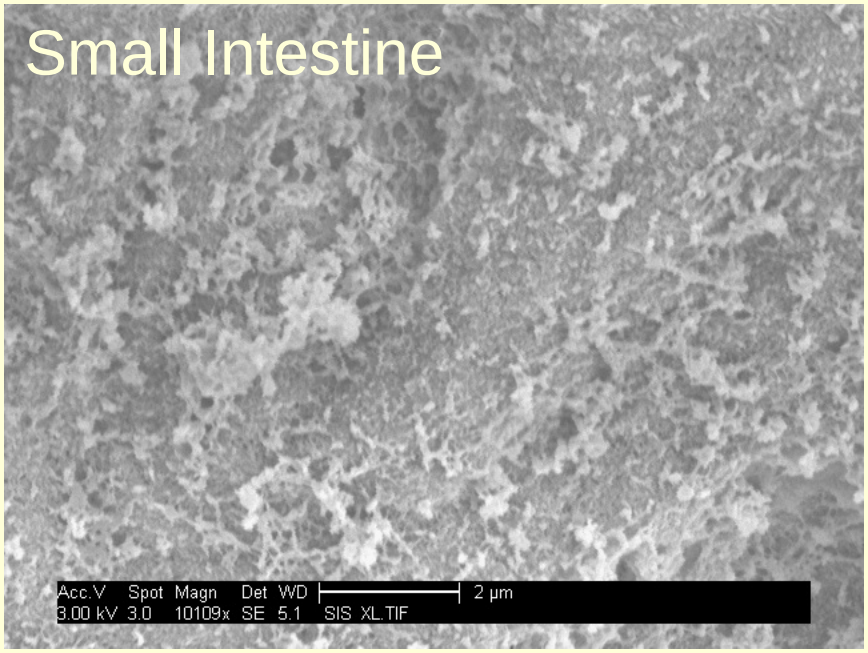


- MTT colorimetric method that measures cell proliferation

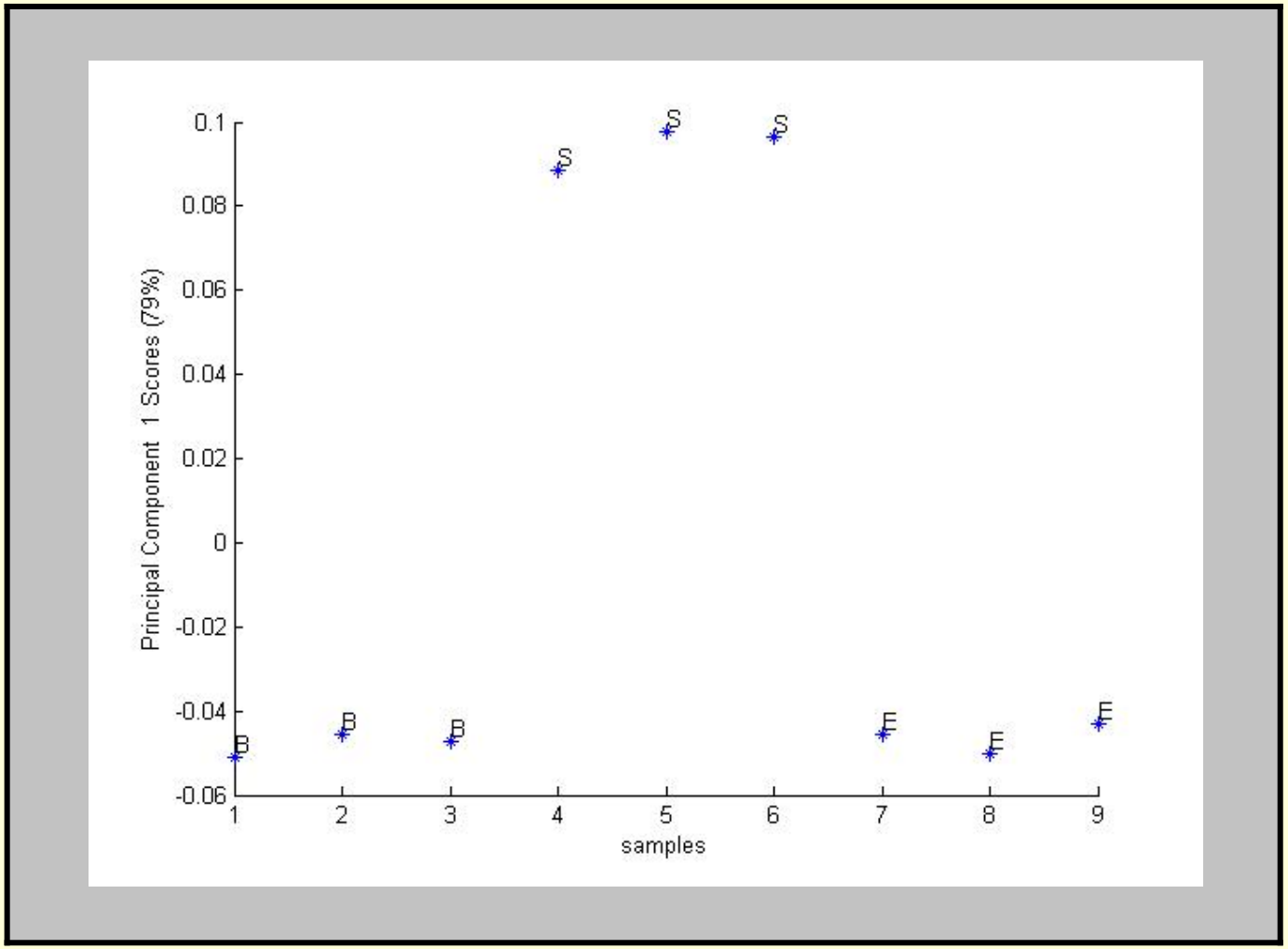
Scanning Electron Micrographs of Surfaces of Decellularized Tissues





