



Role of PAX-7 as a tissue marker in mangled extremity: a pilot study

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Received: 28 November 2018 / Accepted: 1 March 2019 / Published online: 9 March 2019
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Abstract

The rising incidence of mangled extremity seen in modern trauma has led to significant patient mortality. A lot of research is going on at microcellular level for a better understanding of tissue injury, repair and regeneration. PAX-7 is one such transcription factor, a marker of satellite stem cells in skeletal muscle. Though few studies have shown concrete evidence of increased expression of PAX-7 in the nearby injured zone in skeletal muscle post-injury, none has studied its expression in an event of mangled injury of limb in humans. We, hereby, attempted to identify whether PAX-7 expression of tissue near the zone of injury, after grievous trauma like mangled injury of extremities, actually increases, decreases or remains unaffected. A pilot study was conducted on 30 cases at a level 3 trauma centre; patients were segregated into two groups—group I with MESS score ≥ 7 and group II with score < 7 . For group I patients, amputation was planned, and for group II, limb salvage surgery was planned. Skeletal muscle samples from three different zones (A, B and C) in group I, while pre- and post-debridement skeletal muscle samples in group II were sent for microscopic examination and IHC staining with PAX-7 antibody. A definite increase in PAX-7 expression, post-trauma near the zone of injury (Zone B and C in group I and post-debridement in group II), was noted. Increased expression of PAX-7 signifies increased recruitment of satellite stem cells near the injury zone, thereby reflecting the activation of skeletal muscle regeneration cascade. Hence, increased staining of PAX-7 in tissues could be a viable marker for identifying potential regeneration of skeletal muscle post-injury.

Keywords Mangled extremity · Immunohistochemistry · PAX-7 · Muscle regeneration · Satellite stem cells · Haematoxylin and eosin stain · Mangled extremity severity score

Introduction

Mangled extremity is defined as a severe injury to a limb that often leaves its viability in doubt. It has also been defined as involvement of at least 3 out of 4 systems, i.e. bone, blood vessels, nerves and soft tissue [1]. In the twenty-first century, the incidence of mangled extremity has risen in proportion to the increased number of motor vehicles on roads [2, 3].

In the present scenario, with the advent of increasing skills of surgeon in handling such trauma, along with the availability of microscopic vascular and nerve repair surgeries, innovative techniques of soft tissue cover and better antibiotics, to curb the infection, it is now possible to

reconstruct even those limbs which in the past used to be amputated. Nevertheless, there is still a chance of postoperative complications like infection, wound dehiscence and healing problems, non-unions, all of which could necessitate secondary amputation after prolonged morbidity [3–7].

A lot of research is going on at microcellular level that has increased our understanding of concepts of tissue inflammation, repair and regeneration in recent decades. Understanding genetics and gene expression is one such pathway that has thrown some light in this aspect. PAX-7 protein is one such transcription factor, which is known to be expressed in quiescent satellite stem cells in skeletal muscle. This PAX-7 protein continues to express in satellite cell as it undergoes activation and proliferation and finally get lost when these satellite cells undergo differentiation. These satellite stem cells are actually the mesenchymal cells which gets stimulated and significantly multiply in the event of muscle trauma and differentiate into new muscle cells during muscle regeneration [8–12]. Hence, PAX-7 can be used as a marker of satellite cells, thereby indicating the muscle

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regeneration potential. Some preclinical and clinical studies have also shown the role of PAX-7 as a marker of muscle regeneration, and therefore, we have assumed that PAX-7 may play a significant role in muscle regeneration in the event of trauma [13–22]. Based on the available literature and facts, we hypothesised that expression of PAX-7 should also be altered near the zone of injury in case of mangled injuries. These altered expressions of PAX-7 in such zone may give an idea about the regeneration potential of muscle tissue and about its reparative capacity and viability.

We, thus, planned a pilot study with the aim of evaluating the expression and significance of PAX-7, if any, in traumatic limb. The staining of PAX-7 antibody was graded, and the results were compared in different specified zones, and the results are presented.

