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Original Article

Cord-Blood Mesenchymal Stem Cells and Tissue Engineering

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Abstract

Cord-blood-derived stem cells have proven clinically useful for numerous disease states, as have mesenchymal stem cells (MSCs) derived from bone marrow and adipose tissue. The recent identification of MSCs in cord-blood heralds cord-blood as an untapped resource for nonhematopoietic stem cell-based therapeutic strategies for the replacement of injured or diseased connective tissue. This review discusses the potential for tissue engineering applications of MSCs, highlighting the development of vascularized tissue engineering constructs using microvascular free flaps as a novel tissue engineering strategy.

Index Entries: Cord-blood; mesenchymal stem cells, tissue engineering; intravascular free flaps.

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Introduction

Cord-blood has proven to be a clinically valuable source for hematopoietic stem cells with many valuable clinical applications. From the recognition of its potential value to its early proponents and pioneering clinical successes, the field of cord-blood hematopoietic stem cell transplantation represents a phenomenal example of the power of transplantational biomedicine *(1–8)*. It also represents a quintessential "bench-to-bedside" achievement shared by numerous physician-scientists *(1–8)*. The current list of diseases treated by cord-blood hematopoietic stem cell transplantation is both voluminous and diverse in pathophysiology *(9–11)*.

However, perhaps equally exciting is the fact that cord-blood contains other stem cell types which might prove just as valuable to patients. Much recent work has identified cord-blood as a rich source of various stem cell phenotypes with the potential to regenerate nearly all the tissue types of the body. Already recognized by the lay press, "fetal stem cells," as cord-blood stem cells have been deemed, skirt the ethical and political quandary associated with embryonic stem cells, and may yet exhibit their highest value through their utility in tissue engineering, regenerative medicine, and organogenesis.

The purpose of this article is to provide a brief review of the tissue-engineering potential of mesenchymal stem cells (MSCs), a nonhematopoietic stem cell phenotype identified within cord-blood that exhibits enormous potential for tissue engineering, regenerative medicine, and organogenesis applications. The potential use of this stem cell phenotype to these ends will be illustrated through presentation of data from our laboratory's approach to tissue engineering using MSCs.

Mesenchymal Stem Cells

MSCs constitute an extremely important and versatile stem cell type for the replacement of many diseased or damaged tissues.

