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UNSEEN ASPECTS OF WOUND HEALING: AN OVERVIEW

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ABSTRACT

Wound and its healing process are thought to be complicated process in the world. In fact, a wound heals in an orderly effective and in a systematic manner. The process of wound healing can be divided into four distinct phases but seen to be overlapping with each other. To distinguish the various phases of wound healing biological markers are used. Although the wound is accidental or surgical, it follows the basic healing procedure and the factors governing the healing of wound are also the same. This review describes the details regarding the wound and its types, phases of healing, biological process involved in normal and pathological wound healing. The feature of the review article is that we have touched the unseen aspects of nutrition support in wound healing. Nutritional deficiencies can impede wound healing, and several nutritional factors required for wound repair may improve healing time and wound outcome. To promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient, it is important to explore nutritional and botanical influences on wound outcome.

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KEYWORDS

Wound healing, Phases of wound healing, Types of wound, Mineral, Nutrition support.

INTRODUCTION

A wound is a disruption in the continuity of cells, anything that causes cells that would normally be connected to become separated, or a wound is a type of injury in which skin is torn, cut or punctured , or where blunt force trauma causes a contusion[1]. In pathology, it specifically refers to a sharp injury which damages the dermis of the skin. Wounds are classified as open and close wounds. Open wounds are further classified according to the object responsible such as incised wounds caused by knife of a razor, Lacerations, irregular tear-like wounds caused by some blunt trauma, Abrasions in which the top most layer are scraped off, Puncture wounds, caused by an object puncturing the skin, Penetration wounds, caused by an object such as a knife entering and coming out from the skin and Gunshot wounds, caused by a bullet. Closed wounds are categories as Contusions, more commonly known as bruises, caused by a blunt force trauma that damages tissue under the skin, Hematomas, also called a blood tumor, caused by damage to a blood vessel, Crush injury, caused by a great or extreme amount of force, Chronic and Acute wounds are the result of injuries that disrupt the tissue [2].

Fig.1
An open wound

fig.2
A laceration to the leg

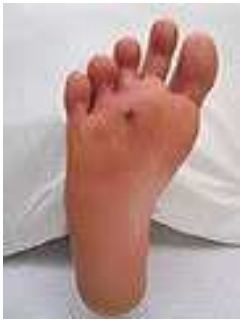

Fig.3
An infected puncture darts. wound to the bottom of the forefoot.

fig.4
A puncture wound from playing

Healing of wounds normally proceeds in a very orderly and efficient manner characterized by four distinct, but overlapping phases. The normal healing response begins at the moment the tissue is injured. As the blood components spill into the site of injury, the platelets come into contact with exposed collagen and other elements of the extracellular matrix. This contact triggers the platelets to release clotting factors as well as essential growth factors and cytokines such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-ß) [3].

Nutritional deficiencies can impede wound healing, and several nutritional factors required for wound repair may improve healing time and wound outcome. Vitamin A is required for epithelial and bone formation, cellular differentiation, and immune function. Vitamin C is necessary for collagen formation, proper immune function, and as a tissue antioxidant. Vitamin E is the major lipid-soluble antioxidant in the skin; however, the effect of vitamin E on surgical wounds is inconclusive. Bromelain reduces edema, bruising, pain, and healing time following trauma and surgical procedures. Glucosamine appears to be the rate-limiting substrate for hyaluronic acid production in the wound. Adequate dietary protein is absolutely essential for proper wound healing, and tissue levels of the amino acids arginine and glutamine may influence wound repair and immune function. The botanical medicines *Centella asiatica* and *Aloe vera* have been used for decades, both topically and internally, to enhance wound repair. Scientific studies are now beginning to validate efficacy and explore mechanisms of action for these botanicals. To promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient, it is important to explore nutritional and botanical influences on wound outcome [4].

Figure 5.
The four possible responses following tissue injury

Phases of wound healing:

The process of healing starts instant at the moment of injury occur, the phases are: *hemostasis*, *inflammation*, *proliferation* and *remodeling*. These phases basically overlap with each other but by using biological markers we can understand them clearly.

