Autologous Bone Marrow Aspirate Therapy for Skin Tissue Engineering and Tissue Regeneration

Gopal Ji Gupta,¹ Kanchan Karki,^{2,*}
Pradeep Jain,³ and Ajit Kumar Saxena⁴

¹Department of General Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

²Centre of Excellence for Mountain Biology, Uttarakhand Council for Biotechnology, Haldi, India.

³Department of Plastic Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

⁴Pathology and Lab Medicine, All India Institute of Medical Sciences, Patna, India.

Introduction: Chronic wounds that are difficult to heal are physical and financial burden to the sufferer and a challenge to the treating physician.

Objective: The main purpose of this study was to develop a novel method of using bone marrow (BM) aspirate, either cultured or fresh, as early solution for healing of chronic wounds and further reduce infection and tissue necrosis.

Approach: In this case-control study conducted on 75 patients with chronic wounds, 50 patients with aspirate or cultured BM were used as cases and 25 with only daily saline dressings were used as controls.

Results: Autologous BM aspirate either as fresh or cultured even without identification, isolation, and selective application of stem cells achieved significant reduction in the wound surface area when compared with control group.

Innovation: The application of cultured BM cells had significantly higher percentage reduction of wound size compared to freshly applied BM aspirate; this reflects a high importance of stem cell therapy.

Conclusion: The acceptability of the procedure among the patients was highly encouraging. The entire procedure was safe and without any complication.

Keywords: bone marrow aspirate, tissue regeneration, stem cells

INTRODUCTION

CHRONIC WOUNDS REPRESENT a growing healthcare burden that particularly afflicts aged, diabetic, vasculopathic, and obese patients.¹ Normal wound healing is a complex, coordinated sequence of events orchestrated by interactions between many cell types, appropriate cytokines, and growth factors, and proceeds from hemostasis through inflammation to organized tissue regeneration. Impaired wound healing results when these processes fail to progress through the sequential stages of healing and instead characterized by chronic inflammation and increased local injury.² Apart from the obvious morbidity to

the patient, these problem wounds can be a major drain on financial and human resources. Therefore, it is very important to stimulate the healing of acute and chronic wounds to a level that is not presently possible with standard care measures or recently developed innovative approaches.

Recent evidence indicates that stem cells derived from bone marrow (BM) have the potential to treat many disorders given their plasticity and ability to differentiate into various types of tissues, including skin cells.³ Stem cells are known to participate in cell migration, cell proliferation, and repair mechanism and rejuvenate or rebuild tissue compartments of injured

Kanchan Karki, MSc, PhD

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*Correspondence: Centre of Excellence for Mountain Biology, Uttarakhand Council for Biotechnology, Biotech Bhavan, Haldi 263146, India (e-mail: kanchan.karki1@gmail.com).

tissue, as observed in chronic wounds.⁴ The BM is an important source of hematopoietic stem cells that regularly regenerate components of the blood and nonhematopoietic stem cells, including mesenchymal stem cells (MSCs). There are several potential mechanisms by which autologous stem cells could significantly contribute to wound healing. Recently, increasing focus is being placed on the use of BM-derived MSC. Previous reports also suggested that such autologous cultured cells could bring about closure of long-standing and hard-to-heal wounds.³

However, even with most current therapies, >50% of chronic wounds remain refractory to treatment.⁵ Therefore, it is very challenging to identify cost-effective actual application of stem cells to reduce the frequency of nonhealing wounds. To achieve these goals, our study not only encompasses the effect of topically applied BM aspirate on chronic wounds but also provides the effect of only saline dressing in chronic wounds.

CLINICAL PROBLEM ADDRESSED

To develop better approach for healing of non-healing chronic wounds.

MATERIALS AND METHODS

Selection of patients and control cases

For the prospective study, 75 patients of chronic wounds, out of which 50 were in the study group and 25 were controls, with male to female ratio ~3:1, from the Department of Plastic Surgery, University Hospital, Banaras Hindu University, Varanasi, were inducted. Three study groups of patients were made: group A: with only BM application, group B: with cultured BM application, and group C: as control, with only daily saline dressings. Specific inclusion criteria considered for this study were all chronic wounds not responding to conventional methods till 6 weeks and having size $\leq 100 \text{ cm}^2$, and exclusion criteria include patients having diabetic wounds, wounds suspected of malignancy, history of hypertension, clinically significant cardiac or respiratory dysfunction, abnormal hematology, and a wound size $>100 \text{ cm}^2$. The study protocol was approved by the ethics committee of the Institute of Medical Sciences, Banaras Hindu University. Informed consent of each patient was obtained and detailed history of the patient was noted in the prepared proforma.