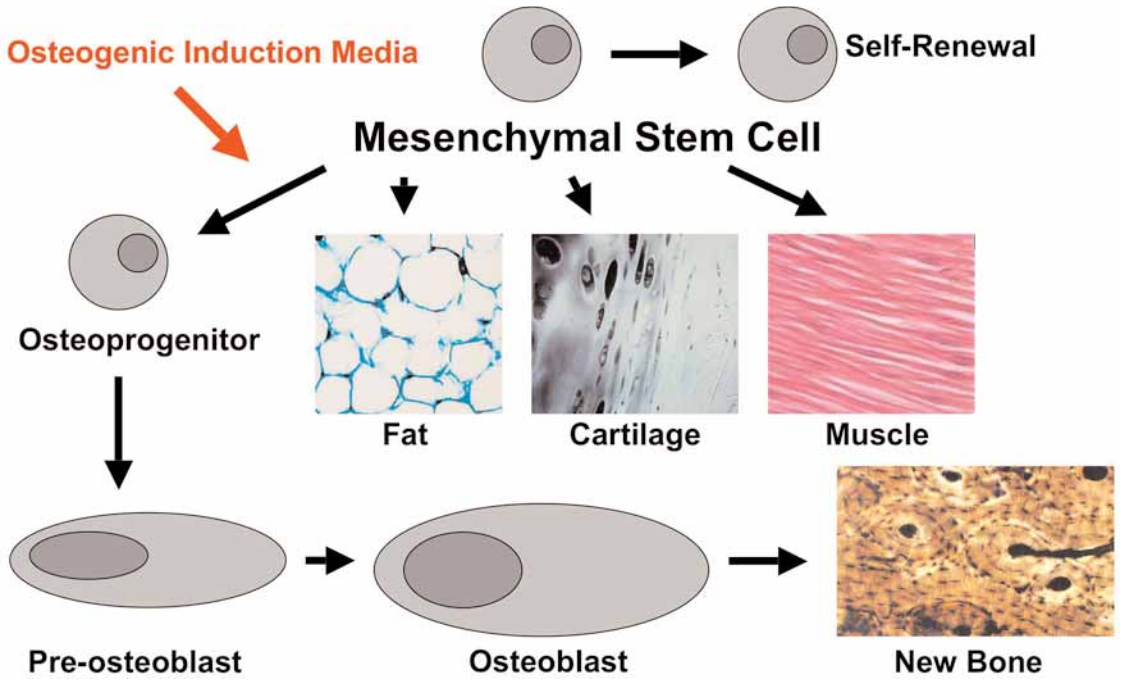

Fig. 1. Multi-lineage potential of mesenchymal stem cells (MSCs) are capable of differentiation into bone, cartilage, muscle, or adipose tissue and can be induced to form each kind of connective tissue by appropriate in vitro manipulation (i.e., here, osteogenic lineage after in vitro culture in osteoinductive media).

- 32 free flaps, n=4 each group,
- Time points: 1, 3, 7, and 21 days
- DiI+ cells in flap sections
- Co-stain for markers of osteoblast phenotype
  - Early (1-3 Wks): *Bone Sialoprotein*
  - Later (2-3 Wks): *Osteonectin*
- Histologic evidence of osteoblasts (3 Wks)
  - Von Kossa stain for mineralization

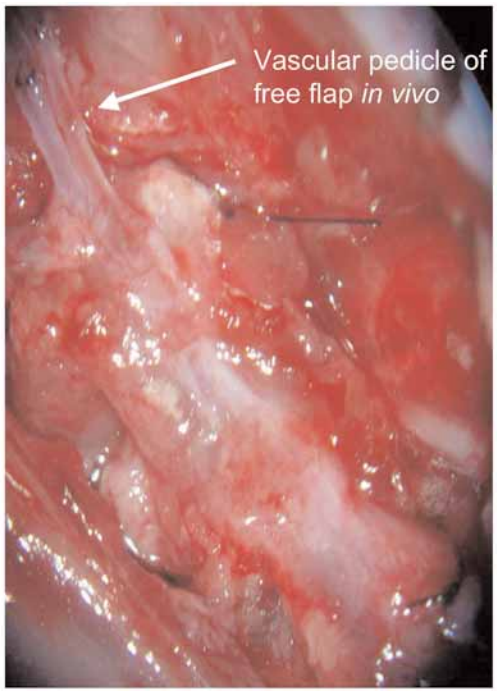

Fig. 2. Synopsis of stem cell seeding of free flaps experimental design.

Since Friedenstein described the ability of "fibroblast-like" cells derived from the bone marrow stroma to differentiate into bone *(12,13)*, MSCs have received a great deal of attention from many researchers regarding tissue-engineering applications and are perhaps the best-studied nonhematopoietic stem cell phenotype. Capable of differentiating into bone, muscle, cartilage, or adipose tissue (Fig. 1), the value of this stem cell lies in its intrinsic capacity both for self-renewal and differentiation into functional connective tissue. This characteristic has already been effectively demonstrated in a number of clinical settings. For example, MSC transplantations have improved hematopoietic stem cell engraftment *(14–16)*, aided

