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Our experience in cell therapy in surgical problems

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Abstract

Aim: To study the effect and efficacy of autologous bone marrow cell therapy in various surgical pathologies of varied etiology which were refractory to conventional methods of management.

Material and method: Ethical committee clearance and informed consent of patients were taken. Two groups of each surgical pathologies were selected randomly. The patients in study group were treated by conventional methods followed by autologous bone marrow cell application locally, whereas patients in control group were treated by conventional methods only. The patients were then followed post-operatively and analyzed by statistical methods.

Result: Post-operatively the patients in study group experienced faster recovery by appearing good quality granulation tissue and rapid epithelisation and there was also significant reduction in local pain. Biopsy showed improved fibroblast, macrophages, neovascularisation and less necrosis per HPF in study group as comparison to control group.

Conclusion: Autologous bone marrow cell therapy is a novel method in the management of various surgical pathologies.

Keywords: Autologous bone marrow cell therapy; Surgical problems; Carcinoma breast; Fistula in ano; Ulcer; Incisional hernia

1 Introduction

In this era of regenerative medicine, use of stem cell in various alignments has shown a very promising result. This paper is used to highlight our experience in the curative measures in various surgical alignments in our department.Stem cells are undifferentiated or non-specialised cells that are able, through cell division, to renew themselves indefinitely [1].Crucially, they are also able, when provided with the appropriate stimuli, to differentiate into one or more of thedifferent types of specialised cell found in tissues and organs. Because of their unique ability to undergo self-renewal whencultured *in vitro* and to be directed to differentiate into specializedcell types, they have enormous potential for use ascell-based therapies [2-4]. There are several different types of stemcell with different characteristics, all of which have potentialuses in regenerative medicine.Stem cells can be classified according to whether they are derived from the early embryo (embryonic stem cells), tissues from the fetus (fetal stem cells), later in development (adult or somatic stem cells/SSC) or whether they are derived by reprogramming adult specialised cells to become pluripotent stem cells (iPSCs). Mesenchymal stem cellS,which are a type of SSC, have potent trophic and anti-inflammatory properties, attributable to their ability to produce growth factors (including vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF)), and prostaglandin E2 [5-8]. Thus it is mostly used in tissue engineering and regenerative therapy. MSCs can be isolates from both bone marrow and subcutaneous fat by liposuction. We preferred he former route.

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With permission of ethical committee and patients consent we have used cell therapy in Complex fistula in ano , Incisional hernia, carcinoma breast following MRM, Chronic diabetic ulcer, Sickle cell disease ulcer, Burger's disease ulcer & others P.V.D, Hansen's ulcer, Complicated burn ulcer with promising results.

A chronic leg ulcer may be defined as a defect in the skin below the level of knee that fails to heal in the expected time which was usually less than 3 weeks and shows no tendency to heal after 3 or more months. Delays in healing occur at any phase but most often occur in inflammatory phase [9-12]. Various etiology of chronic leg ulcers are arterial, venous, neurogenic, lymphatic, hematological disorders, infectious, metabolic(diabetes mellitus) and burn wound etc. chronic leg ulcer affect the daily life of a patient by increasing pain, sleep disturbance, restricted mobility, restricted work capacity, financial loss. So chronic leg ulcer is usually associated with significant morbidity, loss of productivity and reduced quality of life. In past decades, bone marrow cell based therapies have emerged as popular choices in conjunction with other treatment modalities for chronic ulcer healing and regeneration of skin structure and functions [13-16]. Autologous bone marrow cell may offer potential therapies in two broad areas of chronic ulcer surgery: in the enhancement of chronic ulcer healing and closure and in attenuation of immunosuppressant effects of the inflammatory response [17-22].

Management of complex perianal fistula is often challenging in terms of post treatment recovery and associated morbidity(23). Despite recent progress in treatment of the condition, there is considerable recurrence rates after definite surgery for complex anal fistula have been documented [24-27]. Interdisciplinary treatment regimen is essential for disease control and potential long-term remission. Therapeutic goals include symptom improvement, prevention of recurrent perianal abscess, preservation of continence, improvement of quality of life and, finally, healing of the wound. Several reports and randomized studies have demonstrated that stem cell therapy for Crohn's complex anal fistula has raised the healing rates after 12 mo [28-34].