Materials and methods

A pilot study was prospectively conducted on 30 patients that presented with a mangled extremity to a level I trauma centre of North India from 1 January 2016 till 31 December 2016. All patients of age ≥ 18 years who sustained an injury of either upper or lower or both extremities, with either isolated injury or polytrauma and without medical comorbidities, were included in this study.

After initial resuscitation and stabilisation, all patients were segregated into two groups on the basis of MESS score. Group I patients, with MESS score ≥ 7 , were taken up for primary amputation, whereas group II patients, with MESS score < 7 , were planned for limb salvage. Intraoperatively skeletal muscle tissue samples were sent from three different zones in group I patients, whereas pre- and post-debridement muscle samples from the injury zone were taken in group II patients. In group I patients, three different zones were described as follows: Zone A was the zone of mangled tissue containing mostly dead and dying muscle tissues, Zone C was the zone of final amputation expected to have viable skeletal muscle fibres, while Zone B was described as intermediate zone of doubtful viability between Zone A and Zone C (Fig. 1).

The positive control group, used in the study, consists of the normal skeletal muscle tissue samples that were obtained from the resection zone of bone osteosarcoma or from autopsy patients that were available in the histopathology department of our institute. All the samples were stored in sterile containers with 10% buffered formalin solution and were examined under microscope with routine haematoxylin and eosin (H&E) staining. On H&E staining, the extent of necrosis involving individual muscle fibre or large fascicular group was noted along with degree of interspersed inflammatory cells. Viable muscle fibres were clearly delineated from degenerated muscle

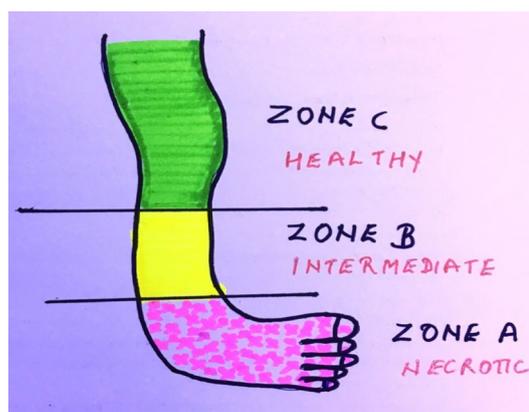


Fig. 1 Pictorial representation of different zones taken, arbitrarily, intraoperatively by the operating surgeon

fibre based on early morphological changes, fragmented sarcoplasm and nucleus.

A 2- μ m-thick paraffin section was made afterwards for each sample, to further carry out immunohistochemistry (IHC) staining by peroxidase and anti-peroxidase method with subsequent staining with PAX-7 antibody (Abcam company, Cambridge, UK). The antibody used in our study was mouse monoclonal antibody and was standardised with control group of muscle with dilution of 1/100.

PAX-7 gives nuclear staining where the positive staining may be located either within or at periphery of the cytoplasm of individual muscle. The positive staining was scored into four groups:

0. When no positive staining is observed.
1. When ≤ 3 nuclei are stained positive.
2. When 4–6 nuclei are stained positive.
3. When > 6 nuclei are stained positive.

On immunohistochemistry staining, the positive staining of nuclei within or at periphery of cytoplasm was noted and graded accordingly as described above (Figs. 2a, b, 3a, b). The sample with higher grade of staining indicates higher expression of PAX7, whereas sample with no expression of PAX-7 is given a score of 0. Thus, higher PAX-7 scores imply significant activation of tissue regeneration cascade with recruitment of more and more satellite mesenchymal stem cells, expressing PAX-7, in the nearby zone of muscle injury.

Observations and results

In group I

It was noted on H&E examination that sections from Zone A showed diffuse necrosis in 100% samples with interspersed

viable muscle fibres in 6.7% of samples. Whereas in Zone C, 73.3% of samples showed viable muscle fibres, out of which 18% samples also showed some amount of patchy necrosis. Rest 26% of samples from Zone C had no viable muscle fibres (Table 1). This could be attributed to incorrect zone determination intraoperatively, inadequate tissue sampling technique and inappropriate storage of samples.

