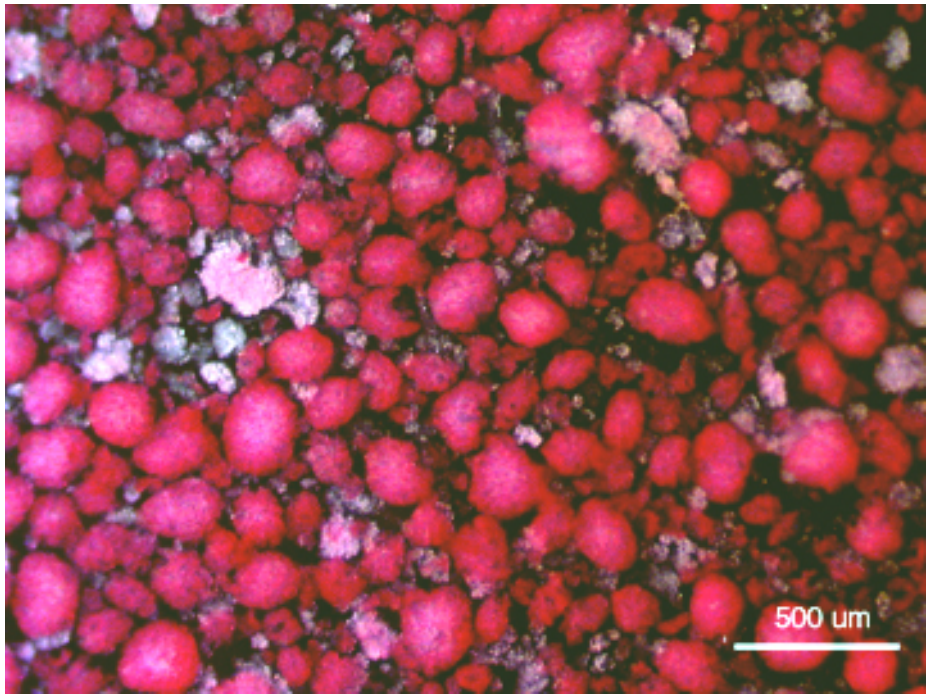
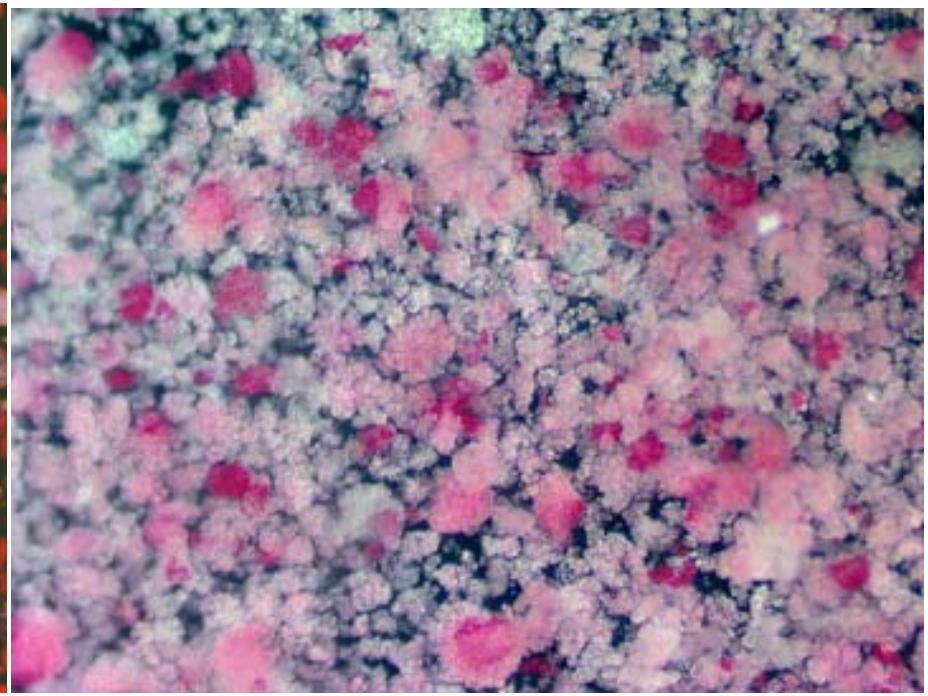




