Published in final edited form as:

J Surg Res. 2017 June 15; 214: 278–285. doi:10.1016/j.jss.2017.03.026.

Evaluating the Efficacy of Different Types of Stem Cells in Preserving Gut Barrier Function in NEC

Christopher J. McCulloh, MD, Jacob K. Olson, MD, Yijie Wang, MS, Jennifer Vu, Sarah Gartner, BS, and Gail E. Besner, MD

Department of Pediatric Surgery, Nationwide Children's Hospital, 700 Children's Drive, Columbus, Ohio, 43205

Abstract

Background—Necrotizing enterocolitis (NEC) is a leading cause of morbidity and mortality in premature infants. Increased intestinal permeability is central to NEC development. We have shown that stem cells (SC) can reduce the incidence and severity of NEC. Our current goal was to investigate the efficacy of four different types of SC in preservation of gut barrier function during NEC.

Materials and Methods—We compared: (1) amniotic fluid-derived mesenchymal SC (AF-MSC); (2) bone marrow-derived MSC (BM-MSC); (3) amniotic fluid-derived neural SC (AF-NSC); and (4) enteric NSC (E-NSC). Premature rat pups received an intraperitoneal injection of 2×10^6 SC or PBS only, and were then subjected to experimental NEC. Control pups were breastfed and not subjected to NEC. After 48h animals received a single enteral dose of FITC-labeled dextran (FD70), were sacrificed 4h later, and serum FD70 concentrations determined.

Results—Compared to breastfed, unstressed pups with intact gut barrier function and normal intestinal permeability (serum FD70 concentration 2.22 ± 0.271 $\mu\text{g/mL}$), untreated pups exposed to NEC had impaired barrier function with significantly increased permeability (18.6 ± 4.25 $\mu\text{g/mL}$, $p=0.047$). Pups exposed to NEC but treated with SC had significantly reduced intestinal permeability: AF-MSC (9.45 ± 1.36 $\mu\text{g/mL}$, $p=0.017$); BM-MSC (6.73 ± 2.74 $\mu\text{g/mL}$, $p=0.049$); AF-NSC (8.052 ± 1.31 $\mu\text{g/mL}$, $p=0.0496$); and E-NSC (6.60 ± 1.46 $\mu\text{g/mL}$, $p=0.033$).

Corresponding author: Gail E. Besner, MD, Nationwide Children's Hospital, Department of Pediatric Surgery, 700 Children's Drive, Columbus, Ohio 43205, gail.besner@nationwidechildrens.org, Phone: (614) 722-3914.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author contributions:

Christopher McCulloh was responsible for design and implementation of the experiments and creation and revision of the manuscript. Jacob Olson contributed to implementation of the experiments as well as review and revision of the manuscript. Yijie Wang assisted with stem cell harvesting and review and revision of the manuscript. Jennifer Vu and Sarah Gartner assisted with implementation of the NEC model and review and revision of the manuscript. As Principal Investigator, Gail Besner oversaw the design and implementation of the experiment, and review and revision of the manuscript.

Disclosure

None of the authors have any conflicts of interest to disclose.

Conclusions—Stem cells improve gut barrier function in experimental NEC. Although all four types of SC reduce permeability equivalently, SC derived from amniotic fluid may be preferable due to availability at delivery and ease of culture, potentially enhancing clinical translation.

Keywords

necrotizing enterocolitis; stem cell; intestine; injury

Introduction

Despite several decades of research, necrotizing enterocolitis (NEC) continues to be a leading cause of morbidity and mortality among premature infants.¹ Several studies in our laboratory and in those of others have demonstrated a reduction in the incidence and severity of experimental NEC *in vivo* through treatment with amniotic fluid-derived mesenchymal stem cells (AF-MSC), bone marrow-derived MSC (BM-MSC), or enteric NSC (E-NSC)^{2–5} (Table 1). It has also been shown that intraperitoneal injection of conditioned medium from AF-MSC is effective in treating experimental NEC.⁵ We have recently compared the following four different types of stem cells and shown them to all have equivalent efficacy in decreasing histologic injury during experimental NEC: AF-MSC, amniotic fluid-derived NSC (AF-NSC), BM-MSC, and E-NSC.⁶ However, no study has investigated the ability of different types of stem cells to maintain gut barrier function. Our goal in this study was to determine whether different stem cell types vary in their ability to preserve intestinal mucosal integrity during experimental NEC.

