

Tissue and Fluid Proteomics

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Sample Sources for Proteomic Analysis

- Cell lines.
- Tissue sections.
- Body Fluids:
 - Blood and urine.
 - Fluids from secretion.
 - Fluids in interstitial spaces.

Fluids from Secretion

- **Saliva**
 - Whole saliva or major salivary gland secretions.
 - 2D gel w/o or w/ MS ID; LC-MSⁿ.
 - Proteome database for biomarkers of specific diseases.
- **Cerebrospinal fluids (CSF)**
 - Fluid surrounding the central nervous system.
 - Total vol ~140 ml, produced at 0.3-0.4 ml/min.
 - Samples were collected by lumbar puncture (10-12 ml).
 - 2D gel w/ MS ID; LC-MSⁿ
 - Studies of the pathophysiological mechanism in frontotemporal dementia, Alzheimer's disease
 - Yuan et al. J Chromatogr B , (2005) 815(1-2),179-89. (review)

Fluids from Secretion

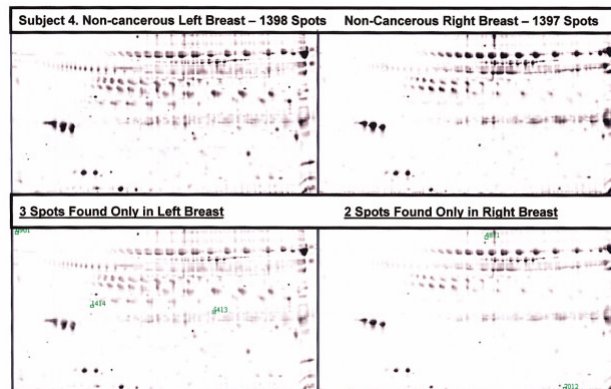
- **Synovial fluid**
 - A dynamic reservoir for proteins originating from serum, synovial tissue, and cartilage.
 - 2D gel / MS ID and LCⁿ-MSⁿ.
 - Study for biomarkers for Rheumatoid Arthritis.
 - Tilleman et al. Rheumatology (Oxford), (2005) 44,1217-26. (review) .
- **Bronchoalveolar lavage (BAL) fluids**
 - Obtained by washing the epithelial lining of lung with PBS.
 - 2D gel / MS ID and LCⁿ-MSⁿ.
 - Studies of fibrosing interstitial lung diseases, such as sarcoidosis, and allergic asthma.
 - Wattiez et al. J Chromatogr B (2005) 815, 169-178. (review)

Fluids from Secretion

- **Lymph**
 - Lymph was collected by cannulation of sheep lymphatic vessels.
 - SELDI and 2D gel w/ MS ID.
 - The 1st proteomic analysis of normal ovine lymph.
 - Leak et al., *Proteomics* 2004, 4, 753-765.
- **Aqueous Humor**
 - AH was collected by 27 G needle (150 μ l) from patients w/ or w/o corneal rejection.
 - 2D gel w/ MS ID.
 - Funding et al., *Acta Ophthalmol. Scand.* (2005) 83, 31-39.
- **Nipple aspiration fluid (NAF)**
 - breast ductal fluid collected by nipple aspiration.
 - Non-invasive way of sample collection.
 - Sample vol: generally ~ 10-20 μ l.
 - 2D gel, SELDI, and chromatography-MSMS.
 - Studies of the early diagnosis of breast cancer.

Protein Profiles of Bilateral Matched Paired NAFs by 2DE-Approach

Non-Cancerous Left Breast Non-Cancerous Right Breast



1398 spots
3 spots found only in
Left Breast

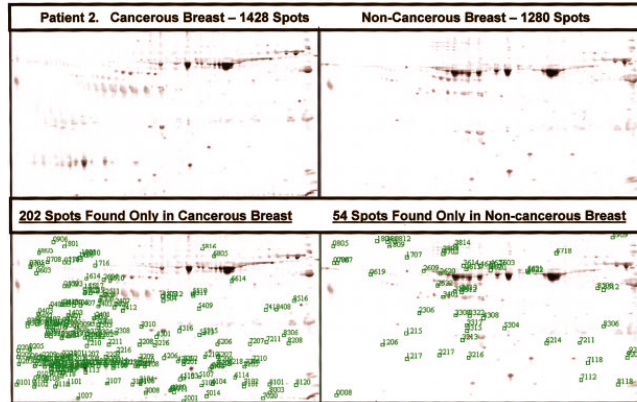
1397 spots
2 spots found only in
Right Breast

Kuerer, HM; et al., *Cancer* (2002) 95, 2276-2282.