Determining cell composition of clinical transplants

Susan Bonner-Weir

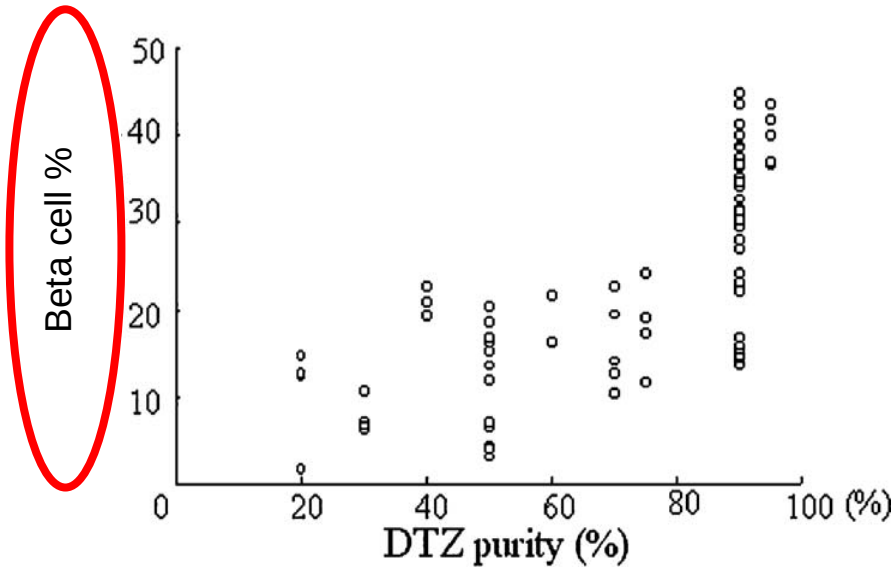
Joslin Diabetes Center

How many islets/ β cells were transplanted?

What was their health at time of transplant?

There are the assessments made at time of transplant, but in order to evaluate outcomes we need rigorous data, even if "after the fact".

Even in experienced hands DTZ overestimates % β cell



Ichii et al, AmJ Tx 05

This is not islet purity but %beta cell of whole preparation

Islet Purity Assessment

By EM : 48.0 ± 2.8 % (range: 16.7 - 86.3%).

By dithizone: 68.2 ± 3.2% (range: 30 - 95%).

Dithizone considerably over-estimates islet purity !

31 pancreases

Assessment of Purity and Amount of Islets/βcells

1. **Dithizone staining before transplant.**

2. **Dispersion of tissue and immunochemical analysis by laser scanning cytometer or Cytospin.**

3. **Morphological (both 1um and ultrastructural) assessment of cell composition after transplant and possibly before.**

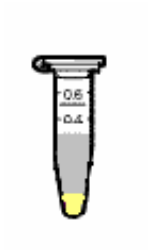
Are Dispersed Cells Accurate for Cell Composition?

Street et al: n= 69, 23.4 ± 1.4% b cells/total prep
Ichii et al: n= 62, 21.6 ± 1.4 % b cells /total prep

Our preps: n= 31, 34.8 ± 2.3% b cells /total prep
(range 13.1-63.7%)

1. **Recovery of cells (30-70%)**
2. **Selective loss of specific cells? β? acinar?**
3. **Identification of all cells?**

