OBSERVATION

# Treatment of Chronic Wounds With Bone Marrow–Derived Cells

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**Background:** Recent evidence indicates that bone marrow contains stem cells with the potential for differentiation into a variety of tissues, including endothelium, liver, muscle, bone, and skin. It may thus be plausible that bone marrow–derived cells can provide progenitor and/or stem cells to wounds during healing. Our objective in this study was to establish proof of principle that bone marrow–derived cells applied to chronic wounds can lead to closure of nonhealing wounds. We applied autologous bone marrow cells to chronic wounds in 3 patients with wounds of more than 1-year duration. These patients had not previously responded to standard and advanced therapies, including bioengineered skin application and grafting with autologous skin.

**Observations:** Complete closure and evidence of dermal rebuilding was observed in all patients. Findings suggesting engraftment of applied cells was observed in biopsy specimens of treated wounds. Clinical and histologic evidence of reduced scarring was also observed.

**Conclusion:** Directly applied bone marrow–derived cells can lead to dermal rebuilding and closure of nonhealing chronic wounds.

*Arch Dermatol. 2003;139:510-516*

RECENT REPORTS describing the plasticity of stem cells may herald a new era in the treatment of many disorders.[1,2] There has been, however, a great deal of debate over the proper and safe use of stem cells derived from human blastocysts. The ability to use somatic stem cells[3-12] could resolve several of the problems posed by the use of embryonic stem cells. For example, if the stem cells were derived from the individual being treated, there would be no rejection of any tissue they generated. Moreover, treating patients with their own cells would limit ethical concerns. It is in this vein that our study was conceived. We chose to use bone marrow aspirate as a source of stem cells to treat chronic wounds that had not responded to conventional and other advanced treatments.

Bone marrow seems a logical candidate for the treatment of chronic wounds as it contains inflammatory cell progenitors, mesenchymal stem cells, and multipotent stem cells. Inflammatory cells have long been known to participate in wound healing, and hematopoietic hormones such as granulocyte-monocyte colony-stimulating factor have been reported to accelerate wound healing.[13,14] Mesenchymal cells fill the dermis of the skin, and evidence suggests that they are phenotypically altered and/or senescent in chronic wounds.[15-17] Given the recent reports of the plasticity of bone marrow stem cells,[4,8,9,18] it is conceivable that they can produce new skin cells.

Here we describe the treatment of 3 chronic wound patients who received both fresh autologous bone marrow aspirate and cultured bone marrow cells derived from those aspirates. The results seem to indicate that engraftment of topically applied bone marrow–derived cells is possible, and can stimulate the healing process.

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## METHODS

### ADMINISTRATION OF BONE MARROW ASPIRATE

This study was approved by the institutional review board of Roger Williams Medical Center, a major research and teaching affiliate of Boston University School of Medicine, and was conducted after appropriate informed consent was obtained. Patients with a prior history of malignancy other than nonmelanoma skin cancer were excluded. Harvesting the bone

**Patient Data**

| Patient No./ Sex/Age, y | Etiology of Wound | Wound Size at Treatment (Tx) Initiation, cm | No. of Cultured Cell Tx | No. of Cultured Cells Administered per Tx | End Point Result |
|---|---|---|---|---|---|
| 1/M/64 | Abdominal hernia (complication of surgery for ruptured gallbladder) | 8 × 3 | 1 | $4.5 \times 10^6$ | Wound healed after 1 Tx with cultured cells and grafting with bioengineered skin |
| 2/F/83 | Arterial disease | 11 × 4 | 3 | Tx 1: $1.1 \times 10^7$<br>Tx 2: $6.0 \times 10^6$<br>Tx 3: $1.0 \times 10^7$ | Wound healed after 3 Tx with cultured cells |
| 3/F/81 | Arterial and venous disease | 2.2 × 0.7 | 1 | $5.2 \times 10^6$ | Wound healed after 1 Tx with cultured cells |