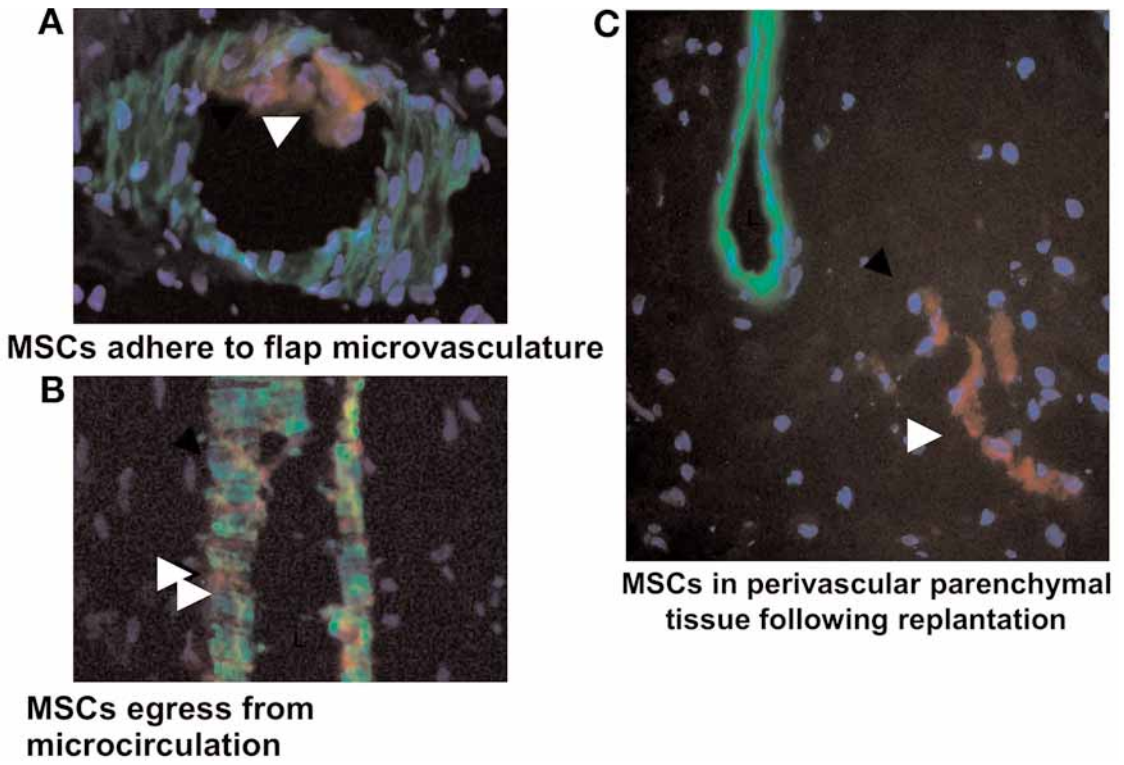

Fig. 3. Mesenchymal stem cells (MSCs) in free flaps: 1, 3, 7 days. **(A)** MSCs seeding of free flaps adhere to flap microvasculature (arrowhead). **(B)** MSCs seeded in free flaps exhibit egress from microcirculation (arrowheads). **(C)** MSCs seeded in free flaps observed in perivascular parenchymal tissue following replantation of free flap (arrowhead).

in the healing of critical sized bone defects *(17)*, effectively treated children with *osteogenesis imperfecta (18)*, and have even been used to create a functional neomandible *(19)*. A number of interesting strategies highlight the versatility of these stem cells for the repair of various tissue deficits.

For example, in an innovative approach to mandible reconstruction, Warnke and colleagues *(19)* reconstructed a large mandibular defect following resection for oral cancer using an "in vivo" tissue-engineering approach. A titanium scaffold reinforced with hydroxyapatite (for structural support) was seeded with MSCs harvested from the bone marrow of the iliac crest of a 56-yr-old patient missing the entire inferior aspect of his mandible following surgical ablation of a squamous cell carcinoma. The scaffold was then implanted into the patient's lattissimus dorsi muscle for 7 wk to allow vascularization and bone growth. Finally, the construct was transferred on the lattissimus dorsi muscle's vascular pedicle to the site of the defect. The outcome resulted in the patient enjoying solid food for the first time in 9 yr and an excellent aesthetic result. Reports of other instances of successful bone regeneration using MSCs are becoming increasingly common *(20–23)*.

Tissue-engineered cartilage using MSCs has been applied clinically for various reconstructive ends, including intervertebral disk replacement, knee-joint resurfacing, digital-joint engineering, and tracheal and auricle reconstruction *(24–30)*. Muscle has also been produced by MSC-based technolgies in various settings *(31,32)*. Finally, an approach to adipose-tissue engineering utilizing MSCs for reconstruction of soft-tissue deficits has been demonstrated. This strategy may revolutionize