Determination of cell composition of human islet preparations by EM

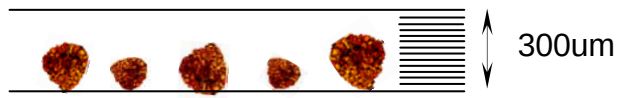


0.5 ml aliquot from
255ml final islet prep

Fix in 2.5% glutaraldehyde

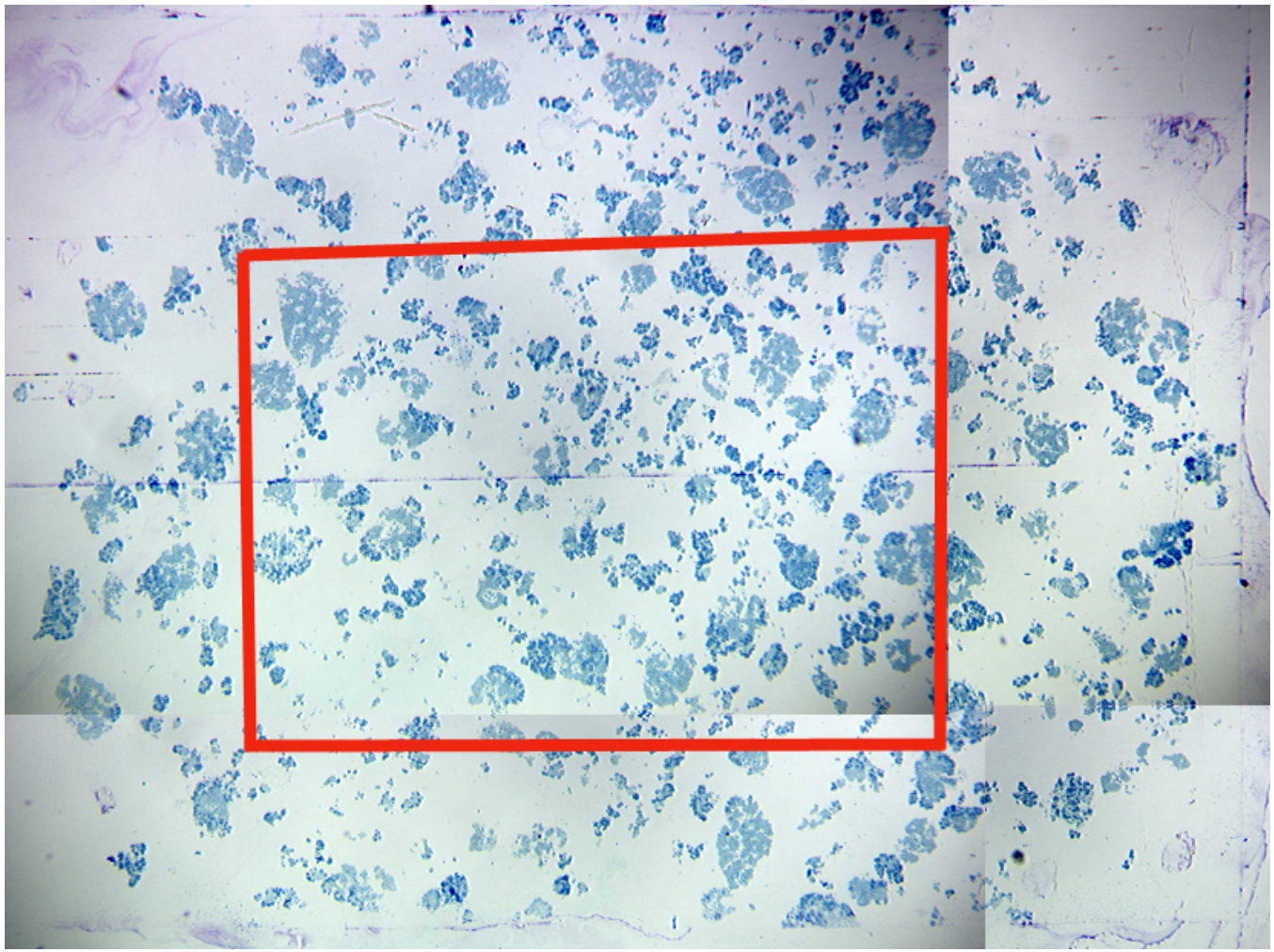
Dehydrate, osmicate, divide into 2 blocks,
Embed, cure, trim, section

I um sections (LM)
60 nm sections (EM)