Materials and Methods

Four different types of stem cells were compared: (1) amniotic fluid-derived mesenchymal stem cells (AF-MSC); (2) bone marrow-derived MSC (BM-MSC); (3) amniotic fluid-derived neural stem cells (AF-NSC); and (4) neonatal enteric NSC (E-NSC) (Figure 1). All cell lines were derived from Lewis rats.

2.1 AF-MSC Culture

Amniotic fluid (AF) was harvested from timed pregnant rats on day 14.5 of gestation via aspiration of amniotic sacs using a 25G needle, based on previously described procedures.^{7,8} AF was placed into T175 flasks in culture media composed of: Minimum Essential Medium Alpha with GlutaMAX™ (MEM-α, ThermoFisher, Waltham, MA), supplemented with 10% Embryonic Stem Cell Qualified Fetal Bovine Serum (ES-FBS, ThermoFisher), 18% Chang B and 2% Chang C (Irvine Scientific, Santa Ana, CA), and 1% penicillin/streptomycin/amphotericin B (PSA, ThermoFisher). Cells were passaged in a 1:4 ratio upon reaching 80–90% confluence by removing non-adherent cells and debris with PBS, followed by addition of 0.25% Trypsin-EDTA (Trypsin, ThermoFisher), which was inactivated after 90 seconds by addition of complete medium, followed by centrifugation at 400g for 5 minutes. Cells from passages 4–9 were used for experimental studies.

2.2 BM-MS C Culture

Bone marrow was harvested from the femurs and tibias of dams at the same time as amniotic fluid was harvested, and cultured as previously documented.^{9–11} Bones were cleared of surrounding tissue and the ends clipped with bone cutting forceps. Marrow was flushed with MEM- α and placed directly into T175 flasks in culture media composed of: MEM- α , 10% Mesenchymal Stem Cell Qualified Fetal Bovine Serum (MSC-FBS, ThermoFisher), and 1% PSA. Cells were passaged in a 1:4 ratio at 80–90% confluence using the same process used for AF-MS C, with cells from passages 4–9 used in all experimental studies.

2.3 AF-NSC Culture

Amniotic fluid was harvested as described for AF-MS C. Using a modification of previously described procedures, AF was placed into culture flasks containing NSC medium composed of Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12, ThermoFisher), supplemented with 4% chicken embryo extract (Gemini Bio-Products, West Sacramento, CA), 2% PSA, 1X N-2 supplement (ThermoFisher), 20ng/ml recombinant fibroblast growth factor basic (FGF, R&D Systems, Minneapolis, MN), and 20 ng/ml recombinant rat epidermal growth factor (EGF, R&D Systems).^{12,13} Medium was changed every four days, during which time NSC aggregates known as neurospheres formed. After two weeks of growth, these neurospheres were then dissociated to individual NSC using a combination of chemical dissociation with StemPro Accutase (ThermoFisher) and mechanical dissociation via gentle pipetting.

2.4 E-NSC Culture

E-NSC were obtained from the small intestines of rat pups 4–7 days of age as in previous studies.^{2,4,14,15} Briefly, the entire small intestine was harvested from duodenum through ileum and opened longitudinally, allowing the mucosa and submucosa to be stripped away. The muscular layer was then digested using 1mg/mL Collagenase and 1mg/mL Dispase (Worthington Biochemical Corporation, Lakewood, NJ) in DMEM/F12 for 45–60 minutes. The resulting solution was filtered through a 70 μ m filter, centrifuged, and resuspended in NSC medium. Media was changed every four days, and observed for the formation of neurospheres that were processed in the same fashion as for AF-NSC.

