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## Reinforcing concepts of renal physiology by crossword puzzles

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### Abstract

Medical teachers work tirelessly to develop innovative and effective educational tools that enhance and supplement the conventional didactic lectures. We introduced crossword puzzles prior to didactic lectures, as a supplemental learning tool to help students get primed with upcoming terms and concepts.

Before traditional lectures, the crossword puzzles were administered in groups of 8 students each (n=73) on two different occasions. They were allowed to discuss and refer to books/internet to solve the puzzles. A batch of 82 students was not given crossword puzzles served as the control group. A Multiple Choice Question test (MCQ) was conducted and the scores in crossword group and control group were compared.

Students' perceptions on the crossword puzzles were assessed using a questionnaire.

The average raw scores in MCQ test of the students in crossword group (n=73) and control group (n=82) were  $36.43 \pm 6.14$  and  $31.59 \pm 7.18$  respectively ( $p = 0.000$ ) and the students' satisfaction with this learning method was >75% for most of the items of the questionnaire.

Crossword puzzles constitute an interactive and fun-filled active learning with peers in a non-threatening environment. Given before teaching the topic, they primed the students with the upcoming concepts and later during traditional teaching, the same were reinforced. Solving crossword puzzles enabled students to self-assess their own understanding of concepts and also to identify their weak areas, which can be corrected later through a targeted approach.

**Keywords:** Crossword; Active Learning; Self- directed Learning.

### Introduction

For years, the mode of presenting facts or concepts to the students has been through passive teaching, primarily didactic lectures. Gradually methods are evolving that engage students in the learning process. Competency based medical education (CBME) encompasses a shift in the teaching-learning (TL) paradigm from teacher-centric to student-centric medical education. With the implementation of CBME curriculum in India, innovation in the active TL method is the need of the hour. The advantages of the use of games or puzzles as learning tools have been widely reported in various medical specialties.<sup>1-3</sup> Puzzles are problem-solving activities that require learners to formulate solutions and are gradually proving their effectiveness in medical education<sup>4</sup>. Crossword puzzles as a learning tool has been used successfully in pathology, pharmacology, physiology, nursing and veterinary sciences as a fun activity.<sup>1,3,5-7</sup> Solving crossword puzzles has shown to stimulate the intellect, intensify the glossary and preclude

passive memorization of facts.<sup>8</sup> Thus, solving of crossword puzzles promotes critical thinking and analytical skills and also improves self-directed learning skills among the learners.<sup>7</sup> The above-mentioned studies that introduced crossword puzzles, examined the students' perceptions on crosswords but most of them did not compare the scores of the students. Also, in all of the studies, the crosswords were given after the didactic lectures. The current study was planned to introduce crossword puzzles prior to the didactic lectures, as a supplemental learning tool to help first year undergraduate medical students get primed with and understand the terms and concepts of renal physiology by a collaborative approach. The objectives of the study were to evaluate the effectiveness of crossword puzzles in reviewing and understanding the concepts of renal physiology when used along with traditional teaching and to describe students' perceptions on introduction of crosswords in Physiology teaching.

## Materials & Methods

The study was commenced after taking approval from Institutional Ethics Committee. A total of 155 first year undergraduate medical students participated in the study.

Before traditional lectures on some topics of renal physiology, the crossword puzzles were administered to 73 students, in a non-testing environment in groups of 8 students each, on two different occasions. The crossword puzzles were made online at the Discovery School's puzzle maker Web site <http://puzzlemaker.school.discovery.com>. The puzzles (Fig. 1 and 2) had vertical columns with words running from top to bottom and horizontal rows with words running from left to right. None of the words ran diagonally or were inverted. Before introducing the crossword puzzles to students, the contents of both the crossword puzzles were validated by experts from the Physiology Department. Each crossword contained 20 clues covering two topics - glomerular filtration and tubular reabsorption. Since the students were given these crosswords before teaching the renal system, many of the questions were factual (lower levels in Bloom's taxonomy) but some were conceptual testing higher degrees of Bloom's pyramid. The students were given one hour to complete the crossword puzzle; for solving these puzzles, they were allowed to discuss among themselves and refer to books/internet. A teacher and two residents were present in the class during the activity. Thereafter the answers of the crosswords were discussed. The activity was followed by didactic lectures in the given topics over a period of three days where the facts and concepts which were asked in crosswords were reinforced. After finishing all the teaching lectures on renal physiology, a system completion test of 50 MCQs was organized. The test consisted of a mix of factual and conceptual questions; testing all the levels of Bloom's hierarchy.

A group of 83 students, were not given crossword puzzles. However they appeared for the same system completion MCQ test after their didactic lectures were over. They served as the control group of our study.

A feedback on the crossword activity was obtained from the students (n=73) by administering a pre-designed and validated questionnaire.<sup>9</sup> The responses were measured on a Likert scale. The Likert scale was reversed for items 6 and 9; this was intentionally done because people have a tendency to check the best responses in feedback forms without sometimes reading the questions. So to ensure that they read the questions and then answer, the scale in these items was reversed. Students were asked to complete the survey during class time in the presence of the tutors. They were asked not to record their names for anonymity. Feedback responses of the students were recorded and satisfaction index for each item was calculated with the following formula:-

$$\frac{[(n1 * 1) + (n2 * 2) + (n4 * 4) + (n5 * 5)] * 20}{(n1 + n2 + n4 + n5)}$$

Where, n is the total number of students gaining the score mentioned in the subscript for that particular item (n3 is not included because it is the neutral response).

Statistical analysis was performed using the IBM Software (Statistical Package for the Social Sciences) SPSS 21. The data was tested for normality. The mean and standard deviation of marks obtained were calculated and scores in both the groups were compared using an Independent Student t test. A  $p < 0.05$  was taken as statistically significant.

### Glomerular Filtration Rate



Fig. 1: Crossword puzzle: GFR.

### Tubular Reabsorption

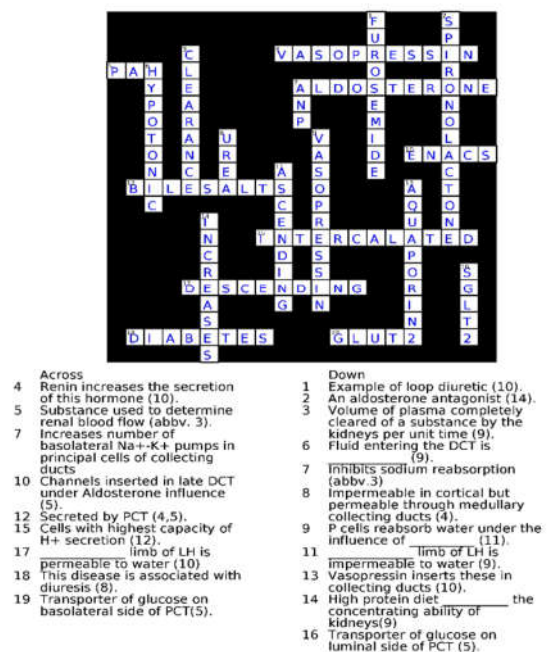


Fig. 2: Crossword puzzle: Tubular Reabsorption.