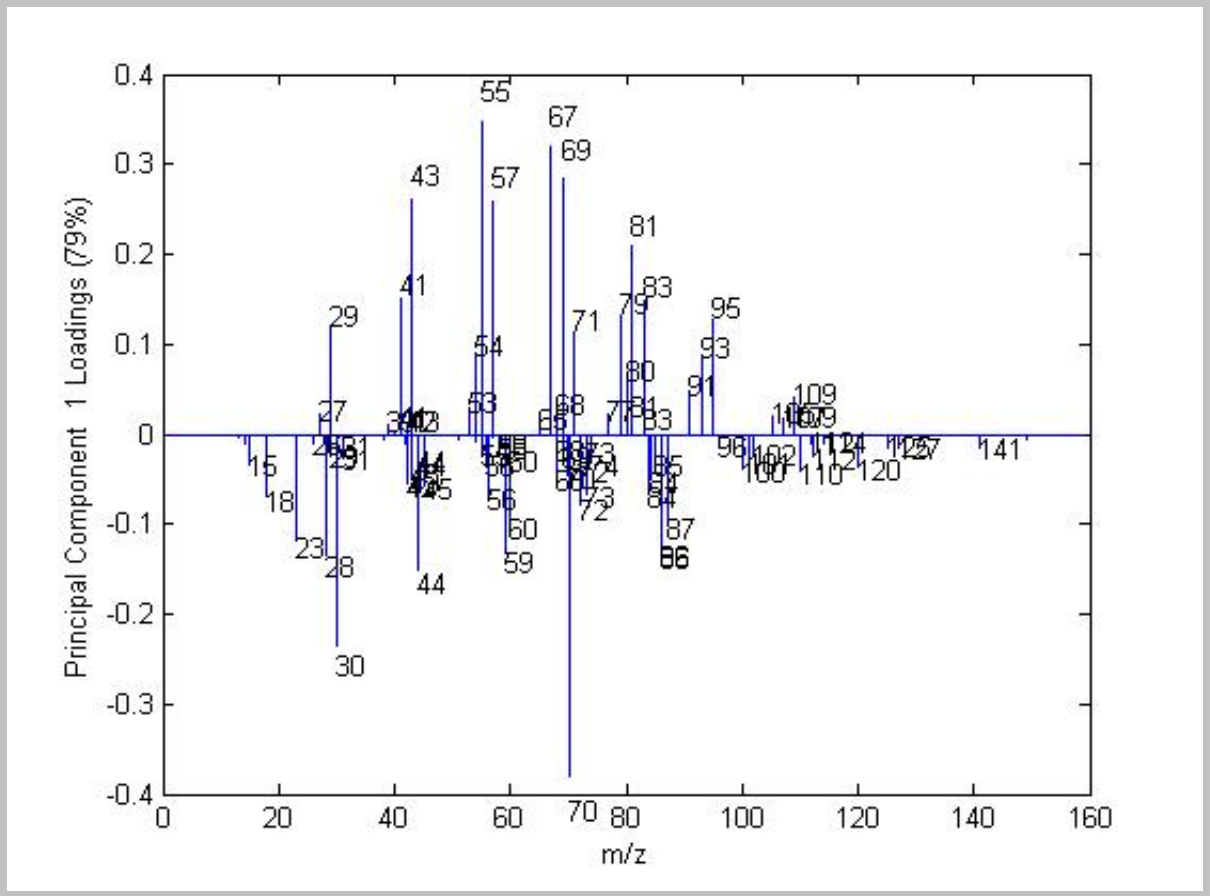


ToF SIMS Scores for Decellularized Tissues



Chris Barnes

ToF SIMS Loadings for Decellularized Tissues



Conclusions

We have an impressive tool chest of methods to bring to bear on scaffold characterization

We can distinguish scaffold types, observe degradation and measure contamination

What do we really need for optimal tissue engineering?

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Investigators, researchers and students

The NIH for funding BEAT and NESAC/Bio

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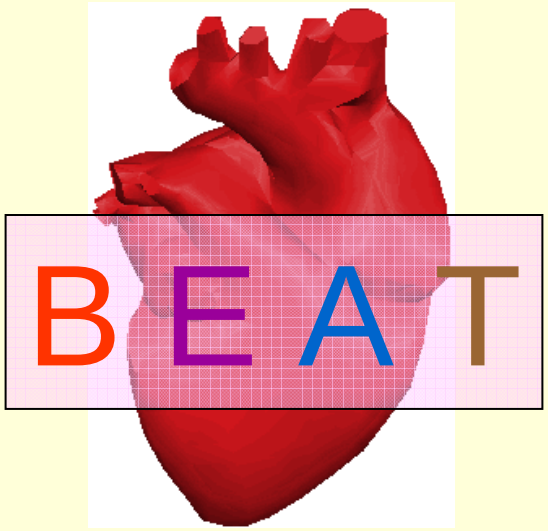
An NSF Engineering Research Center



NIH BRP Grant