Hemostasis:

Injuries initiate the molecular and cellular responses that establish hemostasis. The healing process cannot proceed until hemostasis is accomplished. Primary contributors to hemostasis include vasoconstriction, platelet aggregation, and fibrin deposition resulting from the coagulation cascades. The end product of the hemostatic process is clot formation. Clots are primarily composed of fibrin mesh and aggregated platelets along with embedded blood cells [5]. The process of clot formation is very essential. This process prevents further fluid and electrolyte loss from the wound site and limits

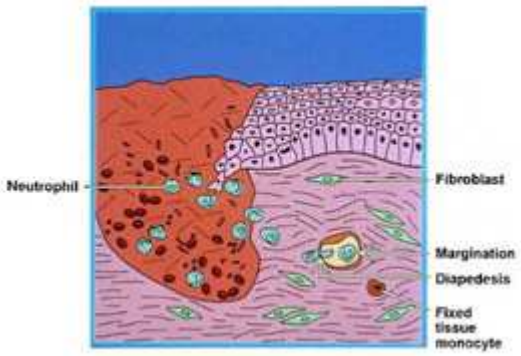

Figure 6.

At the time of injury, the tissue is disrupted and the platelets adhere to the exposed collagen and to each other. The platelets release clotting factors, PDGF and TGF-ß to initiate the repair process

contamination from the outside environment. Fibrin is also the mesh material in the provisional wound matrix onto which fibroblasts and other cells migrate as the healing process proceeds.

Inflammation:

Inflammation is characterized by the erythema, edema, heat, and pain as first described by Hunter in 1794. At the tissue level, increased vascular permeability and the sequential migration of leukocytes into the extra vascular space characterize inflammation [6]. One of the primary functions of inflammation is to bring inflammatory cells to the injured area [7]. These cells then destroy bacteria and eliminate debris from dying cells and damaged matrix so that the repair processes can proceed [8]. Although inflammation is often thought of as the second phase of wound healing, signs of inflammation, including erythema and heat, develop soon after injury as a result of vasodilatation. Vasodilatation follows the initial vasoconstriction that reverses 10 to 15 minutes after injury. Simultaneously, the endothelial cells lining the capillaries in the vicinity of the wound develop gaps between them, which permit the leakage of plasma from the intravascular space to the extra vascular compartment [9]. The migration of fluid into the injured area generates edema, which contributes to the sensation of pain that characterizes inflammation.

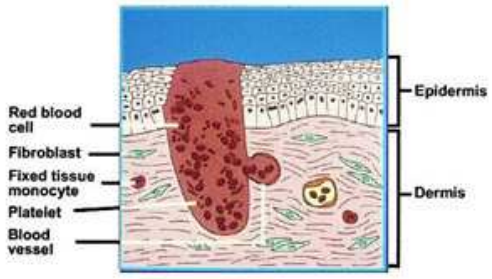

Figure 7

By the first day following injury, neutrophils attach to endothelial cells in the vessel walls surrounding the wound (margination), then change shape to move through the cell junctions (diapedesis) and migrate to the wound site (chemotaxis). This is the beginning of the inflammatory phase.

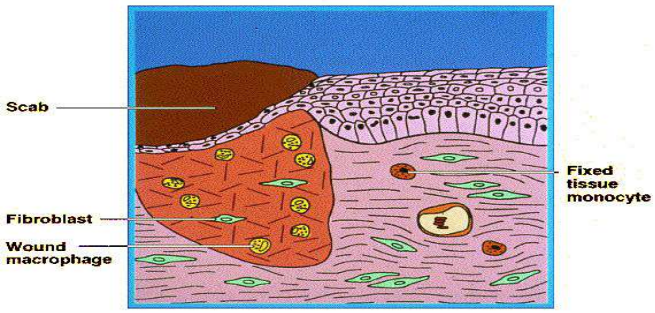

Figure 8.