Protein Profiles of Bilateral Matched Paired NAFs by 2DE-Approach

Cancerous Breast

Non-Cancerous Breast



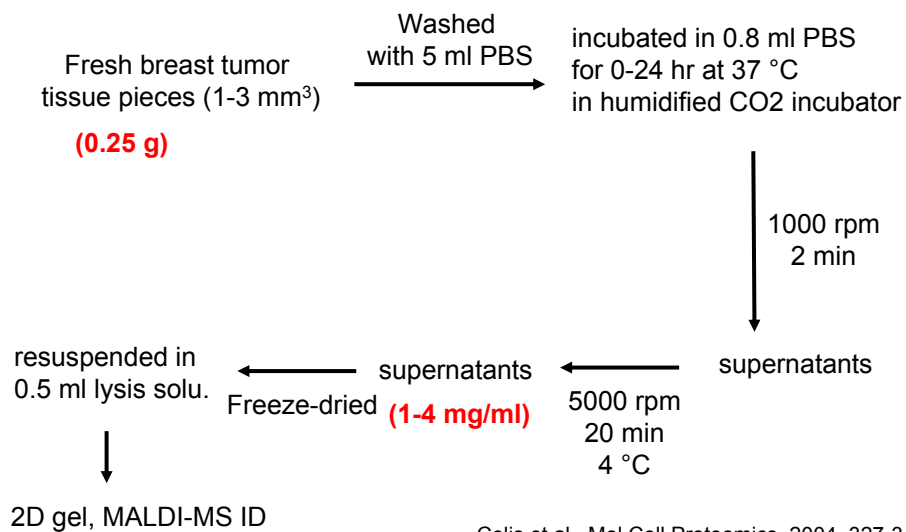
1428 spots
202 spots found only
in Cancerous Breast

1280 spots
54 spots found only in
non-Cancerous Breast

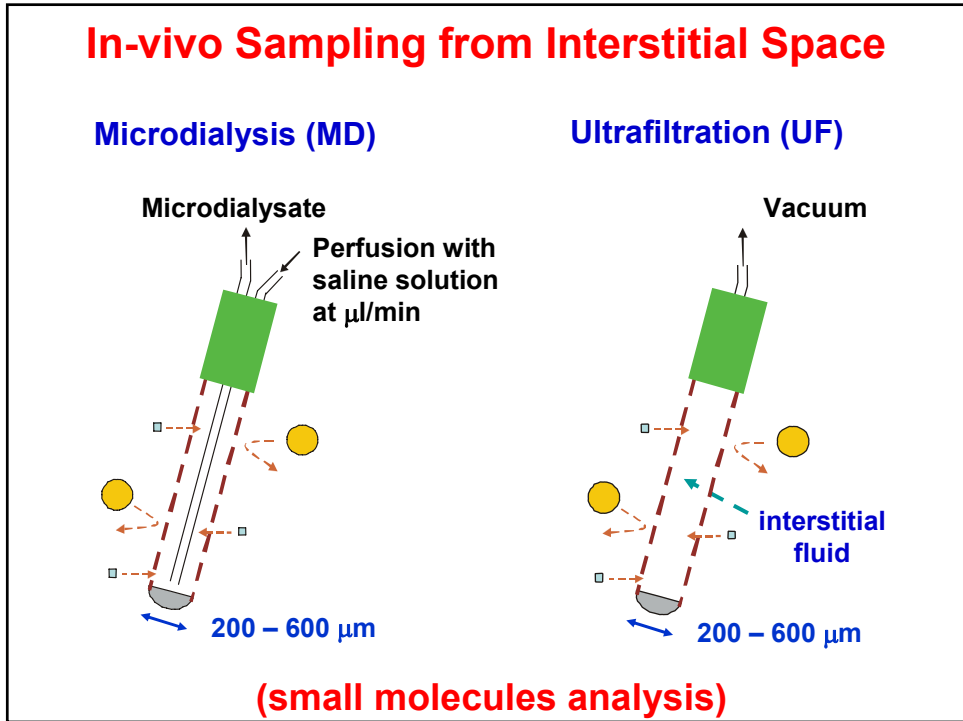
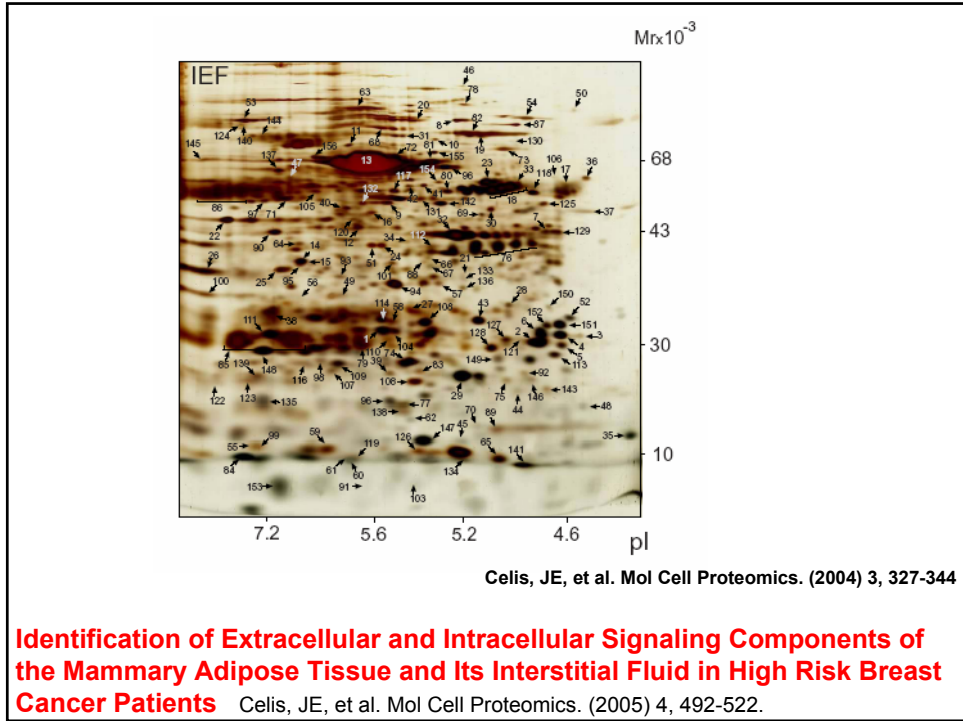
Kuerer et al., *Cancer* (2002) 95, 2276-2282.