Methods

BM aspiration procedure and application. This is done under a fully aseptic condition. Patient was

Figure 1. BM aspiration from iliac crest. BM, bone marrow. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

made to lie in lateral position (for aspiration from iliac crest) (Fig. 1) or prone (for aspiration from sacrum). Half of the aspirated BM was applied (as instillation over wound bed) instantaneously in the same sitting (Fig. 2), while the remaining half was stored in refrigerator at 4°C to be applied on third day for second application. Just after instillation, wound was covered by nonadherent paraffin-impregnated gauze followed by occlusive dressing.

Culture procedure. BM aspirate was taken in heparinized syringe under all sterile conditions. The aspirate was then centrifuged and the pellet was redissolved in the small amount of media (DMEM). BM cells were cultured for 72 h (Fig. 3A,

Figure 2. BM applied as instillation over the wound. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 3. (A) BM aspirate cell cultured for 72 h showing confluency of cells. (B) Bilayer membrane is formed after successful culture in sterile condition. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

B). Serial monitoring of the wound with recording of various parameters was done as per the already prepared “Wound Assessment Chart.” Wound measurement was done by tracing the surface on formalin-sterilized translucent graph paper (Fig. 4).

Statistical analyses

Database management and all statistical analyses were performed using SPSS for Windows (version 16.0; SPSS, Inc., Chicago, IL). Qualitative data were expressed as percentages, and quantitative data were expressed as mean $\pm$ standard deviation. All categorical variables, including clinical parameters, were compared by χ^2 test.

Figure 4. Wound size measurement using translucent graph paper. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Table 1. Study cohort

Clinical Symptoms	Chronic Wounds Patients (n = 75), n (%)
Age (years)	
10–20	17 (22.7)
>20–30	16 (21.3)
>30–40	24 (32)
>40–50	10 (13.3)
>50	8 (10.3)
Sex	
Male	57 (76)
Female	18 (24)
Etiology	
Posttraumatic	29 (38.7)
Postthermal burn	13 (17.3)
Postelectric burn	10 (13.3)
Venous insufficiency	10 (13.3)
Pressure ulcer	5 (6.7)
Postinfective	8 (10.7)
Duration	
6 weeks–3 months	41 (54.7)
>3 months–6 months	22 (29.3)
>6 months–1 year	7 (9.3)
>1 year	5 (6.7)
Location	
Upper limb	30 (40.0)
Lower limb	34 (45.3)
Trunk	10 (13.33)
Head and neck	1 (1.33)
Size (in cm ²)	
Upto 25	16 (21.6)
>25–50	39 (52)
>50–100	20 (26.7)
Hb (in gm%)	
Upto 10	51 (68)
>10	24 (32)
Site of BMA	
Iliac crest	38 (76)
Sacrum	12 (24)
Wound swab culture	
Culture +ve	54 (72)
Culture –ve	21 (28)

BMA, bone marrow aspirate.

Table 2. Correlation between various etiologies of chronic wounds patients using χ^2 test

Etiology	BM, n (%)	cBM, n (%)	Control, n (%)	χ^2	p
Posttraumatic	11 (44.0)	8 (32)	10 (40)	6.488	0.773
Postthermal burn	6 (24.0)	3 (12)	4 (16)		
Postelectric burn	3 (12.0)	5 (20)	2 (8)		
Venous insufficiency	3 (12.0)	3 (12)	4 (16)		
Pressure ulcer	1 (4.0)	2 (8)	2 (8)		
Postinfective	1 (4.0)	4 (16)	3 (12)		
Total	25 (100)	25 (100)	25 (100)		

p -value <0.05 was considered statistically significant.
BM, bone marrow; cBM, cultured bone marrow.