The inflammatory phase continues as fixed tissue macrophages become active and move into the site of injury and transform into very active wound macrophages. These highly phagocytic cells also release PDGF and TGF-ß to recruit fibroblasts to the site and thus begin the proliferative phase

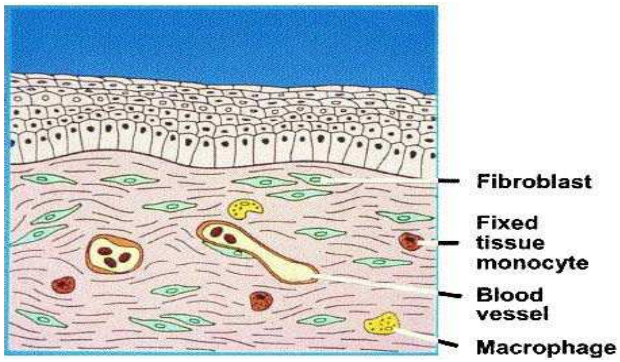

Figure 9.

The remodeling phase is characterized by continued synthesis and degradation of the extracellular matrix components trying to establish a new equilibrium.

Proliferation:

The cellular milieu in wounds changes dramatically in the first week post acute injury. The initial fibrin–fibronectin matrix is heavily populated by inflammatory cells, whereas fibroblasts and endothelial cells will predominate as healing progresses. Reestablishment of the epithelial surface is also initiated within the first several days after injury, as is revascularization of the damaged area. Cytokine networks continue to be a part of the process as cytokine release contributes to fibroplasia, epithelialization, and angiogenesis [10]. Although much is known about the signals that stimulate the predominant activities during this phase of healing, less is known about the signals that bring these activities to a controlled end. Negative feedback mechanisms that deactivate cells after they have completed their work are also essential for normal healing. Additional fibroblasts are required in the healing wound, in that native cells are lost or damaged in any injury. Repopulation of the wounded area with fibroblasts occurs as a result of fibroblast migration from adjacent tissues and proliferation of cells in the wound. In addition, undifferentiated cells in the vicinity of the wound may transform into fibroblasts under the influence of cytokines in the wound milieu [11].

Factors that stimulate fibroblast migration include PDGF [12], TGF-b [13], EGF [14], and fibronectin [15]. Upregulation of cell membrane integrin receptors that bind fibronectin and fibrin in the provisional wound matrix is required for

fibroblasts to migrate , and PDGF and TGF-b [12] both contribute to the migratory process in this manner.

Remodeling:

Scar remodeling begins to predominate as the primary wound-healing activity approximately 21 days after injury. The rate of collagen synthesis diminishes and reaches coincidence with the rate of collagen breakdown. The down regulation of collagen synthesis is mediated by g-interferon [16], TNF-a , and collagen matrix itself [17]. Matrix metalloproteinases (MMPs) are intimately involved with the breakdown of collagen molecules that occurs actively during the remodeling process. The MMPs have been alluded to previously and are involved in many aspects of the healing process. MMPs represent a family of at least 25 enzymes that break down different extracellular matrices [18]. They are produced by a variety of cell types, and different cells generally synthesize different enzymes. The MMP activity within tissues is modulated by tissue inhibitors of metalloproteinases (TIMPs) [19]. Four isoforms of TIMPs have been described. The balance of MMPs and TIMPs within tissues is critical to enzyme activity and is regulated by cytokines including TGF-b, PDGF, and IL-1 [20]. All of the functions provided by MMPs during wound healing have not been clearly delineated. Elevated concentrations of MMPs are seen in chronic ulcers [21], yet deletion of MMP-3 in mice causes failure of wound contraction with a significant delay in healing [22], thereby implicating a complex regulatory pathway. The nature of the wound matrix changes with scar remodeling. Immature scar contains a disorganized array of fine collagen fibers, which is gradually replaced by thicker fibers arranged in an orientation paralleling skin stresses. In addition, the number of cross-links both within and between molecules gradually increases. As the nature of the collagen matrix changes, it becomes less cellular through apoptosis of cells involved in the healing process. As mentioned, the ratio of type I to type III collagen changes, and the quantity of water and proteoglycans diminish. Normal skin shows a basketlike weave pattern that is never completely reproduced with scar remodeling. Although seemingly not as complex as other aspects of the healing process, remodeling is essential to the formation of a strong wound. The remodeling process is associated with a substantial increase in wound-breaking strength. Wound strength 1 week after injury is 3% of normal dermis. After 3 weeks, when the remodeling phase begins to predominate, the wound will have only approximately 20% the strength of normal dermis. At 3 months, however, the wound will have 80% the strength of normal dermis, with the significant increase in strength resulting from the contribution of remodeling. Remodeling will continue for up to 12 months after a wound is created, although scars never regain the strength of normal dermis

.