Fluids in Interstitial Spaces

Ex-vivo Interstitial fluid collection:



Celis et al., *Mol Cell Proteomics*. 2004, 327-344.



Microdialysis vs Ultrafiltration for Proteomic Sampling

Advantage:

- Sampling free drug or metabolites (non-protein-bound) in interstitial fluid at the site of interest.
- Excellent temporal resolution for PK studies from single animal.
- Real *in-vivo* sampling from live, freely-moving animals.

Microdialysis:

diffusion-based technique

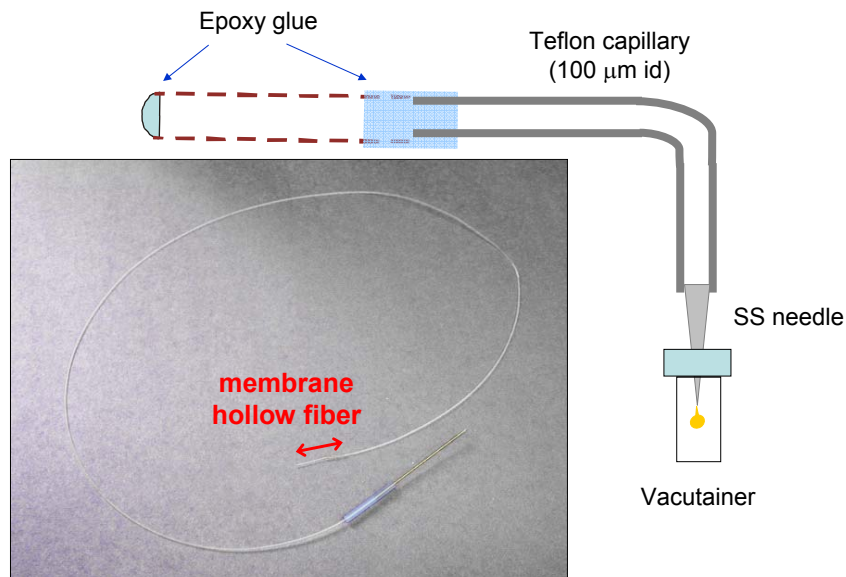
- poor recovery
(for peptides and proteins)
- not suitable for long term in-vivo sampling.

Capillary Ultrafiltration:

non-diffusion-based technique

- better & consistent recovery
- suitable for long term sampling (up to 6 month).