## Results

The average scores of the students of crossword group (n=73) and control group (n=82) were  $36.43 \pm 6.14$  and  $31.59 \pm 7.18$  respectively. Minimum score (out of 50) in crossword group was 22, and maximum 47 while in control group, the minimum and maximum scores were 12 and 47 respectively. The data was tested for normality by plotting histograms and Q-Q plots. The Independent Student t test was applied to compare the scores in both the groups. Additionally, the homogeneity of variances was satisfied by Levine's F test with  $F(153) = 1.87$ ,  $p = 0.173$ . The independent samples t-test was associated with a statistically significant effect  $T(153) = -4.48$ ,  $p = 0.000$ . Thus the students of crossword group obtained significantly higher scores than controls. We also compared the scores of control group and crossword group on MCQ tests on two other systems (GI system and

Respiratory system) in which crossword puzzles were not administered to any of the groups; and the difference in scores was not significant. Perceptions of the students (n=73) on incorporation of crosswords were obtained on a validated questionnaire. (Table 1) In our study, for all of the items of the questionnaire, the satisfaction score was >70%. The best score was given to item 9, which stated that the activity was enjoyable. This was followed by item 1, according to which the crossword activity was useful in comprehending the given topic. However, item no. 3 that stated "the activity helped in enhancing communication skills" was rated the lowest by the students. As the students were not asked to make any presentations about their experiences with the crossword activity, this could be the reason for their rating badly to this item.

**Table 1:** Students (n=73) responses to the feedback questionnaire and the satisfaction index.

Items	Scores					Satisfaction Index (%)
	1	2	3	4	5	
The crossword activity was useful in comprehending the given topic.	5	7	5	33	23	78
The activity enabled in depth coverage of the topic.	6	9	18	26	14	72
The activity helped in enhancing communication skills.	8	9	14	28	14	71
A thorough discussion on the crossword puzzles increased analytical ability.	6	11	3	35	18	74
The activity helped in overcoming shyness and hesitation in the class.	6	5	15	33	14	75
This form of exercise should not be incorporated for all the topics in physiology. *	6	11	16	20	20	73
Are you confident that this knowledge could be applied in clinical practice?	5	11	13	26	18	74
The activity helped me to score better in my formative tests.	6	8	19	25	13	72
The exercise was not enjoyable. *	3	8	5	35	22	79
Crossword puzzle is an effective way of learning.	5	9	10	33	16	75

**Table 1:** Students (n = 73) responses to the feedback questionnaire and the satisfaction index. Scores were determined as follows: 1=strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, and 5 = strongly agree. \*Scores were reversed for items 6 and 9 (5 = strongly disagree, 4 = disagree, 3 = neutral, 2 = agree, and 1 = strongly agree).

## Discussion

The overall goal of medical education is to make an undergraduate medical student competent, and the medical educators strive to make their teaching effective by introducing innovative teaching-learning tools. The current study was planned to evaluate crossword puzzles as a teaching-learning tool to supplement traditional didactic lectures. Use of games and puzzles for teaching concepts have been shown to improve knowledge and identify the gaps in knowledge.<sup>3,10</sup> In addition, games help in developing critical thinking and analytical skills among students.<sup>2</sup> They enhance cooperative and collaborative skills as students work together in groups to reach a common conclusion.<sup>7,11</sup> Gaming, as an active teaching-learning strategy, has been used in gynecology, pediatrics, pharmacology, psychiatry etc. in addition to didactic lectures.<sup>6,12-14</sup> There are many studies involving successful implementation and evaluation of games like crossword puzzles, jigsaw puzzles, quizzes etc. for understanding concepts in Physiology<sup>3,11,12,15-17</sup>, however they mostly evaluated only the perception of students, not the scores.

In our study, the scores in the MCQ test were compared and found significantly higher in the crossword group than controls. The controls were comparable to the crossword group in demographics as well as their level of knowledge because the pattern and difficulty level of the entrance exam to join the medical course was similar for both the groups. However, as compared to controls, the students in crossword group got extra two hours of classroom time and interaction with instructors. The beneficial effect of

crossword puzzles may be attributed partly to this too which is a limitation of our study. The perceptions of the students were obtained on a validated questionnaire.

The students enjoyed the interactive learning session with their friends in a fun filled, non-threatening environment. This is in congruence with findings by Shah S et al., 2010.<sup>3</sup> Crosswords given before teaching the topic, primed them with the upcoming terms and concepts. The students also felt that this intervention aided comprehension of topics, and retention of the concepts. Similar findings were observed in other studies also.<sup>3,6,7</sup> since the crosswords were introduced prior to teaching the system, high order questions were few in number. However, all the concepts that were asked in the crosswords were completely comprehended by the students. Enhanced comprehension could be due to thorough discussion on clues with their peers while searching for answers, and recalling and reviewing the text while solving the puzzle. The discussion on the answers by the instructor immediately after the crossword sessions made them understand the concepts which they read at the time of solving the crossword but could not interpret. As for example in Figure 1, answer to clue number 3 across is "the hydrostatic pressure in glomerular capillaries is higher than that in the capillaries elsewhere in the body". The students read this concept while searching for the answer and understood when the answers of the crosswords were discussed. The same concept was reinforced during traditional lectures.

The students recommended that these activities should be continued as a supplement to didactic lectures. The crossword puzzles have been used successfully as a supplement to traditional didactic lectures in obstetrics and gynecology, psychiatry, and physiology.<sup>1,13,14,17-19</sup>

It is seen that if crossword puzzles are administered before lectures, it can be used as a method of priming the students before the lectures for upcoming terms and if given after lectures, it can be a method of assessing the students' learning. Also, crossword puzzles can be employed for taking a revision class where a fair number of varied concepts can be taught in one enjoyable class.

## Conclusions:

The results of this study show that crossword puzzles incorporated as a supplement to traditional lectures can engage students and encourage them to collaborate learning with their peers. This may also increase the understanding of the concepts. Solving crossword puzzles also enabled the students to evaluate their own understanding of concepts and also to identify their weak areas, which can be corrected later through a targeted approach. This method thus promotes self-directed learning among students. It increases their involvement in the learning process and keeps them motivated.

