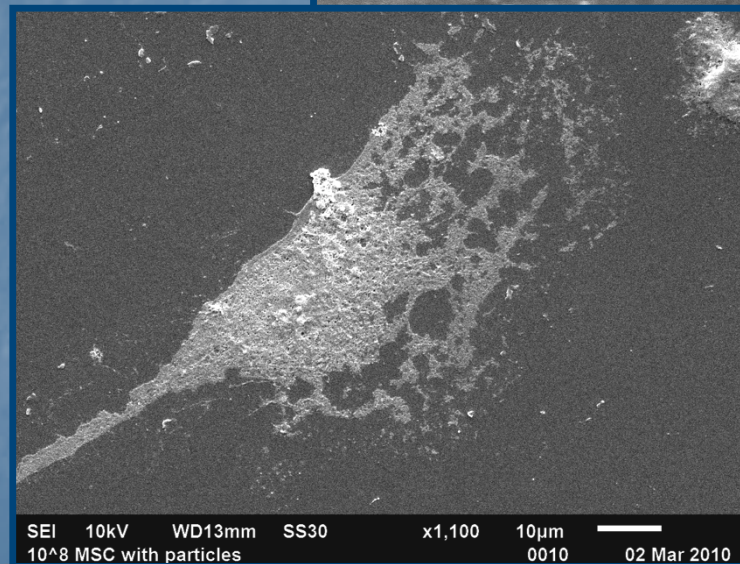
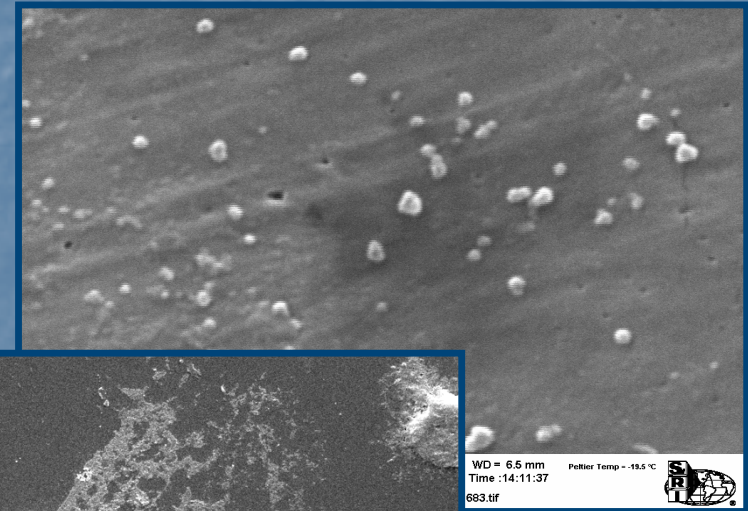


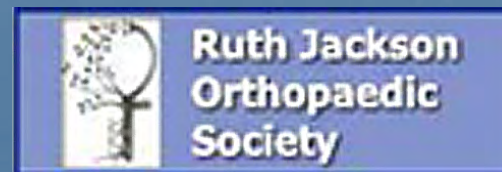
Effect of UHMWPE Particles on Mesenchymal Stem Cell Replication and Differentiation Potential

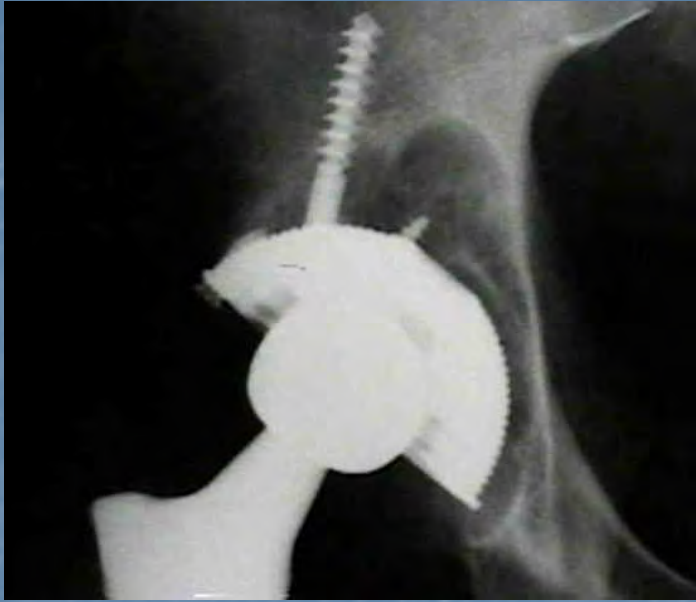
Amanda Marshall, MD
Sarah Smith, MS
Jay Stanley, MD
Roger Lee, MD
David Dean, PhD
Xiao-dong Chen, PhD



Disclosure

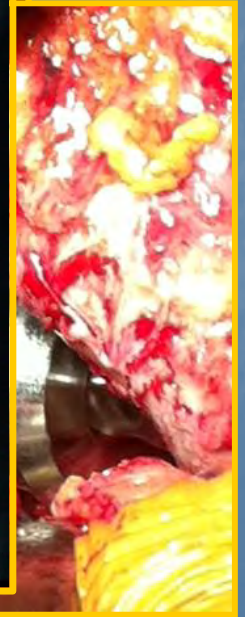
- Personally, have received nothing of value from any commercial entity
- No professional benefit has been accepted including royalties, stock, or honoraria
- 2007 OREF-Zimmer Career Development Award
- 2009 RJSOS Academic Enrichment Award
- 2011 OREF-AAHKS Research Award





- Ultra-high molecular weight polyethylene (UHMWPE) wear debris particles → osteolysis / implant loosening
- Long-term Implant stability
 - Osteoblasts' ability to form new bone in the periprosthetic region
 - *normal bone formation requires adequate availability of OB