On immunohistochemistry, 93% of samples from Zone A had either no or minimal expression of PAX-7 (score 0 and

1, respectively), while only 6% had score of 2 with moderate expression, with a mean score of 0.67 ± 0.617 (Fig. 2a, b). In Zone C, 73% of samples got a score of either 0 or 1, while another 13% of samples, showing moderate staining, had a score of 2. Around 13% of samples also showed significant staining with score of 3 in Zone C, with a mean score of 1 ± 1.069 . In Zone B, around 87% samples had a score of either 0 or 1, while approximately 13% shared a score of either 2 or 3 (Figs. 2a, b, 3a, b; Table 2).

Fig. 2 Photomicrographs showing immunohistochemistry staining for PAX-7. **a** Normal muscle with no nuclear positivity (Peroxidase anti-Peroxidase, $\times 400$). **b** Injured muscle showing three intracytoplasmic nuclear positivity for PAX-7 protein by the traumatised muscles (Peroxidase anti-Peroxidase, $\times 400$)

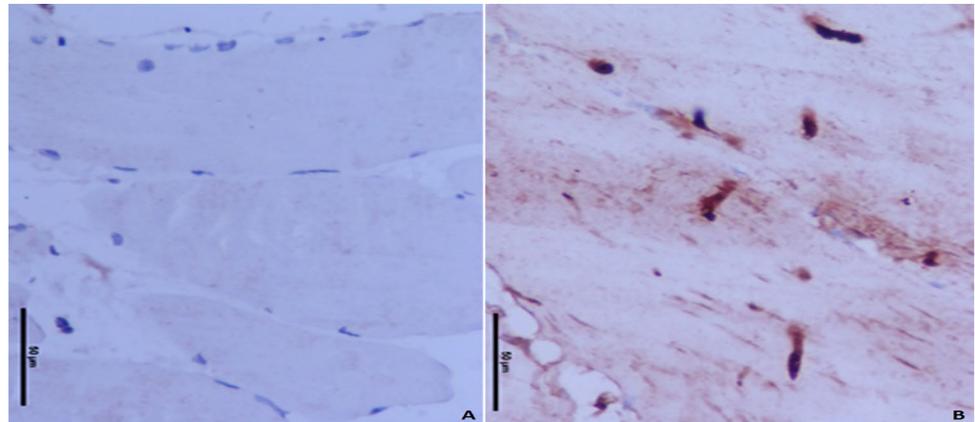


Fig. 3 Photomicrographs showing immunohistochemistry staining for PAX-7 protein. **a** Injured muscle with PAX-7 positive nuclei along the periphery of the muscle indicating a score of 2 (Peroxidase anti-Peroxidase, $\times 400$). **b** Injured muscle showing cluster of PAX-7 positive nuclei in between the degenerated muscle fibres indicating active regenerative activity (Peroxidase anti-Peroxidase, $\times 400$)

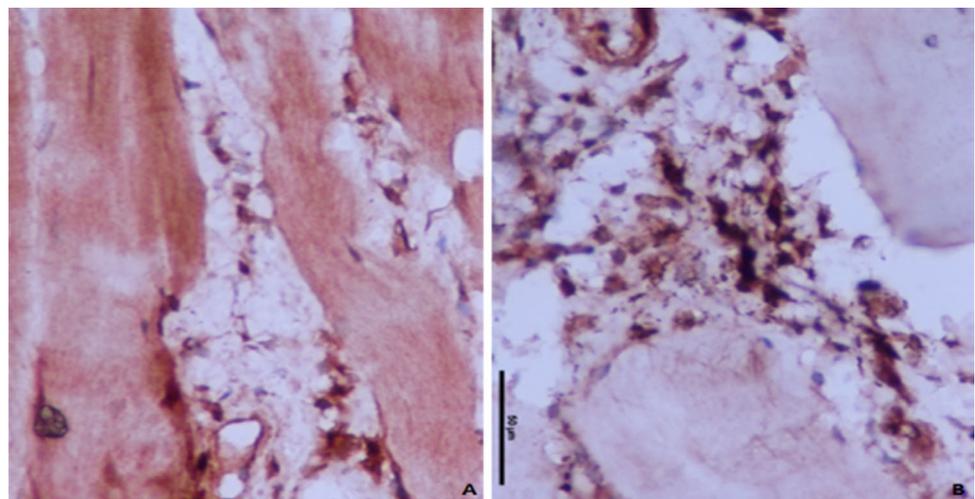


Table 1 Observation and results of histopathological examination in group I and II