2.5 Stem Cell Verification

Flow cytometry was used to confirm known stem cell markers on all cell populations. AF-MS C were confirmed to be positive for CD29, CD49, CD90, and Oct4. CD29, CD49, and CD90 are surface markers of MSC derived from amniotic fluid. Oct4 is a transcription factor suggestive of early stemness, and positivity of some cells was observed. These cells were also confirmed to be negative for CD11 and CD45 (surface markers of hematopoietic stem cells). BM-MS C cells were confirmed to be positive for CD90, again indicating MSCs. These cells were also negative for CD11 and CD45. Both AF-NSC and E-NSC cell populations were confirmed via flow cytometry to be positive for the presence of Nestin, an intermediate filament protein transiently expressed only in NSC but not in mature cells of neural origin.

Additionally, multipotency of AF-MSC and BM-MSC populations was confirmed via adipogenic and osteogenic induction using the StemPro® Adipogenesis Differentiation Kit (ThermoFisher) and StemPro® Osteogenesis Differentiation Kit (ThermoFisher) as previously described.⁶ Cells induced to form adipocytes stained positively using Oil Red O, confirming the presence of lipid droplets, and cells induced to form osteocytes stained positively using Alizarin Red S, indicating calcium deposition in cells induced to form osteocytes (Figure 2).

2.6 Experimental NEC Model

All animal studies were conducted in compliance with protocol #AR15-00012 approved by the IACUC of The Research Institute at Nationwide Children's Hospital. The model used in our laboratory has been extensively documented and is based on a modification of the original model of *Barlow et al.*^{16,17} (Figure 3). Briefly, Sprague-Dawley rat pups were delivered via C-section approximately one-half day prematurely from timed-pregnant dams (Envigo, Indianapolis, IN). Pups were then randomized to one of six groups: (1) Breastfed ($n=10$); (2) NEC + 1% phosphate-buffered saline (PBS, Corning, Manassas, VA) ($n=35$); (3) NEC + AF-MSC ($n=57$); (4) NEC + BM-MSC ($n=21$); (5) NEC + AF-NSC ($n=24$); (6) NEC + E-NSC ($n=22$). All pups exposed to NEC received either a single intraperitoneal (IP) injection of 2×10^6 stem cells in PBS, or PBS alone. Pups were subjected to repeated episodes of hypoxic and hypothermic stress every 8 h. Hypoxia was induced by placement into a chamber containing N₂ gas calibrated to FIO₂ < 1.5% for a duration of 90 seconds. Pups were placed into a 4°C environment for 10 minutes to induce hypothermic stress. Additional stress was induced via hypercaloric gastric gavage feeds of Esbilac milk replacer (PetAg, Hampshire, IL) fortified with Similac 60/40 (Ross Pediatrics, Columbus, OH) every 4 h, for a total of 836.8kJ/kg per day. On the first day of the model, pups also received a single oral dose of 2 mg/kg lipopolysaccharide (LPS, Sigma-Aldrich, St. Louis, MO). Breastfed pups were placed with a surrogate dam immediately after delivery and not exposed to experimental stresses.

2.7 Permeability Assay

After 48 h, pups in all groups received a single enteral dose of fluorescein isothiocyanate-labeled dextran (FD70, molecular weight 70,000, 750mg/ml, Sigma-Aldrich) in 100μL of PBS. Four hours later, all pups were sacrificed via decapitation and serum collected into BD Microtainer SST tubes (Becton, Dickinson and Company, Franklin Lakes, NJ), inverted a minimum of twenty times, and allowed to stand for a minimum of 30 min before centrifugation at 2000g for 15 min. The resulting cell-free serum was then diluted into 270μL of PBS, mixed, and placed into three separate wells in a 96-well plate for reading in a fluorescent plate reader with filter cutoffs of 492/518nm. Serum samples with a volume less than 50μL were discarded and not analyzed. The values read in each of the three samples were averaged and compared to a standardized curve determined by serial dilutions of the original known-concentration FD70 solution to determine serum concentrations.

2.8 Statistical Analyses

All values are reported as mean $\pm$ SEM. Statistical significance of FD70 levels was assessed using one-way ANOVA followed by Student's t-test for pairwise comparison. *P* values < 0.05 were considered statistically significant.