Table no. 1
Phases of healing

Phases of Healing	Day of post injury	Cells involved in phases
Hemostasis	Immediate	Platelets
Inflammation	Day 1 - 4	Neutrophils
Proliferation	Day 4 – 21	Macrophages
	Lymphocytes	
	Angiocytes	
	Neurocytes	
Contracture		Fibroblasts
	Keratinocytes	

Remodeling	Day 21 – 2 yrs	Fibrocytes

Vitamins and Minerals Essential to Wound Healing

Vitamin A

Vitamin A is required for epithelial and bone tissue development, cellular differentiation, and immune system function. Substantial evidence supports the use of vitamin A as a perioperative nutritional supplement [(23)]. In addition to facilitating normal physiological wound repair, Ehrlich and Hunt have shown vitamin A reverses the corticosteroid- induced inhibition of cutaneous and fascial wound healing [(24)]. Vitamin A has also corrected non-steroid induced, post-operative immune depression [(25)] and improved survival in surgically-induced abdominal sepsis. Levenson et al suggest vitamin A benefits the wound by enhancing the early inflammatory phase, including increasing the number of monocytes and macrophages at the wound site, modulating collagenase activity, supporting epithelial cell differentiation, and improving localization and stimulation of the immune response. Animal studies show vitamin A may increase both collagen cross-linkage and wound breaking strength. Levenson and Demetrio recommend vitamin A supplementation of 25,000 IU daily before and after elective surgery [(26).]

Concern among some practitioners regarding the potential toxicity of higher doses of vitamin A has led to uneasiness about using it perioperatively. The vast majority of toxicity cases have occurred at daily vitamin A dosages of 50,000-100,000 IU in adults over a period of weeks to years [(27)] Short-term supplementation of 25,000 IU daily appears to be safe for most nonpregnant adults. Caution must be exercised in supplementing vitamin A in patients for whom the anti-inflammatory effect of steroids is essential, such as in rheumatoid arthritis or organ transplants, as well as in pregnant women and women of childbearing age.

Vitamin C

Ascorbic acid is an essential cofactor for the synthesis of collagen, proteoglycans, and other organic components of the intracellular matrix of tissues such as bones, skin, capillary walls, and other connective tissues. Ascorbic acid deficiency causes abnormal collagen fibers and alterations of the intracellular matrix that manifests as cutaneous lesions, poor adhesion of endothelium cells, and decreased tensile strength of fibrous tissue[(28)] Although ascorbic acid is required for reparation of damaged tissue, researchers have demonstrated the benefit of vitamin C only in vitamin C-deficient individuals using low doses of ascorbic acid[(29)] In a study by Hodges et al, four subjects (ages 33-44) were depleted of vitamin C for 99 days to induce scurvy. On day 100, a 5-cm incision was made in the left thigh of each subject and they began the oral administration of 4, 8, 16, or 32 mg ascorbic acid daily. Healing was measured by histological and electron microscope technique. It was shown that 4 mg daily of vitamin C was just as effective as 32 mg daily for wound healing in these vitamin C-deficient subjects. The efficacy of using vitamin C to improve wound healing in non-deficient individuals remains uncertain.

Zinc

Approximately 300 enzymes require zinc for their activities. Zinc is an essential trace mineral for DNA synthesis, cell division, and protein synthesis [(30)], all necessary processes for tissue regeneration and repair. Zinc deficiency has been associated with poor wound healing and decreased breaking strength of animal wounds [(31)], which can result from decreased protein and collagen synthesis during healing found in zinc-deficient animals [(32)]. Senapati and Thompson found zinc levels were 50-percent higher in

muscle and skin from abdominal wounds of rats during wound healing, but mild deficiency reduced this accumulation[33] Zinc demands are thought to be the highest from time of wounding throughout the early inflammatory phase. Sequential changes in zinc concentrations were studied in the incisional wound model in the rat. Zinc levels increased from wounding and peaked on the fifth day – at a time of high inflammation, granulation tissue formation, and epidermal cell proliferation [34]. Zinc concentrations returned to normal by the seventh day, when inflammation had regressed. It has been suggested that increased local demand for zinc resulting from surgery and wounding exposes otherwise marginal zinc deficiencies in humans [33].