Implant Stability



Clinical Relevance

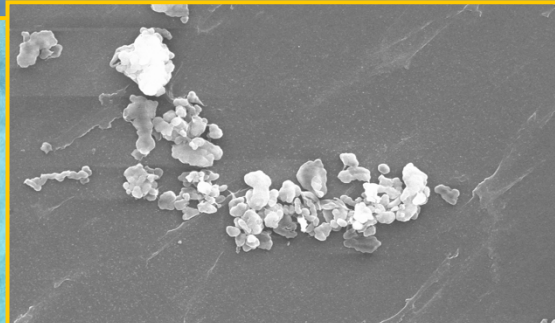
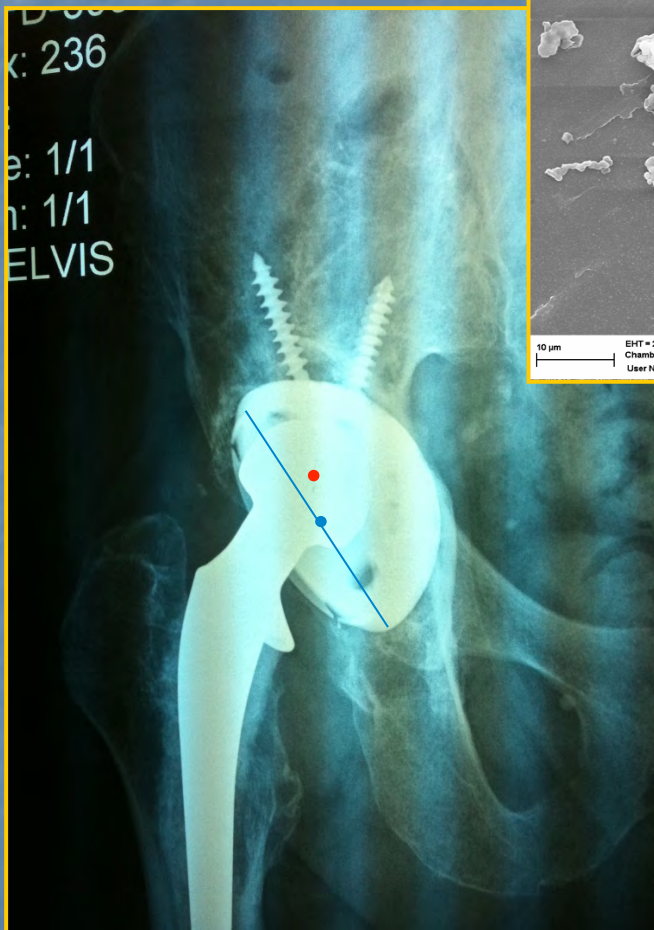
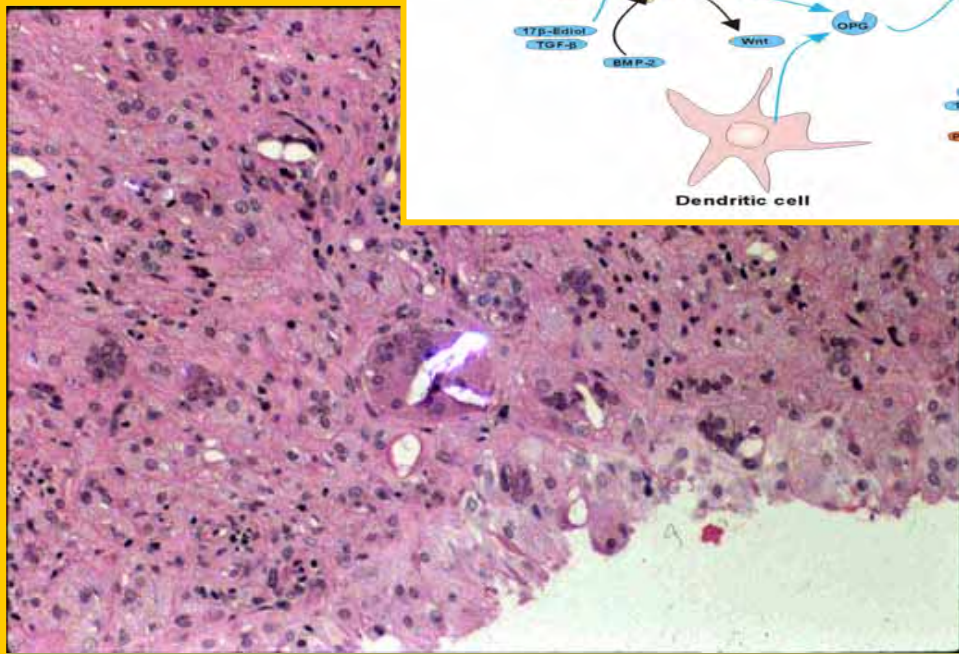
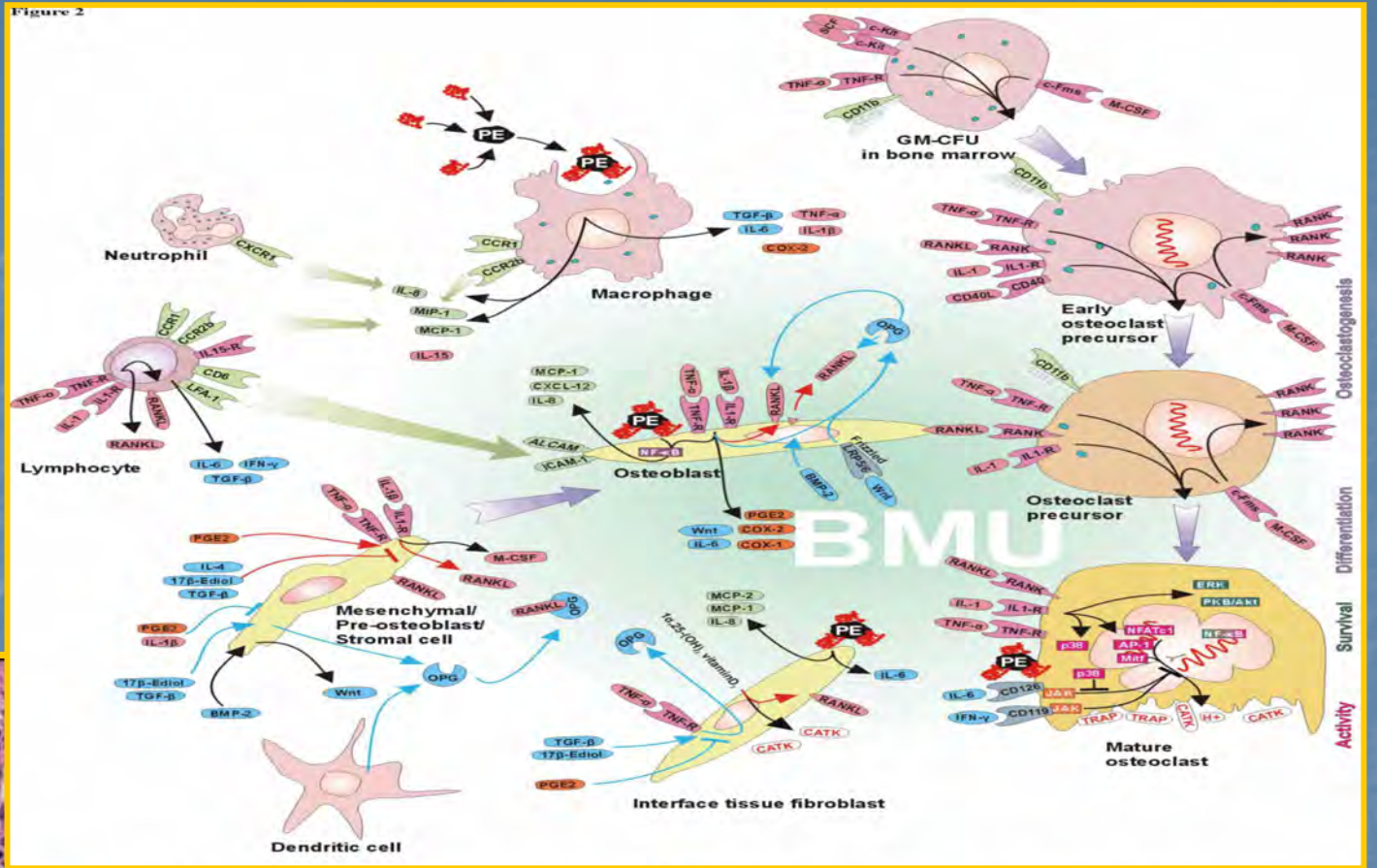


Figure 2

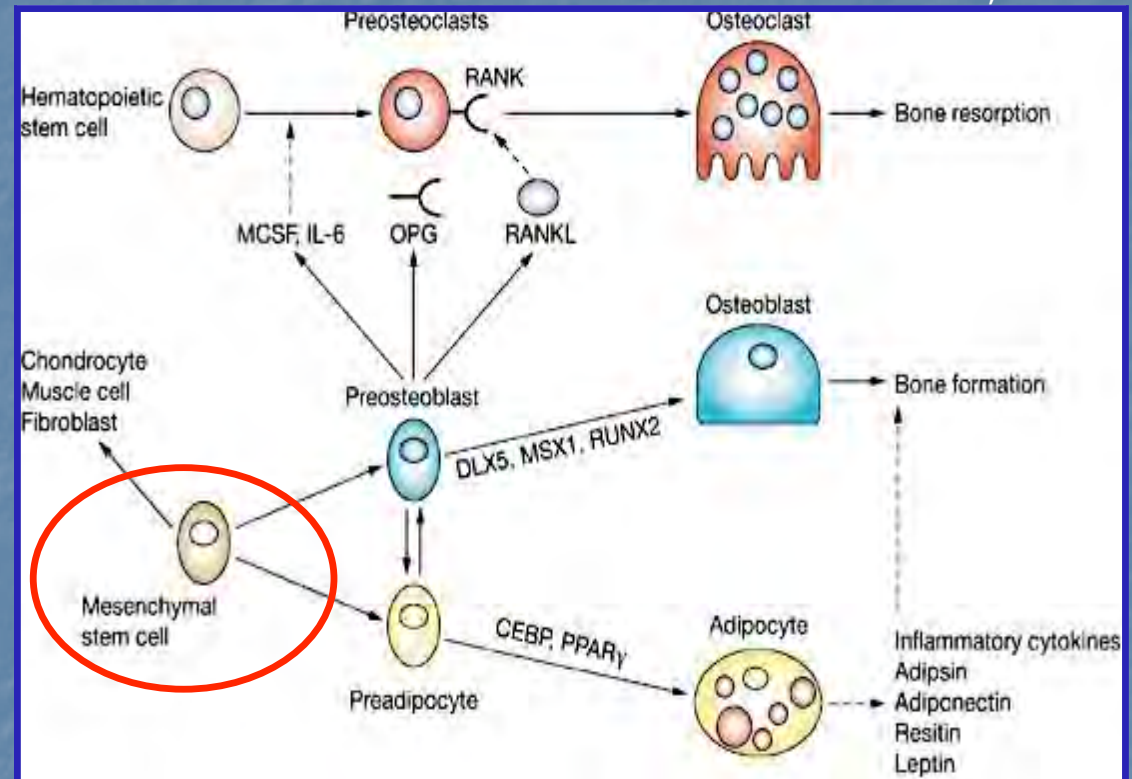


Gallo J et al, 2007

Background

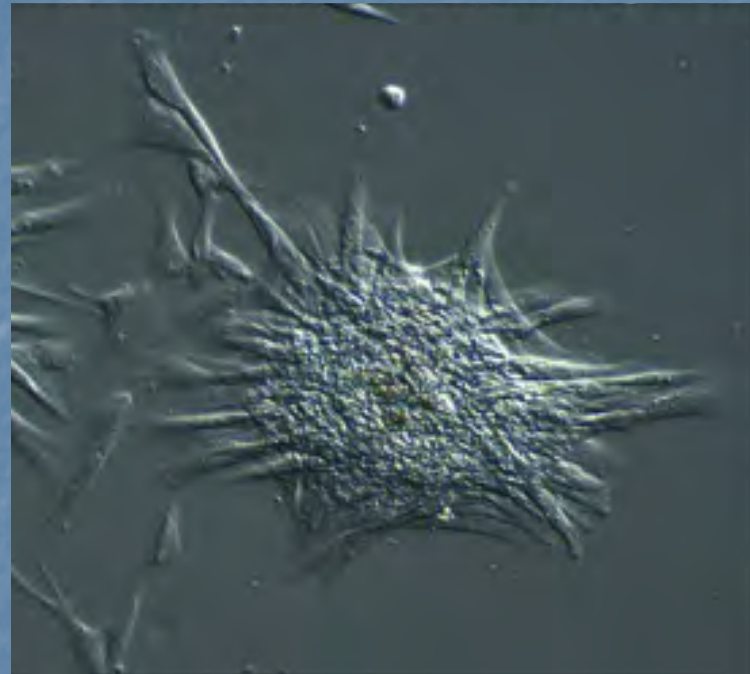
Purdue et al., 2006

- MSC dictates the osteoblast precursor availability in the periprosthetic area



Study Goal

- To better elucidate the biological consequences of wear debris on peri-prosthetic cells



