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Role of Prolotherapy in Electric Scalp Burns Management

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Abstract

Electric burns are known for difficulty in healing and wound management. There is a lack of growth factors in these chronic wounds and needs to be supplemented with adjuvant therapy that allows for faster healing.This article highlights the role of prolotherapy in the management of electric burns.

Keyword: Sprolotherapy; Electricburns; Scalp burns**.**

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Introduction

Adult wound healing is divided into three stages: the inflammatory phase, proliferative phase, and remodelling phase. The three stages have to occur in conjunction to result in wound healing. Wound bed preparation is a novel concept and can be summarized using T.I.M.E with T for tissue: non-viable or deficient. I for infection/inflammation, M for moisture balance. E for epidermis which was changed to E for an edge.[1] Large wounds often require graft or flap for wound coverage, which require wound bed preparation. Prolotherapy is a procedure in which some irritant substance is injected into the wound that initiates an inflammatory reaction that in turn promote healing of the wound.[2] Recently in literature, we came across the use of prolotherapy for use in wound bed preparation.

Materials and methods

This study was conducted in the Department of Plastic Surgery at a tertiary care centre after getting the departmental ethical committee approval. Informed written consent was taken from the patient. The details of the patient in the study are as follows: 14 year old female without any known comorbidities with a history of accidental electric burns from the low voltage source and sustained

circumferential 3rd to 4th degree burns over the scalp frontal region (Fig. 1).

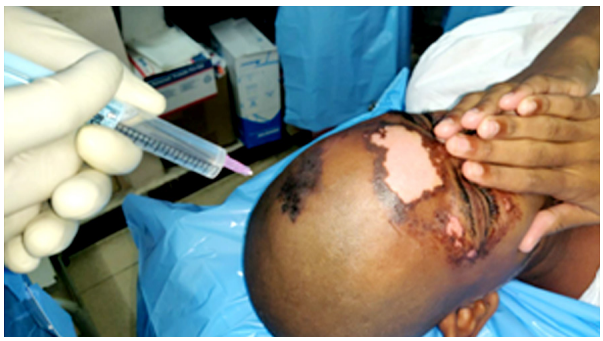

Fig. 1: Prolotherapy for Scalp Burns

Wound bed preparation was done for the patient with prolotherapy with 25% Dextrose (fig. 2) as her ulcer did not show any evidence of healing. Dextrose 25% solution was used as an agent for prolotherapy.

Fig. 2: 25% Dextrose used in prolotherapy

It was injected uniformly on to the wound (10ml) followed by gauze dressing. A repeated session of prolotherapy was given every three days. After 4 sessions of the treatment, the wound was reassessed after 2 weeks for evidence of wound healing.

Results: The wound bed showed good granulation tissue (figure 3).

