1. GENERAL INTRODUCTION

Herbal medicines provide an efficient local aid to healthcare and disease free life. It has been confirmed by WHO that traditional medicine based largely on different species of plants and animals, serve the health needs for a large number of people. Many drugs we use today are based on the folk remedies and ethno pharmacological studies. There are more than 100 drugs of known structure that are extracted from higher plants and are used in allopathic medicine. Different pharmaceutical dosage forms or preparations were originally designed to extract and concentrate the active principles like alkaloids, glycosides, and volatile oils, primarily from plants and used for therapy.

These purified and highly concentrated chemically synthesized medicines has shown pronounced effects because of their increased potency, but in most of the cases when used for long duration and in certain cases when used for acute clinical medications showed drastic side effects which sometimes could be lethal. Crude therapeutic products are less toxic than their synthetic counterparts because they contain the total family of medicinal compounds & hence they offer lesser risk of side effects.

The nature has provided a complete storehouse of remedies to cure all ailments of mankind. WHO has estimated that around 80% of world population even now depends upon the traditional system of medicine for primary medical care. In modern medicine, approximately 50 % of drugs which in clinical use are derived from
natural sources (Products) and of these half of them are plant based. Despite advances in pharmacology and synthetic chemistry, this reliance on natural products, particularly on plant, remains largely unchanged.

1.1 NANOTECHNOLOGY

Nanotechnology is the innovation of 21st century, which offers very promising applications in drug delivery system. Due to their small size (1nm to 100 nm), such drug delivery system are promising tools in therapeutic approaches such as selective or targeted drug delivery across biological barriers or intracellular drug delivery which is interesting in gene and cancer therapy.

Some nanomaterial that are nano medicines are that considered to be potential nano medicines are generally split into several categories based on the types of nanomaterial or the application areas, such as drug delivery, drugs and therapies, in vivo imaging, in vitro diagnostics, biomaterials and implants (Wagner et al., 2008). Regardless of what criterion is used to categorize these nanomaterials, they share a certain degree of commonality in their physicochemical characteristics within and across the categories and the same characteristics in different nanomaterial can be visualized through the use of the same or equivalent techniques described above.

Nano-drug delivery systems aim to optimize bioavailability at particular locations over a period of time, minimizing drug toxicity, increasing drug-therapeutic index and replacing invasive
administration routes with non-invasive ones (Goldberg et al., 2007; Wagner et al., 2008). Nano-drug delivery systems include liposomes, nano suspensions, nano particles, dendrimers, fullerenes, and carbon nanotubes and the drug carriers in nano-drug delivery systems can be devised by regulating the composition, size, shape and morphology (Goldberg et al., 2007; Wagner et al., 2008). In a nano-drug delivery system, the system size can influence bioavailability and circulation time in blood stream, partly resulting from the impact of surface area-to-volume ratios on the solubility of the drug delivery systems (Goldberg et al., 2007; Rabinow, 2004; Vinogradov et al., 2002). Studies have showed that 10–100 nm is an optimal size for nano-drug delivery systems to mostly avoid rapid removal through extravasation or through phagocytosis (Stolnik et al., 1995; Vinogradov et al., 2002). Recent studies have demonstrated that the shape of the drug carrier plays an important role in bio distribution and cellular uptake as well as avoiding phagocytosis and prolonging circulation in blood stream (Champion and Mitragotri, 2009; Geng et al., 2007). In addition, it has been reported that the surface charge of a nano-drug delivery system affects the pharmacokinetics of drugs entrapped/adhered (Hathout et al., 2007; Law et al., 2000), while the structural difference of the delivery systems may influence drug delivery efficiency (Inokuchi et al., 2010). Among the techniques described in this article for physicochemical characterization, DLS, FCS, RS, NSOM, SEM, TEM, STM, AFM, NMR, XRD, SAXS, FS and several separation
techniques are suitable for evaluating the size and size distribution of nano-drug delivery systems. NSOM, SEM, TEM, STM, AFM, XRD and SAXS are proper modalities for shape measurement, while appropriate methods for surface charge measurement include zeta potential measurement (DLS), ATR–FTIR, GE and CE. In addition, TERS, CD, MS, IR, STM, AFM, NMR, XRD, SAXS, FS and some of the thermal and separation techniques can investigate the structural properties of the nanomaterials.

