Stimulated Healing of Recalcitrant Wounds by Topical Application of Enriched Cell Culture Medium: A Clinical Report

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This study was designed to test the efficacy of enriched cell culture medium as a wound dressing. The rationale was to create within the wound space an optimal microenvironment, conducive to cellular proliferation, vascular granulation tissue formation, and epithelialization. This study was performed on various wounds that failed to respond to previous conventional treatments.

A total of 288 wounds were within the inclusion criteria, with only contaminated and neoplastic wounds excluded. Most of the patients (80 percent) were ambulatory, and the wounds were examined by the attending physician once every 7 to 14 days at an outpatient clinic. The remaining 20 percent of patients were admitted to the study while hospitalized.

Cell culture medium MCDB, supplemented with insulin, thyroxin, and growth hormone, was gelled. The gel was self-applied once a day to freshly washed wounds, covered with a gauze pad, and anchored with netting.

Healing started 7 to 14 days after the initiation of treatment with enriched cell culture medium. However, the criterion for success of the treatment was determined on complete wound closure, which was achieved in 189 of 288 wounds (65.6 percent). Wound closure was correlated with the initial wound volume, stage, and origin. The average time required for closure of wounds caused by systemic pathologies (n = 181) and those based on regional status (n = 107) were 12.0 and 4.4 weeks, respectively, compared with 290 and 10.3 weeks of the previous conventional treatment. In 19 extensive wounds, when vascularized granulation tissue was established, a successful surgical closure was attained.

Most wounds of patients who did not continue the enriched cell culture medium treatment (34.4 percent) manifested reduced wound volume, ranging from 11 to 98 percent of initial volume. Discontinuation of treatment was associated with difficulties in reaching the clinic for the weekly examination, rather than for reasons directly related to the treatment itself, and occurred significantly earlier during the treatment period.

Thus,. enriched cell culture medium was effective in stimulating wound healing in recalcitrant wounds. The healing was rapid with minimum scarring and pain. No side effects or allergic reactions were reported or observed. (*Plast. Reconstr. Surg.* 108: 104, 2001.)

Wound healing as a physiological phenomenon was investigated extensively. The successive phases of the process were studied in detail, and the pathophysiology of wounds that failed to heal was also described.[1–3]

It is well documented that the status of the wound cells depends, to a great extent, on the microenvironment of the wound,[4,5] which in turn is dependent on the supply of blood-borne nutrients and the clearing rate of the metabolic by-products of the wound cells. In wounds in which the vascular supply or drainage is undermined, the lack of nutrients is combined with accumulation of cellular metabolites and tissue debris. As a result, the healing process is impeded and the wound becomes a hospitable site for microorganism infestation. Reversing this process requires antiseptic and/or antibiotic treatment to clear the infection. However, once the wound is clinically clean, it is believed that the purpose of the treatment should be changed to answer the need to improve the wound cellular microenvironment. The repair and remodeling require a sufficient supply of precursors and cofactors as well as sufficient energy supply to drive the process.[6]

The use of topically applied enriched cell culture medium as a treatment for wounds

From the Faculty of Medicine, Technion. Received for publication May 10, 1999; revised August 28, 2000.

Presented in part at the Sixth Annual Symposium on Advanced Wound Care and Medical Research Forum on Wound Repair, San Diego, California, April 1993. Winner of the Product-Related Award at the Symposium.

provides the cells in the wound with physiologically balanced substrate consisting of nutritional and hormonal components, which are necessary for cellular activation and growth. Previous animal studies[7] using tissue culture technology in vivo allowed for optimization of the media formulation to achieve acceleration of the wound healing process and was based on use of MCDB medium, which supports keratinocytes growth in vitro. It was found that three nonsteroidal anabolic hormones added to the cell culture medium acted synergistically to accelerate the wound healing. Previous pilot clinical trials[8] showed that topical application of this enriched medium formulation stimulated healing of chronic ulcers.

In this study, enriched cell culture medium was employed as an accelerator of wound healing in various wound types, both chronic and nonchronic, which previously failed to respond to commonly accepted treatment modalities. Unlike most methods that target the elimination of wound-healing suppressors, this treatment introduces a novel approach—the stimulation of the healing process, overcoming factors that hinder healing such as undermined blood supply.

PATIENTS AND METHODS

Patient Population

This study was approved by our affiliated hospital institutional review board (Helsinki Committee). All participants signed an informed consent form.

Participants were male and female patients who suffered from wounds on any part of the body. These patients were under continuous medical care in outpatient clinics or hospitals. All the wounds were previously treated with conventional methods, such as topical antiseptics, antibiotics, anti-inflammatory medications, surgical debridement, split skin grafting, hydrocolloids, occlusive dressings, hyperbaric oxygen therapy, and other modalities, but failed to reach closure. In fact, the physicians referred only patients who did not respond to conventional treatments. During the screening, the patients were examined by the attending physician, and most of the wounds were included in this study. The exclusion criteria stipulated neoplastic and contaminated wounds.