Incisional hernias are result of common complication of abdominal midline incision and wound dehiscence [35-38]. Once patient developes such a hernia, they are at risk for recurrent hernia formation via the same wound even after repair , and the best mesh for incisional hernia is yet to be discovered. The best surgical techniques for years have not answered to this question. At the present more than hundred surgical meshes are available in the market, however the ideal mesh does not yet exist and still needs to be developed [39-42]. Postoperative complications like seroma formation, adhesion, enterocutaneous fistula, surgical site infection and recurrence still occurs despite advancement in prosthetic technology [43-45]. In this recent scientific study we have concentrated on improving prosthetic biocompatibility such as coating mesh with the patient's own bone marrow cells to reduce mesh related complicationsand for a strong repair [46-48].

Carcinoma breast is now the most common cancer worldwide. In our country where early diagnosis of breast cancer is often delayed, many patients go into locally advanced stage or metastatic stage [50-52]. Thus majority of them require modified radical mastectomy rather than breast conservative surgery. But after modified radical mastectomy they suffer from the common immediate complications like wound pain, marginal necrosis, flap necrosis, seroma, wound infection, lymphoedema, stiff shoulder and also from delayed complications like hypertrophic scar, keloid and local recurrence of tumour [53-55]. Due to these complications the duration of hospital stay increases and the adjuvant therapy for such patients also gets delayed. Many studies have been done all over the world to prevent these complications [56-57]. None of the methods alone is sufficient to prevent such problems thus a combined approach is used. Autologous bone marrow therapy is a new concept which is based on the facts that bone marrow contains mesenchymal stem cells which stimulates the local stem cells, fibroblast and macrophages for their enhanced activity [58-62]. They also liberate cytokines and growth factors which helps in rapid wound healing and preventing early flap complications like wound pain, flap and marginal necrosis and seroma formation.

Aims and objective

Comparison between autologous bone marrow derived cell therapies based treatment to conventional management in the following conditions.

- Complex fistula in ano
- Incisional hernia
- In carcinoma breast following MRM
- Chronic diabetic ulcer
- Sickle cell disease ulcer
- Burger's disease ulcer & others P.V.D
- Hansen's ulcer

- Complicated burn ulcer

2 Material and methods

Ethical clearance and informed consent of the patients were taken before the study among the patients admitted in our surgery unit during the year January 2016 to September 2021 at VSSIMSAR, Burla, Odisha, India were randomly selected for study.

2.1 Inclusion criteria

Arterial ulcer, venous ulcer, sickel cell ulcer, diabetic ulcer, neuropathic ulcer, infective ulcer, post burn ulcer, incisional hernia, ca breast patients requiring modified radical mastectomy.

2.2 Exclusion criteria

Unmotivated patient: A thorough history was taken then clinical examination and investigation (culture from ulcer, routine blood investigation, serum total protein, albumin, usg Doppler of lower limb, x-ray of that part, edge biopsy, FNAC of lymph node, ECG,) were done to diagnose the cause of ulcer.In complex anal fistula patients MR fistulogram done preoperatively. Incisional hernia patients requiring surgery, undergonerelevent investigations including a pre-operative CT scan in selected cases. Proper pre-operative work up done in carcinoma breast patients requiring modified radical mastectomy. Conventional surgical intervention performed in all patients. In the study group autologous bone marrow cell derived from bone marrow was infiltrated locally at the site of pathology after conventional surgical intervention.

3 Results

3.1 Cell therapy in chronic ulcers

A thorough history was taken then clinical examination and investigation (culture from ulcer, routine blood investigation, serum total protein, albumin, usg Doppler of lower limb, x-ray of that part, edge biopsy, FNAC of lymph node, ECG,) were done to diagnose the cause of ulcer. Out of 80 diagnosed case 40 patients kept in study group and rest 40 in control group. Debridement and primary ulcer care were performed in all patients. But in study group autologous bone marrow cell derived from bone marrow was injected at the ulcer base, floor, margin, edge and surrounding tissues of the ulcer site after debridement was done in study group then the wound was covered with normal saline soaked gauge. Post-operatively the patients were followed and parameters like wound pain, quality of granulation tissue from wound on day-3 onwards were studied. Wounds in study group showed better result.