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## Role of Hand Held Low Level Laser Device in Treatment of Lymphedema Our Experience

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### Abstract

Lymphedema is a disease affecting lymph flow causing symptoms of pain, oedema, and increased chance of infection. There are different modalities of treatment of lymphedema and includes decongestive compression therapy and methods to cause debunking of the oedema. Recently we came across use of LLLT in lymphedema treatment as well as a hand-held low-level laser machine. We share our experience of the application of the hand-held laser device in the management of lymphedema.

**Keywords:** Low Level Laser Device; Lymphedema.

### Introduction

Lymphedema is a disease caused by obstruction to lymph flow.<sup>1</sup> Primary lymphedema arises from aberrant lymphatic development. The damaged lymphatics cannot transport lymph in adequate quantities and the lymph accumulates in the lymphatic spaces.<sup>2</sup> Low Level Laser Therapy (LLLT) is reported to have beneficial effects on cells and tissues in a broad range of conditions, including lymphedema, through encouraging formation of lymphatic vessels (lymph angiogenesis), promoting lymphatic flow and stimulating the immune system. LLLT uses waves between 650-1000 nm.<sup>3,4</sup>

### Materials & Methods

This is a prospective case study of a 29-year female admitted in the department of Plastic Surgery in August 2019, with c/o swelling of the left lower limb for 16 years. The patient was assessed and investigated thoroughly. The patient was diagnosed to have primary lymphedema of the left lower limb associated with secondary skin changes (WHO grade-7). The patient was given standard care as per International Society of Lymphology (ISL) guideline. As the patient was not willing for any microsurgical or excisional procedure, a decision was made to perform liposuction. The patient was given low level laser device (fig. 1) for prevention of recurrence over the affected limb. The patient was

instructed to use the device once a day for 15 minutes over the affected limb every day for 6 weeks and the cost of the laser comb devices range from 400 to 400 to 5000 INR.

### Results

There was no recurrence of the lymphedema and the patient was satisfied with the treatment after 6 weeks of follow up. (Figure 2)







**Fig. 2:** after liposuction at 6 weeks of follow up.

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## Basics of Neural Regulation of Respiration

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### Abstract

The nervous system normally adjusts the rate of alveolar ventilation as per the demands of the body so that partial pressure of oxygen (PO<sub>2</sub>) and partial pressure of carbon dioxide (PCO<sub>2</sub>) in the arterial blood are hardly altered, even during heavy exercise and other respiratory stress situations.

Respiratory centres are classified into two types i.e. medullary and pontine. These centres contain various neurons like Dorsal respiratory group of neurons (DRG) controls inspiration and Ventral group of neurons (VRG) controls expiration along with other centres controlling the rate and depth of respiration. Reflexes like cough reflex, sneeze reflex and J reflex are mediated through these centres. Central and peripheral control of respiration is mainly regulated by PCO<sub>2</sub> and PO<sub>2</sub>.

**Keywords:** Alveolar Ventilation; Respiratory Centres; Cough Reflex; Sneeze Reflex; J Reflex.

### Introduction

The nervous system normally adjusts the rate of alveolar ventilation as per the demands of the body so that partial pressure of oxygen (PO<sub>2</sub>) and partial pressure of carbon dioxide (PCO<sub>2</sub>) in the arterial blood are hardly altered, even during heavy exercise and other respiratory stress situations.<sup>1</sup>

#### Role of Respiratory centres

The respiratory centres are classified into medullary and pontine centres. Medullary includes dorsal respiratory group of neurons (DRG) which controls inspiration and ventral group of neurons (VRG) controls expiration. Pontine includes pneumotaxic centre which controls rate and depth of respiration and apneustic centre controls inspiration.<sup>2</sup> Dorsal respiratory group of neurons (DRG) arises from Nucleus tractus solitarius (NTS). Rhythmic discharges from DRG leads to generation of inspiratory ramp: Make – 2sec and Break – 3sec. Ventral respiratory group of neurons (VRG) functions both in inspiration and expiration. Pneumotaxic centre is located dorsally in the upper pons. It controls the “switch off” point of the inspiratory ramp thus controlling the duration of inspiratory signals. Strong-inspiration might be lost in as little as 0.5 second i.e. shallow breathing. Weak, the ramp continues to rise for as long as 5 to

10 seconds i.e. slow breathing.<sup>3</sup> Apneustic centre is situated in the lower part of pons. It discharges tonically upon the DRGN- promoting more sustained respiratory activity. Two factors normally control and lessen this tonic influence are Pneumotaxic centre and vagal afferent impulses (Hering-Breuer inflation reflex). An animal with midpontine transection and vagotomy develops apneustic breathing i.e. prolonged inspiration- brief expiratory period- another sustained inspiration.<sup>4</sup> Lung inflation signals limit inspiration by Hering-Breuer inflation reflex. When the tidal volume becomes more than 1.5 lit/ breath, stretch receptors situated in the walls of bronchi and bronchioles become stimulated thus transmitting signals through vagi and inhibit DRGN which switches off inspiratory ramp. This reflex acts as a protection from excessive lung inflation.<sup>5</sup>

#### Role of Cough Reflex

Cough reflex begins with the irritation of the bronchi and larynx. The impulses pass through vagus nerve to medulla (Inspiratory Center) for initiating inspiration and thus 2.5 litres air is inspired. Epiglottis and vocal cords close completely to obstruct the air below vocal cords. The expiratory muscles contract forcefully and create a pressure of 100 mm Hg in the lungs and respiratory passage below

the vocal cords and the vocal cords suddenly open widely. Air in the lungs explodes out widely with a velocity of 500 miles/hour thus removing the irritating foreign particles, present in the respiratory passage.<sup>6</sup>

#### **Role of Sneezing Reflex**

In sneezing reflex, all the steps are almost the same as that of cough reflex except that the irritation occurs in nasal mucosa instead of bronchi and lungs. Sensation travels through Trigeminal nerve instead of vagus nerve and soft palate and uvula are closed instead of vocal cords.<sup>7</sup>

#### **Role of J- reflex**

"J receptors"- are sensory nerve endings in the alveolar walls in juxtaposition to the pulmonary capillaries. They are stimulated when the pulmonary capillaries pressure is increased or the pulmonary capillaries become engorged with blood or when pulmonary oedema occurs in such conditions as congestive heart failure, high altitude and exercise. Excitation may give the person a feeling of dyspnoea.<sup>8</sup>