marrow samples was performed on an outpatient basis. Patients were premedicated with 1 to 2 mg of lorazepam intravenously or intramuscularly. The procedure produced minimal discomfort and afterward all patients reported that they would not object to having the procedure done again. Bone marrow was aspirated (10-25 mL) from the iliac crest in heparin-rinsed syringes. Within 5 minutes, approximately 2 to 4 mL of the aspirate was applied directly to the wound and 1 to 3 mL of the aspirate was injected into the edges of the wound. The remainder of the aspirate was placed in culture as described below. Prior to the application of the bone marrow aspirate, the wound had been debrided to allow bone marrow cells to come in contact with viable wound tissue. In patient 2, bone marrow aspirate was also injected into an exposed tendon. The aspirate was held in place overnight with an occlusive film dressing (Tegaderm; 3M Corp, Minneapolis, Minn). A bolster of rolled gauze pads was placed over the film dressing and around the wound to prevent leakage of the aspirate. An absorbent pad was placed over the wound and bolsters, and this dressing was then wrapped with rolled gauze and elastic self-adherent bandage. The following day the entire dressing was removed and the wound was irrigated with isotonic sodium chloride solution. The wound was then covered with a foam dressing (Allevyn; Smith & Nephew, Largo, Fla) and wrapped with rolled gauze and elastic self-adherent bandage.

### ADMINISTRATION OF CULTURED CELLS

Patients received up to 3 additional treatments with cultured bone marrow cells. Cultured cells were harvested by scraping the adherent cells and centrifuging the supernatant and adherent cells. The harvested cells were then washed 3 times in preservative-free saline solution, suspended in a small amount (0.1-2 mL) of isotonic sodium chloride saline solution and applied topically to the wound. Cultured cells were only placed on the wounds, not injected. Wounds were dressed as described above for application of bone marrow aspirate.

### CELL CULTURE

At the time of harvest, a portion of the bone marrow aspirate was placed in modified Dexter cultures.[19] Separation of nucleated cells was not carried out prior to culture. The culture medium consisted of the McCoy's 5A formulation, 1% sodium bicarbonate, 0.4% minimal essential medium (MEM) nonessential amino acids, 0.8% MEM essential amino acids, 1% L-glutamine, and 1% penicillin-streptomycin (Gibco BRL, Grand Island, NY), with 12.5% fetal calf serum and 12.5% horse serum (Stem Cell Technologies, Vancouver, British Columbia). Half of the cultures also contained 0.1μM of hydrocortisone (Sigma-Aldrich Corp, St Louis, Mo). The addition or omission of hydrocortisone to the cultures was performed to potentially maximize the number and types of stem and progenitor cells grown. The cultures were incubated at 33°C and 5% carbon dioxide in 25- or 75-mL Corning flasks. Each time cells were prepared for readministration to the patient, cultures containing and not containing hydrocortisone were harvested and administered together.

## RESULTS

Three consecutive patient volunteers having chronic cutaneous ulcerations with more than 1-year duration were enrolled to receive treatment with bone marrow cells. The demographics of these patients are listed in the **Table**. None had healed with standard conventional wound treatments, which were administered by several physicians at different institutions including an advanced wound care facility; none had responded to 2 or more applications of bioengineered skin (Apligraf; Organogenesis, Inc, Canton, Mass) despite adequate debridement; and patient 1 had also not responded to several attempts at autologous skin grafting. All patients were determined not to be candidates for surgical correction. There were some clinically distinctive features of the wounds at the starting point of the study. The wound in patient 1 was overlying an abdominal wall hernia, the complication of an emergency surgery for a ruptured gallbladder. Clinically, the wound and surrounding skin had little underlying dermal and subcutaneous tissue, and peristaltic activity of the intestines could be easily observed. Patient 2, with inoperable arterial insufficiency, had a necrotic wound with an exposed necrotic Achilles tendon (**Figure 1**A). Loss of the tendon appeared certain and amputation of the leg was being considered. Patient 3 had a nonhealing venous ulcer complicated by arterial insufficiency; the punched-out ulcer had a sclerotic base exhibiting no evidence of healing or granulation tissue.

### BIOPSY RESULTS

Biopsy specimens were obtained from patients 1 and 2. Evidence that engraftment of at least some administered cells occurred was based on the observation of (1) a dramatic increase in the cellularity of the wound, (2) the presence of cells of different morphological features very quickly after the administration of bone marrow cells, and (3) the appearance of immature cells not seen in chronic wounds.