Data were analyzed statistically by independent Student's t -test and one-way ANOVA. p -value <0.05 was considered statistically significant.

RESULTS

A case-control study was conducted on 75 patients with chronic wounds, out of which 50 patients with aspirate or cultured BM were used as cases and 25 with only daily saline dressings were used as controls. Table 1 shows the general characteristics of chronic wounds in patients inducted in the study.

Correlation between various etiologies of chronic wounds in patients using χ^2 test

Chronic wounds with no application of BM or cultured were taken as control for comparison with various wounds of different etiologies. There was no significant difference with respect to different etiologies of study groups (Table 2).

Correlation between various durations of chronic wounds in patients using χ^2 test

There was no significant difference with respect to various durations of study groups (Table 3).

Change in wound dimensions

Results of ANOVA analysis show that there was no significant change in wound dimension on day 1 and 3 in all the studied groups. However there was a significant correlation ($p < 0.05$) between all three groups and change in wound dimension on day 7 and in the fourth week (Table 4 and Fig. 5).

Table 3. Correlation between various durations of chronic wounds patients using χ^2 test

Duration	Hb <10 gm%, n (%)	Hb >10 gm%, n (%)	χ^2	p
6 weeks–3 months	11 (45.8)	30 (58.8)	4.117	0.249
>3 months–6 months	9 (37.5)	13 (25.5)		
>6 months–1 year	1 (4.2)	6 (11.8)		
>1 year	3 (12.5)	2 (3.9)		
Total	24 (100)	51 (100)		

p -value <0.05 was considered statistically significant.

Table 4. Change in wound dimensions

Time	BM, Mean $\pm$ SD	cBM, Mean $\pm$ SD	Control, Mean $\pm$ SD	f	p
Day 1	39.56 $\pm$ 17.09	40.33 $\pm$ 13.87	39.03 $\pm$ 11.10	0.052	0.949
Day 3	33.42 $\pm$ 11.34	31.33 $\pm$ 8.58	37.65 $\pm$ 10.77	2.443	0.094
Day 7	30.20 $\pm$ 12.37	24.90 $\pm$ 7.34	33.33 $\pm$ 8.66	4.831	0.010 ^a
Week 4	22.54 $\pm$ 6.32	18.42 $\pm$ 7.55	30.54 $\pm$ 8.62	13.872	<0.001 ^a

^a p -value <0.05 was considered statistically significant.
SD, standard deviation.

Group comparison. Group comparison analysis depicts that wound dimension was significantly changed between BM and cultured bone marrow (cBM), BM and control, and cBM and control in the fourth week. However, there was no significant change in wound dimension on day 1 (Table 5).

Progress of the wound: percentage reduction

There were appreciable differences in the surface area of chronic wounds in patients where BM was applied in either form (aspirate or cultured). This was more significantly observed in patients where cultured BM was applied over wounds compared with control groups with only saline dressings (Table 6).

Group comparison. There was a progressive and significant percentage reduction of wound surface area from day 3 onward when compared with the control group. This was a more dominant feature in the group where cultured BM was applied throughout the study (Table 7).

Follow-up (after 4 weeks)

Follow-up study shows that 60% of patients in the BM group and 76% of patients in the cBM group did not require any further intervention, whereas, 80% of the control cases required surgical interventions in the form of grafting after 6 weeks (Table 8). Figure 6 shows postelectric burn non-healing raw area around the right knee. After

Figure 5. Change in wound area (cm²). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Table 5. Group comparison of wound size

Time Interval	p		
	BM vs. cBM	BM vs. Control	cBM vs. Control
Day 1	0.980 ^a	0.991 ^a	0.944
Day 3	0.928 ^a	0.174 ^a	0.049 ^a
Day 7	0.077 ^a	0.157 ^a	<0.001 ^a
Week 4	0.041	0.004	<0.001 ^a

^ap-value <0.05 was considered statistically significant.

application of cBM, wound healing was expedited with noticeable reduction in wound size. Almost 10% of the area was yet to be healed at week 6. At 16 month follow up, the wound was healed with the scar showing no contracture at knee joint. Another case of electric burn with nonhealing area over nape of neck is shown in Figure 7. cBM was applied, following which wound dimensions were reduced with achievement of almost 70% wound healing in 3 weeks. Wound closure occurred after 1 month. A follow up image after 2 months and 16 months show soft, supple skin without any contracture and contour defect like hypertrophy.