#### **Role of CO<sub>2</sub>, H<sup>+</sup> and O<sub>2</sub>**

Direct or central control of respiratory centre activity is by CO<sub>2</sub> and Hydrogen ions. Chemo sensitive area of respiratory centre is situated on the ventral surface of medulla. Primary stimulus is H<sup>+</sup> for stimulation of chemo sensitive area and CO<sub>2</sub> has a weak direct effect. Potent indirect effect occurs through H<sup>+</sup> Decrease stimulatory effect of CO<sub>2</sub> occurs due to renal readjustment by increasing bicarbonate which binds with H<sup>+</sup> ions in blood and cerebrospinal fluid. Changes in PO<sub>2</sub> have little direct effect on control of respiratory centre.<sup>9</sup> Peripheral chemoreceptor system for control of respiratory activity is mainly by PO<sub>2</sub>. Role of O<sub>2</sub> in respiratory control is carried by Carotid bodies and aortic bodies. Impulses are transmitted along glossopharyngeal nerve and vagal nerve. Decrease arterial O<sub>2</sub> stimulate chemoreceptors when PO<sub>2</sub> is 60-30 mm Hg.<sup>10</sup>

#### **Mechanism of stimulation of chemoreceptor by O<sub>2</sub> deficiency**

Glomus cells located in peripheral chemoreceptors have O<sub>2</sub> sensitive K<sup>+</sup> channels which are inactivated when blood PO<sub>2</sub> decreases. As a result of depolarisation there is increased opening of voltage gated calcium channels leading to increase in intracellular calcium concentration and release of neurotransmitter (ATP) resulting in activation of afferent neurons to CNS leading to stimulation of respiration.<sup>11</sup>

#### **Conclusion:**

Spontaneous respiration is produced by rhythmic discharge

of motor neurons orchestrated by brain that innervate the respiratory muscles. The rate and depth of respiration change appropriately in response to alterations in metabolic demands without any voluntary effort on our part. This involuntary process is mainly controlled by various respiratory centres located in pons and medulla.

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## Wound Healing

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### Abstract

Wound represents one of the biggest surgical dilemmas with great variation in care. The reason is due to the different variants in the nature of wound & treatment, limited evidence convincing in wound care and disparate opinions among treating doctors & nurses. Tissue injury leading to a wound is a platform for multidisciplinary interventions. A wound, whether created by a scalpel, trauma or infection, undergoes a reparative process in a definite sequential manner. Hence, evidence based care should be followed thereby ensuring optimum care to the patient as well as the wound.

**Keywords:** Wound Healing; Evidence Based Medicine; Wound Management.

### Introduction

Wound healing is a series of complex events that resurface, restore and reconstitute the tensile strength to damaged skin. A good understanding of the sequence of events, which includes molecular signals, and cells involved in wound healing, can aid in a better optimization of wound care. If the process of wound healing goes askew in any phase, it results in exuberance of fibroblastic proliferation called hypertrophic scars, keloids and contractures leading to aesthetic and functional problems. Hence the primary goal in wound care should be prevention of complications and preservation of functions.<sup>2</sup> any available national and international guidelines for different wounds can be followed.<sup>1</sup>

### History

Hippocrates first defined healing by first and secondary intention.<sup>3</sup> Alexander Flemming discovered the first antibiotic, Penicillin.<sup>4</sup> The cardinal signs of clinical infection - rubor (erythema), tumor (swelling), dolor (pain) and calor (heat) was described by Celsus. Indian contribution to wound healing was done by Sushruta, the Father of ancient plastic surgery, when he described 14 different types of dressings and described ancient Indian surgical technique and wound management and noted in his book the 'Sushruta Samhita'.<sup>5</sup> Ambroise Pare (1510-1590) proposed use of various dressings for traumatic gunshot wounds like rock alum, verdigris, vitriol, rose honey and vine.<sup>6</sup> Lorenz

Heister from Germany wrote 'general system of surgery' and described wounds of all kinds, operations and bandages.<sup>7</sup> Henri Francois Le Dran (1685-1770) gave concept of debridement.<sup>8</sup> The routinely used Gamgee pads were described by Joseph Sampson Gamgee (1828-1886) as cotton wool covered with unbleached gauze to make dressing pads.<sup>9</sup> Louis Pasteur, a French Scientist, 'The Father of Microbiology' developed sterile surgical procedures and Joseph Lister, an English surgeon used gauze pieces with carbolic acid leading to significant reduction in mortality rate.<sup>10</sup>

The present modern era has been overwhelmed with the ever increasing number of high technology, engineered advanced wound care products like 'Living Skin equivalents', Growth factors, Cultured keratinocytes and Stem Cell Therapy.<sup>11,12</sup> The nervous system normally adjusts the rate of alveolar ventilation as per the demands of the body so that partial pressure of oxygen (PO<sub>2</sub>) and partial pressure of carbon dioxide (PCO<sub>2</sub>) in the arterial blood are hardly altered, even during heavy exercise and other respiratory stress situations.<sup>1</sup>

### Pathophysiology

*The pathophysiology of the wound healing varies according to the type of wound, if it is:*

1. Acute wound (or)
2. Chronic wound

### Pathophysiology of Acute Wound Healing

Acute Wound healing is characterised by three phases i.e., inflammatory phase, Proliferative phase and Maturation (remodelling) phase.

#### Inflammatory Phase

This phase comprises vascular and cellular events which begin immediately post injury and lasts for 2-5 days. The immediate vascular event is characterised by a brief period of vasoconstriction which can last from 3-5 seconds to 5 minutes. Followed by vasoconstriction, a persistent and progressive vasodilatation initiates within half an hour, which leads to an increased blood flow through the area, which increases the warmth at the injury site. The progressive vasodilatation causes an increase in hydrostatic pressure, causing fluid leak, thereby causing local swelling.

The cells that are released during this phase are neutrophils, macrophages and monocytes. The neutrophils help in opsonisation of the bacteria. Monocytes are converted to activated macrophages, which produce growth factors and cytokines, aiding in scavenging non-viable tissue and bacteria.<sup>13</sup>

#### Proliferative Phase

The proliferative phase lasts from 2 days to 3 weeks. This phase is characterised by formation of granulation tissue. The macrophages from the infiltrative phase recruits fibroblasts, which in turn stimulate the synthesis of a network of collagen fibrils. The fibroblasts get converted into a myfibroblast (with smooth muscle) which aids in wound contraction, thereby bringing the wound edges closure and decreasing the size of the wound.<sup>13</sup>

#### Remodelling Phase

This phase begins at 3 weeks of the injuries and can last upto 2 years. The soft, gelatinous, immature collagen laid during the proliferative phase is replaced by an organized mature collagen, this provides tensile strength to the wound, which gradually increases over years, but is never as same and strong as the original tissue.<sup>13</sup>

### Pathophysiology of Chronic Wound Healing

Chronic wound healing is characterised by an increased duration of inflammatory or proliferative phase. This impairment is due to changes in one or more of the components that aid in wound healing like growth factors, cytokines, etc.,. The prominent cytokines that are increased and thereby contributes to a chronic wound are Matrix metalloproteinases (MMPs) like MMP-1 (collagenase), 2 (gelatinase A) and 9 (gelatinase B), serine proteases, pro-inflammatory cytokines such as TNF- $\alpha$  and interleukin-1 $\beta$  (IL-1 $\beta$ ), and TGF- $\beta$ . There is also a decrease in the level of Tissue Inhibitor of Metalloproteinases (TIMP).