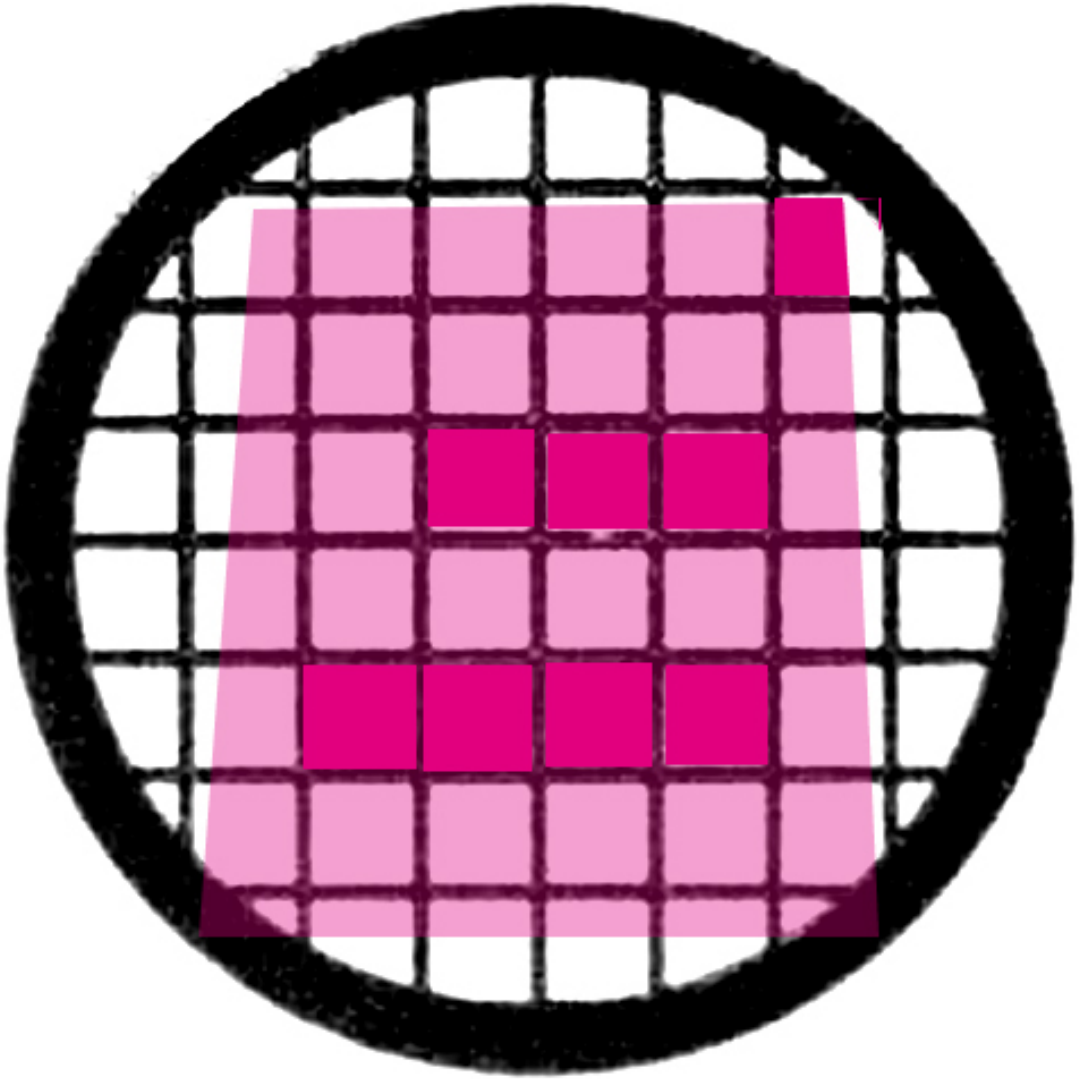
sections parallel to surface

EM section usually includes 70% of the sample: random sampling of each of 2 replicates