Wound Categories

The origins of specific wounds were classified into two major categories: (1) vascular insufficiency and systemic diseases and (2) status after surgery, trauma, and others. These categories are related to the conditions that contribute to wound formation and affect the wound-healing process. Wound penetration was classified into four stages: Stage I represents superficial wounds in which epidermis and some dermis were damaged; stage II included the dermis and subcutaneous tissues; stage III denotes exposure of muscle tissues; and stage IV involves deeper damage with injury to muscle, ligament, and/or bone.

Enriched Cell Culture Medium

MCDB medium supplemented with insulin, thyroxin, and growth hormone was gelled in sterile 3% hydroxyethylcellulose and stored refrigerated in 20-ml syringes.

Treatment

The patients were given oral and written instructions to wash the wound daily with antiseptic soap and rinse it under running water. Treatment was self-administered once a day. The enriched cell culture medium gel was applied to the wound bed (1 ml/cm^2), covered with sterile cotton gauze, and anchored with netting. No adhesives were used. In the few cases in which edema was found, pressure garments or elastic bandages were applied. When progress in the healing process was halted, a biogram of the wound exudate was taken. Antiseptic treatment was applied temporarily for 1 to 2 weeks and then the enriched cell culture medium treatment was resumed. These were usually cases in which admission to the study was conditioned on pretreatment with antiseptics.

Evaluation

The patient medical history, specific wound origin, vascular status and specific wound clinical data, and the surrounding skin condition were recorded. The wound was then measured and photographed. The patient was examined in the outpatient clinic every 7 to 14 days, at which time the wounds were evaluated, measured, photographed, and redressed. Follow-up after wound closure extended over 12 weeks. The chi-square test, Student's *t* test, and

survival analysis were used for statistical processing.

Results

A total of 214 patients, 131 men and 83 women, were within the inclusion criteria and admitted into the study. About 25 percent of the patients had more than one wound, resulting in a total of 288 wounds and an average of about 1.3 wounds per patient.

The origins of specific wounds were classified into two major categories: 181 wounds in the vascular insufficiency/systemic disease category and 107 were wounds after surgery, trauma, or other. The various major pathologies accounting for the presence of the wounds and their incidence are shown in Table I. About one third ($n = 58$) of wounds in the first category were diabetic, half of which were associated with vascular insufficiency; the other 68 percent were nondiabetic ulcers associated mainly with venous insufficiency. In the postsurgical/trauma category, about half ($n = 57$) were postsurgical wounds with the other wounds divided equally between trauma and burn. Some of the patients in this category were diabetic, but this was not the primary cause of wound formation.

It is well accepted that the leg and foot are predisposed to chronic ulcers, and this is emphasized by the fact that the vast majority of our patients suffered from chronic wounds of the leg and foot. In the vascular insufficiency/systemic disease group, almost all wounds (97 percent) were located in the leg and foot; in the postsurgical/trauma group, about half of wounds (55 percent) were found at those sites.

Age of participants varied between 2.5 and 90 years. The age and cause of wounds were highly correlated. Patients suffering from wounds caused by vascular insufficiency or systemic disease were significantly older ($p < 0.01$) than patients with postsurgical/trauma wounds, with average ages of 63.9 and 47.6 years, respectively.

Previous treatment duration extended over weeks, months, and, in 28 percent of all wounds, even years. Previous treatment for vascular insufficiency/systemic disease wounds lasted significantly longer ($p < 0.001$) than treatment of postsurgical/trauma wounds, with averages of 64.4 and 2.3 months, respectively.

The wounds failing to reach closure, although previously treated with various modalities for rather extended periods, were then treated with enriched cell culture medium. The onset of the healing process was first noticed 7 to 10 days after the start of the treatment, when the color of the wound bed began to change from whitish/gray to pink, an indication of the vascular response in the wound bed. As the healing progressed, both vasodilatation and onset of neovascularization were observed. Once initiated, the growth of vascularized granulation tissue proceeded, filling the wound space until a viable substratum was formed to support epithelialization. Growth of epithelium started as the wound edges became attached to the substratum and advanced as a front, which eventually covered the entire wound. The healing cascade, once started, rapidly gained momentum until just before the final wound closure. At that time the rate of epithelialization slowed down until final closure was attained. It is important to note that wound healing with enriched cell culture medium was not accompanied by excessive scarring. Even burn wounds yielded smooth, colorless scars.

All 288 wounds treated with enriched cell culture medium demonstrated initiation of healing. However, for 99 wounds—80 of which were caused by vascular insufficiency or systemic disease—treatment was discontinued before reaching a complete closure (Table II). Yet, some decrease in wound volume, ranging between 11 and 98 percent of initial volume, was measured during the treatment period. In 63 wounds, relocation and/or unrelated medical problems were the cause for discontinua-

TABLE I
Classification and Frequency of Wounds

Vascular insufficiency and systemic disease ($n = 181$)	Surgical, Traumatic, and Other ($n = 107$)
Venous insufficiency (83)	Surgical wounds (56)
Vascular + diabetes (34)	Burns (26)
Vascular insufficiency (arterial or venous and arterial) (29)	Trauma (25)
Diabetes (24)	
Other (pressure, scleroderma, radiation) (11)	