- Evaluate the effect of UHMWPE on the mesenchymal stem cell replication and differentiation

Clinical Relevance

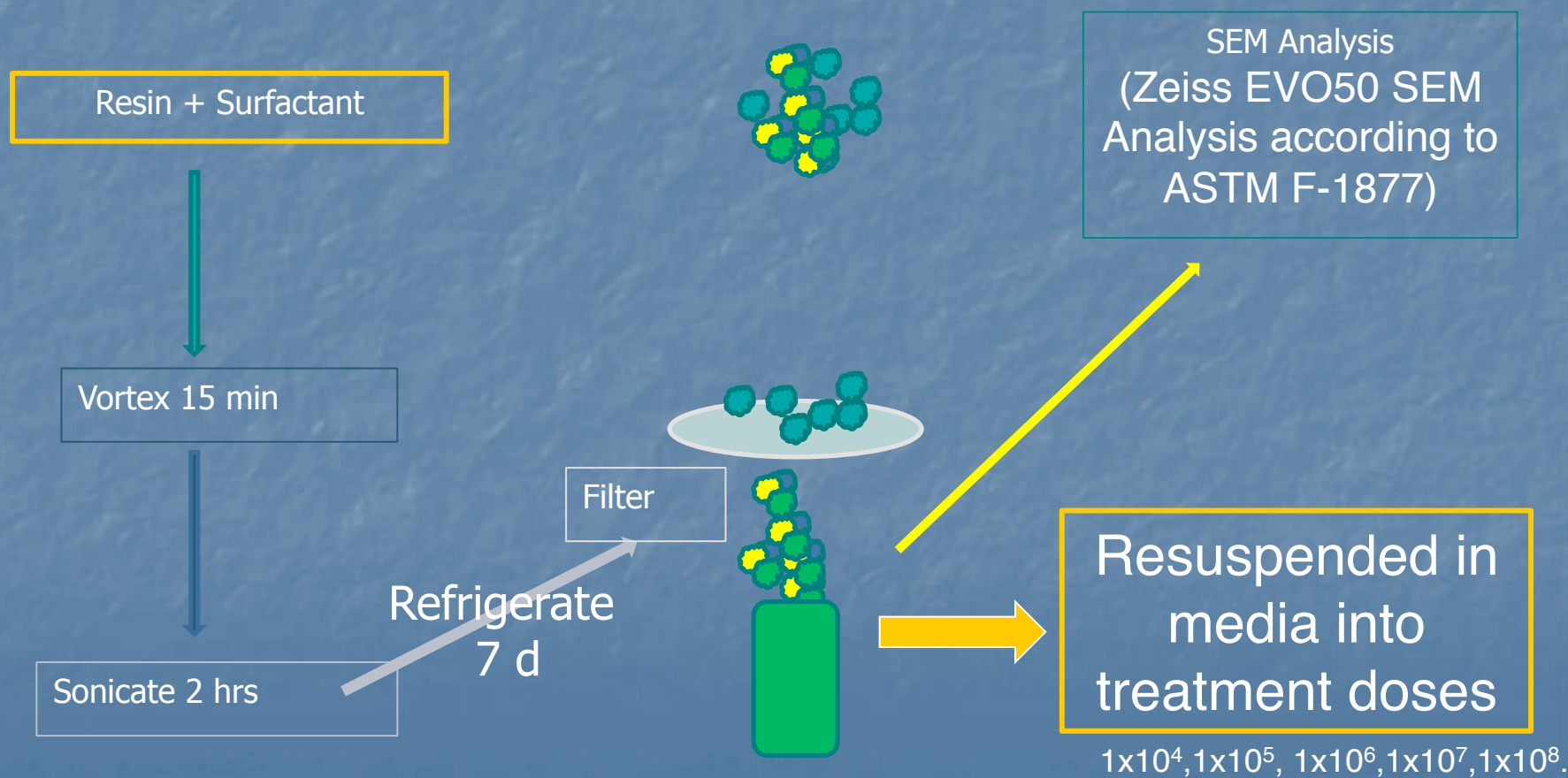
- Develop treatment strategies for existing osteolytic lesions
- Improve the longevity of future implants
- Allow preoperative risk assessment of an individual's response to the anticipated particle load

Outcome Measures

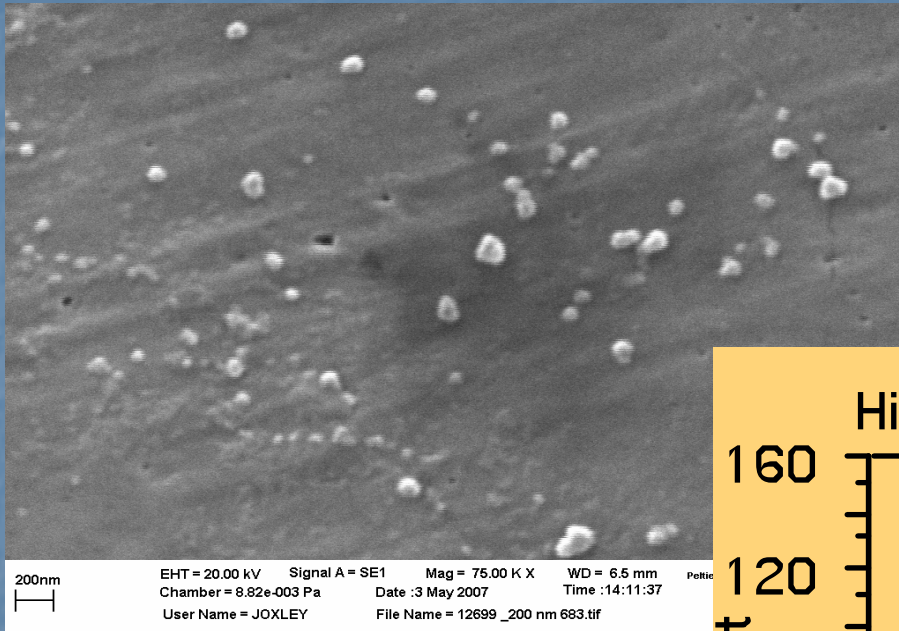
- Proliferation
 - Total Cell Count
- Replication
 - Colony Forming Units - Fibroblast (CFU-F)
- Differentiation Capacity
 - CFU – AD for adipogenesis
 - CFU – OB for osteogenic potential