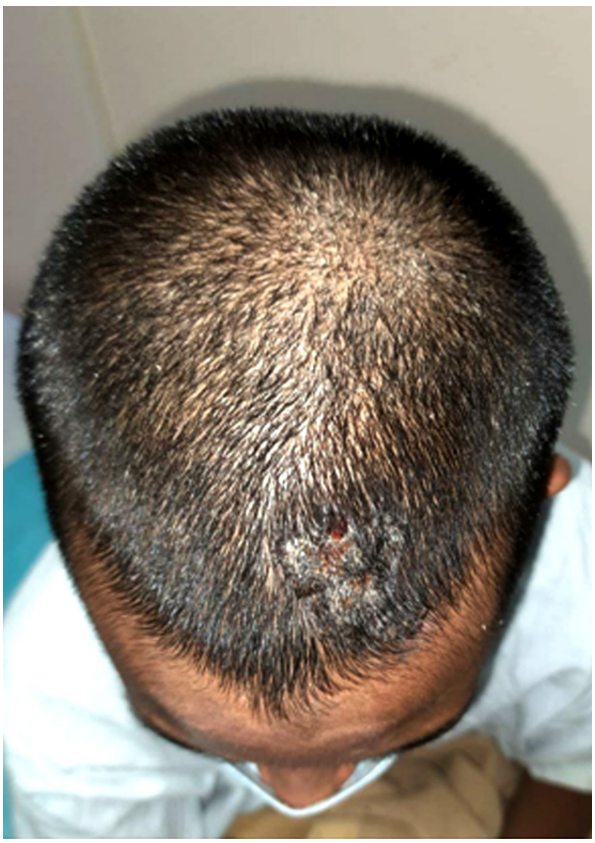

Fig. 3: Healed wound

Prolotherapy is found feasible as an adjuvant modality of wound bed preparation. Its role in wound bed preparation needs investigation by further studies.

Discussion

Burn injury is a major cause of trauma to the human body, with a long healing period. The mortality rate of burn injury has decreased with new treatment modalities, but secondary infections and prolonged healing periods still affect the mortality rates. Many therapeutic methods are available to affect wound healing such as the topical application of insulin, growth factors, negative pressure assisted wound closure, oxidized regenerated cellulose/ collagen, hyaluronic acid conjugated with glycidyl methacrylate or gelatin dressings.

The term prolotherapy was coined by Dr George Hackett in 1956 derived from the Latin word proles meaning offspring or progeny and the English word therapy. It involves injecting an irritant substance into aligament or tendon to improve the growth of new tissue. Multiple agents are used in prolotherapy, such as irritants (phenol), chemo-attractants (sodium morrhuate), and osmotic agents

(dextrose).

However the exact mechanism of prolotherapy is not clear, proponents of the technique believe that the application of hypertonic dextrose causes cell dehydration and osmotic rupture at the injection site that leads to local tissue injury that induces granulocyte and macrophage migration, with the release of the growth factors and collagen deposition.[3] In Vitro studies have shown that concentrations of 5% dextrose have resulted in the production of several growth factors needed for tissue repairs like PDGF, TGF-b, EGF, b-FGF, IGF-1, and CTGF.[4]

In vitro studies have shown that the cultivation of cells in the high glucose culture medium can increase the PDGF expression. PDGF has multiple reparative effects in wounds, including promotion of angiogenesis, fibroblast proliferation, and extracellular production. TGF-b expression is also increased by high glucose.[5,6] TGF-b is involved in different steps of wound healing from inflammation to wound re-epithelialization. Other growth factors increased by high glucose include EGF,b-FGF, IGF and CTGF.

Studies on prolotherapy suggest that there are direct effects on collagen synthesis.[7]A few studies have demonstrated up-regulation of the matrix in response to prolotherapy or in vitro cultivation with high concentrations of glucose. Collagen type-I synthesis is also increased in high glucose cultivation of renal fibroblasts, in a TGF-b-mediated pathway.[8] Cartilage matrix protein aggrecan is increased and reported in chondrocytes cultured inhigh glucose, and in patients who have received intra-articular injections of 12.5% dextrose.[4-8] There were no adverse effects for the prolotherapy with 25% dextrose solution. No local or systemic side effects were demonstrable.

Prolotherapyhas been shown effective in treating many musculoskeletal conditions such as tendinopathies, ligament sprains, back and neck pain, tennis/golfers elbow, ankle pain, joint laxity and instability, plantar fasciitis, shoulder, knee pain and other joint pain. Prolotherapy is useful in the chronic wound and allows to hasten the healing time.Prolotherapy provides analgesia to the patient although the mechanism is not known.

Limitations

The study was done on a single patient and needs a large population-basedstudy to apply in practice

Declarations

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Status of Indian States and Union Territories in PubMed Central

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Abstract

Introduction: The PubMed Central (PMC) is a free digital repository of archived open access full-text scholarly articles published in biomedical and life sciences journals. It was developed by the National Center for Biotechnology Information (NCBI) PMC is a free digital archive of full articles, that can be accessed by anyone from anywhere via a web browser.