Table 1 Patients distribution in Cell therapy in chronic ulcers

diagnosis	Number of patients in Study group	Number of patients in control group	Total number of patients	percentage
Diabetes mellitus	15	15	30	37.5
Venous ulcer	11	11	22	27.5
Sickle cell ulcer	5	5	10	12.5
Buergers disease	4	4	8	10
Burn wound	3	3	6	7.5
Hensen’s disease	2	2	4	5

Table 2 Postoperative pain in study vs control group

Post-operative days	Number of patients required analgesic in study group	Number of patients required analgesic in control group
1	32	33
2	28	30
3	12	30
5	5	28
7	1	15
10	0	10
11	0	0

Biopsy was taken from floor of ulcer on post-operative day 3 and histopathological examination done; average number of fibroblast per HPF in study group was 14.8, but in control group it was 3.2. similarly, macrophage per HPF was 18.1 in study group but it was 5.3 in control group; neovascularisation and necrosis in study group were 18.3 and 0.8 respectively however in control group it was 6.5 and 5.1 respectively.

Table 3 Comparison of wound healing of study group and control group

	Fibroblast/HPF	Macrophages/HPF	Neovascularisation/HPF	Necrosis/HPF
Study group	14.8	18.1	18.3	0.8
Control group	3.2	5.3	6.5	5.1