Along with the development of nano-drug carriers, certain types of nanomaterials have been used to design active pharmaceuticals, such as a dendrimer-derived microbiocide for preventing HIV infections and fullerenes for binding and scavenging or inactivating free radicals, which are associated with the induction of neural and cardiovascular diseases (Wagner et al., 2008). Super-paramagnetic iron-oxide NPs coated with amino silane, for example, can be used in hyperthermia treatment of cancer by subjecting the tumour tissue to high temperatures in order to destroy neoplastic cells (Wagner et al., 2008). Magnetic NPs bound to antibodies can be specific to certain targets, e.g., stem cells, and allow sorting via magnetic field for cell therapy (Wagner et al., 2008). In addition to the physicochemical properties, including size, shape, surface charge and structure mentioned already, the stability, particularly thermal stability, of the nanomaterial plays a crucial role if nano drugs and nano-formulations are to retain and exert consistent therapeutic efficacy. In this article, the modalities
capable of characterizing the stability of nanomaterial are zeta potential measurement, CD, HPLC, HDC and several thermal techniques including TGA, DSC, ITC and thermophoresis. Molecular diagnostics is aimed at diagnosing disease at a molecular level before symptoms manifest (Wagner et al., 2008), and compared with conventional molecular imaging agents, employment of nanomaterial based contrast agents generally increases the signal intensity of a single particle (Rosenblum et al., 2010; Thomas et al., 2013). The strong signal generated by the nanomaterial-based contrast agents, in fact, helps overcome the essential disadvantages of low sensitivity in MRI and limited depth penetration of optical imaging to a certain degree (Lam et al., 2013; Rosenblum et al., 2010; Thomas et al., 2013). Given the novel properties of nanomaterial, several distinct nanomaterial are commonly designed as nano scale imaging probes, including quantum dots with specific electronic and optical properties, up conversion phosphors consisting of phosphor nano crystals doped with rare earth metals, and super-paramagnetic iron oxide particles containing an iron oxide core of magnetite and/or maghemite encased in polysaccharide, synthetic polymer or monomer coatings, or other soft materials like dendrimers (Biju et al., 2010a; Liang et al., 2008; Rosenblum et al., 2010; Wang et al., 2011). In addition to the characteristics of conventional imaging probes, such as structure, purity and solubility, certain physicochemical properties of nanomaterial-based imaging contrast agents also have to be
considered, including size, shape, composition, zeta potential and dispersion (Leung et al., 2012). Techniques that can characterize the property of purity include NMR, HPLC and HDC, while the property of composition can be characterized by MS and NMR. Furthermore, the EM- and SPM-derived techniques, such as ESEM, TEM, STM (scanning tunneling microscope) and AFM, can be implemented to characterize the dispersion of nano-based imaging probes.

Even _in vivo_ nanomaterial-based imaging contrast agents are continuously under development, nanomaterial toxicity in the body has not been comprehensively studied (Chi et al., 2012). While toxicity of being a minor concern leads to various types of nanomaterials widely used in the context of _in vitro_ diagnostics (Chi et al., 2012), the applications of _in vitro_ diagnostics have attracted a large amount of research interests, mainly split into NP-based biomarkers and novel sensor platforms composed of nanomaterials (Chi et al., 2012; Wagner et al., 2008). Among the physicochemical characteristics, stability is a key property in the applications of biomarkers.

Compared to drug delivery studies, the developments of nano scale biomaterials and implants are still in their infancy. Still, nanomaterials have been used in a wide spectrum of applications, including tissue regeneration and medical implants (Liu and Webster, 2007; Wagner et al., 2008). Nano materials have been considered for a variety of implant applications, such as bone
substitute materials, cartilage regeneration, vascular graft endothelialisation, bladder replacement, dental restoratives, neural prostheses and antibiotic materials (Liu and Webster, 2007; Wagner et al., 2008).

1.2 WOUND HEALING

Wounds are visible signs of disruption of the cellular and anatomic or functional continuity of normal living cells. It is caused by trauma, physical, chemical, electrical or microbiological means. Wound could be due to accidents or at times, as in the case of surgical wounds can be intentionally inflicted. Whichever may be the case, wounds need individual care and attention. Healing is the restoration of the integrity of the injured tissues.