DISCUSSION

Contrary to the common belief that the wound healing is delayed only in old age, we, in our study, found the involvement of all age groups, with 76% of the patients below 40 years of age (Table 1). The male to female ratio of ~3:1 indicates the fact that the male population is more active and prone to sustain trauma. In a nondiabetic scenario, trauma was the leading cause (~39%), followed by burn (~30%). Venous insufficiency and pressure ulcer were other causes of chronic wounds. Majority of the patients (54.7%) had wounds for 6–12 weeks at the time of seeking our help. As expected, the lower limb was the most common site to be affected (45.3%), followed by the upper limb (~40%). Majority of the patients (52%) had large wounds (>25 to 50 cm²), but a smaller chronic wound (25 cm² or less) was equally disturbing and forced the patient to seek treatment for delayed healing. In our circumstances, except on few occasions, we could aspirate only 10 mL on an average from the iliac crest. Besides, on some occa-

Table 6. Progress of the wound

Percent Change	BM, Mean ± SD	cBM, Mean ± SD	Control, Mean ± SD	f	p
Day 3	15.52 ± 3.22	22.31 ± 4.51	3.53 ± 0.41	219.64	<0.001 ^a
Day 7	23.66 ± 5.32	38.26 ± 5.87	14.60 ± 2.51	154.76	<0.001 ^a
Week 4	42.02 ± 6.55	54.32 ± 6.89	21.75 ± 4.55	182.64	<0.001 ^a

^ap-value <0.05 was considered statistically significant.

Table 7. Group comparison of change in wound size

Time Interval	BM vs. cBM	BM vs. Control	cBM vs. Control
Day 3	<0.001	<0.001	<0.001 ^a
Day 7	<0.001	<0.001	<0.001 ^a
Week 4	<0.001	<0.001	<0.001 ^a

^ap-value <0.05 was considered statistically significant.

sions, we failed to aspirate from the iliac crest and had to aspirate from the sacrum in that situation (24%). Seventy-two percent of the chronic wounds in our study had positive culture.

We excluded wounds larger than 100 cm² as it was usually difficult to aspirate more than 10 mL in one attempt and our planning was to apply 0.1 mL of aspirate/cm² of the wound. We deliberately avoided aspiration from the sternum because of likelihood of associated complications and requirement of special needle. We found aspiration from the iliac crest as well as sacrum to be absolutely safe and without any complication and morbidity in our study.

The BM is an important source of hematopoietic stem cells and nonhematopoietic stem cells including MSCs. We used BM aspirate from the same individual in an autologous manner to avoid the risk of rejection, prevent a chance of blood-borne transmission of diseases, and avoid ethical concern.

While some authors⁶ injected the BM aspirate into the wound margins, others⁷ used it as a topical application. Badiavas and Falanga³ used it for topical application and also injected into the wound margins. However, we used freshly aspirated BM for topical application without its centrifugation. To retain BM aspirate or its cultured form on the wound bed, we applied it slowly and carefully preventing its run off from the wound. To retain the MSCs present in BM aspirate in the chronic wounds, some other methods have also been described such as applying “Integra,”⁸ Biological skin substitute,⁹ collagen sponge,^{10,11} and occlusive film dressing (Tegaderm) as done by Badiavas and Falanga.³

Throughout the study at every point of evaluation, the mean percentage reduction in wound size was significantly higher in BM/cBM group compared to the control group. This clearly shows that the BM application whether freshly applied or following culture does have some elements to expedite wound healing. We presume this element to be

Table 8. Follow-up (after 4 weeks)

Percent Change	BM (n = 25), n (%)	cBM (n = 25), n (%)	Control (n = 25), n (%)
No further intervention	15 (60)	19 (76)	5 (20)
Surgical intervention required	10 (40)	6 (24)	20 (80)

Figure 6. Postelectric burn nonhealing raw area around right knee. After application of cBM, wound healing expedited with noticeably reduction in wound size (A–D); almost 10% area remained to be healed at week 6 (E); 16 months follow up picture (F) showing healed scar without any contracture at knee joint. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

none other than stem cells as we did not have the facility and resources to immunologically mark the stem cells in particular and isolate them, which would have been an ideal step.