Histopathological examination	Group I			Group II		P value			
	Zone A (%)	Zone B (%)	Zone C (%)	Pre-debridement (%)	Post-debridement (%)	Zone A versus B	Zone B versus C	Zone A versus C	Pre-versus post-deb
Necrosis	100	40	6.70	100	53	0.5	0.016	0.004	0.015
Viability	86.60	73.30	20	7.00	80.00	0.5	0.008	0.002	0.001
Inflammation	40	60	73.30	93	87	0.063	0.5	0.37	1

An increasing trend towards the positive expression of PAX-7 staining was observed from Zone A to C. Despite relatively higher scores of PAX-7 in nearby zone of injury (Zone B and C) clearly indicating towards the increased expression of PAX-7 in these zones, it was not found statistically significant (Zone A–C: p value of 0.272).

In group II

On H&E examination, 100% of sections from the pre-debridement group showed diffuse necrosis with 6% of them also showing interspersed viable muscle fibres, whereas in post-debridement group, around 80% of samples had viable muscle fibres, with approx. 42% samples also having concomitant patchy necrosis. Also in 20% of samples, no viable muscle fibre could be traced (Table 1).

On immunohistochemistry staining in pre-debridement group, 67% samples had a score of either 0 or 1, while a score of 2 and 3 was seen in another 27% and 6% of samples, respectively, with a mean of 1.07 ± 0.961 . In post-debridement group, around 47% and 33% samples had a score of 2 and 3, respectively, with moderate to significant staining with a mean of 2.07 ± 0.884 . While only 20% showed a score of either 0 or 1 with either no or minimal staining (Figs. 2a, b, 3a, b; Table 2).

The data above clearly showed relatively increased expression of PAX-7 with higher score of 2 and 3 in post-debridement samples in group II patients, thus indicating a more marked tissue regeneration response in nearby healthy muscle tissue in zone of injury. Also, this increase was found to be statistically significant with p value 0.007.

Discussion

The rising incidence of mangled extremity along with aftermath of social, psychological and functional impairment of patient has put a significant economical burden over the society. Not only the surgery, whether amputation

or limb salvage, but also its complications in postoperative period has a great impact on patient and the society as a whole. Along with various conventional measures and innovative techniques like thorough debridement, high-grade antibiotics, early vascular and microscopic nerve repair, etc., many different perspectives are now being considered in order to better tackle these problems [2, 3, 6]. A lot of research work is going on at gene level that enlightens us, altogether, with new concepts of tissue healing and reparative process. PAX-7 is one such transcription factor that attracted many researches in recent decades based on its potential to recruit and stimulate more and more mesenchymal satellite stem cells following muscle injury. Few studies have clearly demarcated that PAX-7 protein is able to drive transcription in quiescent, activated and proliferating satellite cells. Further, the expression of PAX-7 is downregulated in those satellite stem cells that undergo terminal differentiation but is maintained and remains transcriptionally active in those that opt out of immediate differentiation [8–13, 23]. Various English studies have shown that the increased expression of PAX-7 in skeletal muscle post-injury may predict the good outcome after surgery and may fasten the recovery process [16–23].

Keeping the future potential of PAX-7 in mind, we conducted a pilot study including 30 patients that reported to the emergency department of our institute. In this study, young male constitutes the majority, with 56.6% of patients falling in age group of 20–40 years. Out of 30, only two were female and rest were males. Road traffic accident was being the most common mode of injury (Table 3).