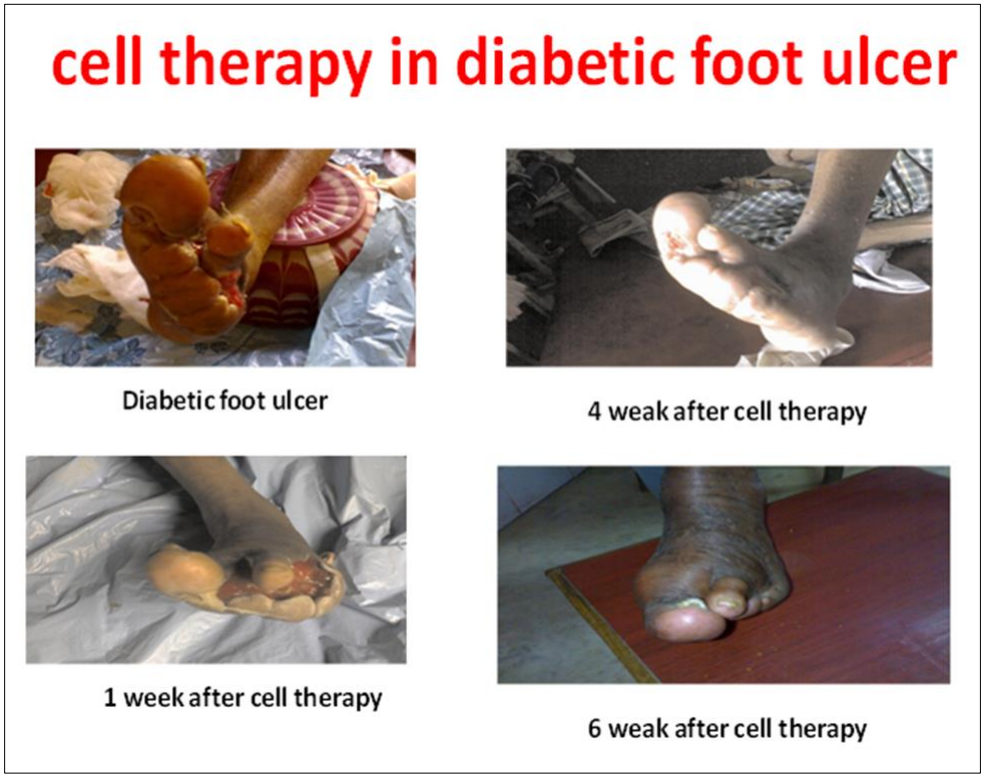

Figure 1 Cell therapy in diabetic foot ulcer

Figure 2 Cell therapy in sickle cell ulcer

Figure 3 Cell therapy in peripheral vascular disease

Figure 4 Cell therapy in burn injury

3.2 Cell therapy in complex fistula in ano

- In our study, 50 patients of complex anal fistulas were studied. In the first group (control group) of 25 patients were underwent surgical therapy only. The second group (study group) of 25 patients underwent surgical therapy followed by local infiltration of bone marrow derived cells in fistulectomy bed. In the study group, wound is laid open and dressed with normal saline soaked gauge piece, no antiseptic lotion applied. In control group only fistulectomy done, wound laid open and dressed with providone iodine soaked gauge piece. Both groups were compared in post-operative follow up. Biopsy of granulation tissue taken from the wound of both control and study group on post-operative day 5 for Histopathological examination. Post-operative wounds in the study group showed better quality of granulation tissue,less post-operative discharge, less local pain and tenderness.

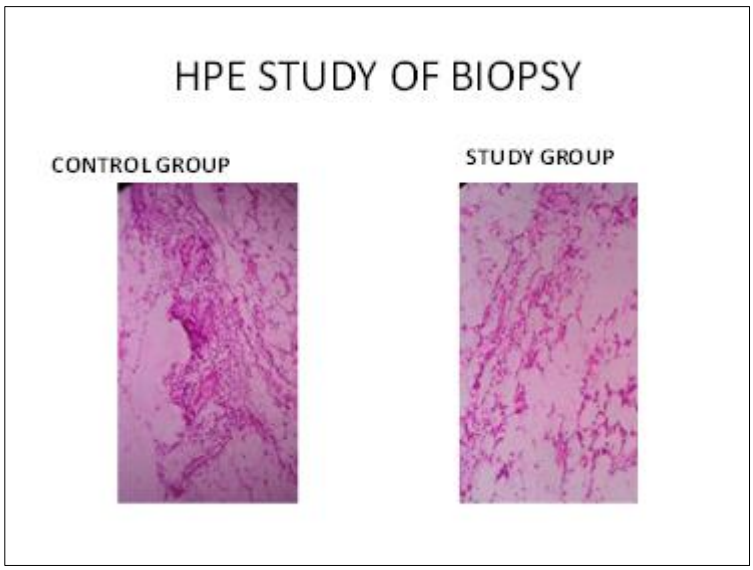

Figure 5 Histopathology of biopsy specimen from wound site post operatively on post-operative day

Table 4 Comparison of post-operative wound study

	Control	Study
necrosis	More	Less
Neutrophils, lymphocytes	Less	More
neovascularization	Less	More
macrophages	Less	More
fibroblasts	Less	More

Table 5 Comparison of post-operative wound study

	Day 2 control	Day 2 study	Day 5 control	Day 5 Study	Day 7 control	Day 7 study	Day 21 control	Day 21 study
Local pain	++++	++	+++	+	++	-	-	-
Local tenderness	++++	++	+++	-	++	-	-	-
Discharge	++	++	++	-	++	-	-	-
Floor of ulcer	poor	healthy	poor	Healthy	poor	healthy	poor	healthy

Figure 6 Cell coated mesh in incisional hernia

Total 40 patients were included in our study. All patients underwent relevant investigations. CT scan was performed in selected cases. 20 patients were included in control group and rest 20 patients included in study group. Post operatively the patients experienced less pain as compared to control group. In the study group patients did not require analgesia after post-operative day 3 whereas in control group patient required analgesia up to postoperative day 7.

Table 6 Comparison of post-operative pain in incisional hernia

Post-operative days	Number of Patients required analgesia in study group	Number of Patients required analgesia in control group
1	20	20
2	20	20
3	8	18
4	3	10
5	0	8
6	0	5
7	0	3

In study group drain was removed earlier as compared to control group as seroma collection is more in control group as compared to study group. Drain was removed when there is ≤ 10 ml seroma collection in both study and control group. And in study group no patients required drain after 5th post-operative day whereas in control group drain was present till 7th post-operative day.

Table 7 Comparison of post-operative seroma formation in incisional hernia

Post-operative days	Average Seroma collection in Study group in ml	Average Seroma collection in control group in ml
1	75	80
2	40	60
3	35	55
4	20	30
5	10	20
6	0	15
7	0	10

Out of 20 patients in control group recurrence was observed in 1 patients but in study group there was no recurrence.

