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DEVELOPMENT OF A MINIMALLY INVASIVE BONE MARROW HARVEST DEVICE AND METHOD FOR THE RAPID EXTRACTION OF BONE MARROW FOR USE IN BONE MARROW TRANSPLANTATION AND STEM CELL THERAPY

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The bone marrow contains a rich supply of hematopoetic, mesenchymal and other stem cell populations. Bone marrow and peripheral blood–derived hematopoetic stem cells are currently used in > 40,000 bone marrow and peripheral blood stem cell transplants each year worldwide. Adult bone marrow–derived stem cells may soon be used in various stem cell–mediated regenenerative therapies, resulting in an increased need for a simplified, economical acquisition of bone marrow. Allogeneic marrow, or G-CSF mobilized marrow, may prove to be superior to PBSC for several transplantation indications, and thus improved harvest methods are needed.

Current methods of acquiring stem cells are tedious and expensive. Traditional methods for harvesting bone marrow from patients are crude and generally require 100 or more separate insertions of a large trocar needle into the donor's ileac crest. Serial trocar insertions allow aspiration of a small volume of bone marrow each time, eventually obtaining a volume of marrow containing an adequate number of stem cells. Marrow donors usually require general anesthesia, multiple staff, and often an overnight hospital stay. Peripheral blood stem cell harvests, alsthough less invasive, require the donor to undergo several days of expensive G-CSF administration, followed by many hours of apheresis. Traditional bone marrow harvests and peripheral blood transplants incur average charges approaching $15,000 per harvest.

We have developed a novel harvest device, the MarrowMiner, for rapid, minimally invasive extraction of bone marrow. This device requires only 1 or 2 separate bone marrow entry sites, because after entry into ileac crest, the device can access most of the accessible marrow space. We anticipate that the MarrowMiner device will enable a single operator to harvest bone marrow in a short period with only local anesthesia required in the outpatient setting, significantly facilitating convenient, on- demand stem cell collection while significantly reducing harvest costs. Collected marrow could be used immediately or undergo stem cell enrichment and manipulation for various current and potential future therapeudic indications. Results from the MarrowMiner device's preclinical development in miniature swine and human cadavers will be presented.

HEMATOPOIESIS/MESENCHYMAL CELLS

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IN VITRO UMBILICAL CORD BLOOD EXPANSION RESULTING IN UNIQUE CD34^{BRIGHT} CELL POPULATION THAT ENGRAFTS IN NOD/SCID MICE

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Umbilical cord blood (UCB) has gained recognition as a viable alternative to bone marrow or mobilized peripheral blood for hematopoietic stem cell transplantation and is being used for treatment of mainly pediatric patients with various malignant or genetic blood disorders. The limited number of cells contained in a single cord blood unit remains the major bottleneck for broader application of this treatment in adult patients. Extensive research is being focused on strategies for overcoming this bottleneck. Expansion of cells from a single cord blood unit or combining 2 cord blood units for a single transplantation are the 2 most common strategies currently in clinical evaluation.

Our research effort is focused on the expansion of umbilical cord blood cells. At BD Technologies, we have developed a unique higher-throughput technology platform that we have used successfully to identify a serum-free in vitro culture condition that supports expansion of UCB-derived cells. Expansion of UCB cells in this condition results in a unique cell population, characterized by a high level of glycoprotein CD34 expression. Characterization of this expanded population using flow cytometry, colony-forming assays, and gene array analysis indicate that this expanded cell population contains more primitive stem/progenitor cells than the remainder of the CD34+ population after expansion. When transplanted into sublethally irradiated NOD/SCID mice, these cells were found to engraft in the bone marrow and to develop CD19+ B-cell, CD33+ myeloid, CD34+ stem and progenitor, CD14+ monocyte, and CD42+ megakaryocyte lineages. Secondary transplantation studies indicate the long-term engraftment potential of the expanded cells.

In addition, when transplanted subcutaneously on porous polymer carriers into NOD/SCID mice, the expanded cord blood cells are found to initiate neovascularization within the carrier. Immunohistochemistry revealed that human cord blood cells were incorporated into the new vasculature. We conclude that our culture condition supports expansion of UCB cells that may have relevance for therapeutic applications in humans.

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BONE MARROW–DERIVED MESENCHYMAL CELLS DELAY ALLOGENEIC REJECTION

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Bone marrow–derived mesenchymal stem cells (BM-MSCs) have been demonstrated to have immunosuppressive effects in mice and humans. These findings suggest that they may be exploited in organ transplantation settings. We investigated immunoregulatory aspects of rat BM-MSCs. Bone marrow was extracted from 6- to 8-week-old DA rats and plated in 10% complete medium. Every 48 hours, nonadherent cells were discarded. The immunosuppressive effects of these cells in an allogeneic mixed lymphocyte reaction were investigated. MSCs dramatically suppressed MLR response (99%) when added at a concentration of 10^5 MSCs. Suppression was dose-dependent. In ConA cultures, MSC suppression of lymphoid cells was maximal at 10^5 cells. MSC suppression was not MHC-restricted, because Lewis-derived MSCs also suppressed MLR and ConA responses (> 90%). Immunoregulatory levels of IL-6, IL-10, and TNF-α were detected in supernatant of MSCs (BioPlex assay). The ability of MSCs to suppress allogeneic skin grafts was evaluated in an allogeneic rat model. Allogeneic skin grafts survived for 7 days without immunosuppressive therapy. In preliminary experiments, MSCs (derived from DA) were injected either as a single dose (10×10^6/kg) or as multiple doses given every 2 days (on days 0, 3, and 5). MSC injections, either a single injection or multiple injections, delayed skin graft rejection (days 8–12 or 14). Multiple MSC injections (10×10^6/kg) suppressed intestinal transplantation rejection in a rodent heterotopic intestinal transplantation model as well. The results suggest that MSCs may have clinical utility in transplantation settings.

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HUMAN MESENCHYMAL STEM CELLS AFFECT IGG PRODUCTION INDUCED BY LIPOPOLYSACCHARIDE, CYTOMEGALOVIRUS AND VARICELLA ZOSTER VIRUS IN HUMAN SPLEEN CELLS

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Human mesenchymal stem cells (MSCs) suppress T-cell proliferation and formation of cytotoxic T lymphocytes in vitro. In vivo, MSCs prolong skin allograft survival and mitigate severe graft-versus-host disease. The immunomodulatory properties of MSCs have focused on T cells. We have examined the effects of MSCs on human splenic B-cell IgG secretion. IgG response was analyzed using the ELI-spot assays, after stimulating of human spleen cells with lipopolysaccaride (LPS), cytomegalovirus (CMV), or varicella zoster virus (VZV) antigens with or without 10% irradiated MSCs. Unstimulated spleen cells gave 30 ± 9 Spot-forming units (SFUs)/