I um section

Sampling of EM Section in Systematic Manner

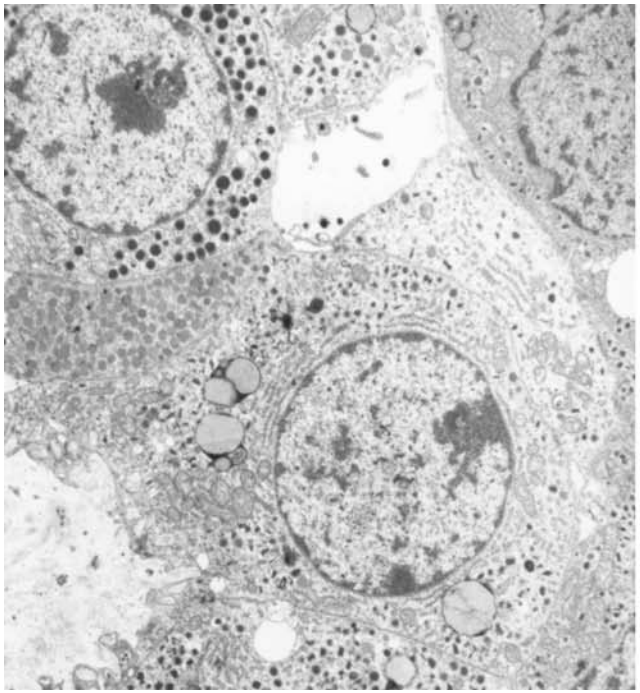


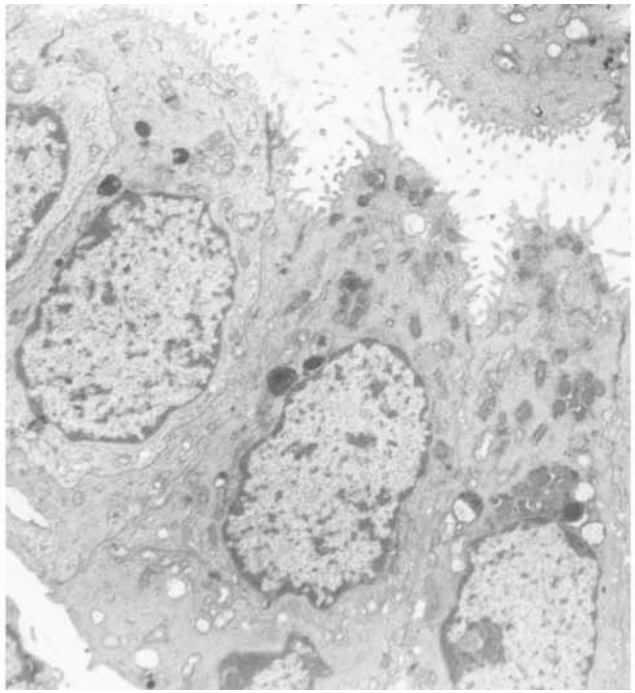
16 images of each of 2 blocks

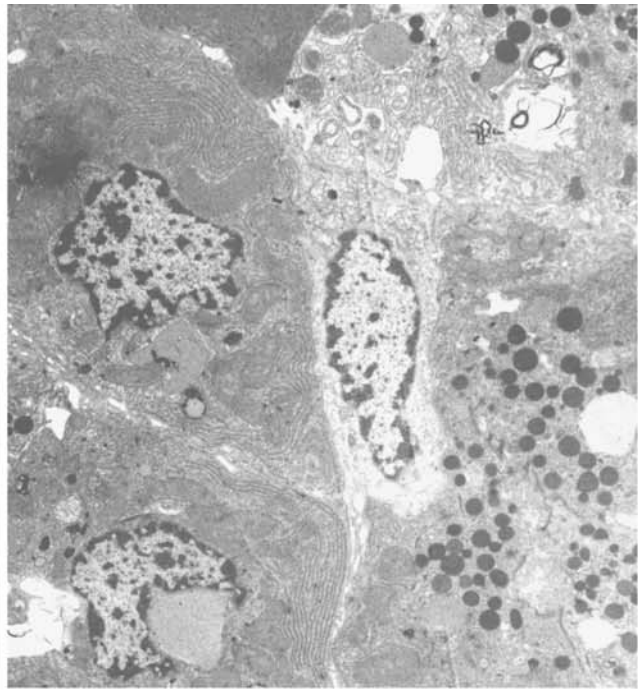
1900 X (negative)
4000 X final mag

Total: 500-800 cells assigned to β or non β endocrine, acinar, duct, dead or endothelial.