Particle Preparation

- GUR 1050 suspended in water (pH 5.5) + 500ppm Pluronic



Methods - UHMWPE Particles

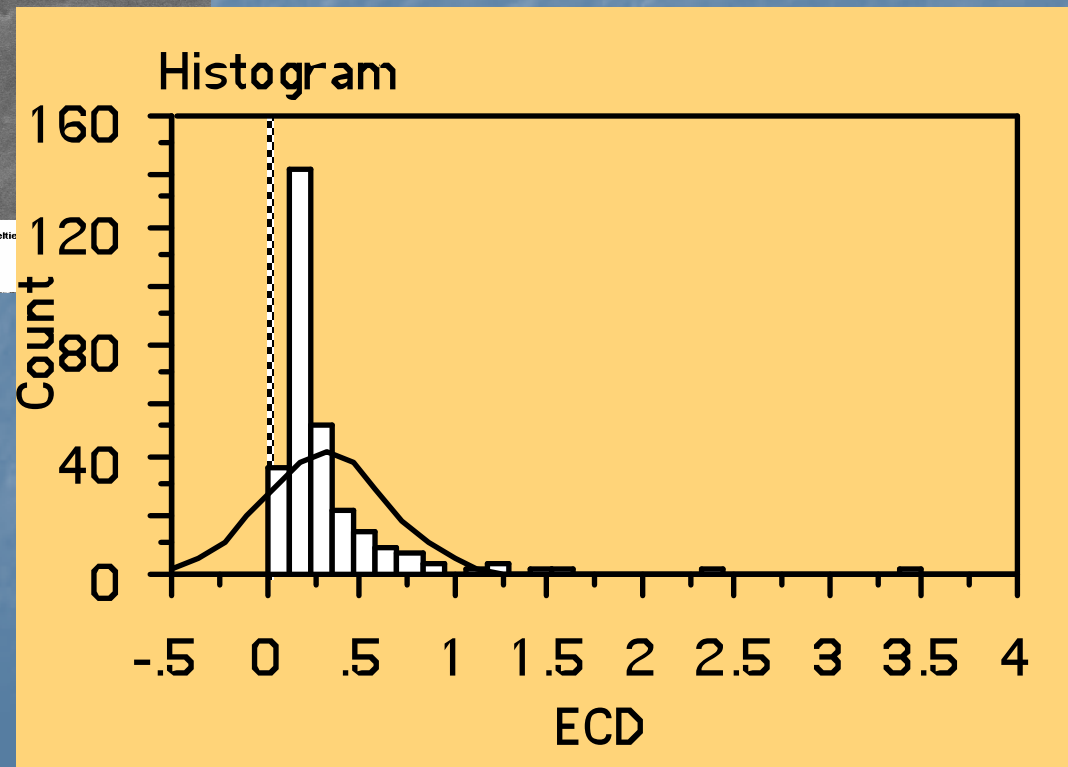


75,000x

Mean particle size =
0.4 μm
{range: 16 nm - 4 μm }

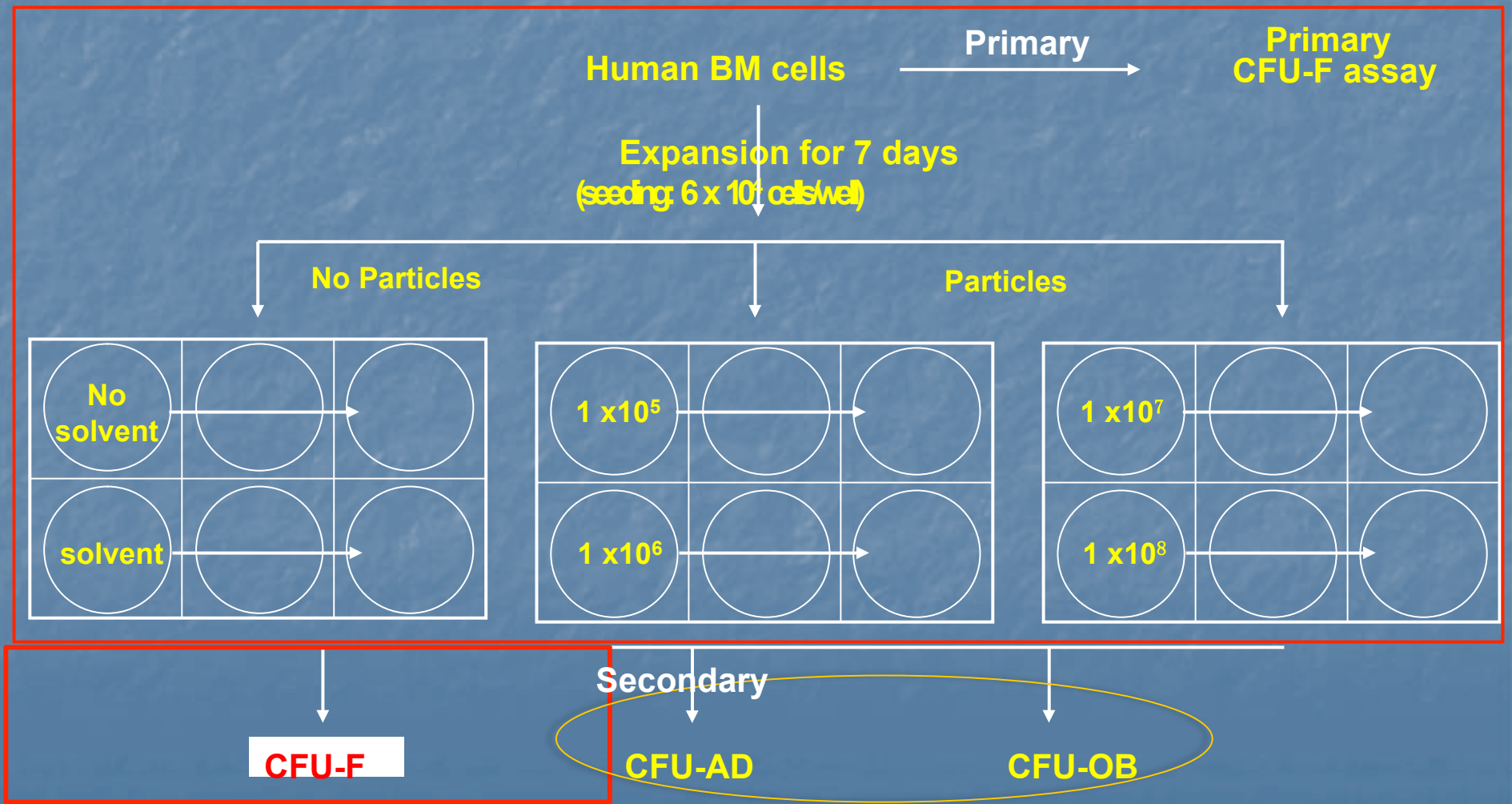
GUR 1050

Suspended in water, pH
5.5, containing 500 ppm
Pluronic



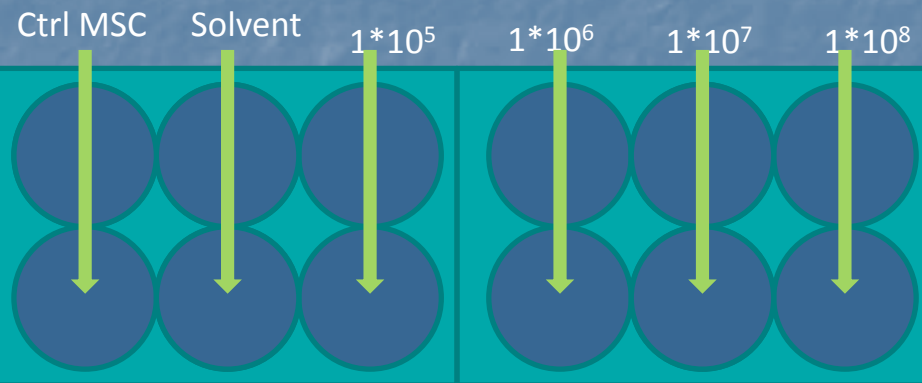
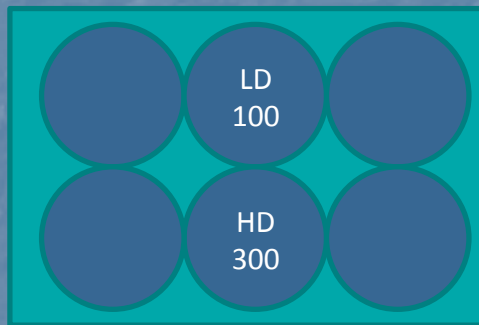
Methods