TABLE II
Reasons for Discontinuing Treatment with Enriched Cell Culture Medium

Reason	No. of Wounds	% of All Wounds
Noncompliance	34	11.8
Relocation	32	11.1
Nonrelated medical or psychological problems	31	10.8
Hypergranulation	1	0.35
Infection	1	0.35

tion of treatment. In about half of wounds (n = 53), treatment with enriched cell culture medium was discontinued within 15 days after admission to the study. In only two cases did the attending physician terminate the treatment: one because of infection and the other because of hypergranulation. A local treatment with cortisone reversed the hypergranulation. Discontinuation of treatment was associated with aging. In the postsurgical/trauma category, average age in the discontinued group was 54.5 years compared with 45.2 years in patients reaching a complete closure (p = 0.031). However, in the vascular insufficiency/systemic disease group—in which the average patient was older than in the postsurgical/trauma group—wounds that were closed and those where treatment was discontinued exhibited similar average age.

All remaining wounds in the study (n = 189; 99 from vascular insufficiency/systemic disease and 90 after surgery, trauma, or other) reached complete closure after treatment with enriched cell culture medium. Wound closure was found to be closely correlated with wound origin. Frequency of wound closure was significantly ($p < 0.001$) higher in the postsurgical/trauma group compared with the vascular insufficiency/systemic disease group; complete closure was attained in 84.1 percent of wounds in the former group and in 58.9 percent of wounds in the latter group.

In 19 cases of large vascular insufficiency/systemic disease wounds, split skin grafting was successfully enabled after a substratum of vascularized granulation tissue was formed. Two of the successfully grafted patients had dehiscent split skin grafting before treatment with enriched cell culture medium. Because the closure of these 19 wounds involved a surgical intervention in addition to this treatment, they were excluded from the statistical analysis. The remaining 170 wounds treated with cell culture medium attained complete closure with no additional intervention. The latter included three patients with dehiscence of previous split skin grafting.

The time required to achieve complete wound closure with enriched cell culture medium was significantly ($p < 0.001$) shorter in postsurgical/trauma wounds than for vascular insufficiency/systemic disease wounds (Fig. 1). Average duration of cell culture medium treatment resulting in complete closure was 12 and 4.4 weeks for vascular insufficiency/systemic disease wound types and postsurgical/trauma wound types, respectively. Previous treatment duration of wounds varied as well, with averages of 64.5 and 2.3 months (290 and 10.3 weeks), respectively. With enriched cell culture medium treatment, the long-standing chronic wounds finally closed in a relatively short period of time. When treatment was discontinued, its duration was longer than the average time of treatment required for complete closure (Fig. 1, *insert*). Whereas complete closure was attained in vascular insufficiency/systemic disease wounds on the average after 12 weeks, in wounds in which treatment was discontinued, thus recorded as not closed, treatment duration was only 5.5 weeks ($p < 0.001$). Similarly, closure of postsurgical/trauma wounds

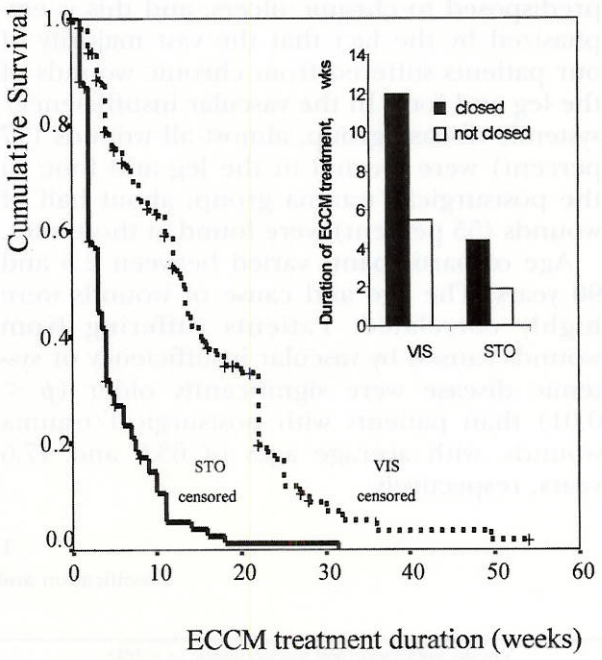

FIG. 1. Enriched cell culture medium (ECCM) treatment duration in postsurgical/trauma (STO) and vascular insufficiency/systemic disease (VIS) wound groups. Insert denotes average treatment duration in closed and nonclosed wounds.