Methodology: The name of each of the Indian state and union territory was put in the search box on the webpage of PubMed Central (https://www.ncbi.nlm.nih.gov/pmc/) on 11 May 2021 and the total number of publications that were reflected was noted.

Observations: The data presents the ranking of various States and Union territories (UT)of India on the basis of number of PMC publishedarticles. All the PMC publications since the launch of PMC in Feburary 2000, till May 2021have been included in this study. So, the data reflects an aspect of advancement made by different states/ UT in last two decades, in field of biomedical and life science research. The capital city of the country holds the first position in the list with the highest number of total publications (over 74,000), Ladakh acquires lowest position with only 4 PMC articles.

Conclusion: There is a need of attention to be paid by the Central as well as respective State Governments for introducing such polices that may uplift the research environment in the States/UTs which are lagging behind in contribution so that the variations are reduced.

Keywords: PubMed Central; NCBI; Indian states; Union territories.

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Introduction

The PubMed Central (PMC) is a free digital repository of archived open access full text scholarly articles published in biomedical and life sciences journals. It was developed by the National Center for Biotechnology Information (NCBI).[1] PMC was launched in February 2000, and has grown rapidly as the NIH Public Access Policy is designed to make all research funded by the National Institutes of Health (NIH) freely accessible to anyone.[2] The contents are linked to other NCBI databases this enhances the public's ability to discover, read and build upon biomedical knowledge.[3]

PMC is distinct from PubMed. As PMC is a free digital archive of full articles, that can be accessed by anyone from anywhere via a web browser. While PubMed is a searchable database of biomedical citations and abstracts where the full text article resides elsewhere (in print or online, free or behind a subscriber paywall).[4]

As of December 2018, the PMC archive contained

over 5.2 million articles,[5] with annual deposit of more than 103,000 papers.[6] PMC identifies about 4,000 journals which deposit their published content into the PMC repository.[7]

Methodology

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While the capital city of the country holds the first position in the list with the highest number of total publications (over 74,000), Ladakh acquires lowest position with only 4 PMC articles. Despite the fact that the mean±SEM number of publications per unit (state/UT) is 10,124±2215, the number is highly variable from unit to unit. Other than holding the first place, Delhi, has shown an exclusive position in the list by succeeding the unit with second highest ranking (Karnataka) with a huge gap. (Table1, Fig.1)

Table 1: Ranking of States and Union Territories as per number of PubMed Central Publications.

Ranking	State	Number of PubMed Central publications
1.	Delhi	74115
2.	Karnataka	26205
3.	Maharashtra	21980
4.	Punjab	21702
5.	Tamil Nadu	21644
6.	West Bengal	21077
7.	Uttar Pradesh	17263
8.	Kerala	16268
9.	Chandigarh	14016
10.	Gujarat	13442
11.	Andhra Pradesh	12608
12.	Goa	11329
13.	Haryana	11050
14.	Rajasthan	10190
15.	Assam	9891
16.	Bihar	7428
17.	Madhya Pradesh	6889
18.	Odisha	5624
19.	Telangana	5085
20.	Himachal Pradesh	4478
21.	Uttarakhand	4419
22.	Puducherry	4358
23.	Jammu & Kashmir	3824
24.	Jharkhand	3064
25.	Chhattisgarh	2674
26.	Manipur	2409
27.	Sikkim	2365
28.	Meghalaya	1959
29.	Arunachal Pradesh	1494
30.	Tripura	1355
31.	Mizoram	1272
32.	Nagaland	1243
33.	Andaman and Nicobar Islands	1158
34.	Lakshadweep	414
35.	Dadra and Nagar Haveli and Daman & Diu	165
36.	Ladakh	4