- Young MSCs
 - ALLCELLS - (Emeryville, CA) Passage 1



Methods

Primary
CFU-F



Secondary
CFU-F



Secondary
CFU-AD

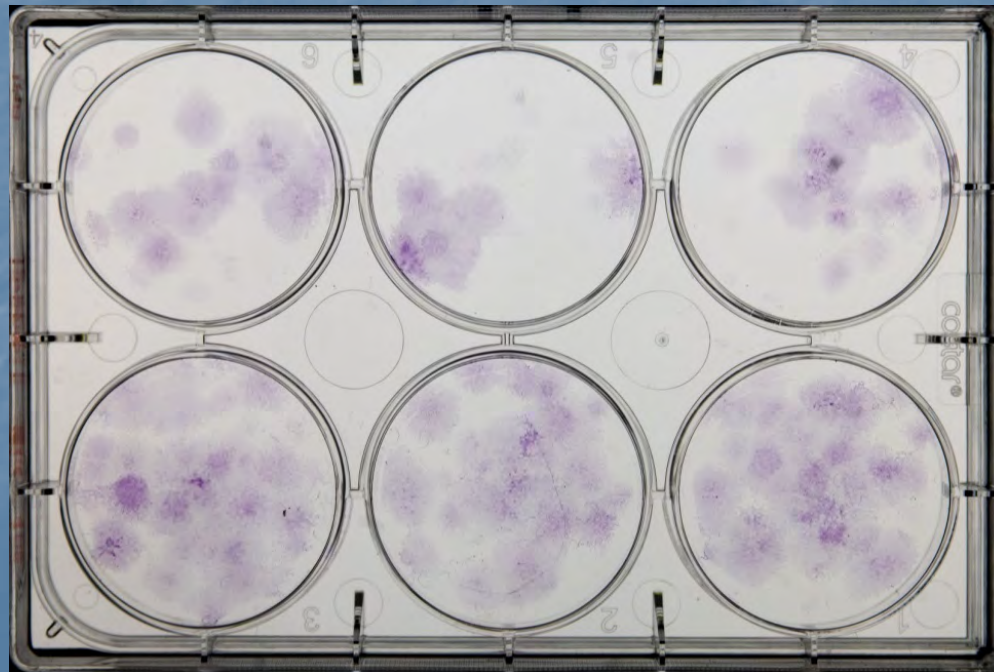


Secondary
CFU-OB



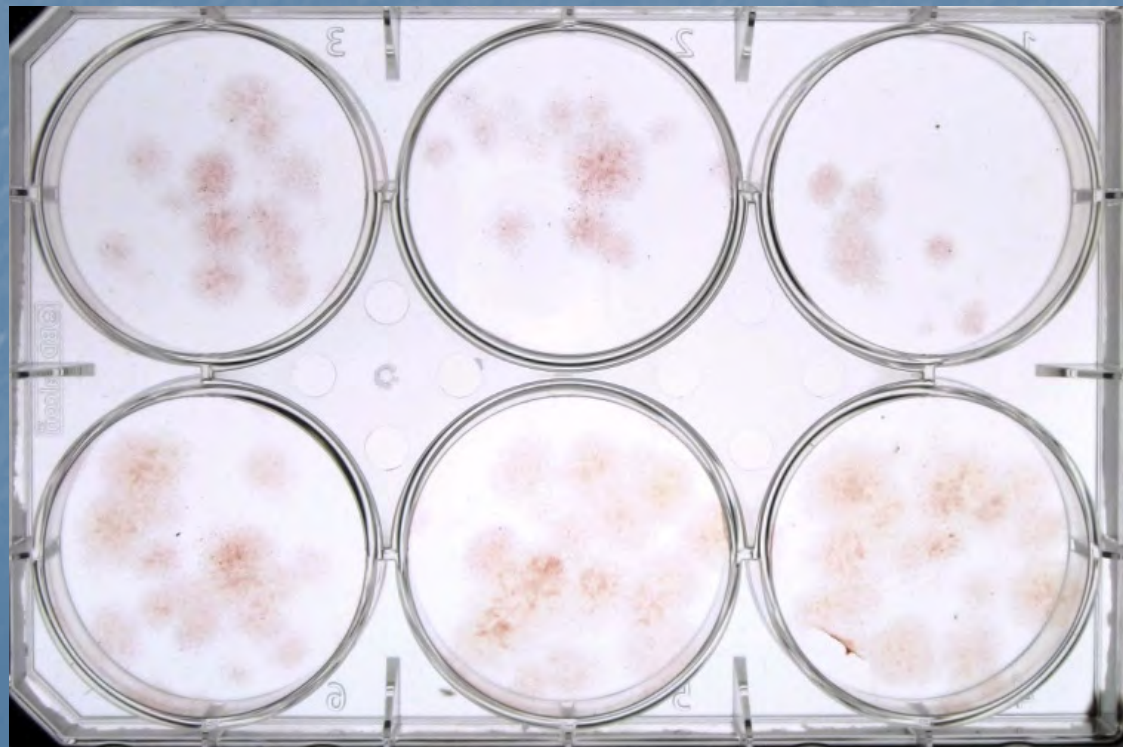
CFU-F Replication Assay

- After 14 days of culture, colonies visualized utilizing crystal violet
- View colony number and size



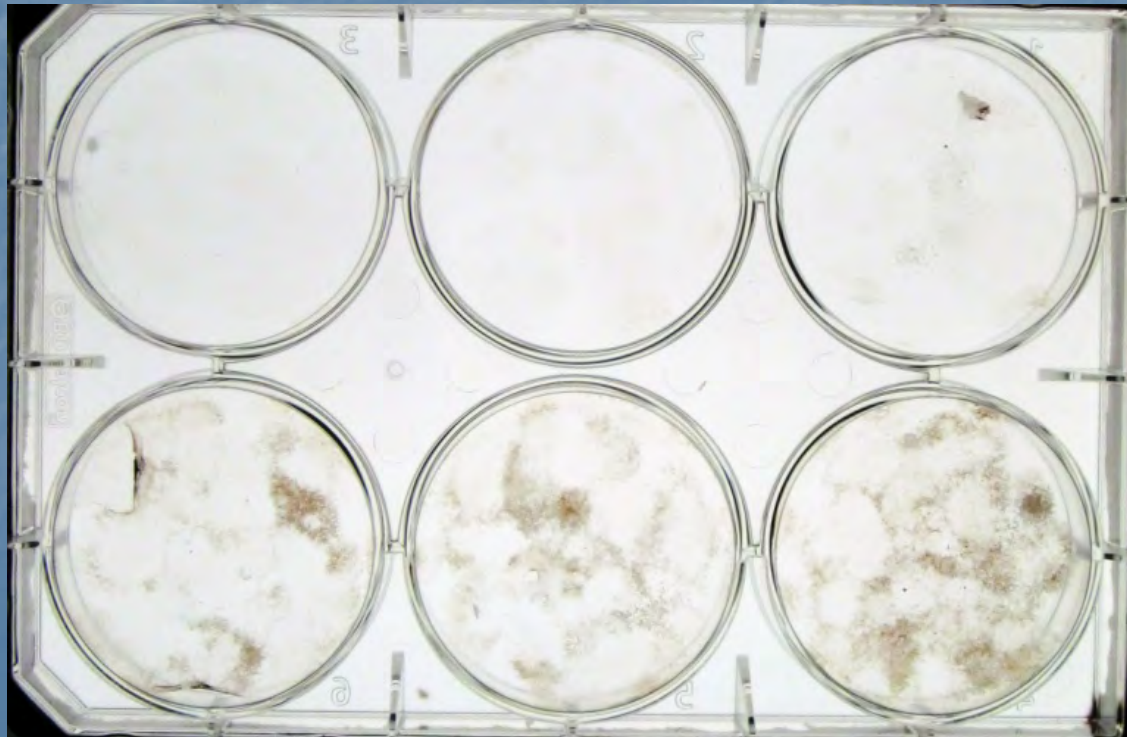
Differentiation Potential - Adipocyte

- CFU – AD
- Stained with Oil-Red-O
- View colony number and size

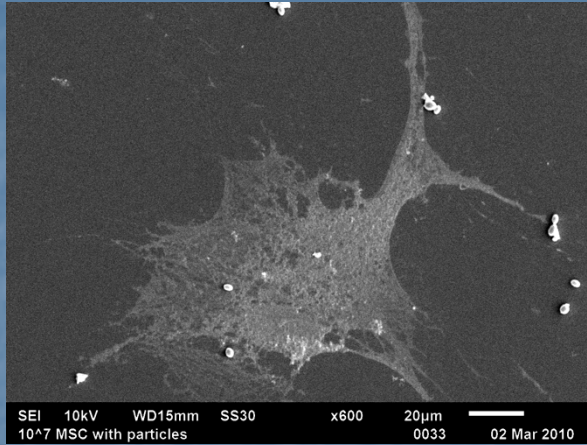


Differentiation Potential – Osteoblastic Lineage

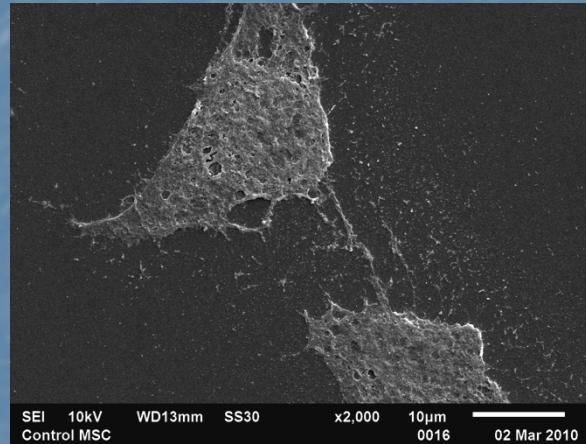
- CFU – OB Assay
- Von Kossa Staining for calcified matrix
- View colony number and size