Results

Breastfed pups not exposed to experimental NEC had intact gut barrier function with normal intestinal permeability (serum FD70 concentration 2.22 ± 0.271 $\mu\text{g/mL}$) (Figure 4). Pups exposed to experimental NEC that received IP PBS alone had impaired gut barrier function with significantly increased intestinal permeability (18.6 ± 4.25 $\mu\text{g/mL}$, $p=0.0005$).

Compared to pups treated with PBS alone, pups treated with SC had significantly reduced intestinal permeability as follows: AF-MSc (9.45 ± 1.35 $\mu\text{g/mL}$, $p=0.017$); BM-MSc (6.73 ± 2.74 $\mu\text{g/mL}$, $p=0.049$); AF-NSC (8.052 ± 1.31 $\mu\text{g/mL}$, $p=0.0496$); and E-NSC (6.60 ± 1.46 $\mu\text{g/mL}$, $p=0.033$). When comparing each of the stem cell groups to one another, no significant differences were found in intestinal permeability.

Discussion

The morbidity and mortality of NEC continue to be significantly high despite improvements in neonatal care and improved understanding of the pathology of the disease.^{1,18} We have previously shown that the four stem cell types investigated in this study equivalently reduce the incidence and severity of NEC from 61% to 19–23%.⁶ However, it remains unclear how these stem cells that are from different sources lead to similarly improved outcomes. We have previously demonstrated that different types of stem cells can affect different areas of the intestine, with transplanted NSCs localizing within the myenteric plexus rather than within regions of epithelial origin.⁴ Based on these observations, we sought to determine whether differences in SCs would lead to differences in mucosal barrier protection. However, the current study suggests that different types of stem cells equivalently preserve intestinal barrier function through mechanisms that remain unclear. *Zani et al.* showed that AF-MSc exert their effects through a COX-2-dependent pathway that modulates the stromal cells themselves.^{5,19} We and others have previously demonstrated that engraftment levels of different types of SC (AF-MSc, BM-MSc, and E-NSC) are higher in injured tissues than non-injured tissues, however overall engraftment rates are relatively low and may not account for the protective effects of SC even in injured tissues.^{2,5} We are currently investigating the patterns of engraftment between these different stem cell populations, with the goal of identifying differences in how these cells incorporate into intestinal tissue.

Our current results demonstrate that treatment with different types of SC preserves and maintains the integrity of the intestinal barrier during injury. However, it is unclear whether the enhanced gut barrier function observed is due to pathways that are shared or different between the different stem cell types. Previous studies have shown that stem cells can modulate inflammatory cytokines during injury, which may result in improved healing and decreased apoptosis in injured tissues.²⁰ These anti-inflammatory pathways may be essential to the improved intestinal barrier function seen in all cell types in this study.

While we have shown that stem cell therapy can improve gut barrier function in NEC, there are a number of challenges posed with bringing this therapy to the bedside. Stem cells have the potential to trigger tumorigenic and immunologic responses in humans that present a significant challenge to translation.²¹ In addition, in previous studies, we have demonstrated significant entrapment of stem cells in the lungs of newborn rat pups with intravenous administration.²² Due to many challenges faced by stem cell therapy, we are currently investigating the potential for non-cell-based therapies for NEC. One very attractive possibility is the use of stem cell-derived exosomes. Exosomes are nanovesicles ~40–100 nm in size that contain DNA, RNA, microRNA and proteins.²³ These nanovesicles are released by all cells in the body via exocytosis and act as intercellular messengers that can affect changes in both neighboring and remote cells throughout the body. We have previously shown that BM-MSC-derived exosomes have the same protective effect on the intestines during experimental NEC compared to BM-MSC themselves.³ Our future studies will investigate exosomes derived from different stem cell types to identify an optimal cell-free treatment for NEC.

Conclusions

All four stem cell types investigated in this study equivalently improve gut barrier function and reduce intestinal permeability in an animal model of NEC. This is consistent with prior experiments in which we have shown that all four of these types of stem cells equivalently reduce the incidence and severity of experimental NEC. Although the efficacy of protection afforded by these different cells appears equivalent, further work is needed to elucidate the pathways and mechanisms by which these different stem cell types exert their effects to determine whether they are common or divergent between different types of SC. It is our hope that in the future, stem cell-based therapy using stem cells themselves or their secreted products may be used to prevent damage to the intestine with loss of intestinal mucosal barrier function due to NEC (Figure 5).