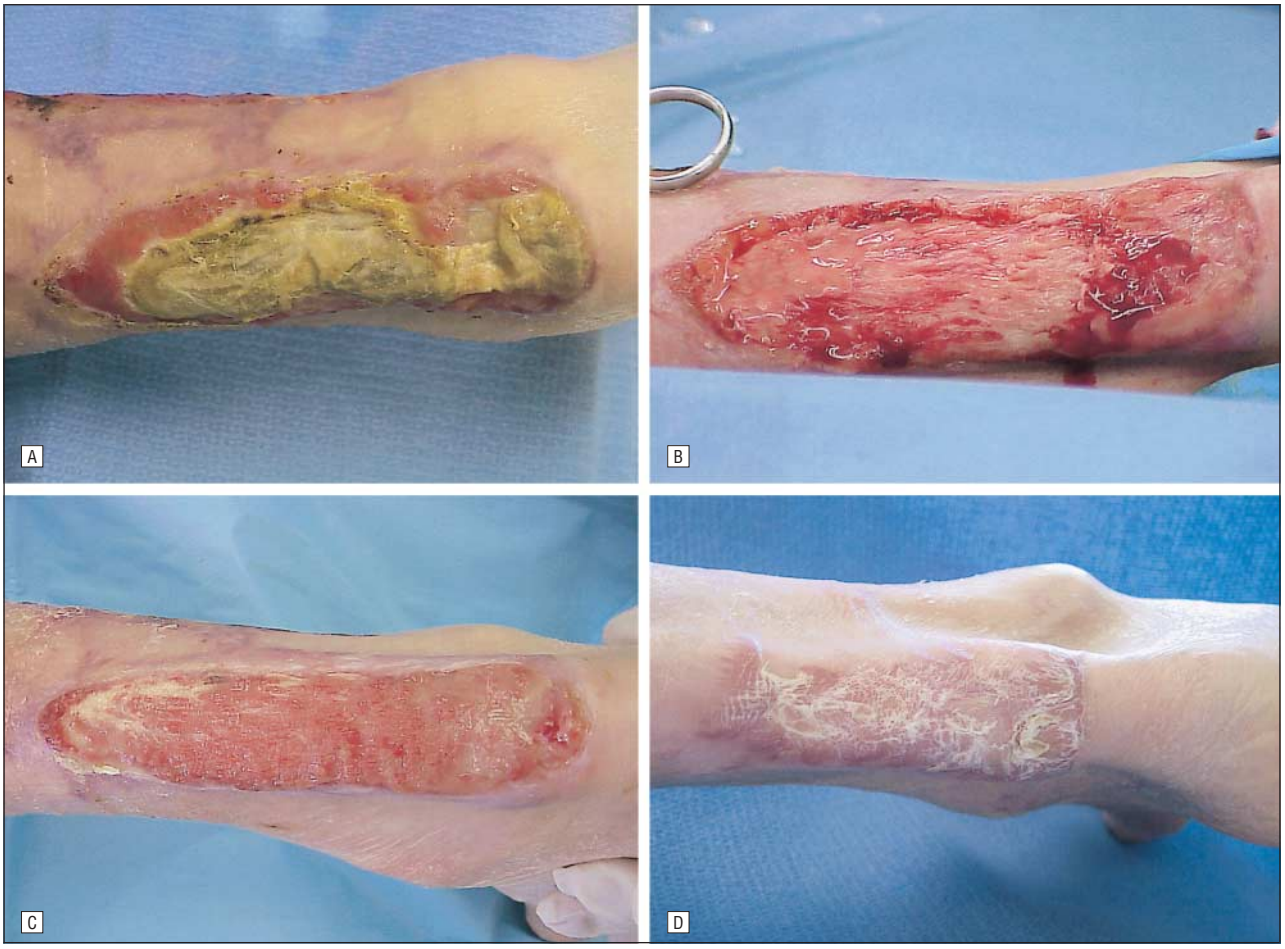


**Figure 1.** A, Visible necrotic Achilles tendon prior to treatment and before wound debridment in patient 2; B, appearance immediately after debridment and prior to application of bone marrow aspirate; C, increased vascularity and evidence of good granulation tissue 5 weeks after application of bone marrow aspirate; and D, healed wound following administration of bone marrow aspirate and cultured bone marrow cells.