An extensive literature search was done beforehand (Table 4). Around 1297 and 1139 articles in Pubmed and Medline database could be found on PAX-7, respectively. On further refining our search, total of 69 articles with MeSH (PAX-7 and muscle regeneration and immunohistochemistry) could be traced in Pubmed. Out of these 69 studies, 10 most relevant studies were included in the final review after screening all abstracts, removing duplicates and obtaining full text. These studies highlight the different

Table 2 Observation and results of PAX-7 in group I and II

PAX-7 staining	Frequency	Mean	SD	Median	P value			
					Zone A–B	Zone B–C	Zone A–C	Pre versus post-deb
GROUP I								
Zone A	15	0.67	0.617	1	0.257	0.671	0.272	
Zone B	15	0.87	0.834	1				
Zone C	15	1	1.069	1				
Group II								
Pre-deb	15	1.07	0.961	1				0.007
Post-deb	15	2.07	0.884	2				

Table 3 Demographic data of patients included in this study

S no.	Name	Age	CR no.	MOI	Diagnosis	MESS	Group	Surgery done
1	Kishan	27/M	201602354314	Railway tract injury	Lt traumatic amputation at knee joint and Rt B/K mangled limb	10	I	B/L A/K amputation open stump
2	Gayatri	40/F	201605845416	RTA	Lt mangled foot	11	I	B/K amputation closed
3	Ranjana	45/F	201602301342	RTA	Rt B/K mangled limb with Lt crush foot	11	I	Rt B/K amputation and lt toes ampu and K wire for 1st and 4th toe
4	Chajju	65/M	201603192474	RTA	Rt mangled upper limb	11	I	Rt A/E amputation open stump
5	Rambaksh	60/M	201603498697	RTA	Lt B/K mangled limb	13	I	Lt knee disarticulation closed
6	Ramesh	35/M	201605695783	RTA	Rt B/E mangled limb	9	I	Rt A/E amputation closed
7	Girdhari	55/M	201604670159	Railway tract injury	Lt A/k mangled limb with Rt B/K traumatic amputation	13	I	Lt high A/K amputation open with Rt A/k revision amputation
8	Dharamveer	36/M	201604671023	RTA	Lt B/E mangled limb	10	I	Lt A/E amputation closed
9	Gurinder	26/M	201605761042	RTA	Rt B/K mangled limb	11	I	Rt A/K amputation closed
10	Gaurav	23/M	201605729793	RTA	Rt femur and both bone leg open Gd IIIC	12	I	Rt A/K amputation Closed
11	Rajbir	29/M	201604381963	RTA	Rt mangled foot c Rt femur # open Gd IIIA	11	I	RT B/K amputation and Rt LFPTCS for I/C Femur
12	Ravi Prakash	20/M	201605816890	RTA	Rt B/k mangled limb c Rt supra pubic Rami #, pubic diastasis with SI joint disruption	13	I	Rt A/K amputation open
13	Shyam lal	70/M	201604233344	RTA	Rt mangled foot	11	I	RT B/K amputation open
14	Baljeet	40/M	201604898761	RTA	Lt mangled Lower Limb	7	I	Lt knee disarticulation closed
15	Pawan	31/M	201604398750	RTA	Rt mangled foot with Rt femur # open Gd II	7	I	Rt B/K amputation and debridement with primary closure of femur
16	Jaspal	45/M	201603901548	RTA	B/L both bone leg open GdIIIB	6	II	B/L debridement + external fixator
17	Kasim	46/M	201605975972	RTA	Rt both bone leg Gd IIIB with cut ATA and CPN palsy	6	II	Debridement + external fixator
18	Moja Ram	70/M	201605857724	RTA	Rt proximal BB Leg open Gd IIIB	6	II	Debridement + external fixator
19	Santosh	23/M	201606399126	RTA	Lt proximal tibia open Gd IIIB and Lt MM # open Gd I	6	II	Debridement + external fixator
20	Subhash	28/M	201604574435	RTA	Rt # dislocated elbow open GdIIIB	5	II	Debridement + external fixator
21	Ranjit	35/M	201606256031	RTA	Lt both bone Leg open GdIIIB	6	II	Debridement + external fixator
22	Ram Singh	40/M	201605761565	RTA	Lt both bone Leg open GdIIIB c Rt shaft femur # closed	6	II	Debridement + ExFix for BBleg
23	Rajbir	35/M	201604095529	RTA	Rt # dislocated elbow open GdIIIB c extensive degloving from shoulder to mid forearm	6	II	Debridement + external fixator
24	Brijesh	20/M	201606284019	RTA	# Lt 3/4/5th metatarsal with lateral cuneiform with cuboid# open Gd3B	6	II	Debridement + K wire fix + SFPTCS + JESS fixator
25	Sarwan	46/M	201605857783	RTA	# Rt segmental both bone leg gd IIIB	6	II	Debridement + external fixator