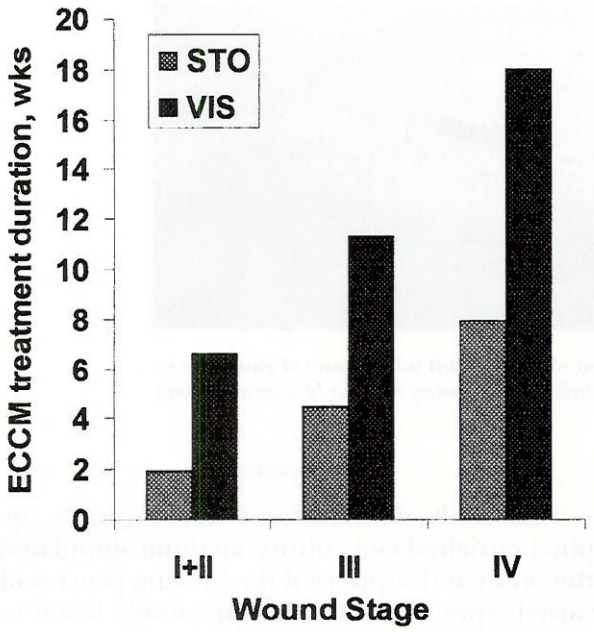

FIG. 2. Stage-dependent duration of enriched cell culture medium (ECCM) treatment. STO, postsurgical/trauma wounds; VIS, vascular insufficiency/systemic disease wounds.

required 4.4 weeks of treatment, and nonclosure was associated with a shorter duration of treatment with enriched cell culture medium of 1.9 weeks ($p = 0.041$). Neither duration of treatment with cell culture medium nor probability of closure was related to previous treatment duration.

Average duration of treatment with enriched cell culture medium was highly correlated with the wound stage (Fig. 2). Stage I ulcers, in which epidermis and some of the dermis were damaged, required significantly ($p < 0.001$) shorter treatment duration than stage IV ulcers in which muscle, ligament, and/or bone was exposed. This trend was common to both types of wounds, although actual duration of treatment was longer in the vascular insufficiency/systemic disease group. Time to complete healing was three times longer in stage IV compared with stage I in vascular insufficiency/systemic disease ulcers and four times longer in postsurgical/trauma ulcers.

The average initial wound volume was found to be partially related to closure. Initial volume of closed wounds was smaller than nonclosed wounds. In the postsurgical/trauma group, initial volume of closed wounds was significantly ($p = 0.024$) smaller than that of nonclosed wounds, with volumes of 7.5 and 37.6 ml, respectively. On the other hand, in closed and nonclosed wounds caused by vascular insufficiency or systemic disease, closure was independent of the initial wound volume, with values of 3 and 4.1 ml, which did not differ significantly.

CASE REPORTS

Case 1

A 44-year-old man was hospitalized with multiple wounds on the dorsum of the right foot due to arterial and venous insufficiency (Fig. 3, *left*). Initial antiseptic treatment lasted 4 weeks, and 3 weeks of treatment with enriched cell culture medium yielded complete closure of all wounds (Fig. 3, *right*).

Case 2

A 57-year-old man with diabetes mellitus and gangrene of the foot presented with a postsurgical wound after amputation of the first left toe (Fig. 4, *left*). One week of previous treatment consisted of systemic antibiotics and topical antiseptics. After 9 weeks of treatment with enriched cell culture medium, complete closure was attained (Fig. 4, *right*).

Case 3

A 68-year-old man suffering from non–insulin-dependent diabetes mellitus and varicose veins of the right leg was operated on for great saphenous vein extirpation. He developed an infected scar wound (postsurgical) that did not heal for 9 weeks despite surgical debridement and treatment with an-

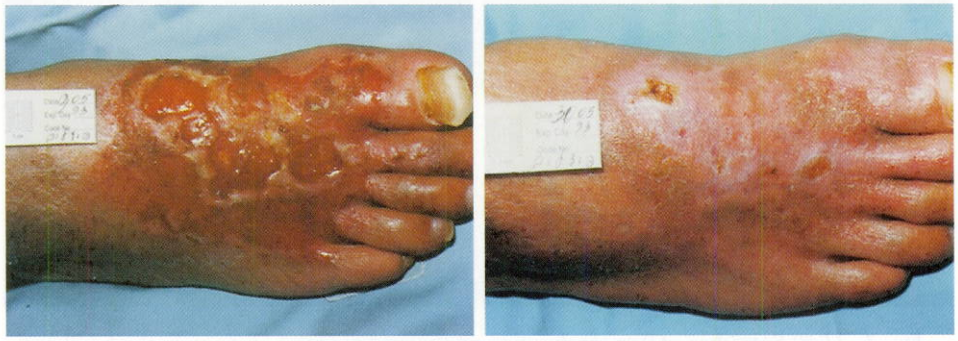

FIG. 3. (*Left*) Multiple arterial and venous insufficiency ulcers of 4 weeks duration on the dorsum of the right foot. (*Right*) Completely healed wounds following 3 weeks of treatment with enriched cell culture medium. Note the smooth skin and minimal scarring.