### Specimens From Patient 1

A baseline biopsy was performed at the edge of the ulceration in patient 1 just prior to bone marrow aspiration (day 0). Histologic examination of the specimen revealed few dermal cells (mainly fibroblasts), fibrosis, an absence of reticulin fibers, and a decrease in elastic fibers consistent with a dermal scar. On day 11, a second biopsy specimen from the wound edge revealed a dramatic increase in the number and variety of cell types within the dermis (**Figure 2**), including mature and immature inflammatory cells. Engraftment of bone marrow cells was evidenced by the appearance of immature hematopoietic cells within the dermis. These immature cells are not found in the skin except in the case of an underlying hematopoietic malignancy or a myeloproliferative disorder, and the patient did not have these diseases. The observed immature hematopoietic cells included bandlike and blastlike forms that stained positive for myeloperoxidase, which identifyed them as granulocytic progenitors. Mature inflammatory cells included neutrophils, eosinophils, mast cells, plasma cells, and histiocytes. Many immature, elongated, spindle-shaped cells were noted, particularly in the mid- and deep dermis (Figure 2). These spindle cells had an immature mesenchymal morphology and stained positive for vimentin, which is consistent with a mesenchymal origin. They were not likely to be tissue macrophages, as they did not stain for CD68 or S100 proteins. The immature, mesenchymal-appearing cells may then have been engrafted bone marrow cells, possibly bone marrow stromal cells. The CD34 stain highlighted several blood vessels but did not stain the new immature cells observed in the dermis. Some dendritic cells in the dermis stained with factor XIIIa. However, the immature-appearing, elongated, spindle-shaped cells did not stain for factor XIIIa. The CD31 stain, a marker for endothelial cells, highlighted several cells within the dermis and also revealed many new blood vessels. This finding supported our clinical observation of increased vascularity of the wound bed at that time. In addition, the biopsy specimen was filled with newly laid-down reticulin fibers, and such fibers were absent from the specimen obtained before treatment (**Figure 3**).

### Specimens From Patient 2

A biopsy specimen obtained prior to the administration of bone marrow aspirate showed mild chronic inflammation, an absence of reticulin fibers, and dermal fibrosis, all consistent with dermal cicatrix formation. However, there was focal necrosis of the superficial dermis and necrotic Achilles tendon. Additional specimens were