Healing could be normal or times abnormal. The prime objective of wound management programs is to keep the wound healthy, so as to make the healing progress in a normal pace. For this, depending upon the severity of the wound, a number of measures are used, for instance, aseptic techniques (dressing, suture materials etc.) and drug factors. Another concept that is gaining importance is to accelerate the normal healing process, as it reduces the patient’s hospitalization and discomfort due to prolonged bearing of wounds. However, it is important to note that healing is a complex process which involves a number of intimately linked biochemical, cellular and molecular events. This complexity offers a large avenue for a large number of agents, which are part of the wound management program to affect the healing either positively
or negatively. Thus, the field of wound Pharmacology, has two main objectives viz. to study the influence of pre-and post-wound management on healing and to discomfort the healing enhancers (pro-healing agents).

Tremendous advancement has been made in the first instance. A number of agents that are employed in pre-and post-surgical wound care, for example dressing material ointment bases, chemotherapeutic agents, reports are just to mention a few. We do not have a marketed drug that can be used safely and confidently to promote all the phases of healing, after the discovery of epidermal growth factor (EGF) in 1962, besides PDGF, FGF, and TGF. These were recognized to stimulate angiogenesis, fibroblasts proliferation and collagen production experimental wounds of normal healing.

A number of plants have been described to have healing promoting potential in varieties of folklore medicines prevailing in different parts of India.

1.2.1 General considerations

Generally wound is a disruption of normal tissue structure and function. Wounds can result from injurious processes beginning either internally or externally to the involved organs. These can range from controlled acute disruption of local environment around the normal structures by damaging its mechanical, physiological and biochemical events resulting in an intricate process of wound healing.
Wound healing is a response of the living to the injury and represents an attempt to maintain normal structure and function. One of the most important attributes of life is its capacity for self-repair. With a few exceptions, cellular and biochemical mechanisms help wound heal quickly and effectively. The difference between acute wounds and chronic wounds is that in the former healing occurs through an orderly and timely process leading to restoration and functional integrity, while, on the other hand, chronic wounds fail to follow this course and are often associated with underlying pathology.

The response to injury consists of two processes. Repair and regeneration—restore functions in the pre-existing structure. Repair is the replacement of lost tissue by granulation tissue, followed by fibrosis and scar tissue formation which occurs when the surrounding specialized cells do not possess the capacity to proliferate as seen in neurons and muscles. Regeneration is the replacement of lost tissue similar in type as occurs due to proliferation of surrounding undamaged specialized cells as seen in the cells of liver and intestine. In many cases both repair and regeneration contribute to the healing process.

1.2.2 Types of wounds

There are several types of wounds seen in patients. They may be

1. Incised wounds: A sharp instrument usually causes these, which are relatively clean.
2. Lacerated wounds: These wounds are common following road traffic accidents. These wounds usually have jagged edges with lacerated and devitalized structures inside the wound.

3. Penetrating wounds are similar to incised wounds, except that its depth is more.

4. Crushed wounds occur due to industrial, road traffic and war injuries.

1.2.3 Wound repair reaction

The process of wound repair is highly dynamic and complex consisting of integrated series of cellular, physiological and biochemical events, leading to reestablishment of structural integrity, functional restoration and regain of strength of injured tissue. It starts whenever there is a damage to living tissue, with the following events:

Hemorrhage leads to the formation of a clot with fibrin framework which eventually dries and contracts and this may act to some extent as glue to hold the wound edge and to cover wound surface. It also leads to the activation and aggregation of platelets. There will be the release of platelet derived growth factor which plays an important role in healing process.

Then follows an inflammatory response to bring various constituents that is required to the affected area. The extent and severity of the inflammation depends on the type of wound for example in burns, the cellular phase of the inflammatory reaction may be delayed or even almost absent. After a period of 24 to 36
hours of wounding, the first sign of proliferative connective tissue activity; can be seen. This consists of division of fibroblasts and endothelial cells which along with the macrophages, make up the majority of granulation tissue. A key role in the healing of all the organs except those of epithelial origin is played by granulation tissue. As granulation tissue fills the wound cavity, the older parts of it become organized in such a way that the fibroblasts are left behind to synthesize more collagen around them.

Fibrillar collagen polymerized from the extra cellular tropocollagen, which later matures and leads to scar changes to give real strength to the healed wound. While the granulation tissue is growing or even earlier, the epithelial cells begin to migrate from the wound edges, either under the clot, across or through it, depending on how much dehydration has taken place, to completely cover the wound with epithelium—epithelization.