The significant difference in the wound dimensions between the BM aspirate and cBM cells group

could be observed only at the end of the study. While the BM aspirate group showed significant reduction in wound dimensions compared to control at 4 weeks, the cBM group significantly reduced the wound size in comparison to control from as early as day 3, which may provide a chance for their serial application over

Figure 7. A case of electric burn with nonhealing area over nape of neck (A); cBM was applied, following which marked reduction noted in wound dimensions (B & C) with achievement of almost 70% wound healing in 3 weeks (D); wound closure occurred after 1 month (E). Follow up image after 2 months (F) and 16 months (G) showing soft, supple skin without any contracture and contour defect like hypertrophy. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

the chronic wounds without bothering the patients with repeated aspiration.

The endpoint of our study was 4 weeks as reported also by Falanga *et al.*¹² We could not afford to do so because we could not keep our control patients indefinitely without any specific measure for healing of their wounds. However, as a natural curiosity, we continued to follow up our patients following application of BM/cBM as long as the patients did not insist for intervening surgically.

Stem cells are one of most studied and the main cellular component of this therapy. Transplanted MSCs are thought to impact locally through increased angiogenesis, antiapoptotic signaling, or paracrine signaling. MSCs have the potential to stimulate and differentiate the resident progenitor cells and secrete growth factors to promote recovery of chronic wounds. The delivery method and the number of cells delivery should also be taken into consideration during the stem cell therapy for wound management. Although no attempt was made by us to isolate the various cellular elements in the BM aspirate and using any particular delivery method, we achieved comparable results in terms of mean percentage reduction of wound size at the endpoint of 4 weeks. The wounds that completely healed following application of BM did so with soft, supple, and durable scar without producing any contour deformity or contracture of the wound or nearby joint at as much a late follow-up as 16 months (Figs. 3 and 4). This probably hints at dermal regeneration side by side with epithelialization of the wound.

INNOVATION

Our study highlights the importance of the effective use of bone marrow aspirate either as fresh or cultured. Even without identification, isolation and selective application of stem cells it achieved a significant reduction in the wound surface area when compared with the control group.

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KEY FINDINGS

- Chronic wounds are costly and stressful burden for patients and healthcare professionals; urgently needs novel and highly effective treatments.
- Cultured BM cells had significantly higher percentage reduction of wound size compared to freshly applied BM aspirate and normal saline dressing patients.
- We further recommend a biopsy examination from the wound in follow-up to throw some light on the mechanism of healing chronic wounds.

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AUTHOR DISCLOSURE AND GHOSTWRITING

No ghostwriter was booked for preparation of this article.

ABOUT THE AUTHORS

Kanchan Karki, MSc, PhD, is currently working as a Senior Scientific Officer at Uttarakhand Council for Biotechnology, Govt. of Uttarakhand, Haldi, after her successful completion of PhD from Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi 221005. She has more than 25 research articles in journal of repute such as translational research, clinical nutrition, and DNA cell biology. **Gopal Ji Gupta, MS (General Surgery)**, is a Senior Resident at Department of General Surgery IMS, BHU, who completed his thesis research work on wound healing under the supervision of **Pradeep Jain, MBBS, MS (General Surgery), MCh (Plastic Surgery), PhD (Plastic Surgery), INSA, MNAMS**, Professor Department of Plastic Surgery, IMS, BHU, and under cosupervision of **Ajit Kumar Saxena, MSc, PhD, FIMBS, MAMS**, Professor and Head Pathology and Lab Medicine, All India Institute of Medical Sciences (AIIMS), Patna. He was previously working as Professor at Centre of Experimental Medicine & Surgery, IMS, BHU. All authors have given substantial contributions during execution of research work as well as formulation of this article.

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Abbreviations and Acronyms

ANOVA = analysis of variance
 BM = bone marrow
 BMA = bone marrow aspirate
 cBM = cultured bone marrow
 DMEM = Dulbecco's modified Eagle's medium
 MSCs = mesenchymal stem cells
 SD = standard deviation