Table 8 Comparison of incidence of recurrence in incisional hernia

Recurrence in study group	Recurrence in control group
0/20	1/20

There is no enterocutaneous fistula and mesh migration observed in both study and control group. Patients in study group discharged earlier (post-operative day 5) than control group (postoperative day 8).

Figure 7 Cell therapy in carcinoma of breast after Modified radical mastectomy

3.3 Cell therapy in carcinoma breast after mrm

Then total 60 breast cancer patients with stage T1-3 N0-1 M0 in this study underwent modified radical mastectomy surgery at our surgical unit. Out of 60 patients, 30 patients were kept in study group and rest 30 in control group. Auchincloss type modified radical mastectomy was performed in all patients. The bone marrow stem cell concentrate was kept aside in a sterile syringe. Then skin flaps were raised as per standard procedure, mastectomy and axillary clearance and hemo stasis was obtained in all the cases. Bone marrow stem cell concentrate was infiltrated to under surfaces of breast flaps and breast bed and to axillary bed. Skin flaps were closed with a suction drain. Pressure garment and suction drains were used routinely in both study and control group. Post operatively the patients were followed and parameters like wound pain, marginal necrosis, flap necrosis, wound infection, seroma collection, shoulder joint stiffness, hypertrophic scar, keloid and local recurrences were studied.

Post operatively the patients in study group experienced less pain as compared to control group. Out of 30 patients in control group marginal necrosis was observed in 5(16 %) patients whereas in study group it was seen in 2 (6.6 %) patients. Flap necrosis was seen in 2(6.6%) patients in control group whereas no such complication was observed in study group. In control group 5(16%) patients were observed to have seroma collection after removal of drain which required aspiration but in study group none of the patient had seroma collection after removal of drain. In control group shoulder stiffness was observed in 8 patients (26%) whereas in in study group it was observed in 3(10%) patient. local recurrence of tumour was only seen in 1 patient in control group. No local recurrence of tumour, hypertrophic scar and keloid was seen in study group.

Table 9 Comparison of post-operative complications after MRM

Post op days	Post op pain (study)	Post op pain (control)	Seroma collection in ml (study)	Seroma collection in ml (control)	Flap complication (study)	Flap complication (control)
1	mild	severe	80	110	Nil	nil
3	mild	mild	50	85	Nil+	Marginal2,flap1
5	nil	Mild	20	60	Marginal2, flap nil	Marginal5,flap 2
7	nil	Mild	10	35	Marginal2,flap-nil	Marginal5,flap 2
10	nil	mild	5	20	Marginal2,flap-nil	Marginal5,flap 2

Table 10 Comparison of incidence of complications after MRM

	Study group	**Control group**
General well being	Good	moderate
Seroma collection	0/30	5/30
Shoulder stiffness	3/30	8/30
Local recurrences	0/30	1/30