Roughly the process of healing and its phases are similar in all mammals, but the optimal rate may depend from tissue in the same species. All the events in the above process of repair except scar remodeling run concurrently and independent of each other. Further a given drug may influence one event with or without influencing other events.

1.2.4 Types of wound healing

1. Primary healing: This is also known as healing by first intention. This occurs in closed wounds, which are wounds with the edges approximated, such as, clean skin incision closed with
sutures. But primary healing is also seen in a closed with superficial wound involving only the epidermis and in abrasions involving entire loss of epidermis with the formation of a crust i.e. healing under a scab. The wound needs only minimal cell proliferation and hence there is minimal scarring. Minimal epithelization occurs in 24 hours.

2. **Secondary healing:** This is also known as healing by secondary intention or healing by granulation. This type of healing is seen in a wound whose edges cannot be brought together because of loss of a considerable amount of tissue. Many traumatic wounds and burns possess the above characteristics. It requires wound contraction extensive cell proliferation, neovascularization to heal. The end result is a prominent scar, which may be deforming functionally and cosmetically unsatisfactory.

3. **Delayed primary healing:** This is also known as healing by third intention and occurs when a wound is secondarily closed several days after injury. Grossly contaminated wounds are given a specific time for closure, also enabling the host inflammatory and immune response to control contamination. Delayed primary closure does not impair development of wound strength but decreases morbidity from wound infection.

### 1.2.5 Phases of wound healing

Healing occurs by two ways i.e. the replacement of lost tissues by similar tissue and “Repair” the replacement of the lost tissues by new structure called “Granulation tissue”. Wound healing
thus involves knitting of disrupted surface by collagen, obliteration of dead space and restoration of normal function. Injury triggers an organized and complex cascade of cellular and biochemical events resulting in a healed wound. The wound healing response can be divided into four separate, but overlapping phases:

1. Homeostasis and inflammation
2. Proliferation
3. Maturation and remodeling
4. Wound contraction

1.2.5.1 Homeostasis and Inflammation

The inflammatory phase is an essential phase of healing, characterized by increased vascular permeability, chemotaxis of cells from the circulation into the wound milieu, local release of cytokines and growth factors and activation of migrating cells. Homeostasis precedes inflammation. The obligatory rupture of vessels that accompanies injury exposes the sub endothelial collagen to platelets, which results in aggregation of platelets and activation of the intrinsic part of the coagulation cascade. The contract between collagen and platelets as well as the presence of thrombombin, fibronectin and their fragments results in the release of cytokines and growth factors from platelet α-granules, such as PDGF, TGF-β, PAF, fibronectin and serotonin (5HT). The locally formed fibrin clot serves as scaffolding for invading cells such as neutrophils, monocytes, fibroblasts and endothelial cells. Inadequate clot formation, such as observed in factor XIII (fibrin
stabilizing factor) deficiency, is associated with impaired wound healing secondary to either decreased adhesion of cells into the inflammatory area or decreased chemotaxis. Within 6 hours of injury, circulating immune cells migrate into the wound.

Polymorphonuclear leukocytes (PMNLs) are the first blood leukocytes to enter the wound site and their numbers increase steadily, peaking at 24-48 hours. Increased vascular permeability caused by inflammation and release of prostaglandin, together with a concentration gradient of chemotactic substances such as compliment factors, interleukin 1 (IL-1), tumor necrosis factor --α (TNF-α), TGF-β, platelet factor 4 and bacterial products stimulate neutrophils migration. The main function of PMNLs is phagocytosis of bacteria and tissue debris.

The next cellular immune elements to enter the wound are the macrophages, which migrate within the wound 48-96 hours after injury, and their number reaches a peak around the third day after injury. Their appearance is followed by that of lymphocytes, which occur in significant numbers around the fifth day after injury, with peak numbers on about the seventh day after injury. In contrast to PMNLs, the presence and activation of both macrophages and lymphocytes in the wound is critical to progression of the normal healing process.

Chemotaxis of cells into the wound milieu are followed by cellular activation, which signifies the phenotypic alteration of cellular, biochemical and functional properties induced by local
mediators. Activation of macrophages has fundamental implications in several aspects of wound healing, such as debridement, matrix synthesis and angiogenesis. Activated macrophages, release cytokines, which mediate angiogenesis and fibroplasias, and synthesize nitric oxide, which has antimicrobial properties and stimulates collagen synthesis. Many other cells, including endothelial cells, fibroblasts, monocytes and lymphocytes, can be activated to produce nitric oxide. Synthesis of NO is reduced during impaired wound healing. The inflammatory phase of healing is vital to the proper evolution of the subsequent phases of healing. Reduced inflammatory responses profoundly affect subsequent healing as observed clinically in patients who have diabetes mellitus or secondary to corticosteroid treatment.