Vitamin E

Vitamin E is popular among consumers for skin care and to prevent scar formation. It functions as the major lipophilic antioxidant, preventing peroxidation of lipids and resulting in more stable cell membranes. The antioxidant-membrane stabilizing effect of vitamin E also includes stabilization of the lysomal membrane, a function shared by glucocorticoids[35]. Systemic vitamin E and glucocorticoids inhibit the inflammatory response and collagen synthesis, thereby possibly impeding the healing process. The effect of vitamin E on wound healing is complex; it may have alternate effects in different types of wounds and in the presence of other nutrients, as well as different functions for water soluble versus lipid soluble preparations of vitamin E. Animal studies of vitamin E supplementation on surgical wounds show conflicting results. Greenwald et al showed flexor tendon repair in chickens treated with vitamin E had breaking strength less than half that of controls measured after days 7 and 45 from surgical repair. Another animal study showed impaired collagen synthesis in rats treated with vitamin E after wounding [36]. The researchers cite the glucocorticoid-like effect of vitamin E as the cause of the negative results. However, these effects are mitigated by vitamin A, as vitamin A is a lysomal destabilizer that reverses several of the deleterious effects of glucocorticoids.

Other Dietary Supplements and Wound Healing

Bromelain

Bromelain is a general name given to a family of proteolytic enzymes derived from *Ananas comosus*, the pineapple plant. Throughout the 1960s and 1970s a series of studies found the effects of orally administered bromelain include the reduction of edema, bruising, pain, and healing time following trauma and surgical procedures [37-40]. More recently, researchers from the Czech Republic found that patients with long bone fractures administered a proteolytic enzyme combination containing 90 mg bromelain per tablet had less post-operative swelling compared to patients given placebo[41]. Fractures were treated by surgically inserting rods through the long axis of the fractured bone (intramedullary fixation) or by constructing an external framework of pins and rods going through the skin and muscle to connect to the fractured bone (external fixators). The treatment group was given three 90-mg tablets three times daily for three days after surgery and, subsequently, two tablets three times daily for two weeks. On the fourteenth post-operative day the limb volume of the treatment group was reduced by 17 percent compared with nine percent in the control group. The total number of analgesics consumed by the treatment group was also significantly reduced in comparison to the control group [41].

Protein and Wound Healing

Adequate protein intake is essential for proper wound healing. Protein depletion appears to delay wound healing by prolonging the inflammatory phase; by inhibiting fibroplasia, collagen and proteoglycan synthesis, and neoangiogenesis (proliferation phase); and by

inhibiting wound remodeling[42,43]. Experimental protein depletion in animals caused a decrease in the tensile strength of wounds. Rats fed a diet deficient in protein exhibited decreased wound integrity and strength versus control animals [44]. In a study of 108 human patients with experimental wounds, individuals with either low serum protein or serum albumin had significantly weaker wounds than those with normal protein values [45].

Protein supplementation of elderly patients with liquid protein formulas significantly enhanced healing of pressure ulcers. The change in ulcer area was significantly correlated with the amount of protein in the diet [46]. The surgical or trauma patient exists in a state of metabolic stress, with the severity of the stress depending on the severity of the wounded state. An injured patient requires more protein than a non-injured patient because of the increased metabolic activity of wound healing, acute-phase protein production in response to stress, and amino acid mobilization from muscle used for hepatic gluconeogenesis.

Amino Acids in Wound Healing

It is well accepted that sufficient protein is necessary for wound healing. This appears to be due to the increased overall protein need for tissue regeneration and repair. Researchers have investigated the effects of specific amino acids on the healing process and determined that arginine and glutamine appear to be necessary for proper wound healing.