Free radicals like Nitric Oxide (NO) can cause oxidative damage, contributing to the non-healing nature of the wounds. Non-viable tissue (slough) promotes bacterial colonisation, thereby increasing the inflammatory phase of wound healing. In addition to colonisation, with time, biofilm is produced by the bacteria, which are bacterial colonies in a polysaccharide matrix secreted by the bacteria itself. This creates a great hindrance to wound healing as it is resistant to host defence as well as extraneously applied antimicrobial agents.

Apart from external factors, various internal factors also lead to non-healing wounds. For example, diabetes is characterised by micro neuropathy; which leads to trophic

changes in the skin; microangiopathy which involves thrombosis of small arteries causes ischemia and gangrene. In addition to these, the hyperglycemic state in the blood provides an excellent environment for bacterial proliferation as well as lowering resistance to infection.<sup>14</sup>

### Types of Wound Healing

Wound healing can be of three types:

When a clean wound or a surgical incision is closed with sutures or skin adhesives it is termed as primary healing. When a wound is left open to heal on its own by means of granulation, epithelialization and wound contracture it is termed as secondary healing. When wounds are not ideal for primary closure, eg), infected or grossly contaminated, they are left open initially for a period of 5-10 days, and then closed after the wound is clear from infection. This is termed as delayed primary closure.<sup>14</sup>

### Anatomy

The skin acts as an inherent protective mechanism against the harshness of the external environment. It has two layers - epidermis and the dermis. The epidermis is characterised by 5 layers, from deep to superficial - stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum, stratum corneum. Stratum corneum is an accumulation of desquamated keratinocytes synthesized by the stratum germinativum, which gradually ascends up to the superficial layers and sheds. The dermis lies under the epidermis, is further divided into papillary and reticular dermis. It consists of a rich dermal vascular network, which serves the important function of thermoregulation and also supports the avascular epidermis metabolically. The other structures are fibroblasts - which synthesize structural polymers, including ground substance, collagen, and elastin. Skin appendages include sebaceous glands, hair follicles, and sweat glands (figure 1).<sup>15</sup>

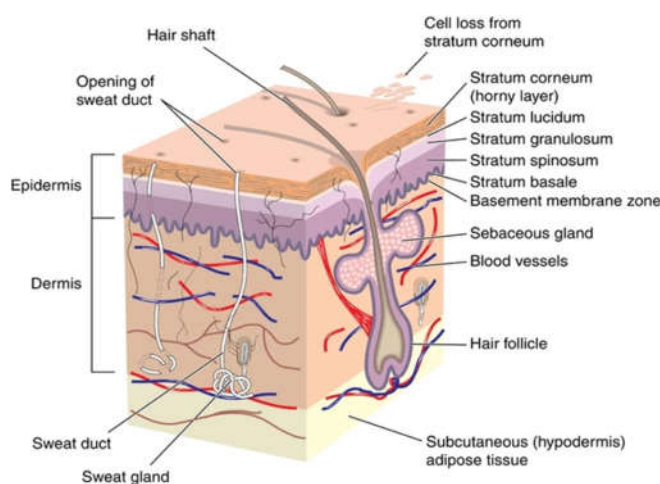


Fig. 3: Anatomy of skin.

Skin is a dynamic organ which undergoes various modifications and has a variable thickness. It is thickest in the soles and palm (1.5 mm thick), and thinnest behind the ears and the eyelids (0.05 mm thick). Children have thin skin which thickens as the child ages. This continues till the 4th - 5th decade of life when there is loss of epithelial appendages, elastic fibres and ground substance, which leads to thinning of the skin.

## Classification

Based on the thickness of the skin involved, wounds can be classified into superficial (epidermal loss), Partial thickness wounds (epidermal and dermal loss), and full thickness wounds (epidermis, dermis, subcutaneous fat, with or without underlying muscle or bone).

**Wounds can also classified based on:**

### **Etiology**

- Traumatic (Sharp/Blunt).
- Iatrogenic (Surgical).
- Burns (Thermal, Electrical, Chemical, Radiation etc.).

### **Duration (Length of time)**

- Acute (< 6 weeks).
- Chronic (> 6 weeks) (includes diabetic ulcer, venous ulcer, pressure ulcer, malignant ulcers, traumatic ulcers etc.).

### **Depth**

- Superficial (above sub dermis).
- Deep (traverses sub dermis).

### **Mode of Injury**

- Abrasion: superficial skin involvement.
- Ulceration: Defect in epithelial lining (skin or mucosa).
- Incisions: By sharp objects.
- Lacerations, Contusions and Bruise: By blunt objects.
- Degloving, Traction and Avulsion Injury: shearing of skin from underlying fascia.
- Puncture Wounds: eg) needle stick injury.
- Bite Wounds (By animals, human, Snake etc.).
- Crush Injury (when body part caught between two objects).

### **Based on amount of contamination at the time of surgery**

- Clean (Class I)
- Clean-contaminated (Class II)
- Contaminated (Class III)
- Dirty-infected (Class IV)

A wound is a breakdown in the protective function of the skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue following injury to the skin or underlying tissues/organs caused by surgery, a blow, a cut, chemicals, heat/cold, friction shear force, pressure or as a result of disease, such as leg ulcers or carcinomas.<sup>16, 17</sup>

## Epidemiology

The epidemiology of various wounds are different in different ethnicities. For example, the western population has a high prevalence of post traumatic wounds, whereas the developing Asian countries have a high prevalence of wounds due to acute and chronic conditions.<sup>18</sup> India being the world capital of diabetes has a high incidence of diabetic ulcers.<sup>19</sup> The prevalence of pressure ulcers worldwide has been reported as 6-18.5%.<sup>20</sup> In the USA, 80% of the lower limb ulcers are diagnosed to be venous ulcers.<sup>21</sup>

## Etiologies

**Infection:** Examples Surgical site infection, leprosy, Cancrum, tuberculosis etc.,<sup>22</sup>

**Arterial Insufficiency:** Arterial insufficiency, leading to localised ischemia, hypoxia, and thereby ulceration of the area. Eg., are thromboangiitis obliterans, atherosclerosis, atheroembolisms etc.,<sup>2</sup>

**Venous Insufficiency:** Venous ulcers can occur due to faulty valves and thereby reflux of blood, like in varicose veins or due to thrombosis of the deep veins which can also cause chronic venous insufficiency.<sup>24</sup>

**Lymphedema:** Ulcerated lymphangiectasia, lymphorrhea, inflammation and pustule formation, skin maceration, infection, papillomatosis and hyperkeratosis.