1.2.5.2 Proliferation

The proliferation phase involves a number of events and important cellular elements.

Angiogenesis

Revascularization of the wound proceeds in parallel with fibroplasias. Capillary buds sprout from blood vessels adjacent to the wound and extend into the wound space. On the second day after injury endothelial cells from the side of the venule closest to the wound begin to migrate in response to angiogenic stimuli. These capillary sprouts eventually branch at their tips and join to form capillary loops, through which blood begins to flow. New sprouts then extend from these loops to form capillary plexus.
Angiogenesis occurs by a combination of proliferation and migration. Putative mediators for endothelial cell growth and chemotaxis include cytokines produced by platelets, macrophages and lymphocytes in the wound, low oxygen tension, lactic acid and biogenic amines. Both basic and acidic FGFs, TGF-α, EGF and TGF-β have all been shown to be potent stimuli for new vessel formation. Mesenchymal cells themselves can be induced to release growth factors and cytokines in an autocrine manner, so continuing the angiogenic process. Two growth factors that are particularly needed for new blood vessels are vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF). While VEGF initiates vessel growth, the PDGF promotes maturation by giving the vessel a strong interior lining. Besides, the continued presence of VEGF also prevents vessels from regressing as they mature.

**Fibroplasia**

Fibroplasia is a process of fibroblast proliferation and matrix synthetic activity. Fibroblasts in the surrounding tissue need to become activated from their quiescent state in which they are non-replicative. Growth factors such as PDGF or EGF induce chemotaxis and proliferation of fibroblasts.

Fibroblasts are attracted to the wound and induced to proliferate by cytokines released initially from platelets and subsequently from macrophages and lymphocytes. Fibroblasts are the primary synthetic element in the repair process and are responsible for producing the majority of structural proteins.
necessary for tissue reconstruction. The main protein product of fibroblasts is collagen, a family of triple-chain glycoprotein that form the main constituent of the extracellular wound matrix; these are ultimately responsible for imparting tensile strength to the scar. Collagen is first detected in the wound around the third day after injury. The levels then increase rapidly for approximately 3 weeks, it continues to accumulate at a more gradual pace for up to 3 months after wounding. Fibroblasts are also responsible for the production of other matrix constituents, including fibronectin, hyaluronic acid and the glycosaminoglycan.

**Epithelization**

Re-epithelization of the wound begins with in a few hours of injury. Epithelial cells arising from either the wound margins or residual dermal epithelial appendages with in the wound bed begin to migrate under the scab and over the underlying viable connective tissue. The epidermis immediately adjacent to the wound edge begins thickening within 24 hours after injury. Fixed basal cells in a zone near the cut edge undergo a series of rapid mitotic divisions and migrate by moving over one another in a leapfrog fashion until the defect is covered. Re-epithelization is complete in less than 48 hours in approximated incised wounds, but may take substantially longer in larger wounds in which there is a significant tissue defect. Re-epithelization process is mediated by a combination of loss of contact inhibition, exposure of fibronectin and cytokines produced
by macrophages and lymphocytes. In particular EGF, TGF-β, basic FGF, PDGF, IGF-1 have been shown to promote epithelization.

1.2.5.3 Maturation and Remodeling

Matrix deposition in the wound

Initially, the wound matrix is mainly composed of fibrin and fibronectin that originate from homeostasis and macrophages; another early expressed protein is thrombospondin-1, which also supports cellular recruitment in wound milieu. Glycosaminoglycans, proteoglycans and other proteins such as “Secreted Protein Acidic Rich in Cysteine” (SPARC) are synthesized next and support future matrix deposition and remodeling.

The intact dermis is predominantly composed of collagen I (80-90 %). In granulation tissue the proportion of type III collagen is increased (30 %), whereas in the mature scar the proportions of type III constitute the early matrix, collagen type I accumulates later and corresponds to the increase in wound breaking strength. The normal dermis shows a pattern like basket weave, while in a scar the thinner collagen fibers are arranged parallel to the skin. These thinner collagen fibers gradually thicken after wounding and are accompanied by increased scar tensile strength indicating a positive correlation between fiber thickness and orientation with tensile strength. Maturation or remodeling can be defined as the process by which the fragile soluble fibrils of collagen change into strong, insoluble fibers. By this process the unorganized polypeptide gets organized into a proper form which provides
mechanical strength to the tissue. During the early process of repair, the total amount of collagen increases and reaches a maximum between 2 to 3 weeks of injury.