Table 3 (continued)

S no.	Name	Age	CR no.	MOI	Diagnosis	MESS	Group	Surgery done
26	Bhupinder	50/M	201603397671	RTA	# Lt S/C humerus and ulna gd IIIB	5	II	Debridement + k wire + square nail
27	Baljit	20/M	201604413881	RTA	Lt open unstable ankle gr IIIB c Lisfranc inj	5	II	Debridement + k wire +PTCS + Ex fix
28	Ghanshyam	35/M	201603001644	RTA	# Rt both bone leg gd IIIB	6	II	Debridement + external fixator
29	krishan	49/M	201603957977	RTA	Rt mangled foot	5	II	Debridement + k wire fix + PTCS
30	Rajesh	21/M	201606437346	RTA	# Rt shaft femur gd IIIB c Rt tibia # closed	6	II	Debridement + rail fixator for femur, external fixator

Table 4 The schematic representation of search methodology used in the study

Database	Results
Pub med (1950–07/10/2017)	
1. PAX-7	1297 articles
2. PAX7 AND satellite stem cells	498 articles
3. PAX-7 AND muscle regeneration	384 articles
4. PAX-7 AND immunohistochemistry stain	224 articles
5. PAX-7 AND muscle regeneration AND immunohistochemistry	69 articles
6. PAX-7 AND mangled extremity	No hits
7. PAX-7 AND mangled extremity AND immunohistochemistry stain	No hits
8. PAX-7 AND MESS score	No hits
9. PAX-7 AND haematoxylin and Eosin stain	No hits
Medline (1966–07/10/2017)	
1. PAX-7	1139 articles
2. PAX7 AND satellite stem cells	266 articles
3. PAX-7 AND muscle regeneration	341 articles
4. PAX-7 AND immunohistochemistry stain	4 articles
5. PAX-7 AND muscle regeneration AND immunohistochemistry	6 articles
6. PAX-7 AND mangled extremity	No hits
7. PAX-7 AND mangled extremity AND immunohistochemistry stain	No hits
8. PAX-7 AND MESS score	No hits

aspects of PAX-7 and PAX-7 positive satellite stem cells of skeletal muscle, in either human or animal study population (Table 5).

These studies, mostly, as indirect evidence enlightens the indispensable role of PAX-7 in regenerative process of skeletal muscle in different physiological and pathological scenarios such as neuromuscular dystrophies and in the stressful event such as muscle injury as a result of trauma and strenuous physical training and detraining (Table 5). Till now, no literature could be traced that highlights the role of PAX-7 in mangled extremity (MeSH: PAX-7 and mangled extremity; PAX-7 and mangled extremity and immunohistochemistry; PAX-7 and MESS score) (Table 4).

Satellite cells are the stem cells present between the sarcolemma of the myofibre and basement membrane, first recognised in frog skeletal muscle by electron microscopy.

The CD 56 and PAX-7 are the two potential surface marker for identification of these satellite cells. The most important feature of satellite cell is their ability to enter the cell cycle, unlike regular myonuclei, and thereby replenishing its own population and regenerating muscle fibre in the event of injury. It will be very unlikely that in resting state, high number of satellite cells is active [8–12]. Mackey et al. in their study observed varying proportion of activated satellite cells in resting state, in single bout of isometric muscle stimulation and in event of light and heavy resistance training. He observed maximum recruitment of satellite cells (mean 10%) in single bout of isometric stimulation group whereas minimum (mean 1.3%) in resting group [13].