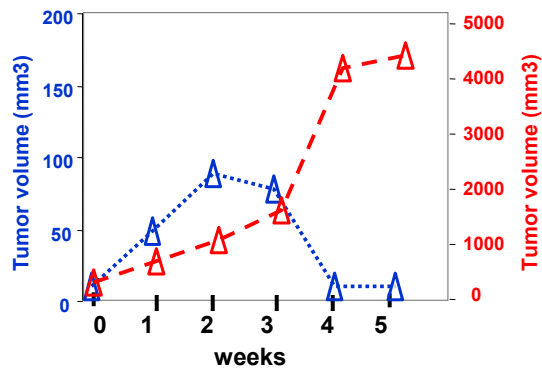
Capillary Ultrafiltration Probe



CUF Sampling from Animal Models

- *In-vivo* UF sampling from interstitial microenvironment in tumor masses at different developing stages.
- Continuous UF sampling from a freely-moving mouse model with chemical-induced Allergic Contact Dermatitis.

Regressive Skin Tumor Model (C2240)



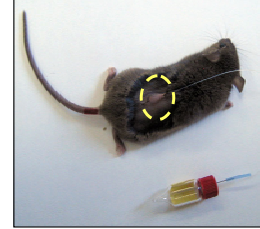
Dynamic interaction
between
Tumor and Host cells

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CUF Sampling from Regressive Tumor Model

C3H/HeN Mice $\xrightarrow{\text{C2240 skin tumor cell}}$ 500,000 cells/50 μ l Inj s.c. on the back

1. Measure tumor size
2. Implant CUF probe in tumor masses for IF collection.



- Tumors grew on WK 1, but tumor masses decreased after WK2.
- Interstitial fluid in tumor was collected by a high MWCO probes for 3 hours.

Protein ID through PROWL & SWISS-PROT database \leftarrow Nano-LC-qTOF MS for peptide sequencing \leftarrow tryptic digestion \leftarrow ~2-5 μ l of IF collected

Secretomes from Regressive Skin Tumors

Tumors progress at 1st week

1. S100A4 (Metastasis-associated calcium binding protein)
2. Thymosin β 4
3. Thymosin β 10
4. Profilin 1 (dendritic exosomes)
5. beta 1-globin
6. Hemoglobin β 2

Tumors regress at 3rd week

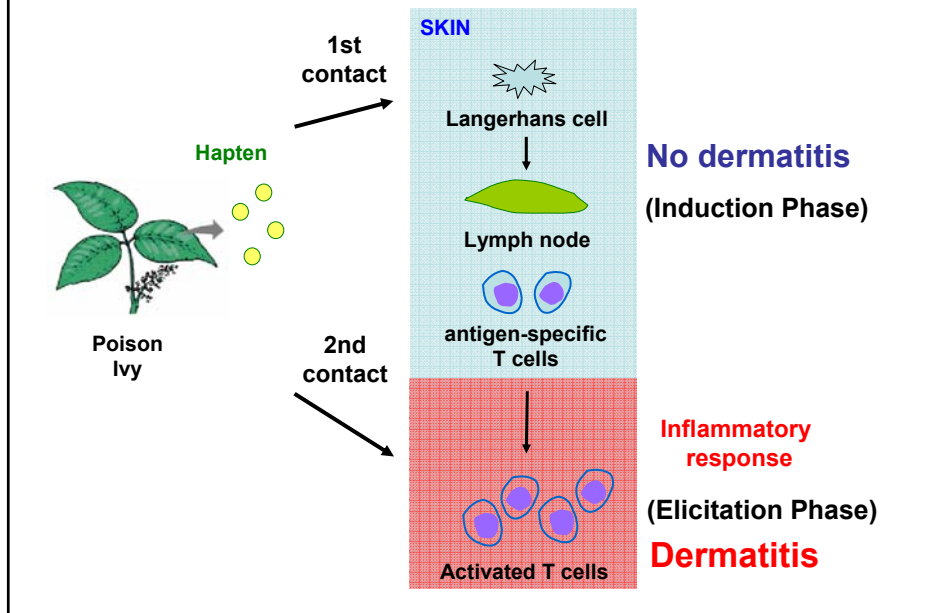
1. Fetuin-A (α -2HS-glycoprotein)
2. Apolipoprotein A-1
3. Alpha-antitrypsin
4. Contrapsin (Trypsin inhibitor)
5. beta 1-globin
6. Hemoglobin β 2

All are secretory proteins

Underline: tumor associated proteins

CUF can be used to sample clean interstitial fluid *in-vivo* from animals at different physiological / disease stages

Allergic Contact Dermatitis



Dynamic CUF Sampling from ACD Model

Animal: C3H/HeN mouse
Hapten: DNFB (dinitrofluor-benzene)
control/control, control/DNFB, DNFB/DNFB