Acknowledgments

We would like to acknowledge Satoru Otsuru MD, PhD, Nationwide Children's Hospital Center for Childhood Cancer and Blood Diseases, for help in developing the BM-MSC culture protocol.

Funding

This work was supported by the National Institutes of Health Diversity Supplement Grant and NIH R01 GM113236.

References

1. Neu J, Walker WA. Necrotizing Enterocolitis. *N Engl J Med*. 2011; 364(3):255–264. DOI: 10.1056/NEJMr1005408 [PubMed: 21247316]
2. Wei J, Zhou Y, Besner GE. Heparin-binding EGF-like growth factor and enteric neural stem cell transplantation in the prevention of experimental necrotizing enterocolitis in mice. *Pediatr Res*. 2015; 78(1):29–37. DOI: 10.1038/pr.2015.63 [PubMed: 25806717]
3. Rager TM, Olson JK, Zhou Y, Wang Y, Besner GE. Exosomes secreted from bone marrow-derived mesenchymal stem cells protect the intestines from experimental necrotizing enterocolitis. *J Pediatr Surg*. 2016; 51(6):942–947. DOI: 10.1016/j.jpedsurg.2016.02.061 [PubMed: 27015901]

4. Zhou Y, Yang J, Watkins DJ, et al. Enteric nervous system abnormalities are present in human necrotizing enterocolitis: potential neurotransplantation therapy. *Stem Cell Res Ther.* 2013; 4(6): 157.doi: 10.1186/scrt387 [PubMed: 24423414]
5. Zani A, Cananzi M, Fascetti-Leon F, et al. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotizing enterocolitis via a COX-2 dependent mechanism. *Gut.* 2014; 63(2):300–309. DOI: 10.1136/gutjnl-2012-303735 [PubMed: 23525603]
6. McCulloh CJ, Olson JK, Zhou Y, Wang Y, Besner GE. Stem Cells and Necrotizing Enterocolitis: A Direct Comparison of the Efficacy of Multiple Types of Stem Cells. *J Pediatr Surg.*
7. Arnhold S, Glüer S, Hartmann K, et al. Amniotic-Fluid Stem Cells: Growth Dynamics and Differentiation Potential after a CD-117-Based Selection Procedure. *Stem Cells Int.* 2011; 2011:1–12. DOI: 10.4061/2011/715341
8. De Coppi P, Bartsch G, Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol.* 2007; 25(1):100–106. DOI: 10.1038/nbt1274 [PubMed: 17206138]
9. Ito M, Kuroda S, Sugiyama T, et al. Transplanted bone marrow stromal cells protect neurovascular units and ameliorate brain damage in stroke-prone spontaneously hypertensive rats. *Neuropathology.* 2012; 32(5):522–533. DOI: 10.1111/j.1440-1789.2011.01291.x [PubMed: 22239468]
10. Otsuru S, Gordon PL, Shimono K, et al. Transplanted bone marrow mononuclear cells and MSCs impart clinical benefit to children with osteogenesis imperfecta through different mechanisms. *Blood.* 2012; 120(9):1933–1941. DOI: 10.1182/blood-2011-12-400085 [PubMed: 22829629]
11. Vemuganti G, Poliseti N, Chaitanya V, Babu P. Isolation, characterization and differentiation potential of rat bone marrow stromal cells. *Neurol India.* 2010; 58(2):201.doi: 10.4103/0028-3886.63789 [PubMed: 20508336]
12. Zhou Y, Besner G. Transplantation of amniotic fluid-derived neural stem cells as a potential novel therapy for Hirschsprung's disease. *J Pediatr Surg.* 2016; 51(1):87–91. DOI: 10.1016/j.jpedsurg.2015.10.016 [PubMed: 26597391]
13. Hartmann K, Raabe O, Wenisch S, Arnhold S. Amniotic fluid derived stem cells give rise to neuron-like cells without a further differentiation potential into retina-like cells. *Am J Stem Cells.* 2013; 2(2):108–118. <http://www.ncbi.nlm.nih.gov/pubmed/23862099>. Accessed November 21, 2016. [PubMed: 23862099]
14. Hagl CI, Heumüller-Klug S, Wink E, Wessel L, Schäfer K-H. The Human Gastrointestinal Tract, a Potential Autologous Neural Stem Cell Source. Zheng JC, ed. *PLoS One.* 2013; 8(9):e72948.doi: 10.1371/journal.pone.0072948 [PubMed: 24023797]
15. Rauch U, Hänsgen A, Hagl C, Holland-Cunz S, Schäfer K-H. Isolation and cultivation of neuronal precursor cells from the developing human enteric nervous system as a tool for cell therapy in dysganglionosis. *Int J Colorectal Dis.* 2006; 21(6):554–559. DOI: 10.1007/s00384-005-0051-z [PubMed: 16267668]
16. Barlow B, Santulli TV, Heird WC, Pitt J, Blanc WA, Schullinger JN. An experimental study of acute neonatal enterocolitis—the importance of breast milk. *J Pediatr Surg.* 1974; 9(5):587–595. <http://www.ncbi.nlm.nih.gov/pubmed/4138917>. Accessed November 21, 2016. [PubMed: 4138917]
17. Barlow B, Santulli TV. Importance of multiple episodes of hypoxia or cold stress on the development of enterocolitis in an animal model. *Surgery.* 1975; 77(5):687–690. <http://www.ncbi.nlm.nih.gov/pubmed/1173200>. Accessed November 21, 2016. [PubMed: 1173200]
18. Henry MCW, Moss RL. Current issues in the management of necrotizing enterocolitis. *Semin Perinatol.* 2004; 28(3):221–233. <http://www.ncbi.nlm.nih.gov/pubmed/15283101>. Accessed November 25, 2016. [PubMed: 15283101]
19. Zani A, Cananzi M, Lauriti G, et al. Amniotic Fluid Stem Cells Prevent Development of Ascites in a Neonatal Rat Model of Necrotizing Enterocolitis. *Eur J Pediatr Surg.* 2013; 24(1):057–060. DOI: 10.1055/s-0033-1350059
20. Tayman C, Uckan D, Kilic E, et al. Mesenchymal Stem Cell Therapy in Necrotizing Enterocolitis: A Rat Study. *Pediatr Res.* 2011; 70(5):489–494. DOI: 10.1203/PDR.0b013e31822d7ef2 [PubMed: 21772224]