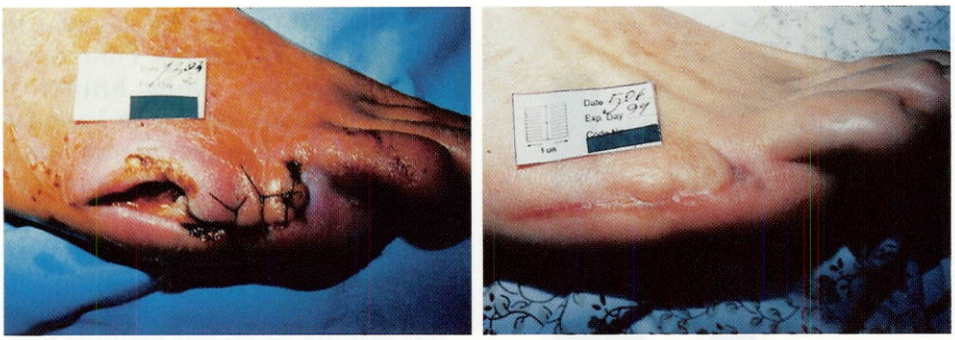

FIG. 4. (*Left*) One week after surgical amputation of the first left toe because of gangrene in a diabetes mellitus patient. (*Right*) Complete wound closure following 9 weeks of treatment with enriched cell culture medium.

tiseptics and systemic and topical antibiotics (Fig. 5, *left*). Six weeks of treatment with enriched cell culture medium achieved complete closure (Fig. 5, *right*).

Case 4

A 62-year-old man was hospitalized with bilateral second- and third-degree burns on the soles of his feet (Fig. 6, *left*). He suffers from insulin-dependent diabetes mellitus with neuropathic changes. He had been treated previously with antiseptic solution for 1 week. Treatment with enriched cell culture medium lasted 6 weeks until complete closure was achieved (Fig. 6, *right*).

Case 5

A 62-year-old man with insulin-dependent diabetes mellitus with neuropathic changes and arterial insufficiency presented with a phase IV heel ulcer (Fig. 7, *left*). Previous treatment using antiseptics and topical antibiotics lasted 14 months. Treatment with enriched cell culture medium for 5 months yielded complete closure (Fig. 7, *right*).

Case 6

A 75-year-old man with non–insulin-dependent diabetes mellitus presented with a 12-year-old chronic atrophic and pressure ulcer (vascular insufficiency/systemic disease) of the metatarsal area of the left foot (Fig. 8, *left*). Previous treatment included antiseptics and topical antibiotics. Complete wound closure was achieved after 4 months of treatment with enriched cell culture medium (Fig. 8, *right*).

DISCUSSION

This study demonstrates that topically applied enriched cell culture medium stimulated the onset and supported the healing process of varied types of wound that previously failed to respond to conventional methods. The cascade of wound-healing process, once initiated, was followed by formation of vascularized granulation tissue and epithelialization, leading to complete wound closure. Duration of wound care was thus significantly reduced using enriched cell culture medium. The time required for complete wound closure was dependent on wound origin, depth of penetration, volume, location, age, general health, and other factors.

All 288 wounds reported in this study responded positively to treatment with enriched cell culture medium. Within 7 to 14 days, a notable color change of the wound bed was observed, indicating onset of vascular reaction. However, although only 65.6 percent of wounds attained complete closure, the results of this study claim higher success in light of the failure of previous treatments to induce heal-

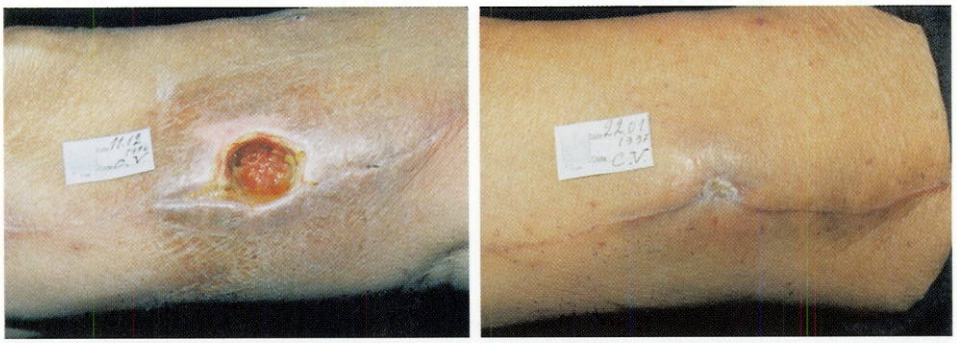

FIG. 5. (*Left*) Scar ulcer of 9 weeks duration on the medial aspect of the right thigh following great saphenous vein extirpation to relieve varicosity. Note skin discoloration around the wound. (*Right*) Complete wound closure was attained after 6 weeks of treatment with enriched cell culture medium. Note the improved skin coloration around the wound.

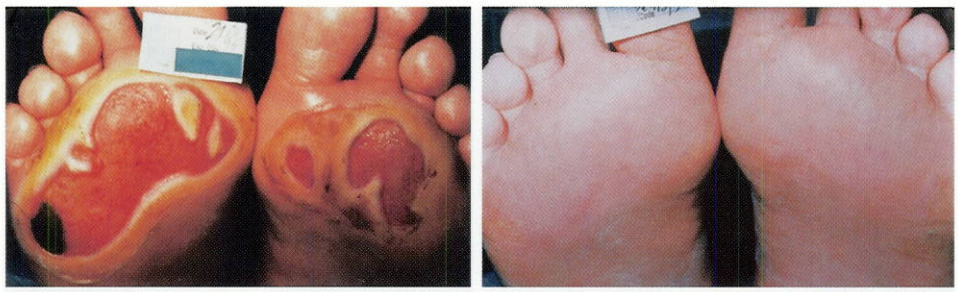

FIG. 6. (*Left*) A bilateral neuropathic second- and third-degree burn of the metatarsal areas in a diabetes mellitus patient. (*Right*) Complete healing of the burns was accomplished in 6 weeks of treatment with enriched cell culture medium. Note the absence of scarring and the smooth skin in the affected areas.