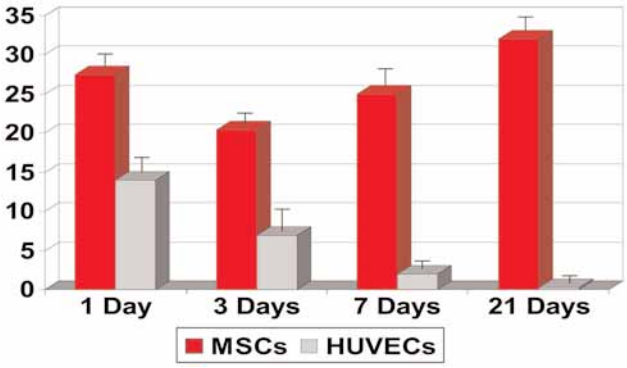

Fig. 4. DiI-labeled MSCs in free flaps vs human umbilical cord endothelial cells control. DiI + cells/HPF.

the treatment of defects that today require large-scale reconstructive surgery. Mao's group has succeeded in developing adipose-tissue constructs that maintain a predefined shape and dimension, the implications of which are enormous for reconstructive surgical applications *(33–35)*.

Initially, it was unclear whether cord-blood contained MSCs in any significant number (likely because of various methods of stem cell harvest and analysis as well as varying immunophenotypic descriptions of MSCs *[23,36]*), until Romanov described the isolation of MSCs from the subendothelial layer of the umbilical cord vein. This fibroblastoid cell population was able to be significantly expanded in vitro *(37)*. Since then, a number of groups have reproducibly isolated and expanded MSC

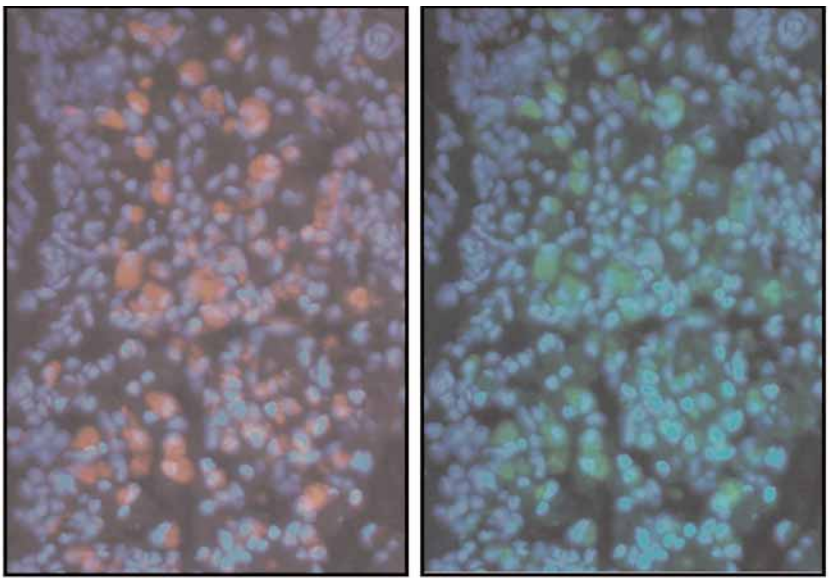

Fig. 5. Immunohistochemical staining for osteoblast phenotype (early): bone sialoprotein. Immunohistochemical staining of free flap sections for DiI-labeled MSCs (left) with costaining for bone sialoprotein (early marker of osteoblast phenotype) (right).