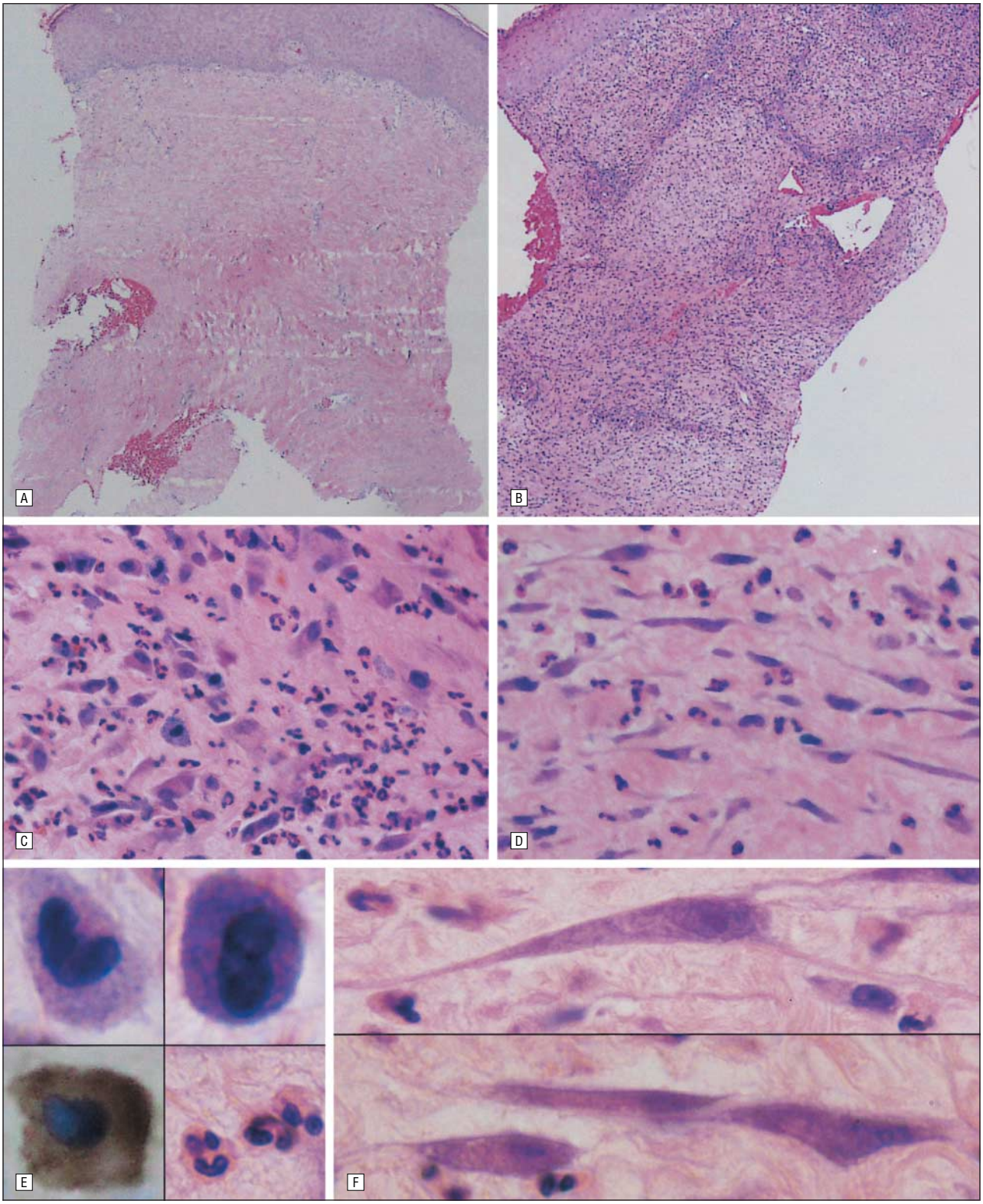


**Figure 2.** Biopsy specimens obtained from patient 1 prior to treatment (A) and following application of bone marrow aspirate (B-F); There were few dermal cells and extensive fibrosis, consistent with dermal cicatrix formation (A, original magnification × 40); a dramatic increase in cell number in the dermis (B, original magnification × 40); several mature (mostly neutrophils) and immature hematopoietic cells (C, original magnification × 200); immature mesenchymal-appearing cells with elongated dendritic processes (D, original magnification × 200); immature hematopoietic cells (upper left and upper right), an immature hematopoietic cell staining for myeloperoxidase (lower left), and many mature blood cells (neutrophils, lower right) (E, original magnification × 1000); and elongated spindle-shaped, immature mesenchymal cells that stained positive for vimentin but negative for CD31, CD34, factor XIIIa, and CD68 (F, original magnification × 1000).