islet duct acinar/duct

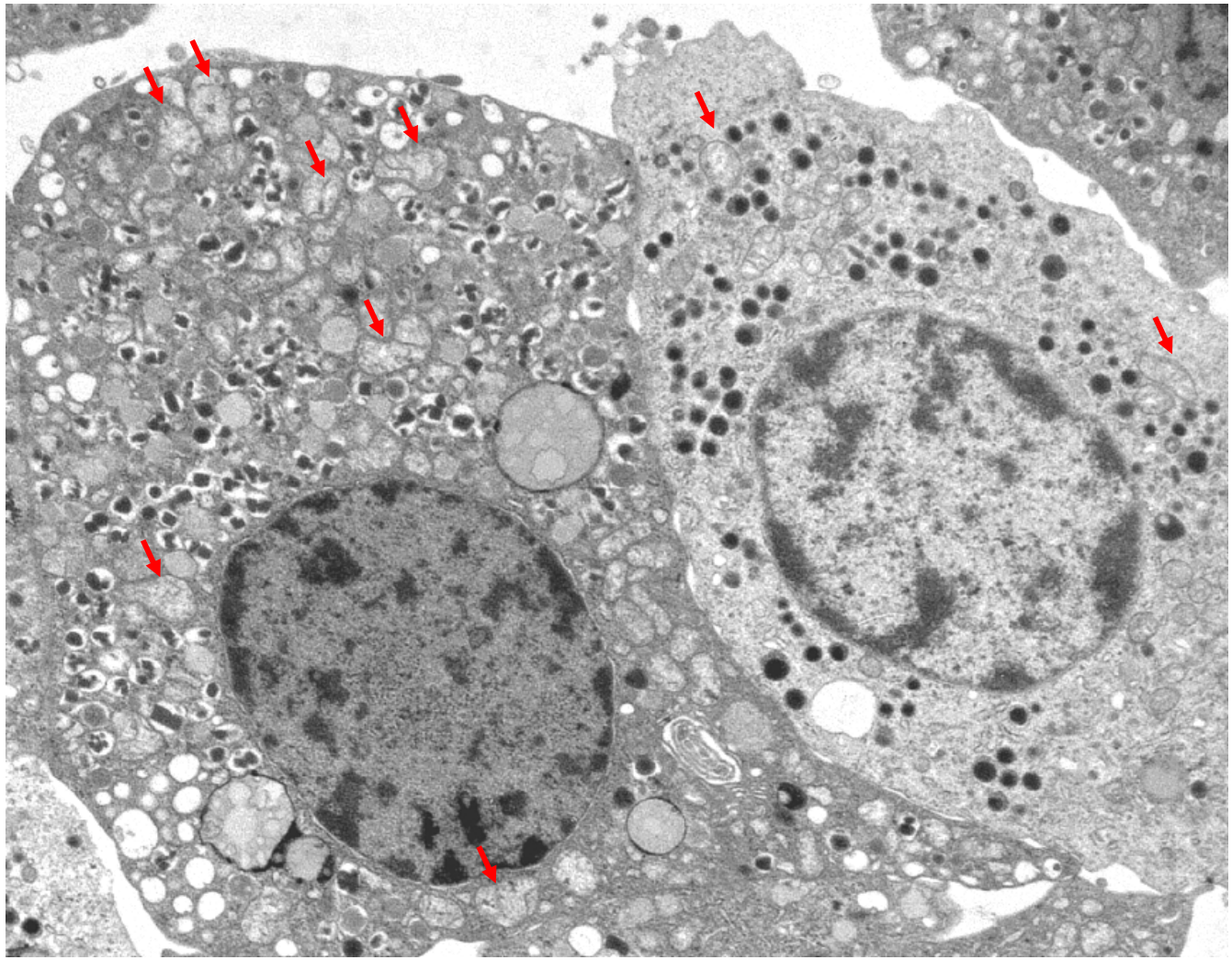






Ultrastructurally one can distinguish cell types of islet preps

All cells can be assigned to cell type by morphology, as well as be assessed for health



Human islet cells: insulin and glucagon

Islet purity assessment

By EM : 48.0 ± 2.8 % (range: 16.7 - 86.3%).