Tensile strength, a functional assessment of collagen increases to 40% one month after injury and may continue to increase for as a year after injury.

However, even at its maximum, the tensile strength is never greater than 80% of its pre-injury strength. Despite a prolonged ongoing remodeling phase (up to 1 year), the orientation of collagen fibers in the healed scar tissue do not become as organized as in the intact dermis.

**Collagen metabolism**

Collagen the most abundant protein in the body plays a critical role in the successful completion of wound healing. Its deposition, maturation and subsequent remodeling are critical to the functional integrity of the wound.

The main types of collagen, which are involved in the wound repair are types I and III. Type I collagen is the major component of extracellular matrix in the skin. Type III which is also normally present in skin, becomes a more prominent component during the repair process. Bio-chemically, each chain of collagen is composed of glycine residue in every third position. The second position in the triplet is made up of proline or lysine during the translation process. The polypeptide chain that is translated from mRNA is called protocollagen. Release of protocollagen into the endoplasmic
reticulum results in the hydroxylation of proline into hydroxyproline and of lysine into hydroxylysine by specific hydroxylases. In the endoplasmic reticulum, the protocollagen chain is glycosylated, which alter the hydrogen bonding forces within the chain, so that the protocollagen chain assumes a α-helical chain to form a right handed super helical structure called procollagen.

Collagen biosynthesis and its post translational modification are highly complex biochemical events. The resultant collagen monomer is further polymerized and cross-linked by the formation of intra-and intermolecular covalent bonds. Collagen breakdown during healing begins early and is very active during inflammation. Collagens are almost exclusively digested extracellular by specific collagenase. These specific enzymes are able to degrade the normally very stable triple helical structure of the collagen at specific sites, rendering the molecule more susceptible for degradation by other proteases. The activity of collagenases is tightly controlled by cytokines (e.g. TGF-β1), which act not only by inducing new gene transcription, but also by decreasing collagenase activity.

1.2.5.4 Wound contraction

Wound contraction is the approximation of the wound edges together, whereas wound contracture is the shortening of the scar itself. Rapid contraction of wound occurs after a lag phase of 1st to 4th day and 14th day, the wound size reduces by 40-80 %. Wound
contraction is found to be independent of new tissue formation, epithelization and collagen synthesis.

Healing by primary or secondary intention determines the role of wound in the healing process. It has been postulated that a special cell, the myofibroblasts, is responsible for contraction, whereas another theory suggests that the locomotion of all fibroblasts lead to reorganization of the matrix and contraction. Typically, the myofibroblasts expresses α-smooth muscle actin in thick bundles called stress fibers, which allow it to contract. The α-smooth muscle actin is non-detectable until day 6 and is then progressively expressed for the next 15 days of wound healing. After 4 weeks, this expression fades and the cell is believed to undergo apoptosis. Fibroblasts placed in a collagen lattice actively move in the lattice and can contract it without expressing stress fibers. More recently, the contraction function has been shown to be preserved in the absence of the myofibroblasts.

1.2.6 Factors influencing wound healing

In many ways, the process of wound healing seems to be physiologically privileged. Repair proceeds, though slowly, even during illness and in the face of advanced life. The factors that are critical in the control of wound healing may be broadly classified as follows

General factors

a) Local factors,

b) Systemic factors
Specific factors
a) Cytokines and growth factors
b) Endocrine factors (hormone)
c) Autocoids

Drugs
a) Anti-inflammatory agents
b) Anti neoplastic agents /cytotoxic drugs
c) Corticosteroids
d) Retinoids
e) Vanadate.

a) Local factors

i) Infection

Wound infection is an imbalance between host resistance and bacterial growth. Bacterial infection impairs healing through several mechanisms. At the wound site, acute and chronic inflammatory infiltrates slow fibroblasts proliferation and thus slow ECM synthesis and deposition. Bacterial contamination results in clinical infection and delays healing if more than 10^5 organisms per gram of tissue are present in the wound.

ii) Adequacy of blood supply

Wounds with inadequate blood supply show slow, prolonged or impaired healing, e.g. patient with varicose veins, healing is prolonged. Ischemia due to pressure causes bed sores and delays the healing. Ischemia in diabetics also impairs healing.
iii) Location, type and size of the wound

Injuries in highly vascular areas (e.g., the face) heal faster than those in poorly vascular areas (e.g., the foot). Moist wounds heal faster than dry ones and a clean aseptic wound made by a scalpel heals faster than a blunt trauma wound. Small blunt wounds heal better than larger ones. Facial incisions regain breaking strength faster than simultaneous dermal incisions.