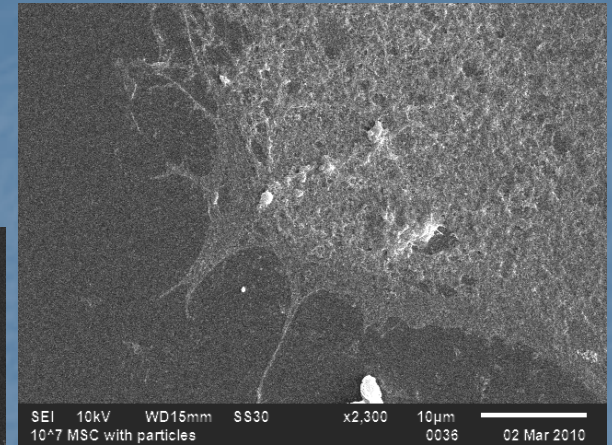
SEM Images



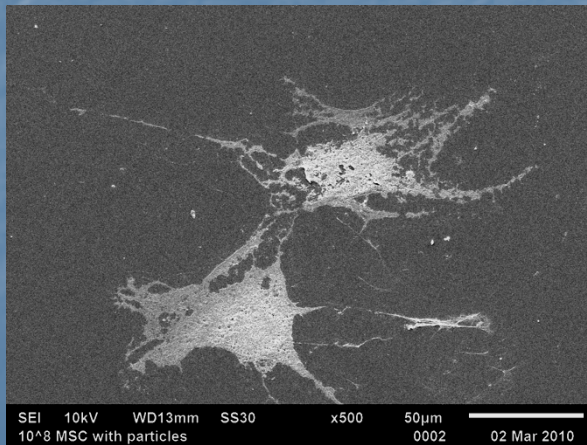
Low mag with 1×10^7 particles



Control without particles

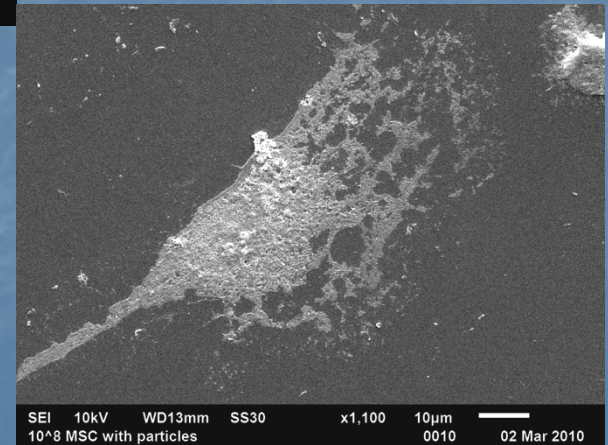


High mag with 1×10^7 particles



Low mag with 1×10^8 particles

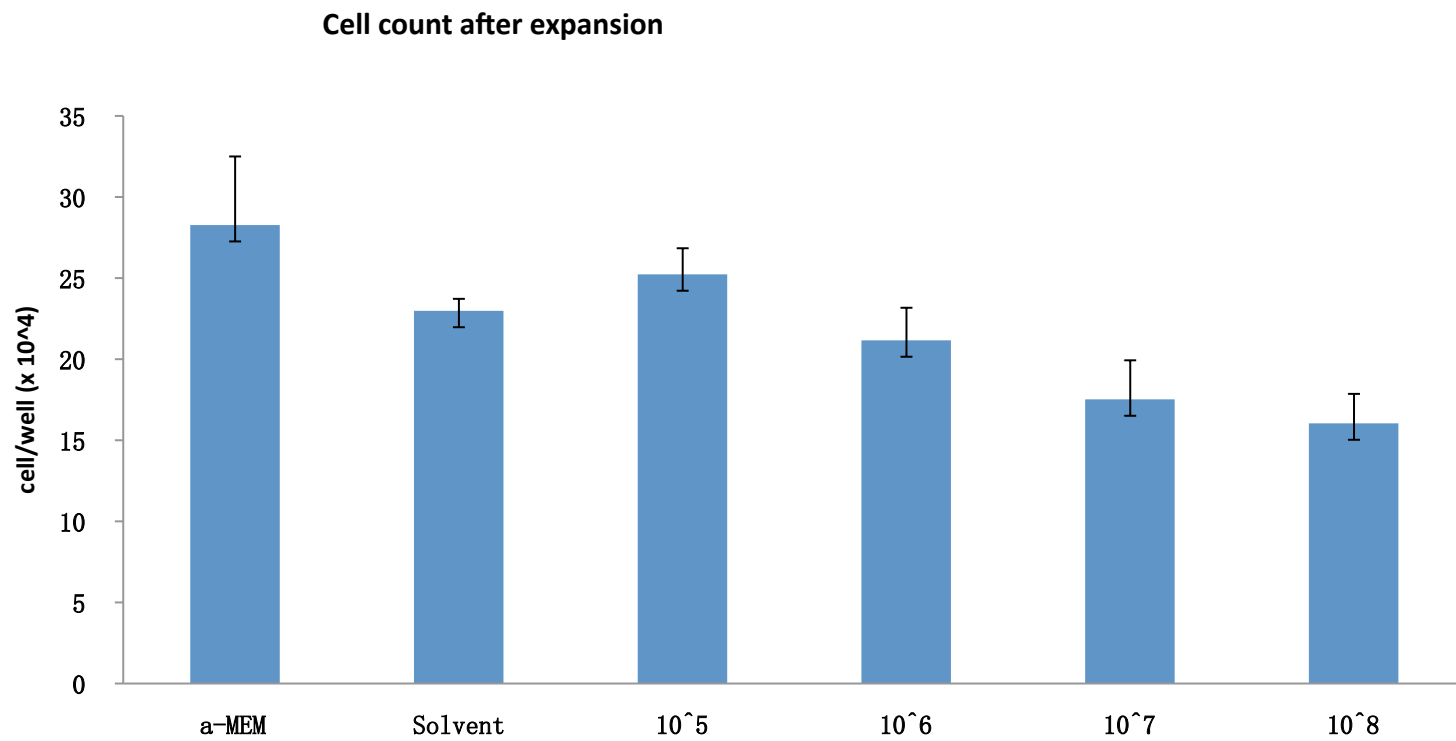
*** Note the cellular disruption at highest treatment dose.**