Lepper et al. [14] in their study demonstrated that “the genetic ablation of PAX-7 positive satellite stem cells completely blocks regenerative myogenesis either following

Table 5 List of studies providing indirect evidence of role of PAX-7 as a marker for muscle regeneration

References	Journal	Country	Species	Study	Conclusion
Seale et al. [15]	Cell	Canada	Mice	Pax7 is required for the specification of myogenic satellite cells	Pax-7 positive muscle cell will give rise to stem cell in cell culture media Stem cells isolated from muscle lacking Pax7 exhibited a strongly increased propensity towards hematopoietic differentiation and were incapable of forming adult myoblasts
Oustamina et al. [17]	EMBO	Germany	Mice	Pax7 directs post-natal renewal and propagation of myogenic satellite cells but not their specification	The number of satellite cells in Pax7 mutant mice declined strongly during post-natal development, although single satellite cells were readily identified in adult Pax7 mutant mice Essential function of Pax7 for renewal and maintenance of muscle stem cells and exclude an exclusive role of Pax7 in satellite cell specification
Seale et al. [18]	PLOS Biol	Canada	Mice	Pax7 is necessary and sufficient for the myogenic specification of CD45+;Scal+stem cells from injured muscle	Infection of Pax7-deficient muscle with adenoviral Pax7 resulted in the de novo formation of regenerated myofibers Pax7 is necessary and sufficient to induce the myogenic specification of CD45+ stem cells resident in adult skeletal muscle
Kadi et al. [19]	Journal of Physiology	Sweden	Human	The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles	Satellite cell content increased by 19% at 30 days and by 31% at 90 days of training. Compared to pre-training values, the number of satellite cells remained significantly elevated at 3, 10 and 60 days but not at 90 days of detraining
Kadi et al. [20]	Pflugers Archiv Eur J. Physiology	Sweden	Human	The behaviour of satellite cells in response to exercise: what have we learned from human studies?	In humans, the satellite cell pool can increase as early as 4 days following a single bout of exercise and is maintained at higher level following several weeks of training Cessation of training is associated with a gradual reduction of the previously enhanced satellite cell pool In elderly, training counteracts the normal decline in satellite cell number seen with ageing
Mackey et al. [13]	Muscle nerve	Denmark	Human	Assessment of satellite cell number and activity status in human skeletal muscle biopsies	Very low levels of active satellite cells (maximum 1.3%) in biopsies taken under resting conditions was observed Significant pooling of activated satellite cells (mean value 10%), 48 h following a single bout of stimulated isometric muscle contractions, was seen Increase in satellite cells (mean value 2.5% and 1.4%, respectively) was seen 12 weeks after light and heavy resistance training

Table 5 (continued)

References	Journal	Country	Species	Study	Conclusion
Lepper et al. [14]	Development	USA	Mice	An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration	Genetic ablation of Pax7(+) cells completely blocks regenerative myogenesis following injury to the muscles in nude mice
Sambasivan et al. [21]	Development	France	Mice	Pax7 expressing satellite cells are indispensable for adult skeletal muscle regeneration	Acute muscle injury provoked by diphtheria toxin in normal mice muscle showed muscle regeneration whereas In PAX-7/DTR +ve mice, there was infiltration of muscle with inflammatory cells, adipocytes and fibroblasts instead of muscle regeneration
Hamilton et al. [16]	Bone and Joint Research	UK	Human	Muscle “regenerative potential” determines physiological recovery following total knee replacement	A strong correlation was noted between satellite cell number (immunohistochemistry) and improvement in patient power output postoperatively Strong correlation was observed between the expression of Pax-7 and power output post-TKR
Noehren et al. [22]	Journal of Bone and Joint surgery	Kentucky, USA	Human	Cellular and Morphological Alterations in the Vastus Lateralis Muscle as the Result of ACL Injury and Reconstruction	Significant reductions, before ACL surgery, in type-IIA muscle cross-sectional area, extracellular matrix, PAX-7 positive satellite cells per fiber, pennation angle, muscle volume, and PCSA in the injured limb compared with the uninjured limb was found These alterations in the injured limb persisted even after surgery and post-op rehabilitation

injury to the tibialis anterior (TA) muscle in a mice". Likewise, Seale et al. [15] in their study showed that the adult satellite stem cells taken from uninjured muscle of mice have no regeneration potential, whereas on the other hand, stem cells isolated from regenerating muscle were capable of giving rise to myoblast expressing PAX-7 as transcription factor. These studies signify the role of PAX-7 as an important regulator of muscle regeneration and repair in the event of muscle injury.