The mechanism for this appears to involve increased facial fibroplasia and collagen production after acute injury.

iv) Movement

Movement causes mechanical stress to the wound and slows healing process.

Granulation tissue is disrupted easily by movement. Early motion, particularly prior to the establishment of the tensile strength, renders the wound to resist trauma thus retarding healing.

v) Ionizing radiation

Exposure to ionizing radiation is deleterious to wound healing. It blocks cell proliferation stimulates contraction and retards the formation of granulation tissue.

vi) Exposure to UV radiation

In wounds exposed to UV radiation, the rate of healing accelerates.

vii) Local heat

Wounds have been reported to heal more quickly at temperature of 300° C than at normal room temperature. This is
because local heat increases blood flow and oxygen tension in wounds.

b) Systemic factors

i) Age

Aging is generally associated with poor qualities of healing because of vascular insufficiency, malnutrition and vitamin deficiency. But, some experimental animal studies have shown that there is no evidence of impaired healing in older animals although there is a general impression to the contrary.

ii) Immune status

In wound healing process, the inflammatory response is very vital. It has been shown that the absence of polynucleur leukocytes during the inflammatory phase can however; predispose to wound infection, which retards healing. Leucopenia at the time of wounding has no effect on wound debridement, fibroblasts proliferation or connective tissue formation.

iii) Oxygenation

Wounds require adequate oxygen delivery to heal. Experimentally, collagen synthesis by fibroblasts is increased with supplemental oxygen.

iv) Nutrition

Wound healing is an anabolic event that requires additional caloric intake.

Several malnourished and catabolic patients clinically appear to have diminished healing; however, no studies have definitely
proved this finding. In vitamin C deficient patients, wound healing is arrested during fibroplasias, since it is necessary for hydroxylation of proline and lysine residues. Without hydroxyproline newly synthesized collagen is not transported out of cells. Without hydroxyllysine, collagen fibrils are not cross-linked. Vitamin A is involved in multiple facets of repair; fibroplasias, collagen synthesis and cross-linking and epithelialization processes.

Animal studies show that vitamin A also reverses the impaired healing that occurs with chouronic steroid treatment. Vitamin B₆ deficiency impairs collagen cross-linking. Vitamin B₁ and vitamin B₂ deficiencies cause syndromes associated with poor wound repair. Vitamin E, antioxidant inhibits or prevents the spread of peroxidation through lipids. It has negative role and it interferes with collagen synthesis and wound repair. Trace metal deficiencies such as zinc and copper have been implicated with poor wound repair because these divalent cations are cofactors in many important enzymatic reactions. Zinc deficiency is associated with poor epithelization and chouronic, non-healing wounds.

V) Metabolic status

It has a profound bearing on healing process. Wounds in diabetics often become infected and in turn make the control of diabetes difficult. Healing is enhanced if glucose levels are well controlled. Obesity interferes with repair independently of diabetes.
c) Specific factors

1. Cytokines and Growth Factors

Cytokines

Cytokines are small, polypeptide protein hormones that are secreted by various cell lines in the body, but predominantly by immune cells. Cytokines are important mediators of host defense and post-injury repair responses. In addition, many cytokines may act as regulators of cell growth and maturation.

Tumor Necrosis Factor –α

TNF-α is released primarily by the macrophage-monocytes lineage of cells and is involved in the recruitment and maturation of the cellular component of inflammation. A variety of stimuli, including parasites, tumors and endotoxins, can stimulate cells to produce TNF-α which is detectable locally within 12 hours after experimental wounding and its level peaks after 72 hours. Recombinant TNF-α applied locally to experimental wounds increased both wound disruption strength and collagen synthesis in normal and doxorubicin-impaired. Additionally, TNF-α has also been implicated in contributing to the poor wound healing seen in septic and chronic disease states.