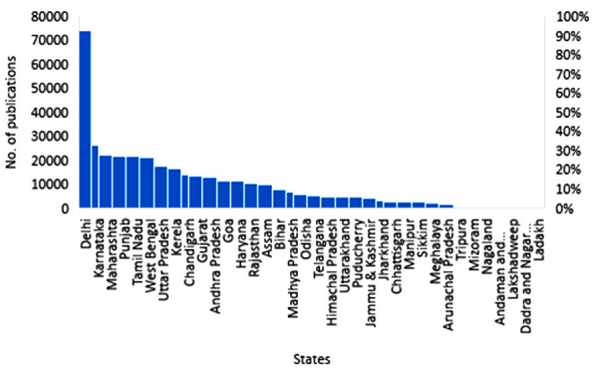

Fig. 1: State/ UTs- wise PubMed Central Publications.

Variations in the number of PMC may be due to a number of factors which are highly variable from unit to unit. Some of these could beper capita income, number of research institutes and/or medical colleges in government and private sector and availability of research funds. Additionally the state/UT wise local factors may also have an impact on contribution of that unit in the field of research. For instance climatic and geographical aspects influence the priorities of the local government or authorities to allocate finances for research or other concerns. Cultural and social norms affect research interests of the faculty and their keen ness for training in research and competitive approach.

Another aspect of our findings is that it includes

numbers of only PMC articles. If otherwise published articles are also included the total number of publications and status of states/ UT in the prepared list may be different. Noteworthy is that PMC is a platform for only valuable authentic and reliable research, which is recognized internationally.

Our data presents that there is much scope and need of quality research advancement in some units as compared to others. For example, Lakshadweep, Dadra and Nagar Haveli and Daman and Diu having less than 500 PMC published in last two decades and Ladakh is with just 4 PMC articles. (Table 2, Fig. 2)

Table 2: States and Union Territories as per Range of Number of PubMed Central Publications.

Group	Range of no of publications	No of states	State
Group 1	70000	1	Delhi
Group 2	30000-70000	0	No State
Group 3	20000-30000	5	Karnataka, Punjab, Maharashtra , Tamil Nadu, West Bengal
Group 4	10000- 20000	8	Uttar Pradesh, Kerala, Chandigrah, Gujarat, Andhra Pradesh, Goa, Haryana, Rajasthan
Group 5	5000- 10000	5	Assam,Bihar, Madhya Pradesh, Odisha, Telangana
Group 6	1000-5000	14	Himachal Pradesh, Uttarakhand, Puducherry, Jammu & Kashmir, Jharkhand, Chattisgarh, Manipur, Sikkim, Meghalaya, Arunachal Pradesh, Tripura, Mizoram, Nagaland, Andaman-Nikobar Island
Group 7	1 -1000	3	Lakshadweep, Dadra and Nagar Haveli and Daman & Diu, Ladakh

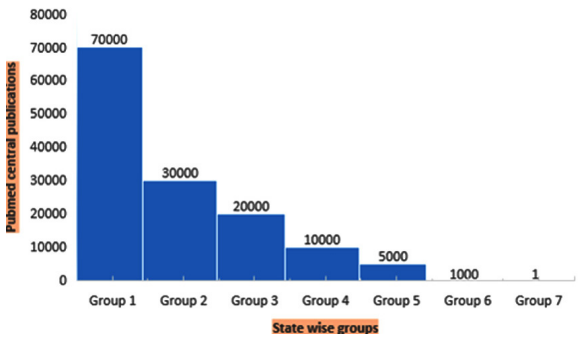

Fig. 2: Groupwise distribution of PubMed Central Publications.

Conclusions

There is a need of attention to be paid by the Central as well as respective State Governments for introducing such polices that may uplift the research environment in the States/UTs which are lagging behind in contribution so that the variations are reduced. Also, the factors associated with better representation of some states than others in regards to number of PMC articles may be explored. This understanding may be incorporated for evolving a better research environment for the States/UTs which are lagging behind.

Although this is a cross sectional observational study, it also opens the door for an elaborate study exploring the growth of the States/UTs in respect to research, prospectively, in retrospect to corelative how the research growth varied with variations in external factors. This may also serve as tool for uplift of the units reluctant in terms of research contribution.

