Harvesting Bone Marrow Without Centrifugation

Obtaining cells from bone marrow

- Normal method is aspiration and centrifugation.
- There’re multiple systems available: Harvest BMAC, Magellan, Biomet, Celling
- Centrifuge systems work by removing lower density plasma in higher density cells
- High density proliferating cells can be discarded with higher density red cells

Obtaining stem cells from bone marrow

- Aspiration technique is important
- Small volumes of bone marrow taken at 1 ml first sight with each site being at least one centimeter apart
- The pockets in the bone hold only so much bone marrow so taking larger volumes extracts peripheral blood
- Also the needle hole itself causes peripheral blood infiltration

References:
Estimating Quality of BM Aspirate: CFU’s vs. Nucleated cell counts

- CFU’s are the most accurate way of counting true adherent/stem cells
- Fresh BM cell counts are all nucleated cells which could be stem cells, or WBC's, or other various cells
- Many Nucleated cell counters can give you same day counts with cell viability measurements
- A clean cell count can be done relatively quickly and in the clinic setting to give the clinician an idea if he or she has adequate cell counts

Study: Marrow Cellution Bone Marrow Aspiration System and Related Concentrations of Stem and Progenitor Cells

- Five patients seen by the same clinician and laboratory
- Marrow aspiration from iliac crest with Marrow Cellution device from posterior orientation
- 2000 unit per mL heparin rinse used prior to aspiration
- No additional heparin or anti-coagulant used
- Primary endpoints included total nucleated cell (TNC) and fibroblast-like colony forming unit (CFU-f)
- Published literature was used for historical values of CFU-f counts from various centrifuge-based systems

Benefits found from the Study

- Automatically repositions the aspiration cannula and aspirates from side ports, mimicking multiple puncture sites
- Does not require filtering or 1% dilution with anti-coagulant
- TNCs and CFU-f was greater than traditional aspirations and comparable or greater than centrifuge products
- Process remains entirely on sterile field
- Reduced the risk of infection
- Cells and growth factors are not discarded as they are in centrifuge-based systems

References:
3. Marrow Cellution Bone Marrow Aspiration System and Related Concentrations of Stem and Progenitor Cells: Five patients seen by the same clinician and laboratory
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References:
### Marrow Cellutions Device

<table>
<thead>
<tr>
<th>Patient Sample</th>
<th>Nuc. Cells/ml</th>
<th>CFU's/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.46 x 10^7</td>
<td>4200</td>
</tr>
<tr>
<td>2</td>
<td>3.16 x 10^7</td>
<td>3600</td>
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<td>3</td>
<td>2.16 x 10^7</td>
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<td>4</td>
<td>1.95 x 10^7</td>
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<td>5</td>
<td>4.81 x 10^7</td>
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<tr>
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<td>310</td>
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<td>8</td>
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<td>5200</td>
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<tr>
<td>13</td>
<td>5.57 x 10^7</td>
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</tbody>
</table>

### Counts from various devices

<table>
<thead>
<tr>
<th>Volume (ml)</th>
<th>CFU's/ml</th>
<th>Total CFU's in Graft</th>
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<tbody>
<tr>
<td>Marrow Cellutation</td>
<td>11</td>
<td>1290</td>
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<tr>
<td>Ceiling</td>
<td>7</td>
<td>2713</td>
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<tr>
<td>Harvest</td>
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<td>1270</td>
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<tr>
<td>Magellan</td>
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<td>514</td>
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<tr>
<td>Biomet</td>
<td>7</td>
<td>154</td>
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</table>

### Nuc. Cell Count vs. CFU (0.54 correlation)
Using CFUs as a Measure of BM Quality

- Advantages
  - Best approximation of number of MSCs in BM sample
  - Much better than nucleated cell counts (NCCs)
    - These can remain high even with significant peripheral blood contamination
    - NCCs can remain high even when BMSCs are low

- Disadvantages
  - 10-14 days until data acquisition
  - Requires ability to culture cells
Getting Reliable CFU Counts

- Serial dilutions of bone marrow
- RBC may inhibit cell adhesion
- Removal of RBCs
  - RBC lysis buffer
  - ~85% recovery of leukocytes
- Removal of RBCs and Granulocytes
  - Ficoll gradient
  - ~70% recovery of mononuclear cells
- Still need to culture 10-14 days in all cases

Reliable CFU Measurements/Comparison

- Dilution of fresh BM gives highest counts
- Length of time for cells to adhere before washing
  - Long or short
- Media type and media change
- Length of time in culture
- Method for scoring
  - What is a colony?

Getting CFU Counts Without a Lab: Stability of BM During Shipment

- Lack of on-site equipment is not a hindrance to measuring BM quality
- Nucleated cell counts remain stable for days at 22°C
- Blood cell forming CFUs are stable as well
- Stability of CFU-f over time
Assaying Fresh BM to Estimate the CFUs: Flow Cytometry

- Are certain cell populations in BM indicative of the CFU levels?
  - These may be measured immediately
- Identifying Cell Types correlated with high CFU counts
  - What are the best markers in vivo for MSCs
    - CD271+
    - MSCA-1
    - CD34+
  - In culture, MSCs have specific markers
    - CD73+, CD90+, CD34-
  - Markers are varied in fresh BM

Using Cell Types to Estimate BM Quality

- Are certain cell types indicative of more MB and less peripheral blood?
- Are megakaryocytes correlated with CFUs and BM quality?
  - Indicate of more marrow and less peripheral blood
- Are CD34+ cells indicative of BM quality?
  - Marker on hematopoietic stem cells and on some MSCs in vivo
  - Less in peripheral blood

Future Questions

- Are higher nucleated cell counts associated with better outcomes?
- Are higher CFU counts associated with better outcomes?
- Is there a quicker way to reliably measure BM quality?
  - Cell surface markers in BM
  - Cell types in BM
- How does quality of BM change with different techniques?