In a study conducted by Hamilton et al. [16] on 29 patients, the preoperative and postoperative muscle power and post-total knee replacement were graded and compared. The results were correlated with the degree of expression of PAX-7 positive satellite cells in biopsies taken from vastus lateralis at the time of surgery. He observed a strong correlation of satellite cell number and expression of PAX-7 with the improvement in patient power output postoperatively. The study highlights the indispensable regenerative reserves of skeletal muscle in the form of PAX-7 positive satellite cells that are recruited and activated in the stressful event like surgery, thereby optimising the adequate muscle power and strength.

Based on these indirect but concrete evidence from the literature, we believe that there may be some potential role of Pax-7 in influencing and guiding the postoperative event up to some extent [8–22]. This study has been conducted in order to further extend the utility of PAX-7 and satellite cells in the clinical setting of trauma and muscle injury.

In our study, all the sections were thoroughly examined under microscope with PAX-7 antibody stain. A differential staining pattern of PAX-7, thus observed in samples from both the groups. In group I patient, higher expression of PAX-7 with scores of 2 and 3 was noted in Zone C followed by Zone B as compared to Zone A. Likewise, the similar trend of staining was observed in group II patients on comparison of post-debridement samples with pre-debridement samples. We also noted this increasing trend of staining as statistically significant in only group II patients. The data from our study clearly showed increased expression of PAX-7 in the nearby areas of injury as a consequence of activation of tissue reparative cascade that subsequently deploy more and more satellite mesenchymal cells.

Moreover, higher expression of PAX-7 indicates greater strength of this reparative cascade that may fasten the recovery process and may lead to early rehabilitation and less wound complications [15–22]. So, we believed that patients with higher staining scores of PAX-7 in Zone C or post-debridement may exhibit lesser postoperative complication due to higher activation of regenerating and repairing cascade. Although no clear cut relation could be established between PAX-7 and the postoperative complications like infection and wound dehiscence in our

study, the potential of PAX-7 in future could not be under-emphasised and neglected.

These shortcomings of studies could be attributed to small study group, limited resources and paucity of literature in existence. More research work, better resources and larger studies need to be conducted in future in order to further strengthen our hypothesis.

Conclusion

It was concluded that the expression of PAX-7 is definitely increased in the muscle tissue near the zone of injury in an attempt to accelerate the repair and regeneration of tissue.

It further strengthens our hypothesis that the PAX-7 has a definite role to play in predicting the regeneration ability and potential of traumatised muscle fibres and thereby may predict for better surgical outcome. Therefore, we strongly believe that, if explored meticulously in future, PAX-7 may have a predictive role, in association with various other blood or tissue markers, in deciding for limb salvage versus amputation or in predicting chances of postoperative complications like infection, delayed amputation, revision surgery, etc. However, many more studies may need to be conducted in near future with larger study cohorts and better resources in order to substantiate the exact role of PAX-7 markers in patients with mangled extremity. This study is just a baby step and a food for thought towards the various discoveries that need to be addressed in future.

Acknowledgements This work has been funded by Indian Council of Medical Research (ICMR) (No.3/2/Sept.2016/PG-Thesis-HRD(8) Dated: 4.10.2016), Department of Health Research, New Delhi, and constituted part of a thesis submitted by the principal author.

Compliance with ethical standards

Conflict of interest There are no conflicts of interest from any of the authors.

Ethical approval The study was approved by the Institute's Ethical Committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent Informed consent was taken from the patients at the time of enrolment and the study procedure was explained in detail to all the enrolled patients and they were made to understand that they could withdraw from the study at any point of the study period. The study has not altered any of the management protocols of these patients. Identity of the participants was kept confidential.

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