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Co-stars for Wound Healing and Novel interacting Partners in the Frontier of Burn Wound Healing Process

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Abstract

Skin is a composite organ with functional coordination between various cell types. Trauma injuries disrupt the skin architecture and in response to the injury, fibroblasts, macrophages, platelets, keratinocytes mobilize to seal the damage. However, in many cases, trauma causes a serious problem. Effective chronic non-healing wounds in burns are still a challenge. There is no infallible solution available that can overcome the various complexities in burn wound healing and its management. Hence, there is a need to develop suitable technologies that could solve burn wound related complexities. An ideal intervention for wound care must involve components that act at different steps in the process of burn wound healing. In this article, we would like to review the role MMPs (Matrix Metalloproteinase in modulating the extracellular matrix proteins (ECM) proteins in burn wound healing. In addition, a clear understanding of molecular interaction among growth factors, cytokines, MMPs, and other ECM proteins may provide a suitable platform to bring precise solutions for the life of suffering burn patients. To build up a wide-scale for therapeutic strategies, co-stars of this review might help in aiming at stimulating the tissue regeneration process.

Keywords: Burn wound healing; MMPs; Collagens; Extracellular Matrix.

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Introduction

According to the World Health Organization, the highest mortality rates are observed in low and middle income countries with most cases seen in Southeast Asia. The mortality rate among low income countries is 11 times higher than in high income countries. For example: In India, over 1000 000 people are moderately or severely burnt every year. Globally, burns related trauma is the fourth most common type of trauma worldwide, following traffic accidents, falls, and interpersonal violence.

The largest organ of our body that is the skin, makes the integumentary system, prevents fluid loss and provides barriers against microorganisms. When there is a wound, after dressings wound does not get healed. Most current treatment options such as laser therapy, cryotherapy, skin grafts, pressure garments, dressings have not yielded significant results. We need to understand the mechanism of burn wound healing and the regulation by ECM and co-stars, which may be able to provide appropriate treatment. Fibroblast cells synthesize and secrete ECM proteins that will guide for new skin tissue to form. Signalling pathways such as TGF β, WNT, NOTCH, SONIC HEDGEHOG play a crucial role during organogenesis as well as maintain homeostasis in adult organisms. The ECM and MMPs function at the molecular level

in every signalling pathway in human and most importantly during the process of burn wound healing. The multi step complex wound healing process comprises of homeostasis, inflammatory stage, Proliferative stage, Remodelling Stage.[1] The epidermal layer guards underlying tissues and dermal layer offers tensile strength and provide cushions for skin through the support of ECM.[2,3] Once haemostasis is attained, interleukins (IL) and other cytokines modulate the inflammatory response and recruit other immune cells to take part together in the inflammatory stage. The proliferative stage is characterized by angiogenesis, collagen deposition, and granulation tissue. Fibroblasts grow and produce a new extracellular matrix by secreting collagen.[4,5] After the proliferation phase, the last stage is the remodelling phase, which takes a longer period. In this phase, collagen production predominantly happens, and previously disorganized collagen fibers are rearranged and cross linked to provide tensile strength.[6] The complex healing process of burn wounds, the cascade of molecular overlapping events happens during healing, finely controlled biological process involving a series of complex cellular interactions and is interrupted by local and systemic factors.

When the right environment created in wound bed and body works in a sensible way, accumulation of collagen and ECM restores tensile strength. The functions of cells participating in the healing process are controlled by cytokines and growth factors and interactions with ECM components, mediated by integrin receptors and adhesive molecules.[7,8] The fundamental role in the healing process is played by extracellular matrix components.