**Neoplasms (Cutaneous):** eg., Basal cell carcinoma, squamous cell carcinoma, melanoma etc.<sup>25</sup>

**Pyoderma Gangrenosum:** It starts as a small papule or collection of papules, and the central area then undergoes necrosis to form a single ulcer develops small painful papule or nodule followed by tissue necrosis and ulcerations.<sup>26</sup>

**Calciophylaxis:** It is a rare condition, most often seen in patients with chronic kidney disease mostly in the lower legs. This is due to calcification and occlusion of cutaneous and subcutaneous arteries and arterioles.<sup>27</sup>

**Necrobiosis lipoidica:** It is a rash that is most commonly seen in the shin in diabetic patients. It may harbor a squamous cell carcinoma.<sup>28</sup>

**Vasculitic wounds:** They can present as painful, erythematous nodules with ulcerations over lower legs in patients with autoimmune diseases.<sup>29</sup>

**Anticoagulant (warfarin) induced skin necrosis:** It commonly occur 3 to 5 days after initiating treatment due to inactivation of factor 2,7,9,10 by warfarin and inactivation of proteins C and S.<sup>30</sup>

**Mucormycosis:** India contributed to approximately 71% of the global cases of mucormycosis in patients with COVID-19 based on published literature from December, 2019, to the start of April, 2021. It can initiate as a sinus infiltration which can progress to a devastating erosion of the skin and bone.<sup>133</sup>

**Burns:** Burns can be due to various etiologies like thermal, electrical, chemical, radiation, frostbite or frictional burns<sup>31-36</sup>

**Trauma:** It can be a single wound or multiple wounds. It can be as simple as a superficial dermal abrasion to a complex injury involving skin, fascia, muscle, vessels, nerves and bones.<sup>37-43</sup>

**Pressure (decubitus) Ulcers:** Pressure ulcers develop due to ischemia induced by prolonged external pressure on tissue which is commonly seen in bed ridden hospitalized, unconscious, and patients on ventilators with or without paraplegia, quadriplegia<sup>44,45</sup>

**Neuropathy (Diabetic):** Diabetic ulcer can be attributed to both autonomic and sensory neuropathy. It can also cause neuropathic osteoarthropathy (Charcot foot) leading to chronic non healing ulcers<sup>46-49</sup>

**Idiopathic:** (eg) Acne Keloidalis Nuchae (AKN) which is characterized by follicular based papule and pustules on the occipital scalp or nape of neck<sup>50</sup>

## Investigations

Investigations are done for finding the cause of the wound, factors leading to a slow wound healing, and for assessment for fitness of the wound for reconstruction.<sup>51-57</sup>

This include Complete Blood count, renal function tests, Liver function tests; Coagulation studies; Tissue cultures;

Pulse volume recordings, Doppler studies, Ankle Brachial Index (ABI); plain radiography of the involved area, Computed Tomography, Magnetic Resonance Imaging for osseous abnormalities; vascular doppler studies, and Duplex studies, Edge wedge Biopsy to rule out malignancy in chronic wounds; Lymphoscintigraphy for Lymphoedema patients.

## Complications

**Acute complications:** Bleeding from the wound, loss of tissue, colonisation by multiple drug resistant pathogens leading to antibiotic resistance, biofilm formation; Chronic complications: Sinus formation, fistula, malignant transformation in the ulcer bed (Marjolin's ulcer), osteomyelitis, Post wound healing complications: Contractures, deformity, systemic amyloidosis, heterotopic calcification, hypertrophic scars, keloids, cutaneous horn, verrucae.

**Systemic complications:** Anaemia, fluid and electrolyte loss, septicemia, etc.

All the above said complications significantly impair the quality of life of the patient thereby adding to the morbidity of the condition.<sup>58-61</sup>

## Documentation and Assessment

Wound assessment includes assessing the wound dimension, type of tissue, colour, shape, depth of the wound and nature of the edges, margins, floor, and base. These can be done by various techniques like estimation by ruler, digital planimetry, wound tracing, stereophotogrammetry, technical procedures (e.g. computer, image analysis, and colour imaging using hue saturation and intensity) and photography.

Two tools commonly used to monitor and quantify wound healing are the Bates-Jensen Wound Assessment Tool (BJWAT) and Pressure Ulcer Scale for Healing (PUSH).<sup>62-68, 146</sup>

Presumptive assessment for the risk of an ulcer development helps in preventing the morbidity that occurs with a formed ulcer. Braden scale is one such tool which helps in assessing risk of pressure ulcer development.<sup>69</sup> Risk of development of diabetic ulcers can be assessed with the help of National Diabetes Education Program (NDEP).<sup>70</sup>

### Wound Bed Preparation (WBP)

As described by Falanga, Wound bed preparation (WBP) is defined as the process of removing barriers to facilitate healing.<sup>71</sup> Principles of wound bed preparation are better understood with the acronym 'T.I.M.E'.

#### TIME stands for:

**T:** Tissue - This is an assessment and management of the tissue in the wound, if it is non-viable or deficient. If the tissue is non-viable (necrotic) it should be debrided or removed.

Different methods of debridement can be used. It can be broadly classified into:

- Medical (enzymatic e.g. papain urea<sup>72</sup>, chemical, larva therapy, biological agents<sup>73</sup>, hydro jet debridement<sup>74</sup> etc.)
- Surgical (sharp, laser, ultrasonic etc.).<sup>75, 76</sup>

After debridement, the Deficient tissues may be covered by either primary or secondary wound closure in the primary setting<sup>77</sup>, or covering initially by biological skin substitute<sup>78</sup> or cover with skin graft or flap later.<sup>79</sup>

**T:** Infection and Inflammation management to restore bacterial balance. Bacterial load when it exceeds  $10^5$  to  $10^6$  organisms per gram adversely affects the wound healing, whatever the organism may be. Wound cleansing by irrigation with saline, use of antimicrobials, super oxidized solution<sup>80</sup>, silver solution, Nano crystalline dressing etc., antibiotics and anti-inflammatories, can reduce the bacterial load and thereby aid in wound healing.