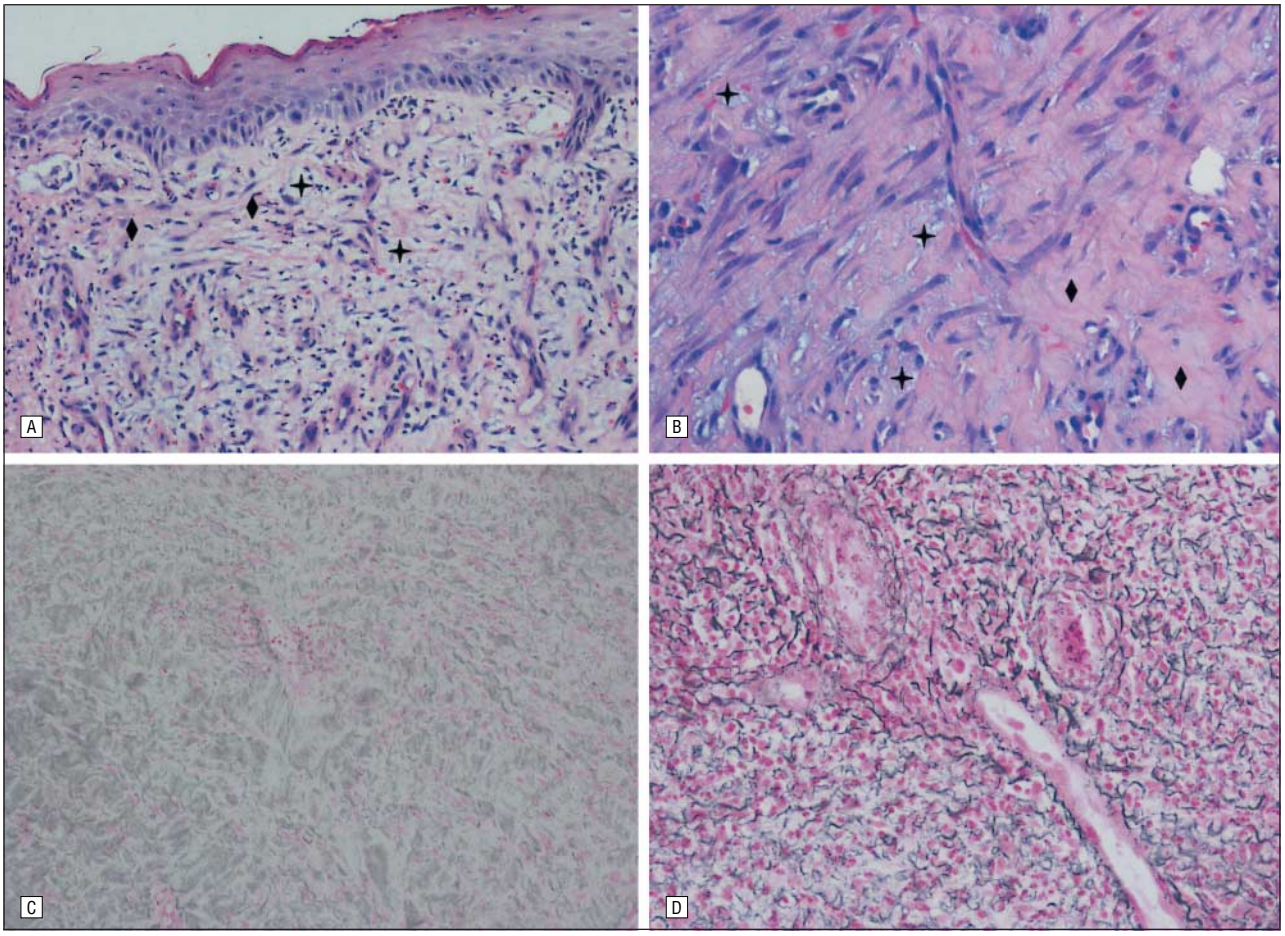


**Figure 3.** Biopsy specimens obtained from patient 2 after application of bone marrow aspirate and cultured bone marrow cells (A and B). Specimens obtained from patient 1 prior to treament (C) and following application of bone marrow aspirate (D). The wound is epithelialized, with new collagen formation (cross) and mucin deposition (diamond) (A, original magnification × 200); mucin deposition (diamond) and collagen formation (cross) shown at a higher magnification (B, original magnification × 400); reticulin stain reveals the absence of reticulin fibers prior to treatment (C, original magnification × 200) and numerous reticulin fibers after treatment (D, original magnification × 200).

obtained 1 week after the first administration of cultured cells and at the time of the second administration. As was observed for patient 1, there were numerous immature-appearing mesenchymal cells in the dermis. And in the posttreatment biopsy specimens, there was also a significant amount of dermal mucin (Figure 3), implying stimulation of the wound bed and deposition of new dermal material (ground substance). Mucin deposition was particularly increased in the later specimens, those taken after administration of cultured cells, but newly laid-down reticulin fibers were also noted in the dermis. As in patient 1, there was no significant increase in CD34 staining cells but a rise in new blood vessel formation. The CD31 stain revealed both an increase in blood vessel formation and a number of CD31 staining cells in the dermis not readily associated with blood vessels.

## CLINICAL OBSERVATIONS

All patients showed clinical improvement in their wounds within days following administration of bone marrow aspirate or cultured bone marrow cells. Wounds showed a steady overall decrease in wound size, and an increase in the vascularity of the dermis and in the dermal thickness of the wound bed. Individual observations for the 3 patients follow.

### Patient 1

Only 1 treatment of cultured bone marrow cells was administered to this patient. At week 27 (11 weeks after the last administration of cells) the wound was less than 50% of its original size. The most dramatic change in the treated wound was the markedly improved thickness, vascularity, and integrity of the dermis. We elected to attempt to close the wound with a meshed bilayered skin equivalent (Apligraf). This treatment had failed several times before the application of bone marrow cells; yet, within 2 weeks, the wound was epithelialized and remains closed at the time of this report more than 2 years later.

### Patient 2

Two months after the last administration of cultured cells the wound had decreased to about 25% of its original size. Each application of cultured bone marrow cells was followed by a burst in granulation tissue and epithelization. Approximately 4 months later the wound com-

pletely closed, and it remains closed at the time of this report (Figure 1D) approximately 2 years later.