Extracellular Matrix in Wound Healing

Fibroblasts are present within the dermal extracellular matrix, and it appears that within an ECM, crosstalk happens between keratinocytes and fibroblasts. The communication occurs between ECM, Keratinocyte and fibroblast are rapid during wound healing. An increase of all dermal extracellular matrices and growth factors happens significantly along with increase of type IV collagen. Type I collagen and elastin increased. A dramatic induction of MMP 1 and MMP 9 was observed shortly after wounding.[9] The increased expression of VEGF, bFGF are critical to angiogenesis, occurred early with peak at days 1 and 4, respectively. Expression of basic fibroblast growth factor (bFGF) strongly promote both fibroblast cells and endothelial cells. To improve the burn wound healing, several developing strategies developed in which correlation between growth factor expression and dermal matrix deposition have been associated.[10] The effects of ECM proteins impact two major signal transduction pathways, intracellular calcium and cyclic adenosine monophosphate (cyclic AMP). The Matrix Metalloproteinase (MMPs) play specific role in wound healing. MMPs are group of enzymes responsible for the degradation of ECM proteins. They are also known as matrixins and can degrade all kinds of extracellular matrix proteins, also can process a number of bioactive molecules. The essential MMPs which play a significant role in wound healing are MMP1, MMP2, MMP3, MMP7, MMP9, MMP12, MMP13.[11] The connection between the cells and extracellular matrix components are integral part of burn wound healing, interacting with cells and growth factors in a dynamic give and take that eventually results in wound closure. Hence it is essential to know the role of ECM in healthy skin.

The extracellular matrix of skin consists of a large number of distinct components, and the predominant ones are collagens (type I, type III, type IV, V, VI, and VII), anchoring fibrils, elastinfibers, fibrillin, heparan-sulphate proteoglycan, basement membranes, laminin and fibronectin which provide various functions. Almost 30 percent of total protein mass of ECM is collagen.[12] In normal human dermis, among several types of collagen, collagen types I and III are considered to be the major interstitial fiber-forming collagens.[13,14] Matrix metalloproteinase belongs to family of proteinases that have been increasingly implicated in normal and pathologic extracellular matrix remodelling.[15] The MMPs are a gene family of enzymes that are produced as inactive zymogens, zinc-dependent for catalytic activity, different in substrate specificity, and inhibited by tissue-derived inhibitors (TIMPs).[16] In the healing process of chronic wounds, MMPs act as a novel target for therapeutic intervention. The production of MMPs results due to the interaction of ECM and growth factors.

In humans, currently, at least 24 known MMPs are there[17] different MMPs are explained. Wound healing is the dynamic biological process that involves many complex interactions at the molecular level. MMPs can cleave laminin to release a fragment that binds EGF receptor on fibroblasts

and stimulates migration and proliferation of keratinocytes. Among many growth factors and cytokines, TGF-ßs play critical roles in regulating the development of the ECM. There are three isoforms (TGF-ß1–3) in humans, with each playing distinct roles in regulating synthesis of the ECM components, and even cellular proliferation or cellular death.[18,19] TGF-ßs are produced in latent forms that need to be activated by cleavage of their pro-peptides, before exerting their activities on the ECM, which include stimulation of cellular production of ECM components.[20] The most well known is TGF-ß1, which can control production and degradation of many constituents involved in wound healing.[21] Once TGF-ß1 binds to its receptor, this interaction stimulates the synthesis of ECM components such as collagen, fibronectin, and hyaluronic acid in many types of cells, including fibroblasts.[21]

In the process of wound healing, MMP and their inhibitors TIMP play a great role. Interaction between Collagen 1, 2, 3 and MMP1 COL1A1 (Collagen type 1) is shown in figure 1, in which inter molecular interactions occuramong MMP1, MMP3 MMP9 and their functional partners such as TIMP1, COL1A2, PLG (Plasminogen), COL3A1 and JUN . TIMP1 interacts with the MMP partners and inactivates them by finding their catalytic zinc co-factors and these interactions regulate cell differentiation, migration.