**M:** Moisture helps in wound healing. But in the form of an exudate it is detrimental. Methods of exudate control includes direct methods like use of absorbent dressings, Negative Pressure Wound Therapy (NPWT)<sup>81, 82</sup> and oedema control.<sup>83</sup>

**E:** Edge of wound (Non-advancing or undermined) management. This involves stimulated the epithelium in the edge of the wound to advance with various adjunctive therapies like Growth factors,<sup>84</sup> Autologous Platelet Rich Plasma (APRP) therapy<sup>85</sup>, Autologous Lipoaspirate therapy.<sup>86</sup>

To conclude introduction of various newer adjunct dressings and state of art technologies helps in a speedy wound bed preparation and thereby allowing a definitive cover of the wound as early as possible.<sup>87</sup>

## Management

Management of an acute or chronic wound can be either Medical or surgical and can be done separately or concomitantly.

### Medical Management

#### General Medical Treatment

- Resuscitation:** The initial management of the patient always follows ABCDE protocol (Airway, Breathing, Circulation, Disability, Exposure). After stabilizing the patient hemodynamically, wound care is started. Tetanus prophylaxis should be given. In case of other associated system injuries, multidisciplinary care should be done.

If the patient presents with a chronic wound, the systemic condition and associated problems should be looked for and have to be addressed before initiating wound management.<sup>88</sup>

- Non-Surgical Debridement:** A clean lacerated wound presenting immediately after the injury (within 6 hrs), primary closure should be done, with no tension across the suture line. But if a patient has a compromised vascularity, it is better to wait, allowing the collateral channels to open.

In a chronic wound, if there is no necrotic tissue, then a medical debridement can be done like with pulse lavage therapy with hydro jet etc. Wet gauze is safer to use than dry gauze as it may not disrupt the healthy granulation tissue.<sup>88</sup>

- Nutrition:** Anemia due to blood loss or preexisting nutritional deficiency which requires blood and nutritional replacement in acute wounds. Hypoalbuminemia and anemia in chronic wound, can be managed by providing 1.25-1.5 g of protein per kilogram of body weight and 30-35 calories/kg gradually increased according to the size of the wound. Micronutrients have to be supplemented.<sup>89</sup>
- Infection:** Certain topical antimicrobials like Hydrogen peroxide are cytotoxic to healthy cells and interfere with epithelialization. Systemic antibiotics according to tissue culture reports can be



added. The bacterial load should be reduced by debridement of dead tissue.

- E. *Dressing*: Dressing can be primary dressing which covers the wound, or a secondary dressing which is used to cover the primary dressing. Dressing serves to protect the wound from the external environment. Nature of dressings can vary. Newer techniques of antibiotic incorporated dressings for infected wounds like antimicrobial coated dressing is useful.<sup>90</sup>
- F. *Adjuvant Modalities*: Growth factor rich autologous platelet rich plasma and other topical agents, Egg membrane, autologous lipoaspirate (Fig. 3) cultured keratinocytes have been described (fig. 2 a, b, c).<sup>91</sup>



Fig. 2a. Egg membrane being applied on the wound.



Fig. 2b: Sucralfate being applied in pressure sore.



Fig. 2c: Vit D applied over the wound.



Fig 3: Autologous lipoaspirate infiltrated in the wound.

- G. *Relieve Pain*: Pain can be avoided by techniques like use of topical lignocaine gel, moistening the gauze with saline before removal to avoid adherence to the wound bed, topical and systemic pain relievers.<sup>92</sup>
- H. *Negative Pressure Wound Therapy (NPWT)*: It refers to any device that tightly seals the wound creating a near airtight environment with a negative pressure of 125 mm Hg. Various modifications of NPWT combination with oxygen and irrigation, known as Regulated Oxygen Enriched-Irrigation Negative Pressure Therapy (ROI-NPT).<sup>93</sup> It can also be used with oxygen and irrigation, known as Limited Access Dressing (LAD) (figure 4).<sup>94</sup>
- I. *Hyperbaric Oxygen Therapy*: HBOT is the use of 100% oxygen at pressures greater than atmospheric pressure while the pressure of the treatment chamber is increased to greater than 1 atmosphere absolute (ATA) or more. It is useful in compromised skin grafts and flaps, crush injuries, arterial insufficiencies, necrotizing fasciitis etc.,<sup>95</sup>



Fig. 4: Limited Access dressing.

- J. *Physiotherapy*: Physiotherapy comprises of correct positioning of the limb within splints, usage of pressure garments, off-loading foot wear, air mattresses, cryotherapy, exercises which may be active or passive, electrical and ultrasonic stimulation of the muscles, laser therapy, hydrotherapy etc.,<sup>96</sup>

#### *Specific Medical Treatment (according to type of wound)*

- A. *Pressure Ulcers*: It includes frequent position change every 2 hours,<sup>97</sup> adequate cushioning at the pressure points eg., air bed<sup>98</sup> avoiding of maceration with urine/stool/sweat,<sup>99</sup> using antiperspirant.
- B. *Venous Ulcer*: It comprises edema management with compression garments and limb elevation, surgical management of the incompetent perforators by ligation of the same.<sup>100</sup>
- B. *Diabetic Foot Ulcers*: Prevention is done by instructing the patient to inspect the foot everyday for wounds, the ulcer can be managed by debridement, dressings, assessment for osteomyelitis followed by antibiotic therapy. Control of blood glucose, management of

peripheral arterial insufficiency is paramount. Wound coverage can be done by Split Skin grafts, or biological dressings or application of growth factors stimulating spontaneous wound healing, negative pressure and hyperbaric wound therapy.<sup>101,102</sup>

- D. *Lymphedema*: It includes Complex decongestive therapy, limb elevation, compression garments.
- E. *Burns*: It includes fluid resuscitation, stabilising hemodynamics. This is followed by wound management, and pain control. Wounds can be managed with Silver sulfadiazine dressings, or biological (collagen) dressings. Secondary infection prevention, pressure sore prevention and avoiding of contractures with splints is mandatory.<sup>103,104</sup>
- F. *Malignant Wounds*: This includes antibacterial impregnated dressing, local antibiotics, exudate control dressings like hydrogel dressing.<sup>105</sup>
- G. *Bleeding Wounds*: Control of bleeding can be by conservative measures-compression and limb elevation, by medical management (topical 1:1000 adrenaline etc) or by surgical management (ligation or cauterization is done).<sup>106</sup>
- H. *Chemo-Extravasation*: Once extravasation of cytotoxic drugs has been recognized the immediate management is to stop the infusion. The cannula should not be removed as it may be required to aspirate the drug and to give neutralizing drugs. This is followed marking of the area to assess progress, cold compression if the compound is non vesicant, hot compression of the compound is vinca alkaloids.<sup>107</sup>