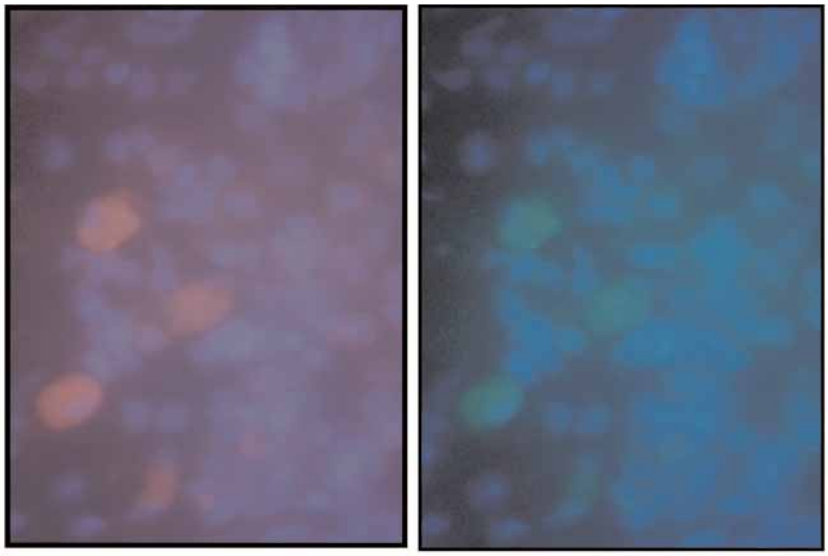

Fig. 6. Immunohistochemical staining for markers of osteoblast phenotype (later): osteonectin. Immunohistochemical staining of free flap sections for DiI-labeled MSCs (left) with costaining for osteonectin (late marker of osteoblast phenotype) (right).

populations from cord-blood using novel in vivo cell-labeling techniques and expansion and isolation protocols *(38–41)*. In addition, numerous investigators have demonstrated the tissue-engineering potential of cord-blood-derived MSCs with production of the chondocyte, osteocyte, myocyte, and adipocyte lineages from cord-blood MSCs *(42–44)*.

Tissue Engineering

Current Approaches

Contemporary strategies for the engineering of new tissue most frequently reflect the Langer and Vacanti tissue engineering paradigm *(45,46)*, which in some form or another, typically involves creating an engineered construct by seeding biocompatible scaffolds with a cell-type of interest. The construct then becomes dependent on vascular ingrowth for survival. This approach has been relatively successful for avascular structures, such as cartilage *(47)* (predominantly imbibition-dependent in vivo anyway), but as would be expected, less successful with three-dimensionally complex tissue, such as muscle, liver, or bone, which require an intact and fully functional vasculature to meet the metabolic demands of a lower surface-area-to-volume ratio (analogous to a tumor with growth that outstrips its ability to survive by diffusion alone and becomes necrotic in its center).

An alternative strategy for tissue engineering and organogenesis aims to provide an intact vascularized scaffold first, which is then seeded with the cell-type of interest. Culturing

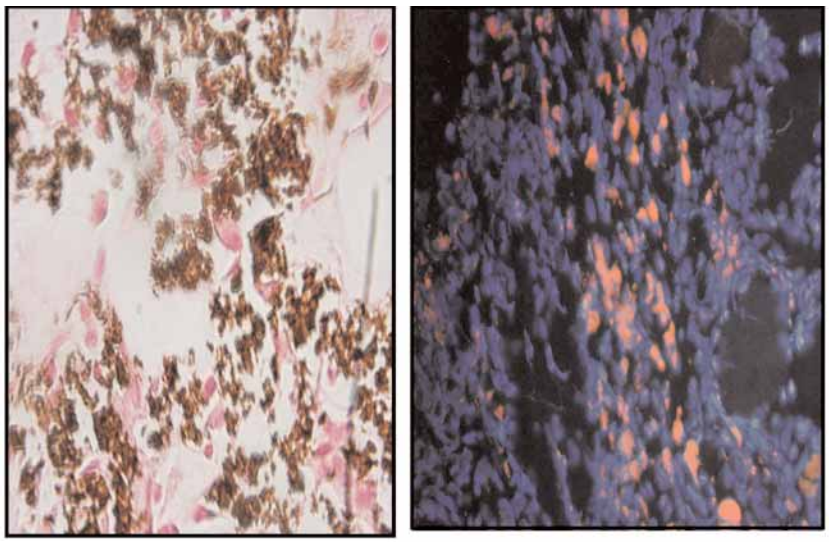

Fig. 7. Staining of free flap sections at 3 wk for DiI-labeled MSCs (right) with costaining for Von Kossa staining (early marker of osteoblast phenotype) (left).

cells in a "prevascularized" environment recapitulates developmental processes, which lay down vascular highways and subsequently, organ-level complex tissue. To this end, the microvascular free flap has been used. Reconstructive surgery has been revolutionized by the concept and application of the "free flap." More than 100,000 free flaps were performed over the last 4 yr. A typical clinical indication for a free flap involves a patient with a traumatic injury to the lower extremity that requires healthy soft-tissue coverage of destroyed muscle. The destroyed soft-tissue is replaced by a free flap in the following manner: a muscle (commonly, the lattissimus dorsi) is surgically harvested and removed from the patient's back, with its artery, capillary bed, and vein intact (the vascular pedicle). The flap, now "free" (i.e., "free" of the body, or ex vivo), is transferred to the site of injury where the flap's artery and vein are surgically connected to a functioning artery and vein near the site of injury. The muscle-containing part of the free flap is then secured in place to cover the defect and the flap now lives off of its "new" blood supply.