Day 1 & 2 Induction:
 0.5 % DNFB (in 20 % olive oil in acetone)
 - 25 µl on shaved abdominal skin and footpads

Day 6 (the day before elicitation) UF probe implantation:
 CUF probe was implanted subcutaneously in ear and housed in a freely-moving system with access to water and food
 - collect interstitial fluid (IF) samples 12 hours before elicitation

Day 7 Elicitation:
 0.2 % DNFB (in 20 % olive oil in acetone)
 - 10 µl on both side of each ear
 - IF samples were collected continuously into 24 hours intervals for 3 days

The diagram also shows a mouse with a "CUF probe implanted" in its ear and a photograph of the experimental setup for fluid collection.

1. Ear thickness were measured daily before and after DNFB elicitation.
2. UF samples were processed with 2DE cleanup kit and analyzed with 75 µl protein load on 3-10 IEF/ 12.5% SDS gel / Sypro staining.

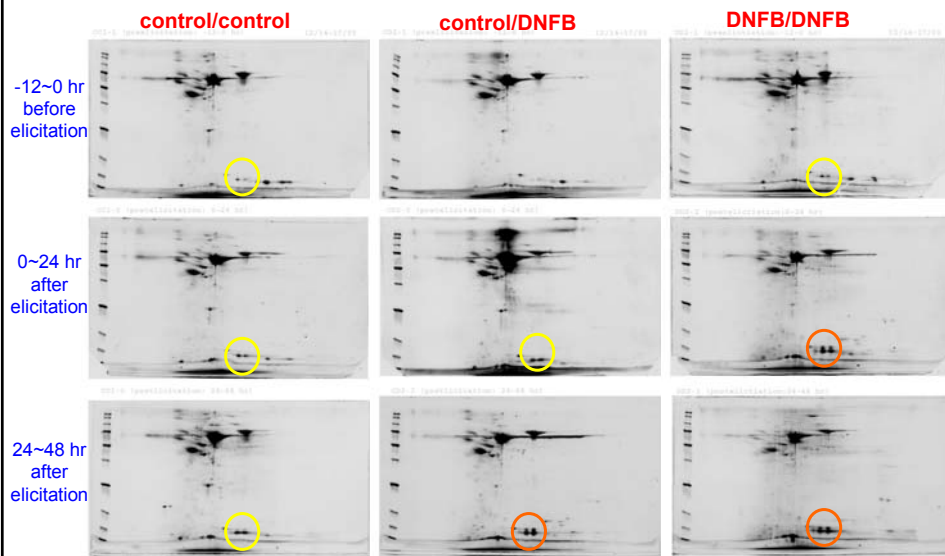
Ear Swelling of ACD Model

	CC-1	CD-1	CD-2	DD-1	DD-2
24 hrs after elicitation	0/1	0/2	0/2	4/-	7/10
48 hrs after elicitation	0/2	0/0	1/2	7/-	5/5

1. Left ear / Right ear (with Probes), unit: 0.01 mm.

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2DE Analysis of IFs from different ACD Stages



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Protein ID of IFs from different ACD Stages

Protein	Mass	MS	MOWSE
Cp protein	121074	MALDI	126
Gsn protein	80712	MALDI	114
Plasminogen	90723	MALDI	88
transferrin	76628	MALDI	140
albumin 1	68678	MALDI	167
vitamin D-binding protein	53051	MALDI	82
kininogen 1	47868	MALDI	74
Serpina1a protein	45593	MALDI	103
apolipoprotein A-IV	44545	MALDI	168
gamma-actin	40992	MALDI	135
apolipoprotein A-I	30569	MALDI	118
trophoblast specific protein beta	13802	MALDI	66
vitamin D-binding protein	53085	Q-TOF	
Calgranulin B	12909	Q-TOF	

* This protein list only represents spots observed in most of 9 gels.
Detail analysis of differences between gels is not shown.

UAB Center of Skin Diseases, ABRF 2006

CUF for Proteomics Analysis of Interstitial Microenvironments

- We have evaluated the use of Capillary Ultrafiltration for proteomic study in interstitial microenvironments by providing both in-vivo and dynamic sampling.
- Challenges in analyzing UF samples by 2DE:
 - **Salty matrix:** may not be a problem; desalt cleanup may lose proteins.
 - **Albumin:** depletion kit from Sigma did not work well.
 - **Sample size:** increase collection area (multiple probes) and longer collection time (lost of temporal resolution).
 - **One shot deal:** DIGE will help.
- Other Multi-dimensional approaches may be more suitable for analysis of IF samples.