## Surgical Management

- A. *Debridement, Incision and Drainage, Amputations*: Dead non viable tissue has to be debrided, which can be either sharp dissection with a knife, or using latest technologies like radiofrequency, ultrasonic waves etc. If an entire limb or body part is non viable beyond salvage, amputation has to be done.
- B. *Primary Closure*: Initially primary closure was done with sutures. With advent of many new technologies, it can be done with steri strips, medical glue etc.
- C. *Delayed Primary Closure*: If the wound is not amenable for suture closure, once it is free of bacteria and filled with healthy granulation tissue, it can be covered according to a reconstructive ladder. Newer modalities like bioengineered skin grafts can be used.<sup>108</sup>
- D. *External Tissue Expansion Wound Closure (ETWC)*: If delayed primary closure is not feasible, then External tissue expansion aided wound closure (ETWC) can be used. This utilizes the biomechanical properties of skin, but the drawback is that it will take time for the wound to close (figure 5).
- E. *Bioengineered Skin Substitutes*: A biological skin substitute can be used as temporary cover which contains collagen from various sources and stimulates the wound bed for healing. eg. Apligraf is a bovine collagen<sup>109</sup> Dermagraft

contains human fibroblast-derived dermal substitute.<sup>110,111</sup>



Fig. 5. Ziplock tags as an external tissue expansion device.

- F. *Fasciotomy*: Compartment syndrome is characterised by a rise in intracompartmental pressure within the limb. It has to be managed immediately. This is done when the pressure rises to 10-30mmhg from the patient's diastolic pressure.<sup>112</sup>
- G. *Escharotomy and Escharectomy*: Eschar is a thick, tough, non-elastic burn tissue, and can contribute to burn induced compartment syndrome. This can be managed by either an escharotomy-where the eschar is surgically divided and the underlying tissues are allowed to relax or escharectomy, -where the eschar tissue if non viable and is excised.<sup>113</sup>
- H. *Tangential Excision*: This technique, similar to a skin graft, but involves excision of unhealthy tissue with a skin graft knife, until healthy dermal bleed is noticed. Following this procedure, the wound either left for healing by secondary intention or can be covered with skin graft, or temporary substitutes.<sup>114</sup>
- I. *Sequential Excision*: Unlike escharotomy or tangential excision, this involves staged excision of the burnt tissue, followed by cover with skin graft. This can be done in areas like face.<sup>115</sup>
- J. *Aspiration or Deroofing of Burn Blisters*: Burn blisters can be aspirated or deroofed with a scalpel, specifically over joints and other vital structures whose function may be affected.<sup>116</sup>
- K. *Autologous Skin Grafting*: If the wound bed is ideal it can be closed with autologous skin graft in various forms.
- L. *Flaps*: The higher rung of the reconstruction ladder comprises flaps. Wounds with exposed bare bone or cartilage or tendon without paratenon are not suitable for coverage with skin grafts. In those scenarios, flap cover can be done. Flap can be either local flap, regional flap, pedicled flap, propeller flap, random flap, or axial flap, perforator flap, free microvascular flap can also be skin only flap, fasciocutaneous flap, muscle flap, or combination.<sup>118</sup>
- M. *Tissue Expander Assisted Wound Closure*: Tissue expansion utilises the expansile property of the skin recruiting adjacent normal skin for wound coverage, expanding it with a tissue expander.



Tissue expander plays a role when there is a paucity of microvascular free tissue for transfer. But the disadvantage is that it takes time.<sup>119</sup>

### Foetal Wound Healing

Wound healing in a fetus differs from adult wound healing in that it heals with very minimal scarring. The exact mechanisms of healing in the fetus are unclear. Minimal inflammatory response with a marked deposition of glycosaminoglycans, particularly hyaluronic acid is seen during foetal wound healing. Foetal wound healing is an organ specific response as organs other than skin and bone are unable to regenerate scarlessly. Sterile, warm amniotic fluid rich in growth factors, hyaluronic acid and fibronectin could modulate healing. The fetus has very low pO<sub>2</sub> and foetal fibroblast achieves repair without macrophages.<sup>137</sup>

### Recent Advances

The most eminent of all is gene therapy, where delivery of a gene into a wound would turn the specific gene on and off and thereby promoting faster wound healing and avoiding abnormal hypertrophic scars or keloids.<sup>120</sup>

Mesenchymal stem cell as a delivery vector to deliver the genes has been proposed.<sup>121</sup> The ultimate goal is to produce artificial skins that are readily implantable.<sup>122</sup> Various other methods described in wound dressings and treatment are quaternary ammonium compounds in wound healing<sup>134</sup>, PolyHexaMethylguanide in wound bed preparation<sup>137</sup>, combination therapy of negative pressure and autologous platelet rich plasma<sup>138</sup>, filling of substances like collagen in wound cavities to promote wound healing<sup>139</sup>, use of Microporous Polysaccharide Hemosphere Technology in wound healing<sup>140</sup>, use of prolotherapy in wound management<sup>138</sup>, Use of hemoglobin<sup>141</sup> and sucalfate<sup>142</sup> in wound bed preparation, Use of Non-Suction Epidermal Blister Grafting (NSEBG) for wound cover<sup>143</sup>, Use of cholecalciferol granules<sup>144</sup> in diabetic ulcers, use of Technology Lipido-Colloidal (TLC) with Silver Impregnated Poly Absorbent Fibre.<sup>145</sup>

### Indian Experience in Wound Healing

Wound healing in India has evolved to a greater extent, beginning from Sushruta to the present century. India has been privy to various techniques like homeopathy, acupuncture, herb mix pastes, yoga and music therapy.<sup>123</sup> Research has been done on the effectiveness of various natural herbs like propolis,<sup>124</sup> *Grewia tiliaefolia*,<sup>125</sup> *Jatyadi Taila*,<sup>126</sup> *Curcumin*,<sup>127</sup> *Carissa spinarum* Linn,<sup>128</sup> *Pyrostegia venusta*,<sup>129</sup> and many herbs.<sup>130</sup> A holistic approach to wound management has also been described.<sup>136</sup>

The Society for Wound Care and Research (SWCR) have come out with general clinical guidelines for wound management. Similar to ABCDE for trauma management, ABCDE approach to wound management has been described.<sup>135, 132</sup>

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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.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. *Acta Odontol Scand* 2003; 61: 347–55.

### Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone-iodine antiseptics. *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

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. *Dent Mater* 2006.

### Personal author(s)

[6] Hosmer D, Lemeshow S. *Applied logistic regression*, 2nd edn. New York: Wiley-Interscience; 2000.

### Chapter in book

[7] Nauntofte B, Tenovou J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

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

### No author given

[8] World Health Organization. Oral health surveys - basic methods, 4<sup>th</sup> edn. Geneva: World Health Organization; 1997.

### 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/HSQ20.pdf](http://www.statistics.gov.uk/downloads/theme_health/HSQ20.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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