Free flaps have been utilized in reconstructive surgery for a decade, yet only now the potential of this concept for tissue engineering, regenerative medicine, and organogenesis is realized. Free flaps are essentially a microcosm of the circulation loop: a three-dimensional vascular scaffold with arterial inflow and venous outflow, which could be used as vascularized tissue-engineering constructs containing various stem cell populations. The "free" or ex vivo period of the free flap harvest affords an opportunity for cellular and/or genetic/cytokine manipulation before replantation of the construct (*48*,*49*).

MSC Seeding of Vascularized Tissue-Engineering Constructs

In the laboratory, the use of microsurgical free flaps as vascular scaffolds for tissue-engineering applications has been investigated by seeding stem cell suspensions through each flap's vascular pedicle in an attempt to design a vascularized tissue-engineering construct, transplantable by standard microsurgical anastomotic techniques. Attaining in vivo stem cell residence in these flaps would permit directed differentiation of a desired cell type for regenerative therapy or organogenesis. MSCs from various sources were examined in this model.

In one study, nude rat thigh adductor free flaps were perfused through the femoral artery and vein with fluorescent dye-labeled MSC suspensions from syngeneic donors or human umbilical vein endothelial cell controls (when the flaps were ex vivo) before replantation of each flap. At various time-points up to 3 wk postoperatively, experimental and control flaps were sectioned and analyzed histologically and immunohistochemically for evidence of donor stem cell residence in the flap (Fig. 2).

At early time-points in experimental flaps, donor stem cell arrest on the vascular endothelium, transendothelial migration of donor cells, and donor stem cell residence in the flap for up to 3 wk postinfusion were observed (Fig. 3). In addition, dil-labeled cells costained for osteogenic markers and exhibited bone morphology, suggesting in vivo terminal differentiation of at least some donor cells. These findings were not observed in control flaps (*50*) (Figs. 4–7).

This preliminary data demonstrated the ability to "culture" multipotent cells in vivo on a vascular scaffold with a surgical pedicle. The applications for such a construct would be wide-ranging, as current tissue engineering techniques are limited by a dependence on vascular ingrowth for construct survival. A relatively simple functional end point was chosen from a cell biology standpoint (formation of bone stock from MSC-derived osteocytes) and these cells were observed in significant numbers. Further studies will address the optimization of in vivo growth and differentiation of this cord-blood stem cell phenotype.

Conclusions

Harnessing the power of the human cell to replenish missing or diseased tissue represents one of the most important

frontiers in medicine in the early 21st century, and this quest will likely consume a significant proportion of biomedical resources of the next few decades. MSCs will likely play a central role in the evolution of therapeutic strategies for the replacement of connective tissue deficits in the near future. The demonstration of this cell-type in cord-blood represents an exciting advance for tissue engineering.

However, before the widespread clinical use of tissue engineering strategies using cord-blood MSCs, methods of MSC harvest that produce large numbers of stem cells that retain their ability for self-renewal must be developed. Despite diverse and growing information regarding MSCs and their use in cell-based strategies, the mechanisms that govern MSC self-renewal and multilineage differentiation are not well-understood and remain an active area of investigation, although recent data has shed light on a pathway, which might allow more controlled manipulation of expansion. Studies of the *wnt* pathway, a gene responsible for significant orchestration of connective tissue morphogenesis during development, has been shown to have a significant role in stem cell self-renewal *(51–53)*. The development of feasible and reproducible methods of expansion of large numbers of MSCs with retained self-renewal capacity would represent a breakthrough of considerable magnitude regarding stem cell biology and constitute a significant step toward making MSC-based tissue-engineering therapies a practical reality for many more patients *(54,55)*.

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