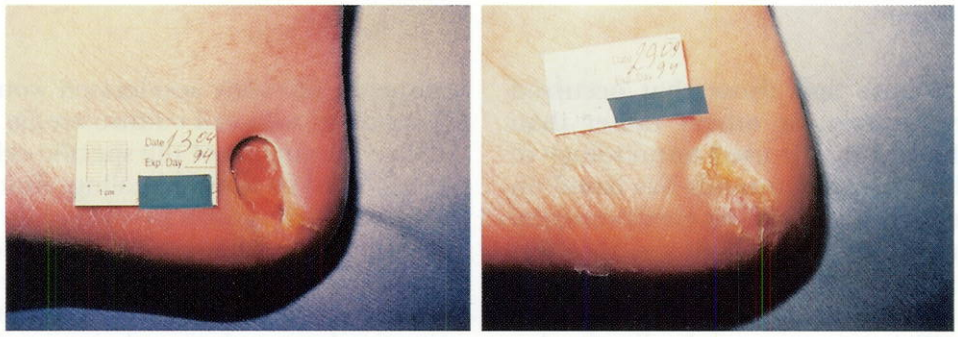

FIG. 7. (*Left*) A deep atrophic ulcer of 14 months duration on the lateral aspect of the left heel in an insulin-dependent neuropathic patient suffering from diabetes mellitus. (*Right*) Complete wound closure was achieved after 5 months of treatment with enriched cell culture medium. Follow-up after 1 year showed that the wound remained closed.

ing. The patients who dropped out and did not attain wound closure had significantly shorter treatment duration compared with patients who continued treatment until complete closure. The majority of those patients (66 percent) discontinued the treatment in less than 1 month.

Despite the discontinuation of treatment, some improvement in wound status and volume was measured. The above might indicate that an extended treatment period would have resulted in an increased success rate of wound healing.

Discontinuation of treatment was attributed to various factors that were not related to the treatment itself but rather associated with difficult in accessibility. Age, nonrelated medical or psychological problems, and location of wounds that in many cases were in the lower extremities are some of the factors that reduced patients' mobility. Their dependence upon adequate transportation limited their ability to regularly visit the clinic for examination by the attending physician. The dropout rate of 34.4 percent observed in this study may also be associated with the broad-based inclusion criteria. The constant suffering, imposed immobility, reduced quality of life, and depression associated with recalcitrant wounds of the lower extremities should not be overlooked. These may also contribute to the patients' reluctance to comply with the prolonged tedious treatment required to achieve complete wound closure.

Healing of wounds is a process that is affected by multiple factors such as anatomic location, wound size, depth, presence of sinuses or tunnels, cause of the wound, and general health. The most important factor that critically influenced both probability and rate of wound closure was, as expected, the cause of the wound. All wounds in this study were considered chronic wounds (static or progressive) and difficult to heal, although they had been treated for extended periods of time before the treatment with enriched cell culture medium. A chronic, problematic wound can result from both local and systemic factors that impair wound healing.[9] Therefore, we employed a different terminology for wound classification. Dividing the wounds into two categories (those caused by vascular insufficiency

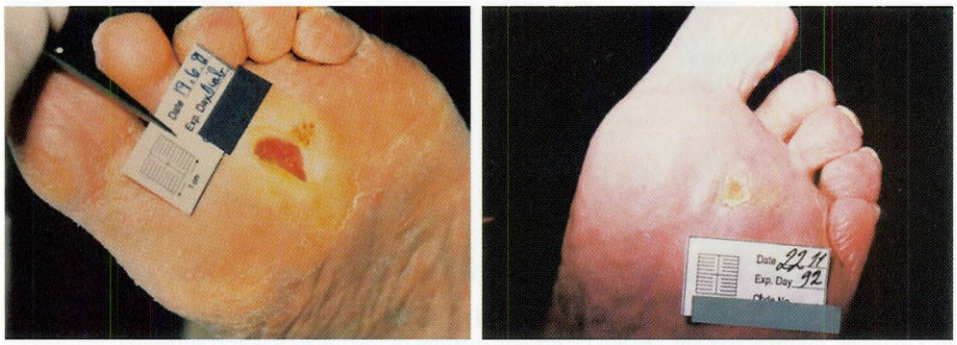

FIG. 8. (*Left*) A chronic atrophic and pressure ulcer of 12 years duration, on the metatarsal surface of the left foot in a patient with non–insulin-dependent diabetes mellitus. (*Right*) Treatment with enriched cell culture medium lasted 4 months when complete wound closure was attained.

or systemic disease and those that occurred after surgery, trauma, or other) rather than relying on the commonly used terminology of chronic and nonchronic wounds was based on the rationale that the prevailing environmental factors in and around the wound greatly affect the healing process. Recalcitrant hard-to-heal wounds are most likely to develop because of vascular insufficiency or systemic disease, because the blood supply and/or metabolism are undermined, whereas conditions for wound healing after surgery or trauma are less hostile. Indeed, both enriched cell culture medium and previous treatments were found to be of significantly longer duration in vascular insufficiency/systemic disease wounds compared with postsurgical/trauma wounds, and this reflects the relevance of the assumption in categorizing the wound types.