### Patient 3

Within 2 weeks of the first administration of cultured bone marrow cells the wound was completely healed, and it remains healed more than 1 year later. Within 2 months, the residual defect, which had been depressed, was almost level with the surrounding skin. This implies dermal regeneration within the wound over a very short time. No posttreatment biopsy specimens could be obtained from this rapidly healed patient.

## ADVERSE EVENTS

There was minor discomfort related to the bone marrow biopsy. No adverse events related to the delivery of bone marrow aspirate or the cultured cells were noted.

## COMMENT

In this report we describe the successful use of bone marrow–derived cells in the treatment of nonhealing chronic wounds. We regard our findings as providing proof of principle that bone marrow contains progenitor cells that can engraft into wounds and contribute to healing and dermal regeneration. The 3 patients described in this report did not respond to traditional modalities of treatment, including compression and offloading. They also did not heal with aggressive wound care administered in an advanced wound care setting, which included several applications of bioengineered skin. In addition, patient 1 did not heal following autologous skin grafting. Admittedly, our report shows heterogeneity in the types of wounds treated and in the actual administration of bone marrow cells. However, this was inevitable because we were trying different approaches within a novel therapeutic modality.

Many new cells were noted in the dermis of posttreatment biopsy samples (Figure 2). Some of these new cells resembled immature, committed hematopoietic progenitor cells. Many spindle-shaped and elongated cells were also noted after treatment. The spindle-shaped cells, however, persisted longer and were more prominent after the application of cultured cells. These changes are different from those seen during treatment with other wound dressings or with bioengineered skin.[20] The cells delivered to the dermis did not appear to be CD34-positive, and thus may not have been hematopoietic stem cells. It is possible that an earlier or mesenchymal stem cell gave rise to the cells noted in the dermis. Several cells in the interstitial dermis stained for the CD31 antigen, suggesting that they may be endothelial progenitors.[21,22]

Evidence of dermal rebuilding was also observed by the laying down of reticulin fibers and the presence of ground substance in the posttreatment biopsy specimens. Pretreatment specimens exhibited histologic features of scarring and did not contain reticulin fibers or appreciable mucin.

Clinically, the effect of delivering bone marrow–derived cells to wounds appeared to affect primarily the dermis and subcutaneous tissues. Significant thickening of the skin was noted in all patients, as well as increased vascularity of the wound bed and surrounding area. Based on our overall findings, we believe that this treatment is most helpful in providing dermal regeneration in a diseased wound bed. Possibly, the introduced bone marrow cells give rise to dermal cells lacking the diseased phenotype observed in chronic wounds.[15-17,23] It is likely that this dermal rebuilding process occurs prior to stimulation of epithelialization. This is supported by our clinical observations that wound epithelialization seemed to lag behind the dermal effects. Dermal rebuilding is also thought to have led to the success of bioengineered skin in healing patient 1, in whom prior applications of bioengineered and autologous skin had failed.

Because new cells are being delivered to the dermis, wound bed preparation is critical in order to allow access of the bone marrow–derived cells to the wound. Appropriate debridement of each wound was performed before all cell applications. Proper wound care and thorough debridement had previously been performed in the patients, but this had not resulted in wound closure. We believe that debridement alone was insufficient in correcting the wound bed and allow epithelization. Recently, there has been increasing recognition that optimal wound bed preparation encompasses not only debridement and proper attention to the bacterial burden, but also correction of the wound matrix and reconditioning of phenotypically altered resident cells.[24] In patient 1, wound bed preparation with bone marrow cells resulted in the successful use of a bioengineered skin equivalent that, although shown to be effective in clinical trials,[25,26] had repeatedly failed to heal this patient.

In this study we treated patients with several applications of bone marrow cells to their nonhealing wounds. The ability to culture the cells allowed us to harvest bone marrow only once in each patient. The culture techniques were designed to optimize stem cell growth. The observation that cultured bone marrow cells seemed to be at least as effective as bone marrow aspirate suggests that progenitor cells for the dermis are not lost in the process of cell culture. This provides us with the potential of greatly expanding these bone marrow cells to deliver a more effective treatment. Characterization and enrichment of the engrafted dermal cells could provide insight into what dermal progenitor subtypes are required for optimal stimulation of the wound.

*Accepted for publication September 4, 2002.*

*This study was supported by grants AR42936 and AR46557 from the National Institutes of Health, Bethesda, Md (Dr Falanga).*

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