High mag with 1×10^8 particles



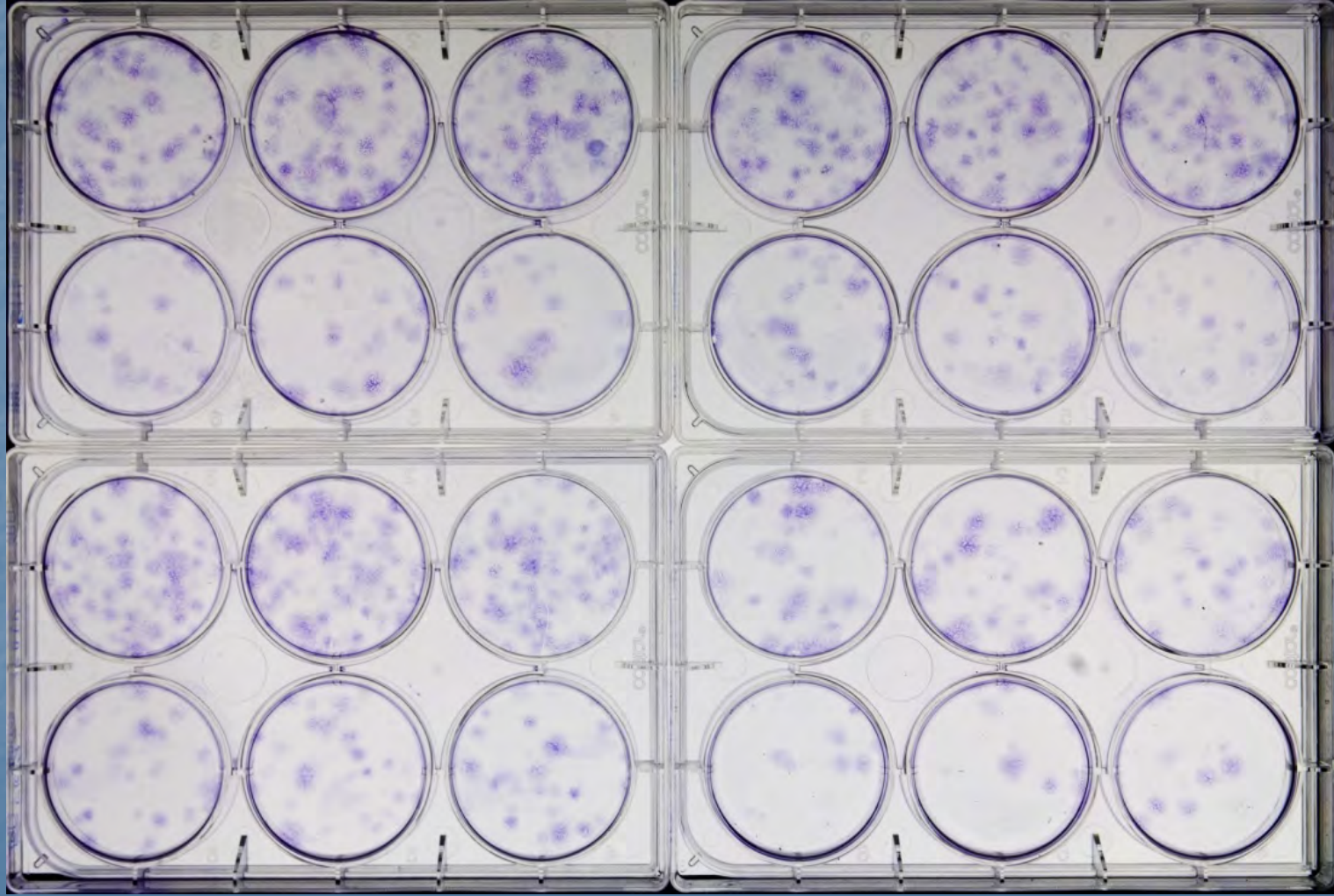
Results – Total Cell Count



Results: CFU-F Replication

10^5

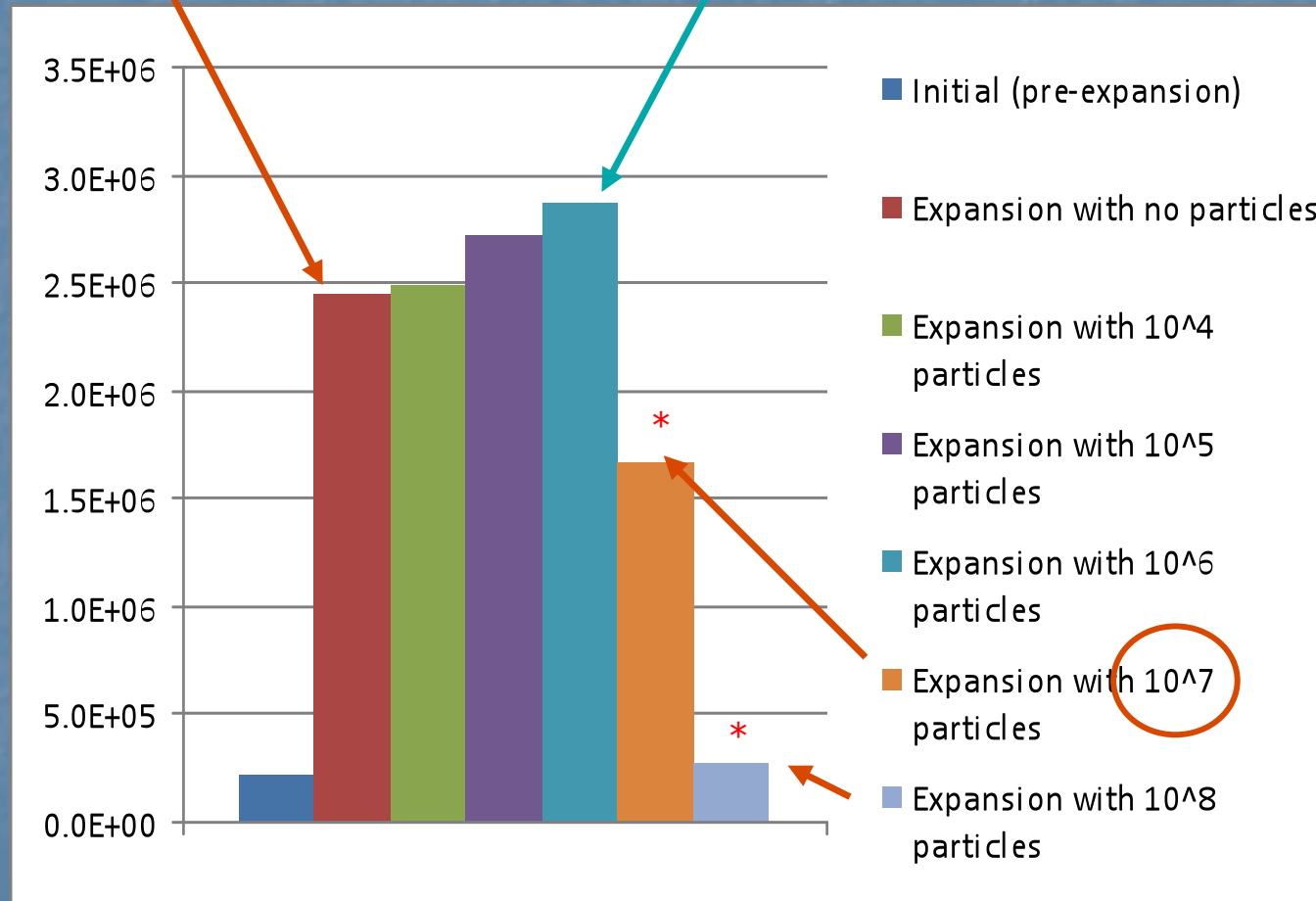
10^7



10^6

10^8

CFU-F Replication



■ Dose- dependent response

* p < 0.05

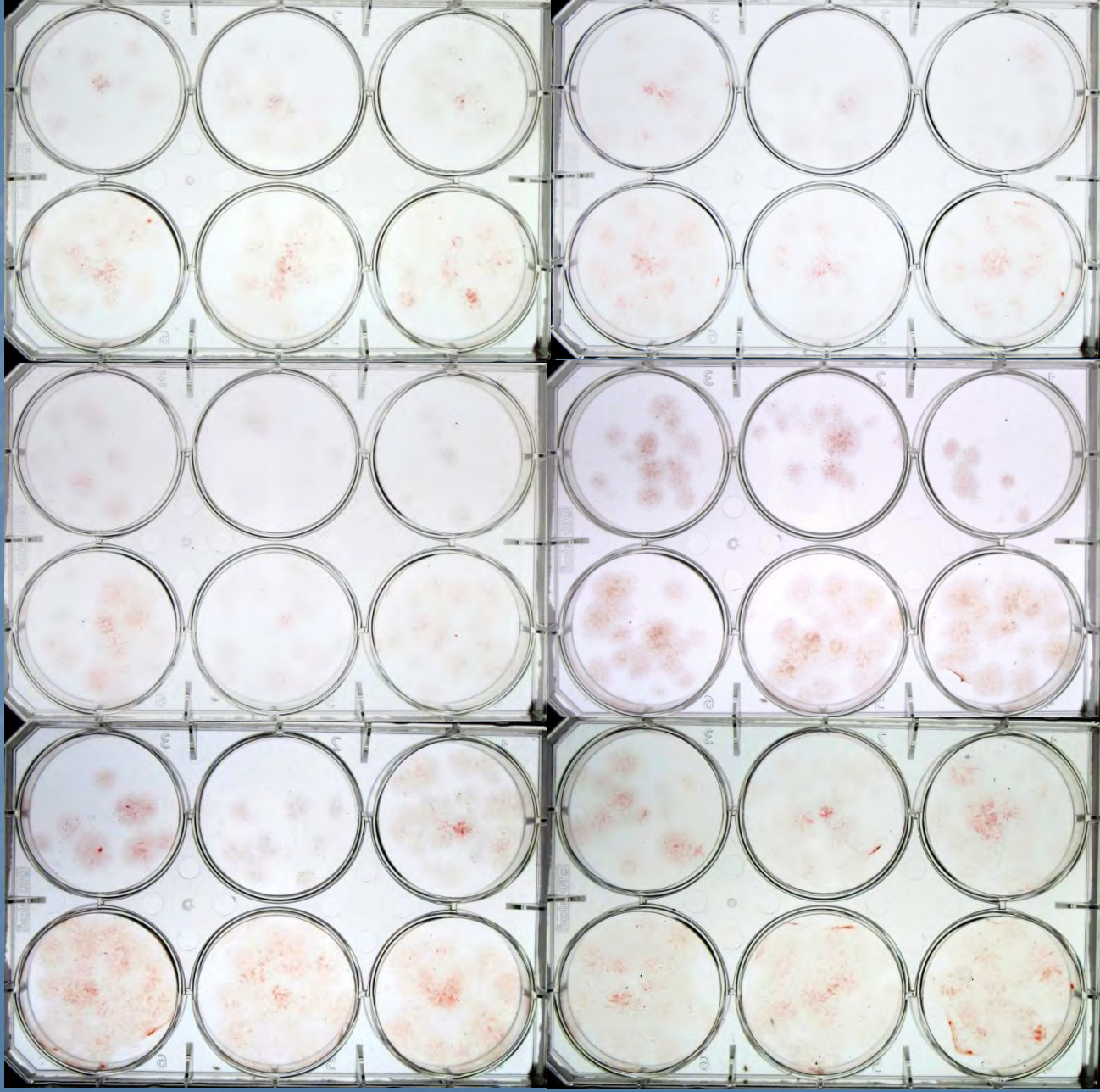
Differentiation Potential

CFU-AD

10^7

10^5

a-MEM

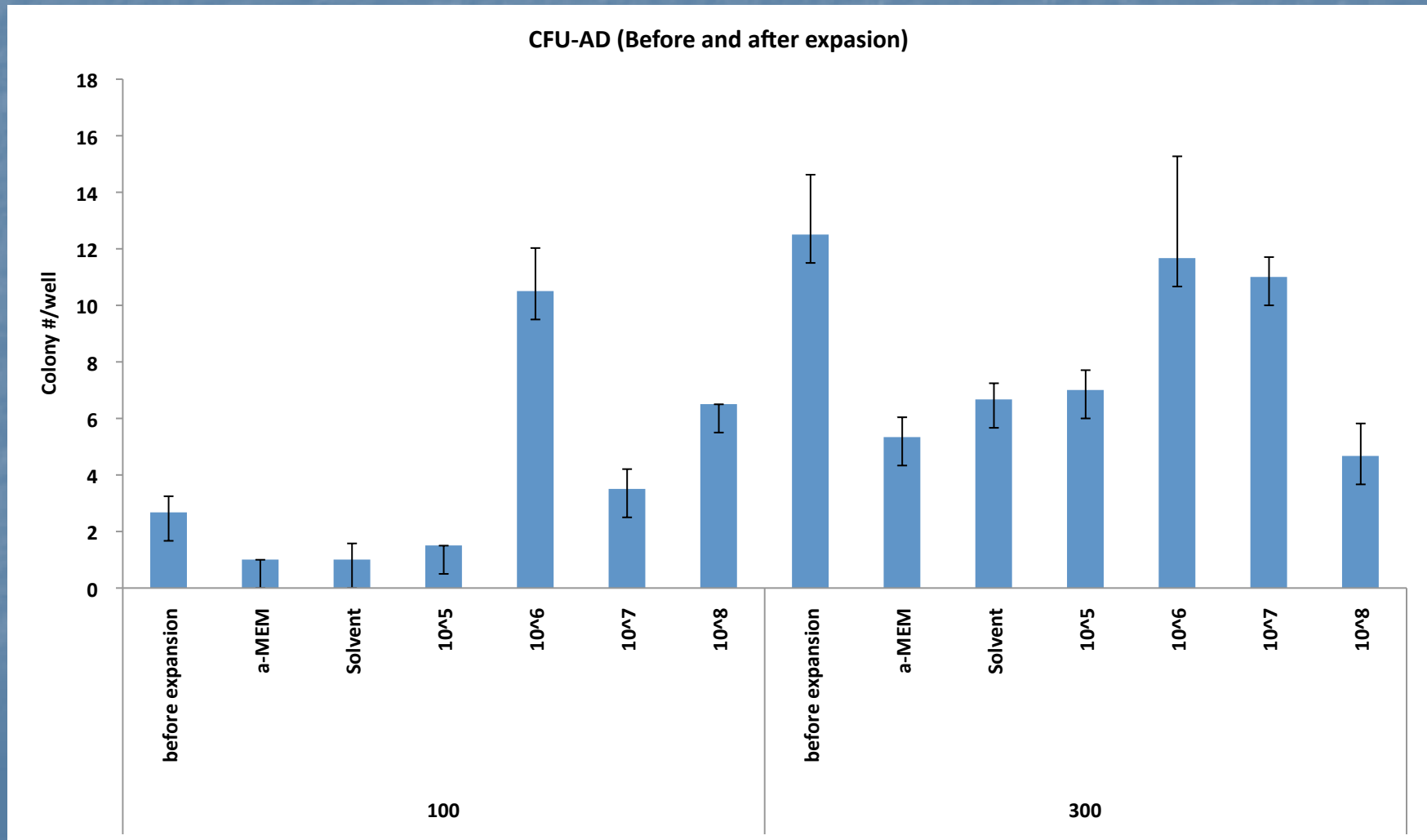


10^8

10^6

Solvent

Results: CFU - AD



Differentiation Potential

CFU-OB

10^7

10^5

a-MEM

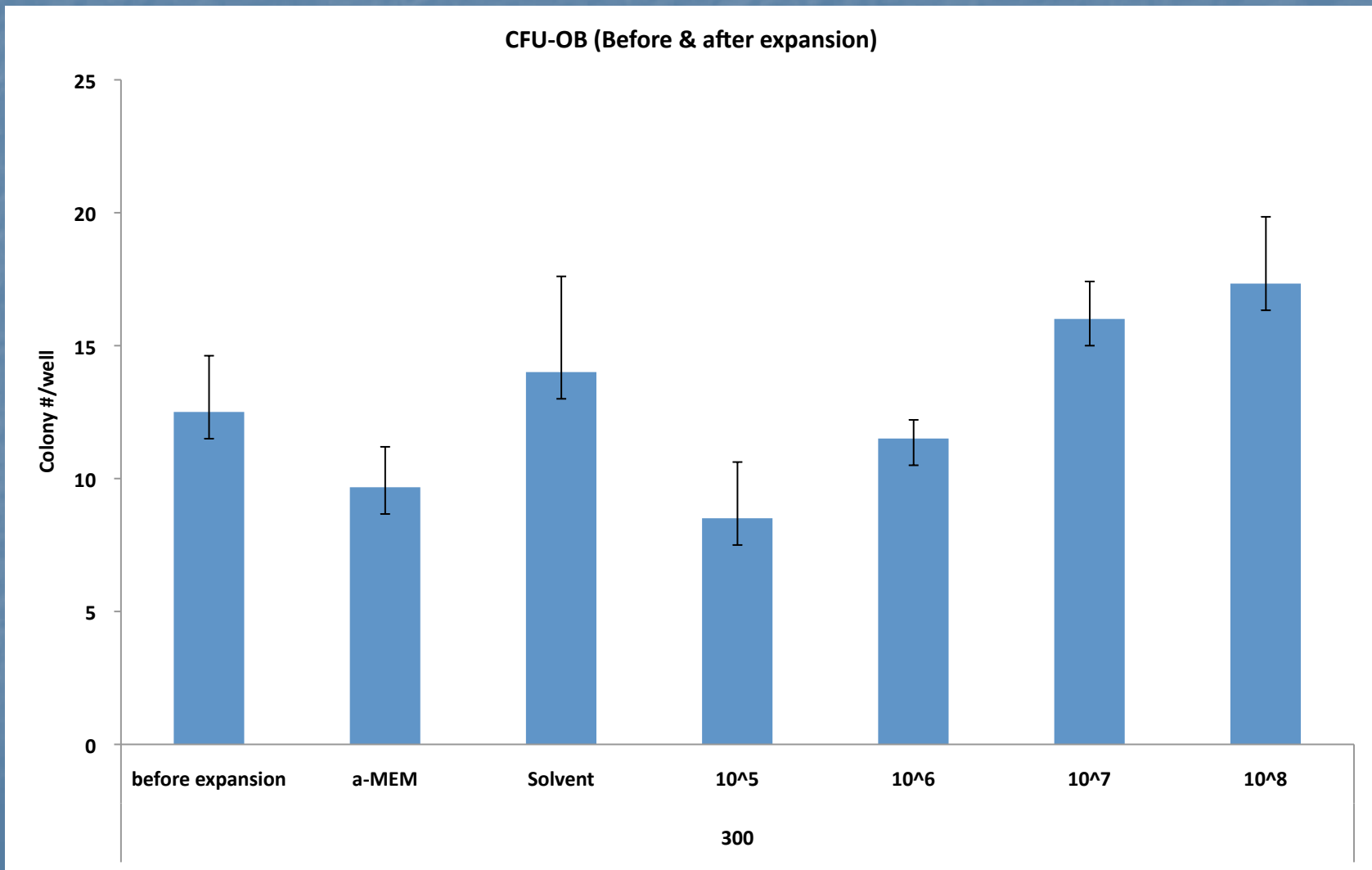


10^8

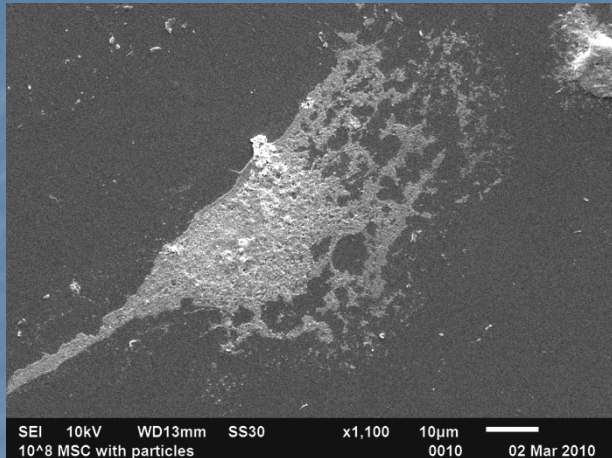
10^6

Solvent

Results: CFU - OB



Conclusions



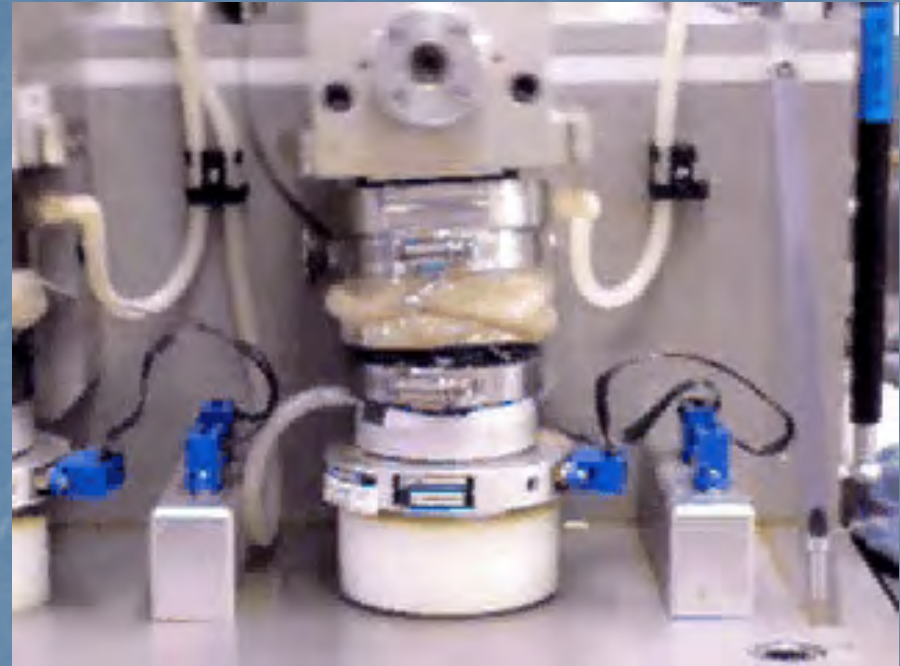
- UHMWPE particles appear to have a direct effect on mesenchymal stem cells
- Revealed a dose-dependent effect of UHMWPE on MSC proliferation
 - Stimulatory up to a threshold dose, then cytotoxic?
- Low dose particle burden with higher adipogenic potential
- Higher dose particle burden with higher osteogenic potential

Limitations

- GUR 1050 Resin, not Wear Debris
 - Simulated Wear Debris
 - Endotoxin Negativity may not translate to actual wear debris
- Cell Surface Markers still under investigation
- Young MSCs

Future Directions

- Wear simulator particles
 - Conventional
 - Cross-linked
 - Vitamin E Poly
- Time Lapse Studies
 - Cellular Uptake Mechanism
- Young vs Aging Stem Cells
- Utilizing Fractionated particles to investigate Size-Dependent Effect
- In vivo Studies



Thank you



- Birthplace of Texas
Independence - 1836



The University of Texas
Health Science Center at San Antonio