In the hard-to-heal wounds, especially those from vascular insufficiency and systemic disease, the wound cells exist in a delicate equilibrium between survival and nonsurvival. It was postulated that treatment with enriched cell culture medium would tilt the balance toward survival by providing an improved microenvironment. Chronic wounds need to be provided with adequate stimulus to healing. The repair process, once initiated, cannot be efficient in the absence of a supportive physiologic environment.[5] The action of enriched cell culture medium is twofold: stimulation and support. The treatment was designed to address these two functional requirements with the three hormones acting as stimulants and the cell culture medium providing the supportive microenvironment. It was previously shown[7] in an animal model that the combination of these two components rather than each of them alone significantly accelerated wound healing. Treatment with cell culture media adapted for the requirements of the specific cells is already being used in wound healing. Autologous keratinocytes are grown in vitro and then grafted to accelerate regeneration of epidermis in extensive burn wounds.[10–13] The enriched cell culture medium treatment is actually a similar tissue culture technique being implemented in the wound site itself. Whether the medium acts directly on proliferation, migration, and biosynthesis of wound cells or indirectly by stimulating the synthesis of locally acting growth factors and cytokines,[14] the result is the same—the wound cells can avail themselves of the newly created substrate and become metabolically active.

Once the wound bed is stimulated and revitalized, final epithelialization proceeds. Epithelial cells migrate from the edge and proliferate to form a seal over the wound. Both rate of healing and wound size dictate the length of treatment needed to attain complete closure. Therefore, success of wound closure was related, among other things, to its initial volume. The larger the volume, the lower the chance of closure. In the postsurgical/trauma wounds, the average initial wound volume of nonclosed wounds was five times more than that found in the closed wound, whereas their treatment duration was about half. However, in vascular insufficiency/systemic disease wounds, treatment time required for wound closure was more than twice that found in the nonclosed wounds, and the volume differences were minor. Furthermore, although the initial volume of closed wounds was similar for both wound categories, the time elapsed until complete healing of vascular insufficiency/systemic dis-

ease wounds was about three times longer than that of postsurgical/trauma wounds. This indicates that the limiting factors of the healing process are different for both types of wounds. The postsurgical/trauma volume-dependent wound healing rate is fast, whereas vascular insufficiency/systemic disease wounds have a slower rate of healing. Thus, the prolonged treatment required for healing of wounds caused by vascular insufficiency or systemic disease is more likely to be attributed to the time needed for stimulation and revitalization of the wound and the adjacent area, rather than to the process of final closure. However, for postsurgical/trauma wounds, the volume-dependent time to healing suggests that given longer treatment duration, the wounds would have attained a complete closure. Alternatively, surgical intervention could, within a reasonable time frame, yield complete closure as well. Indeed, in 11 cases of vascular insufficiency/systemic disease wounds, closure was successfully achieved by split skin graft. The success of the surgical intervention was enabled by treatment with enriched cell culture medium, which stimulated growth of vascularized granulation tissue substratum essential for graft take. Topically applied cell culture medium was reported to increase the percent of skin graft take in athymic mice as well.[12]

Eighty-nine percent of the wounds included in this study were classified as stage III or IV wounds. The stage-dependent prolonged healing is attributed to the pronounced soft-tissue defect that extends into the deep layers, forming sinuses and, in extreme cases, reaching the bone. Such defects are difficult to clean and thus are a source of continuous and repeated infections, often leading to extended treatment periods. In addition, epithelialization will be hindered in the absence of extracellular matrix. The gel stimulation of the granulation tissue proliferation is important in providing scaffolding for the epithelial growth. Indeed, wound healing was stage-dependent and deeper wounds required longer cell culture medium treatment.

In addition to its nutritional benefits, the gel also creates a moist and permeable environment,[15,16] which is the natural substrate for cellular survival. Dehydration, on the other hand, causes scab formation, which in turn interferes with epithelialization and wound closure. Especially in extensive burn wounds, hydration is paramount in reducing the hazard of both topical and systemic dehydration. The patients reported pain relief within a few minutes after application of the gel.