21. Mousavinejad M, Andrews P, Shoraki E. Current Biosafety Considerations in Stem Cell Therapy. *Cell J*. 2016; 18(2):281–287. [PubMed: 27540533]
22. Yang J, Watkins D, Chen C-L, et al. A Technique for Systemic Mesenchymal Stem Cell Transplantation in Newborn Rat Pups. *J Investig Surg*. 2012; 25(6):405–414. DOI: 10.3109/08941939.2012.661519 [PubMed: 23215798]
23. Edgar JR, Harding C, Heuser J, et al. Q&A: What are exosomes, exactly? *BMC Biol*. 2016; 14(1): 46.doi: 10.1186/s12915-016-0268-z [PubMed: 27296830]

Figure 1. Phase contrast microscopy of stem cells, 40×
(A) AF-MSC; (B) BM-MSC; (C) AF-NSC; (D) E-NSC.

Figure 2. Confirmation of MSC multipotency

(A) Representative AF-MSC induced to form adipocytes, stained with Oil Red O, 20 $\times$. Red areas indicate lipid droplets in cells induced to form adipocytes. (B) Representative AF-MSC induced to form osteocytes, stained with Alizarin Red S, 20 $\times$. Red areas indicate calcium deposition in cells induced to form osteocytes. BM-MSCs (not shown) stained positively in the same manner.

Figure 3. Experimental design

Pups are delivered via C-section at time zero and exposed to a series of repeated stresses, including hypoxia, hypothermia, LPS, and hypercaloric gavage feeds. At the time of the final feed, pups are given a single dose of FD70 and 4 h later, animals are sacrificed and serum FD70 levels determined.