Arginine

Arginine is a non-essential amino acid that plays a key role in protein and amino acid synthesis. It is acquired from the diet and derived endogenously from citrulline in a reaction catalyzed by the enzyme arginine synthetase. Adequate tissue arginine appears to be essential for efficient wound repair and immune function [47]. Arginine (17 g/day) was given to 30 elderly patients (>65 years of age) who sustained an experimental surgical injury. Supplemented patients demonstrated significantly greater hydroxyproline (a sign of collagen deposition) and protein accumulation at the wound site, compared to non-supplemented controls. Lymphocyte response, signifying greater immune activity, was elevated in the supplemented group, as was insulin- like growth factor-1, which is a control molecule for wound repair[48] Other studies have found similar results [49. 50].

Glutamine

Glutamine is used by inflammatory cells within the wound for proliferation and as a source of energy [51, 52] Fibroblasts use glutamine for these same purposes, as well as for protein and nucleic acid synthesis. Because optimal functioning of these cells is paramount to the healing process, glutamine is a necessary component of the process of tissue repair. Glutamine is a non-essential amino acid that can become a “conditionally essential” amino acid in certain circumstances, including tissue injury [53]. Glutamine is released from skeletal muscle following injury or surgery, which can cause a relative deficiency of glutamine in skeletal muscle and the gut, as intestinal uptake is frequently diminished as well. Studies utilizing oral glutamine pre- and post-surgery, and in burn patients, have shown mixed results. Oral feeding of glutamine in surgery patients did not affect plasma glutamine or nitrogen turnover. Intravenous glutamine in surgery patients as an alanine- glutamine dipeptide showed consistently better post-operative results, as seen by significantly decreased length of hospital stays (average of four days or less)[52]. A significantly smaller incidence of pneumonia, bacteremia, and sepsis was noted in patients with multiple trauma given enteral glutamine feedings [54]. Whether glutamine supplementation will enhance wound healing in less severely injured individuals is not known.

Botanical Medicines in Wound Healing

Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China have valuable information of many lesser-known hitherto unknown wild plants for treating wounds and burns [(55)]. Traditional forms of medicine practised for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of wounds related disorders [(56)].

According to Biswas and Mukherjee (2003) [(57)], 70% of the wound healing Ayurvedic drugs are of plant origin, 20% of mineral origin, and the remaining 10% consisting of animal products and these drugs are stated to be effective in different conditions such as *Vrana* (wounds or ulcers), *Nadivrana* (sinuses), *Vidradhi* (abscess), *Visarpa* (erysipelas), *Upadamsha* (syphilitic ulcers), *Vranajakrimi* (maggots in wounds), *Dustavrana* (septic wounds), *Vranashotha* (inflammatory changes of wounds), *Vranavisha* (cellulitis), *Ugravrana* (purulative ulcer), *Netravrana* (hordeolum or stye sepsis), *Pramehapidaka* (diabetic carbuncle), and *Bhagandara* (fistula-inano). Sussman (2007) [(58)]reported that, haemorrheologics, pentoxyfilline *(Trental)*, other methyl xanthenes, retinoids, phenytoin, prostaglandins, Vitamin A and C, zinc and some growth factors are the drugs which are having the potential of improving the healing of wounds.

Management

After dealing with the aspects of wound, it's healing and the nutrition required in the healing process in the management of the wound is essential. The treatment depends on the type, cause, and depth of the wound as well as whether other structure beyond the skin are involved. Treatment of recent lacerations involves examination, cleaning, and closing the wound. If the laceration occurred some time ago it may be allowed to heal by secondary intention due to the high rate of infection with immediate closure. Minor wounds like bruises will heal on their own with skin discoloration usually disappears in 1–2 weeks. Abrasions which are wounds with intact skin usually require no active treatment except keeping the area clean with soap and water. Puncture wounds may be prone to infection depending on the depth of penetration. The entry of puncture wound is left open to allow for bacteria or debris to be removed from inside.

CONCLUSION

The ability to heal an injury is a biologic necessity for all organisms, with mammals lagging in proficiency when compared with lower life forms that have the ability to regenerate differentiated structures. Technology and increased scientific knowledge have established a coordinated interplay that has improved the ability to manage wounds in a logical manner, and, on occasion, to accelerate the healing process. Nutritional status of patients at the time of trauma or surgery influences the biochemical processes necessary for the phases of normal healing to occur. Undernourished or malnourished individuals heal less efficiently and are at greater risk for complications during and after surgery.

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