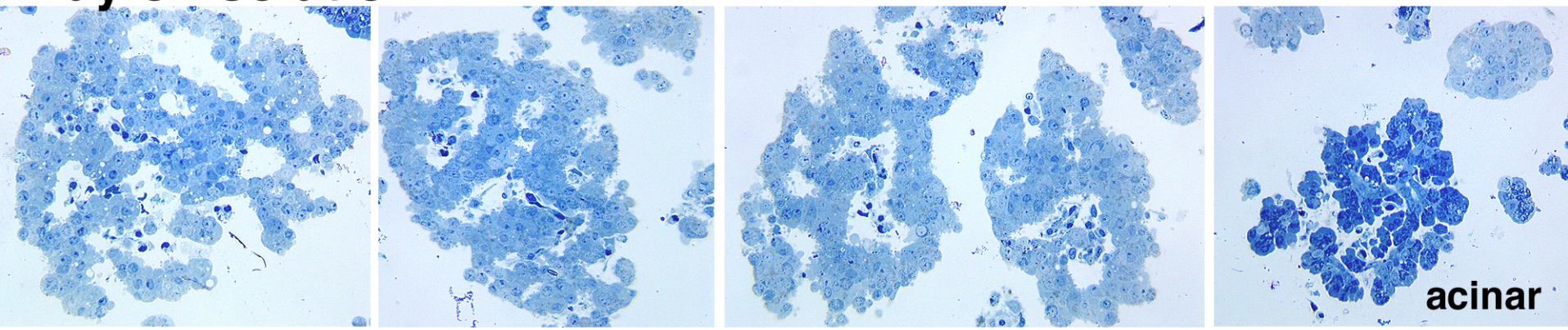
By dithizone-staining: 68.2 ± 3.2% (range: 30 - 95%).

Why does dithizone over-estimate the % islet and number of islet equivalents(IE) ?

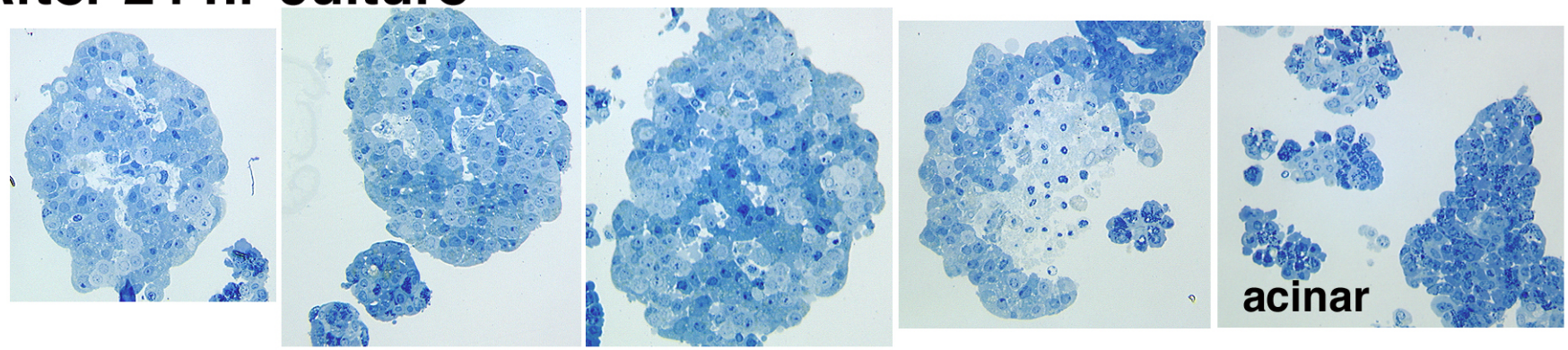
31 pancreases

Vascular channels are enlarged during isolation and distort the volume of freshly isolated islets; they account for 15-20% of the islet cross sectional area.

Day of isolation



After 24 hr culture



Composition of human islets by EM

72.6 ± 1.7% β cells (Range : 40.9 - 83.8 %)

The value of 40.9 % was associated with islet amyloid; the next lowest value was 57.1 %.

31 pancreases.

Is the EM Assessment Accurate for % β Cell/Islet?

Taking 7 clinical preps from 2004,

EM **72.2 ± 3.5 % β cells**
(Range 57.1 - 83. 9)

LM of immunostained pancreas of prep

70.3 ± 3.0 % β cells
(Range 56.3 - 76.5)

These values are cell number, not volume.

Can we develop a new assay for determining islet purity and IE that is more accurate, fast and without need of expensive large equipment?