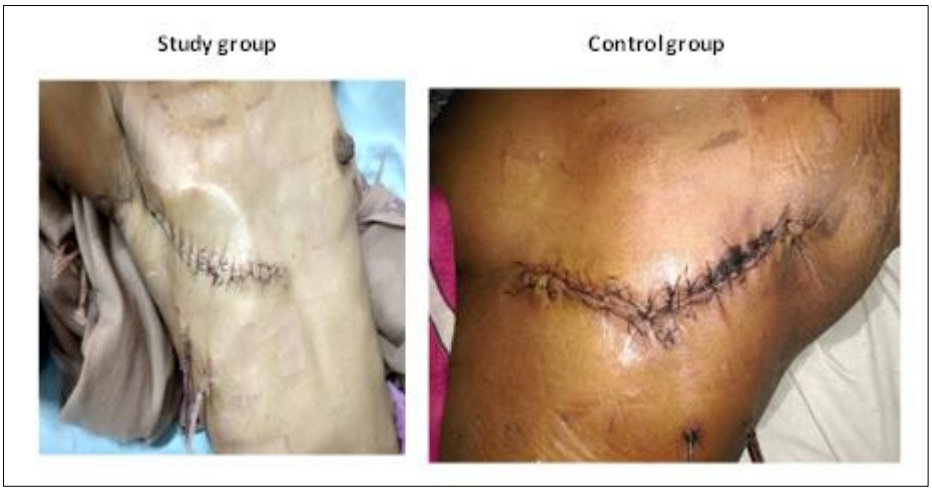

Figure 8 Post-operative wound after MRM in study and control group

4 Discussion

Stem cells are undifferentiated or non-specialised cells thatare able, through cell division, to renew themselves indefinitely.Crucially, they are also able, when provided with theappropriate stimuli, to differentiate into one or more of thedifferent types of specialised cell found in tissues and organs.Because of their unique ability to undergo self-renewal whencultured *in vitro* and to be directed to differentiate into specializedcell types, they have enormous potential for use ascell-based therapies. There are several different types of stemcell with different characteristics, all of which have potentialuses in regenerative medicine. Stem cells can be classified according to whether they are derived from the early embryo (embryonic stem cells), tissues from the fetus (fetal stem cells), later in development (adult or somatic stem cells/SSC) or whether they are derived by reprogramming adult specialised cells to become pluripotent stem cells (iPSCs).Among the best characterized types of SSCs are haematopoeitic stem cells, mesenchymal stem or stromal cells (MSCs), endothelial progenitor cells and neural stem cells. MSCs have potent trophic and anti-inflammatory properties, attributable to their ability to produce growth factors (including vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF)), and prostaglandin E2. Thus it is mostly used in tissue engineering and regenerative therapy.MSCs can be isolates from both bone marrow and subcutaneous fat by liposuction. We preferred he former route.

Mesenchymal stem cells (MSCs) were initially isolated from bone marrow but are now shown to reside in almost every type of connective tissue [16]. MSCs are characterized as a heterogeneous population of cells that proliferate *in vitro* as plastic adherent cells able to develop as fibroblast colony forming-units [17]. MSCs are distinguished from hematopoietic cells by being negative for the cell surface markers CD11b, CD14, CD34, CD45 and human leukocyte antigen (HLA)-DR but expressing CD73, CD90 and CD105. Importantly, the capacity to differentiate into multiple mesenchymal lineages including bone, fat and cartilage is used as a functional criterion to define MSCs [18]. MSCs are clearly capable of responding and modulating their function when exposed to the cells and biochemical factors that are characteristic of an injury environment. Human MSCs migrate preferentially to regions of inflammation [19] and express several chemokine receptors that are necessary to coordinate their homing ability [20]. Furthermore, MSCs have demonstrated chemotaxis toward a variety of wound healing cytokines *in vitro*, including platelet-derived growth factor, insulin-like growth factor-1, IL-8 and TNFα [21,22]. These data suggest that bone-marrow-derived MSCS or

endogenous cells resembling MSCs, such as pericytes, are likely to migrate to and participate in the response to tissue injury. During modified radical mastectomy we iatrogenically injure the lymphatic system, blood vessels and other tissues. Injury to lymphatic system causes seroma formation and lymphoedema.

Studies have shown that mesenchymal stem cells (MSCs) derived either from bone marrow or fat can express LEC markers (prox-1, VEGF-C, VEGF-A) and that stimulation of these cells in cultured media with recombinant VEGF-C, even for brief periods of time *in vitro*, markedly increased their ability to promote lymphangiogenesis *in vivo* [23, 24]. Thus adult mesenchymal stem cells may have an important role in decreasing seroma formation and also lymphoedema after modified radical mastectomy by early healing of injured lymphatic vessels. In our study we have observed less seroma collection in study group than control group and after removal of drain 9 patients out of 20 patients in control group came for seroma collection which required aspiration by needle whereas in study group none of the patient came with complaint of seroma collection after removal of drain. Our this finding supports the above literature there by suggesting the role of autologous bone marrow therapy in early healing of injured lymphatic system resulting into decreased seroma collection.

