UPDATE ON STEM CELL AND PROBIOTIC THERAPY FOR NEC

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ILLUSTRATION OF NEWBORN WITH NEC
NEC is still a disease for which there is currently no known cure. Despite over 6 decades of research, the mortality of NEC remains unchanged and is as high as 50%.
Necrotizing enterocolitis (NEC) is a serious bowel disorder that can occur in premature infants. The figure illustrates the factors contributing to NEC, including prematurity, intestinal immaturity, abnormal intestinal microbiota, genetic predisposition, and highly immunoreactive intestinal mucosa. A novel probiotic delivery system is proposed as a potential therapeutic approach to prevent or treat NEC by modulating the gut microbiota and immune response.
A stem cell is a primitive cell with the ability to:

- Reduce Inflammation
- Self Replicate
- Fight Apoptosis (Cell Death)
- Differentiate into Multiple Tissues
Potential uses of Stem cells

- Stroke
- Traumatic brain injury
- Learning defects
- Alzheimer
- Parkinson
- Wound healing
- Bone marrow transplantation (currently established)
- Spinal cord injury
- Osteoarthritis
- Rheumatoid arthritis
- Crohn’s disease
- Baldness
- Blindness
- Muscular dystrophy
- Diabetes
- Multiple sites: Cancers
Many types of SC exist

Many locations of SC

SC can be induced to form many types of cells

Which type of stem cell will **best** protect the intestines from NEC?
HARVESTING STEM CELLS FROM DIFFERENT SOURCES

- Amniotic Fluid-derived MSC (AF-MSC)
- Amniotic Fluid-derived NSC (AF-NSC)
- Bone Marrow-derived MSC (BM-MSC)
- Neonatal Enteric NSC (E-NSC)
### Stem Cell Verification - Flow Cytometry

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Marker</th>
<th>Purpose</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF-MSC</td>
<td>CD29</td>
<td>MSC</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CD49</td>
<td>MSC</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CD90</td>
<td>MSC</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Oct4</td>
<td>Early AF-MSC</td>
<td>Some positivity</td>
</tr>
<tr>
<td></td>
<td>CD11</td>
<td>Hematopoietic SC</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CD45</td>
<td>Hematopoietic SC</td>
<td>-</td>
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<tr>
<td>BM-MSC</td>
<td>CD90</td>
<td>MSC</td>
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</tr>
<tr>
<td>AF-NSC</td>
<td>Nestin</td>
<td>NSC</td>
<td>+</td>
</tr>
<tr>
<td>E-NSC</td>
<td>Nestin</td>
<td>NSC</td>
<td>+</td>
</tr>
</tbody>
</table>
MESENCHYMAL STEM CELL VERIFICATION

Oil Red O staining for adipocytes, 20x

Alizarin Red S staining for calcium in osteocytes, 20x
2) Hypoxia

3) Hypothermia

4) Hypertonic feeds + LPS
Histological Grading:
0 normal
1 epithelial cell lifting or separation
2 necrosis to mid villus level
3 necrosis of entire villus
4 transmural necrosis
ABILITY OF DIFFERENT TYPES OF STEM CELLS TO PREVENT NEC

- Breastfed (n = 10)
- NEC + PBS (n = 62)
- NEC + AF-MSC (n = 42)
- NEC + BM-MSC (n = 48)
- NEC + AF-NSC (n = 37)
- NEC + E-NSC (n = 36)

NEC Incidence (%)

- Grade 4: 61%
- Grade 3: 16%
- Grade 2: 18%

Intraperitoneal Administration

p < 0.0001  p < 0.0001  p < 0.0001  p = 0.0002
GUT BARRIER FUNCTION

Experimental NEC Protocol

- Hypercaloric Feeds
- Hypoxia
- Hypothermia

48 h

4 h

Serum collected

Serum FD-70 Levels Determined
Intraperitoneal Administration

**ABILITY OF DIFFERENT TYPES OF STEM CELLS TO PRESERVE GUT BARRIER FUNCTION IN NEC**

- Breastfed
  - NEC + PBS (n = 35)
  - NEC + AF-MSC (n = 57)
  - NEC + BM-MSC (n = 21)
  - NEC + AF-NSC (n = 24)
  - NEC + E-NSC (n = 22)

- **Serum FD70 concentration (μg/mL)**

  - Breastfed (n = 10)
  - Intraperitoneal Administration

  - p = 0.017
  - p = 0.049
  - p = 0.0496
  - p = 0.033
Although SC engraft into injured intestine in our animal models, the degree of engraftment is relatively low.

SC therapy may face unique challenges.

Mechanisms other than SC engraftment may mediate the beneficial effects of SC.
Paracrine effects:
• Soluble proteins
• Secreted from secretory granules of donor cells
• Affect cells in close proximity by binding to recipient cell surface membrane receptors

Conventional mechanism of cell-to-cell communication

Exosomes as mediators of cell-to-cell communication
• Bi-lipid membrane vesicles 30-100 nm dia
• Contain miRs, mRNAs, proteins
• Released by fusion of multivesicular bodies with donor cell membranes
• Exert local/remote effects on recipient cells
• Present in blood, urine, saliva, breast milk

Requires SC engraftment

SC engraftment not required
HYPOTHESIS - Exosomes mediate the ability of stem cells to protect the intestine from NEC
METHODS

- Stem cells grown 48 hours in starvation medium
- Differential ultra-centrifugation
- Re-suspended in PBS and identity confirmed by nanoparticle tracking analysis
EXOSOME CHARACTERIZATION

**A**

**B**

**C**

**D**

**AF-MSC**

**BM-MSC**

**AF-NSC**

**E-NSC**
EFFECT OF SC-DERIVED EXOSOMES IN NEC

Exosomes purified from SC and labeled with red fluorescent dye

Exosomes IV or IP

Intestinal injury
Hypoxia
Hypothermia
Hypertonic feeds

Xenogen fluorescent imaging

NEC
NEC + exosomes

Histology

NEC
NEC + exosomes
DOSE DEPENDENCY OF EXOSOME THERAPY

- NEC Incidence (%)
- Exosome Concentration (particles / 50 μL)

Lines represent:
- BM-MSC exosomes
- AF-MSC exosomes
- E-NSC exosomes
- AF-NSC exosomes

Graph shows NEC Incidence (%) across different exosome concentrations.
ABILITY OF SC-DERIVED EXOSOMES TO PREVENT NEC

A

NEC Incidence (%)

* p < 0.05

8 x 10^7 SC administered IP

Breastfed (n = 10)

NEC + PBS (n = 28)

NEC + AF-MSC-exosomes (n = 18)

NEC + BM-MSC-exosomes (n = 15)

NEC + AF-NSC-exosomes (n = 14)

NEC + E-NSC-exosomes (n = 9)

0%

10%

20%

30%

40%

50%

60%

70%
COMPARISON OF MSC vs. MSC-DERIVED EXOSOMES IN NEC

Grade 2-4 NEC

Grade 3-4 (severe) NEC
SUMMARY OF EXOSOME STUDIES

- SC-derived exosomes engraft into injured intestine
- Exosomes significantly reduce the incidence and severity of experimental NEC
- Exosomes are as effective as the stem cells from which they are derived in protecting the intestines from NEC
- Lower immunogenic and tumorigenic risk
- Can cross the blood-brain barrier
- Exosomes may represent a novel cell-free therapy for NEC in the future