Figure 4. Intestinal permeability during experimental NEC

Each bar represents the mean serum FD70 concentration in the different treatment groups, with error bars showing SEM. No significant differences were found when comparing each of the four stem cell treatment groups to one another.

Figure 5. Illustration of potential future stem cell-based therapy for NEC

Although there are several hurdles to overcome before translating this therapy to the bedside, stem cell-based therapy using stem cells themselves or their secreted products may one day be used to prevent damage to the intestine with loss of intestinal mucosal barrier function due to NEC.

Table 1

Study	Model	SC Source	SC Type(s)	Administration method	Treatment effect	Notes
<i>McCulloh et al</i> ²²	SD Rats	Pan-EGFP Lewis rats	AF-MSC, BM-MSC, AF-NSC, E-NSC	IP injection at delivery, 2×10 ⁶ cells	All four SC types equivalently reduced incidence and severity of NEC	
<i>Good et al 2012</i> ²²	EGFR-KO mice	wild-type mice	Amniotic fluid (whole)	Enteral, 50μL/g body weight	Reduction in incidence and severity of NEC and improved survival with AF	
<i>Rager et al 2016</i> ²³	SD Rats	H2K-GFP transgenic mice	BM-MSC, BM-MSC exosomes	IP injection at delivery, 3×10 ⁵ cells (BM-MSC) or 2.5×10 ⁹ exosomes	Equivalently decreased incidence and severity of NEC with either BM-MSC or BM-MSC-derived exosomes	
<i>Siggers et al 2013</i> ²⁴	Pigs	Pigs	Amniotic fluid (whole)	Enteral, 10ml/kg q3h for first 48 h	Decreased NEC score in pigs treated with AF	
<i>Tayman et al 2012</i> ²⁰	SD Rats	human bone marrow	BM-MSC	IP injection, day 3/7, 6×10 ⁵ cells	Improved survival and reduced incidence and severity with BM-MSC	Stress day 1–3, no stress day 4–7 (to mimic recovery)
<i>Wei et al 2013</i> ²	C57/BL6 mice	pan-EGFP mice	E-NSC	IP injection at delivery, 50,000 cells	Improved survival and motility with E-NSC or HB-EGF, with best effect obtained with E-NSC + HB-EGF or with E-NSC engineered to overexpress HB-EGF	
<i>Yang et al 2012</i> ²⁵	SD Rats	eFYP transgenic 7AC5Nagy/J mice	BM-MSC	IP or IV injection, 24 h after delivery, 3×10 ⁵ cells	Reduction in incidence and severity of NEC with BM-MSC or HB-EGF, with best effect obtained with BM-MSC+HB-EGF	
<i>Zani et al 2014</i> ⁶	Rats	pan-EGFP transgenic SD rats	AF-MSC, AF-MSC conditioned medium, BM-MSC	IP injection, 24 h and 48 h after delivery, 2×10 ⁶ cells or 50μL of conditioned medium	Improved survival and gut function with AF-MSC and conditioned medium No survival effect with BM-MSC	
<i>Zani et al 2014</i> ⁹	Rats	pan-EGFP transgenic SD rats	AF-MSC	IP injection 24 h after delivery, 2×10 ⁶ cells	Increased survival and decreased ascites with AF-MSC	

<i>Study</i>	<i>Model</i>	<i>SC Source</i>	<i>SC Type(s)</i>	<i>Administration method</i>	<i>Treatment effect</i>	<i>Notes</i>
<i>Zhou et al 2013⁴</i>	SD Rats	E12.5 c57BL/6-Tg pan-EGFP Mice	E-NSC	IP Injection day 3/7, 50k cells	Longer survival, improved motility, greater villous length with E-NSC	Stress day 1–3, no stress day 4–7 (to mimic recovery)

Abbreviations: SD, Sprague Dawley; EGFP, enhanced green fluorescence protein; IP, intraperitoneal; HB-EGF, heparin-binding EGF-like growth factor; eYFP, enhanced yellow fluorescent protein