In figure 2, the interaction of Matrix Metallopro -teinases (MMP1, MMP3 & MMP9) and Collagens 1, 2 & 3 MMP1 cleaves collagens and do proteolysis of extracellular matrix (ECM). MMP9 cleaves collagen type IV and play an essential role in cell migration, degrading Fibronectin. This signalling helps in wound healing process.

The functional predicted partners of collagens and MMP are TIMP1, JUN, CD44, COL6A3. CD44 interacts with HA (hyaluronic acid) and mediate cell-cell, cell-matrix interaction and it has affinity for collagens and MMPs. These interactions helps for activation of lymphocytes and haematopoiesis. Also, TIMP1 act as a growth factor that regulates functional signalling pathway for wound healing. TIMP1 interacts with the MMP partners (MMP1, MMP2, MMP3 & MMP9) and inactivates them by finding their catalytic zinc co-factors and these interactions regulate cell differentiation, migration.

Observation from Burn Wound

Authors examined type I and III collagen content and expression of Fibronectin in fibroblasts isolated from normal and hypertrophic skin tissue of the burn patients. Also elucidated possible mechanisms of hypertrophic scar through the software interaction study. Normal human skin and hypertrophic scar specimens were obtained from burn patients of with informed consents and fibroblasts were isolated from the skin tissues using standard protocol. The results indicated that collagen I, expression in scar fibroblast increased in comparison to normal. Collagen III expression also checked quantification indicated that level of collagen III expression slightly higher in hypertrophic scar than the normal. Fibronectin expression was also indicated that expression of cells is greater in the hypertrophic scar than the normal. To predict the interacting partners of ECM proteins, "STRING" software has been used and interaction among ECM proteins were discussed. The software analysis is being done which shows the interacting partners of Collagens. The MMP are found to be an active partner involved in it. MMP are present with their inhibitors TIMP and helps in the wound healing process.

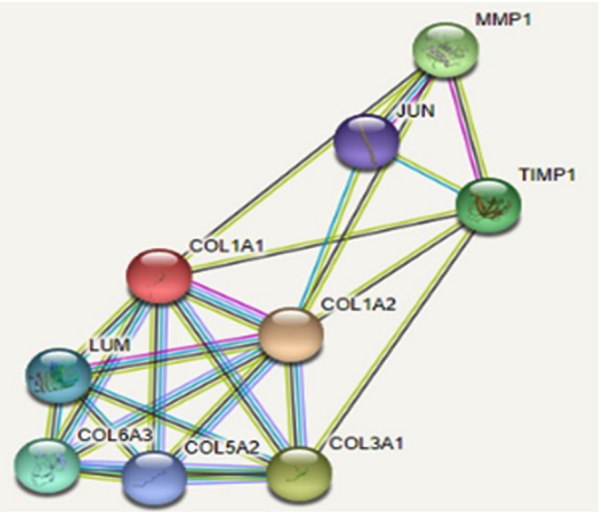

Fig. 1: Interaction between Collagen 1, 2, 3 and MMP1.

COL1A1(Collagen type 1) interacts with MMP1, MMP3 MMP9 and their functional partners are TIMP1, COL1A2, PLG (Plasminogen), COL3A1 and JUN . TIMP1 interacts with the MMP partners and inactivates them by finding their catalytic zinc co-factors and these interactions regulate cell differentiation, migration and plays a role in integrin signalling pathways.

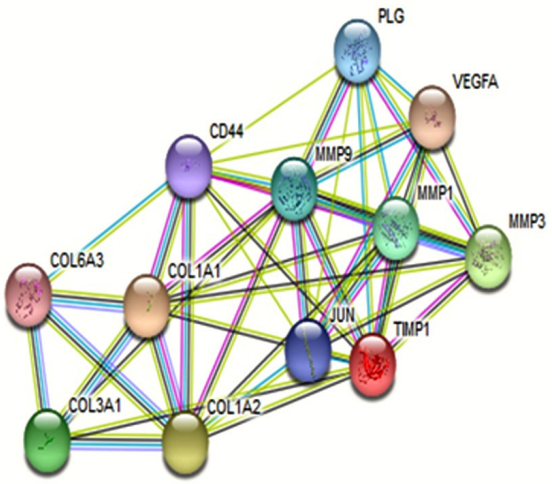

Fig. 2: Interaction of Matrix Metalloproteinases (MMP1, MMP3 & MMP9) And Collagens 1, 2 & 3