There was a remarkably low incidence of contamination during treatment with enriched cell culture medium, especially in light of repetitive contamination manifested in many of these chronic wounds, which were treated with antiseptics before the cell culture medium treatment. Only one patient had to discontinue this treatment because of infection, which was deep-seated and therefore not diagnosed before the start of the treatment. This is even more exceptional because infection, being one of the major factors that interfere with wound healing, is usually the cause for a wound becoming chronic.[17,18] One of the possible explanations is that the daily change of dressing and gel had a sponging action similar to that found with other hydrocolloids, permitting mild debridement and removal of the substrate with the bacteria. In addition, bacteriologic tests conducted in our laboratory (unpublished data) demonstrated that, compared with blood agar, the enriched cell culture medium gel does not readily support bacterial growth. Moreover, the concomitant growth of vascular components within the granulation tissue improved the function of the immune system with increased infiltration of immune system cells involved in the inflammatory process and in the elimination of bacterial contamination. In addition, angiogenesis increases exposure of the wound to circulating cells such as platelets, leukocytes, and macrophages. These cells are activated and release growth factors that further accelerate the healing process.[19]

In conclusion, application of a physiologically balanced nutritional substrate enriched with three nonsteroidal anabolic hormones was effective in stimulating the cascade of wound healing in both vascular insufficiency/systemic disease and postsurgical/trauma wounds. Shortening of the treatment period is an advantage associated with enriched cell culture medium treatment. Further controlled clinical trials are indicated.

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ACKNOWLEDGMENTS

We gratefully acknowledge the contribution of Dr. Ada Tamir for the statistical analysis. We also thank Life Medical Sciences, Inc., Edison, N.J., for support of this work.

REFERENCES

1. Cooper, D. M. The physiology of wound healing: An overview. In D. Krasner (Ed.), *Chronic Wound Care: Clinical Source Book for Healthcare Professionals.* King of Prussia, Pa.: Health Management Publications, 1990. Pp. 6–12.
2. Hunt, T. K. Vascular factors govern healing in chronic wounds. In A. Barbul, M. D. Caldwell, W. H. Eagelstein, et al. (Eds.), *Clinical and Experimental Approaches to Dermal and Epidermal Repair: Normal and Chronic Wounds.* New York: Wiley-Liss, 1991. Pp. 76–84.
3. Nwomeh, B. C., Yager, D. R., and Cohen, I. K. Physiology of the chronic wound. *Clin. Plast. Surg.* 25: 341, 1998.
4. Hunt, T. K. Wound fluid: The growth environment. In A. Barbul, M. D. Caldwell, W. H. Eagelstein, et al. (Eds.), *Clinical and Experimental Approaches to Dermal and Epidermal Repair: Normal and Chronic Wounds.* New York: Wiley-Liss, 1991. Pp. 109–125.
5. Stadelmann, W. K., Digenis, A. G., and Tobin, G. R. Impediments to wound healing. *Am. J. Surg.* 176 (Suppl. 2A): 39s, 1998.
6. Hunt, T., and Hopf, H. Nutrition in wound healing. In J. Fischer (Ed.), *Nutrition and Metabolism in the Surgical Patient,* 2nd Ed. Boston: Little, Brown, 1996. P. 423.
7. Lindenbaum, E. S., Tendler, M., and Beach, D. Serum-free cell culture medium induces acceleration of wound healing in guinea-pigs. *Burns* 21: 154, 1995.
8. Lindenbaum, E., Har-Shai, Y., Ullmann, Y., et al. Preliminary clinical trails of serum free cell culture medium as a treatment for chronic atrophic ulcers. *Eur. J. Plast. Surg.* 20: 115, 1997.
9. Ramasastry, S. S. Chronic problem wounds. *Clin. Plast. Surg.* 25: 367, 1998.
10. McAree, K. G., Klein, R. L., and Boeckman, C. R. The use of cultured epithelial autografts in the wound care of severely burned patients. *J. Pediatr. Surg.* 28: 166, 1993.
11. Cuono, C., Langdon, R., and McGuire, J. Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. *Lancet* 1: 1123, 1986.
12. Boyce, S. T., Supp, A. P., Harriger, M. D., Greenhalgh, D. G., and Warden, G. D. Topical nutrients promote engraftment and inhibit wound contraction of cultured skin substitutes in athymic mice. *J. Invest. Dermatol.* 104: 345, 1995.
13. Compton, C. C., Hickerson, W., Nadire, K., and Press, W. Wound healing potential of cultured epithelium. *Wounds* 5: 97, 1993.
14. Vogt, P. M., Lehnhardt, M., Wagner, D., Jansen, V., Kreig, M., and Steinau, H. U. Determination of endogenous growth factors in human wound fluid: Temporal presence and profile of secretion. *Plast. Reconstr. Surg.* 102: 117, 1998.
15. Levine, R., Agren, M. S., and Mertz, P. M. Effect of occlusion on cell proliferation during epidermal healing. *J. Cutan. Med. Surg.* 2: 193, 1998.
16. Vogt, P. M., Andree, C., Breuing, K., et al. Dry, moist and wet skin wound repair. *Ann. Plast. Surg.* 34: 493, 1995; discussion 34: 499, 1995.
17. Robson, M. C. Wound infection: A failure of wound healing caused by an imbalance of bacteria. *Surg. Clin. North Am.* 77: 637, 1997.
18. Tarnuzzer, R. W., and Schultz, G. S. Biochemical analysis of acute and chronic wound environments. *Wound Repair Regen.* 4: 321, 1996.
19. Dvonch, V., Murphy R. J., Matsuoka, J., et al. Changes in growth factor levels in human wound fluid. *Surgery* 112: 18, 1992.