Combination of:

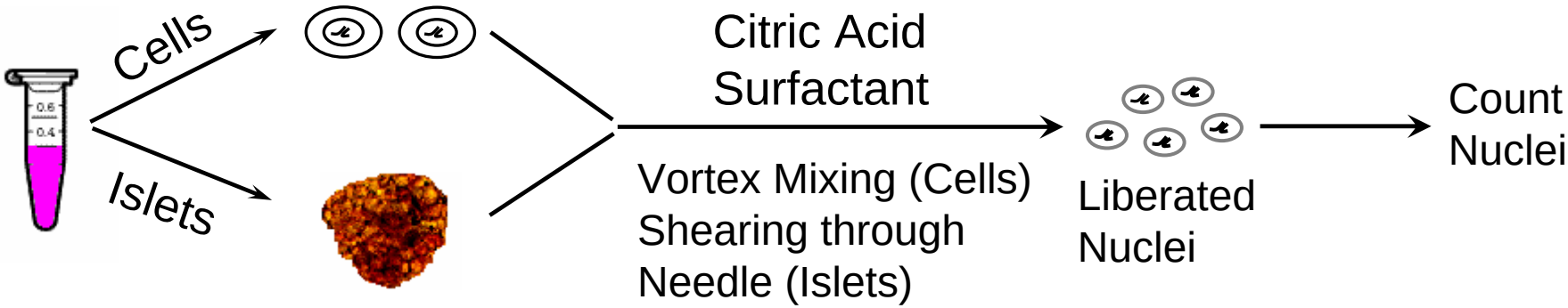
- **nuclei counting (Pisania & Colton)**
- **morphological identification**

We have so far tested the technique using the 1 um plastic sections but now need to validate with frozen sections.

Nuclei Counting Assay

Anna Pisania & Clark Colton

Determine number of cells in preparation, and with modification the number of viable cells.



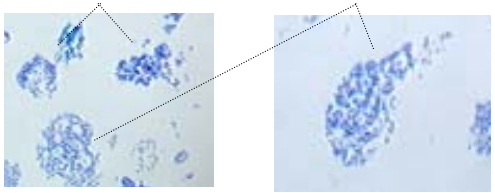
Accurate: using 125 IE: COV~ 6%
Rapid: Guava Flow Cytometer- 10 min
Visual counting - 60 min

Combine Nuclei Counting with Morphological Assay

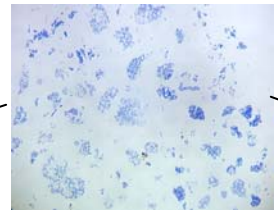
1 μm section

Light Microscopy

Non-islet Islet



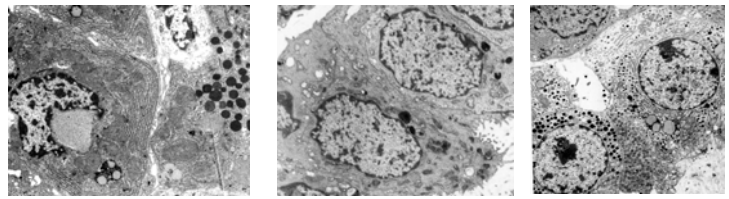
10 min



2 hr

Electron Microscopy

Acinar Duct Islet



Stereological point counting

↓

Volume fraction islets, f_L

Individual cell counting

↓ 500-800 cells

Number fraction islets, f_E

	Fraction Islets				N_{Total}	N_{Islets}	IEQ	
Preparation	Light f_L	EM f_E	DTZ f_{DTZ}	$\frac{f_{L+E}}{f_{DTZ}}$	10^6 cells		Nuclei Counting	Conventional Method*
1	0.60 ± 0.10	0.49	0.85	0.64	-	-	-	-
2	0.56 ± 0.01	0.62	0.90	0.66	-	-	-	-
3	0.66 ± 0	0.68	0.80	0.84	-	-	-	-
4	0.86 ± 0	-	0.95	0.91	10.8	9.3	47,000	100,000
5	0.64 ± 0.01	-	0.80	0.80	6.4	4.1	21,000	55,000

$$N_{Islets} = f_L \cdot N_{Total}$$

$$IEQ = \frac{N_{Islets}}{2000}$$

Can we use frozen sections to do this before transplant?

*DTZ as reported by the isolation center

Anna Pisania

SUMMARY

1. **Purity of islets by EM analysis (31 clinical islet preparations) showed 48.0 ± 2.8 %.**

2. Purity assessed by dithizone staining was 68.2 ± 3.2%.

3. Overestimation of islet equivalents is partly due to dilated vascular channels in freshly isolated islets. (15-20 % of the islet area).

4. Human islets are composed of 72.6 ± 1.7% β cells.

Acknowledgements

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