MMP1 cleaves collagens and do proteolysis of extracellular matrix (ECM). MMP9 cleaves collagen type IV and play essential role in cell migration, degrades Fibronectin. The functional predicted partners of collagens and MMP are TIMP1, JUN, CD44, COL6A3. CD44 interacts with HA (hyaluronic acid) and mediate cell-cell, cell-matrix interaction and it has affinity for collagens and MMPs. These interactions helps in lymphocyte activation, recirculation and haematopoiesis. Also, TIMP1 act as a growth factor that regulates functional signalling pathway for wound healing. TIMP1 interacts with the MMP partners (MMP1, MMP2, MMP3 & MMP9) and inactivates them by finding their catalytic zinc co-factors and these interactions regulate cell differentiation, migration and plays a role in integrin signalling pathways.

Discussion

Wound healing involves various cellular events, secreted growth factors and cytokines. This complex process involves interaction among extracellular matrix components which are essential for wound repair phenomenon. ECM creates a provisional matrix, providing structural integrity with various types of cells. The delicate balance between repair system and Extracellular components play key role in proliferation, differentiation and remodelling of tissue. Moreover, ECM components such as Fibronectin, proteoglycans, vitronectins, and collagen all together bring together a state of healing of wound. The ECM elements such as Collagen-I, Collagen-III, Fibronectin and Matrix Metalloproteinase have indispensable role and the "scaffolding" created by ECM provide the structural integrity during the stages of wound healing. Since wound healing is a dynamic process of the molecular interaction among cytokines, growth factors, MMPs, ECM full fill a function of signal transduction and the interactive sequence of biological reactions are connected with the healing process. Scaring in burns and contracture formation inversely proportional to the dermal tissue component available for healing, which is directly related to the support of fibroblast infiltration, new vascularization and epithelialization. Therefore the interaction of MMPs, and other essential components make significant contribution in dermal tissue remodelling and burn wound healing.[22,23,24] In fig. 3, Model showing, Factors Associated with Burn wound healing which provide future prediction to target the molecule for better understanding for healing of burn wound scar.

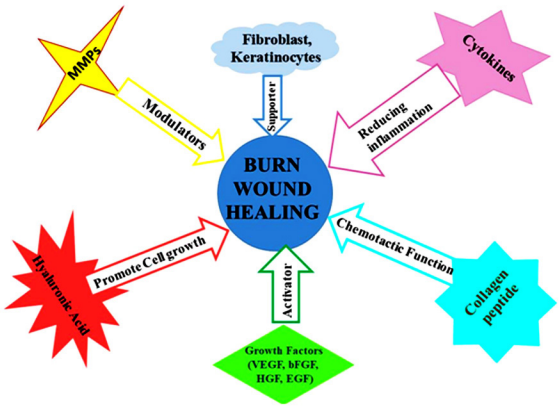

Fig. 3: Model showing, Factors Associated with Burn wound healing.

Summary

The precise balance between the restoration system and ECM components plays a key role in tissue repair. Regardless of all recent advances in burn wound healing, burn wound represents a major challenge throughout the world. Basic needs of specific types of wounds and degree of burns such as nutritional optimization and advanced suitable burn wound healing agents need to be focused on. ECM proteins and MMPs could open a better avenue. Thorough knowledge of understanding of the molecular intervention is required for better understanding of healing of burn wound scar.

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Subject Index

Author Index

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Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors

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Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540–7.

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Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone-iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3–9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792–801.

Unpublished article

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Personal author(s)

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Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. pp 7–27.

No author given

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Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979–2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7–18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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