MSCs produce basic FGF and VEGF-A, which provide powerful mitogenic cues to promote proliferation, migration and differentiation of microvascular endothelial cells [25,26]. MSCs also express paracrine factors to promote vascular stability and vasoprotection [27,28], including adrenomedullin [29]. It has been hypothesized that these functions are unique to MSCs due to their possible perivascular origin, and they are able to exploit these functions to recreate their perivascular niche as theprocess of vasculature remodeling is concluded [30]. Enhancement of vascular formation by bone-marrow derived MSCs has been demonstrated *in vitro* [31] and to facilitate the development of long-lasting functional vasculature as perivascular progenitor cells [32]. Thus autologous bone marrow therapy may facilitate neovascularisation and thereby prevent marginal necrosis and flap necrosis. Though proper surgical technique like tension free suturing, not too thin flap, less thermal injury by electro cautery have an important role in preventing marginal necrosis and flap necrosis but despite the use of all these techniques marginal necrosis and flap necrosis are very frequent in patients after modified radical mastectomy.. In our study we have seen marginal necrosis in 4 out of 20 patients in control group and 2 out of 20 patients in study group and flap necrosis in 2 out of 20 patients in control group and none of the patients in study group. Though such finding is not statistically significant still relatively we have seen less marginal necrosis and no flap necrosis in study group as compared to control group. Regarding postoperative pain , patients experienced very less pain as compared to control group may be due to the anti- inflammatory activites of mesenchymal stem cells. MSCs have anti-inflammatory effects because they inhibit dendritic cell [DC] maturation and B and T cell proliferation and differentiation, that they attenuate natural killer [NK] cell killing, and that they also support suppressive T regulatory cells [Tregs] [33-35]. MSCs also decrease the amount of IL-10 and TNF-α secreted by DC cells, and increase the amount of the anti-inflammatory IL-4 produced by T cells [33-35]. MSCs provide significant benefit during dermal wound healing, as they can,

- Accelerate the rate of wound closure and re-epithelialization,
- Improve the quality and strength of the regenerated tissue,
- Recover wound healing pathologies that might otherwise result in a chronic, non-healing wound, and
- Minimize the visual appearance of scar tissue.

In adult cutaneous wound healing, inflammatory cells are recruited to the wound and produce proinflammatory mediators such as monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1 beta (MIP-1β), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). These mediators can not only induce additional inflammation but also contribute to excess extracellular matrix (ECM) deposition and fibrosis. Moreover, the inflammatory cells can produce growth factors such as transforming growth factor-beta 1 (TGF-β1) and platelet-derived growth factor, which stimulate fibroblast proliferation, myofibroblast differentiation, and excess ECM deposition, leading to scar formation. We have not seen any local recurrence and it need to be studied more to prove the anti-tumour effects of mesenchymal stem cells (Figure 4). This study is purely clinical and we have only seen the effects of autologous bone marrow therapy and the rationale behind them are still being studied at molecular level. At present our sample size is small and we will continue our research in more number of patients in future.

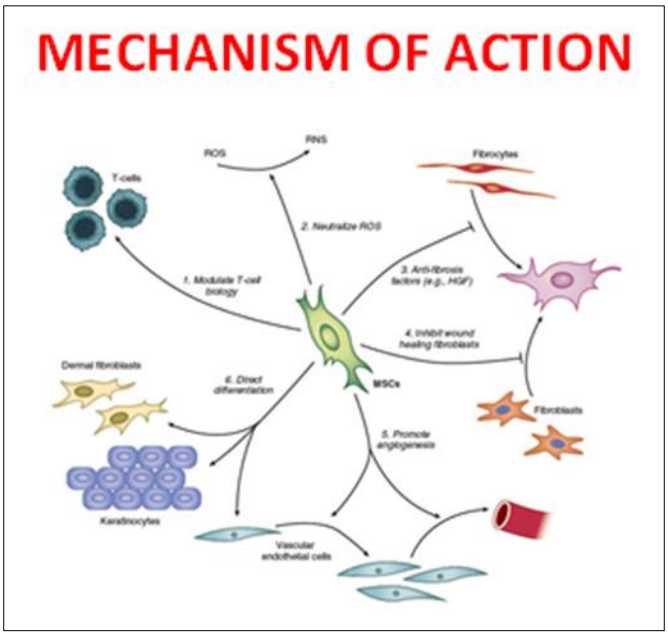

Figure 9 Mechanism of action of mesenchymal stem cell

5 Conclusion

Bone marrow cells act as a novel and feasible technique for treatment of complex fistula in ano , chronic ulcers , carcinoma breast and for incisional hernia repair(mesh coating) by decreasing the post-operative morbidities, complications and recurrence.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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