Interleukin-1: IL-1 is produced primarily by cells of the monocytes-macrophage lineage of cells but also by keratinocytes in active wounds. IL-1 levels become detectable within the first 24 hours of experimental wounding, peak between the first and third days, and then rapidly decline throughout the first week. IL-1 has
been shown to increase fibroblast and keratinocyte growth as well as collagen synthesis.

**Interleukin-2**: IL-2 is an important cytokine produced by T-lymphocytes and is involved in T-cell activation. IL-2 has been shown to increase fibroblast metabolism in *in-vitro* models

**Interleukin –6**: Within wounds Polymorphonuclear cells (PMNs) and fibroblasts secrete IL-6. IL-6 is detectable within 12 hours of experimental wounding and may persist at high concentrations for longer than a week. IL-6 production is diminished in fetal wounds; whereas the exogenous administration of IL-6 to these wounds has been shown to lead to scarring.

**Interleukin –8**: IL-8 is primarily by macrophages but also by fibroblasts in the acute wound. Recent experiment have demonstrated that low energy cutaneous laser irradiation increases local IL-8 levels in a dose-dependent fashion and suggests that the laser’s beneficial effect on enhanced wound healing may be partially mediated through IL-8 production

**Interferon – γ**: IFN-γ is an important cytokine produced primarily by T Lymphocytes and macrophages and it plays an important role in tissue remodeling of wounds. In addition, IFN-γ has been shown to locally reduce wound contraction.

**Interleukin-4** is a potent cytokine produced by T cells, promotes fibroblasts proliferation and collagen synthesis. IL-10 in the acute wound that seems to be an important counter-regulatory cytokine.
1. Growth factors

Growth factors are cytokines whose primary function is directing the maturation of cells during the normal turnover and in the past injury tissue repair response. Over the past two decades much research has been conducted in characterizing the role and potential treatment applications of individual growth factors in impaired wound healing states.

**Platelet-derived growth factor:** PDGF is essential in initiating and sustaining the wound healing response. PDGF release platelet alpha granules soon after injury. Its immediate effects include the recruitment and activation of immune cells and fibroblasts. Thereafter, PDGF is also secreted by macrophages and stimulates collagen and proteoglycans synthesis. The PDGF-BB isomer is the most widely clinically studied. PDGF-BB has improved wound closure in chronic and diabetic non-healing ulcers and did not improve wound healing or contraction in steroid-impaired animals.

**Transforming growth factor-β:** Platelets, macrophages and fibroblasts within wounds release TGF-β. TGF-β exists as at least 3 isomers-TGF-β1, β2 and β3. All 3 isomers induce extracellular matrix. TGF-β1 and TGF-β2 promote extracellular matrix and cutaneous wound scarring, while TGF-β3 may in fact prevent scarring.

**Fibroblast growth factor:** FGF family of proteins is an important mediator of wound angiogenesis and epithelialization. FGF exists largely as two forms, an acidic and a basic isomer. Both isomers are
released by macrophages and endothelial cells within wounds and stimulate fibroblast and keratinocyte proliferation and migration. Basic FGF plays an important role in preventing wound contraction and in collagen remodeling.

**Keratinocytic growth factor:** KGF is a member of the FGF family of polypeptides. Two forms KGF-1 and KGF-2, have been identified. Both KGF isomers are important regulators of keratinocyte proliferation and maturation. The profound mitogenic effect of KGF on epithelization makes it particularly attractive in modulating wound healing.

**Epidermal growth factor:** EGF is secreted by keratinocytes and directs epithelization in an autocrine fashion. In addition, EGF stimulates fibroblast collagenase secretion and thus may be important in wound remodeling. Recent evidence suggests that aged dermal fibroblasts have decreased EGF-receptor expression and may contribute to the impaired healing seen during aging.

**Vascular endothelial growth factor:** VEGF is released primarily by keratinocytes but also by macrophages and fibroblasts. VEGF levels rise steadily after wounding and serve as a potent angiogenic factor. VEGF administration improved granulation-tissue formation in both normal and hypoxic tissues during experimental wounding.

**Insulin−like growth factor**

IGF also known as somatomedian-exists as two isomers, IGF-1 and IGF-2, which help regulated cell metabolism and growth. The primary effects of IGF include stimulating fibroblast and
keratinocyte proliferation, as well as collagen synthesis. The exogenous administration of TGF-1 improved wound healing in both diabetic and steroid-impaired subjects.

2. **Endocrine hormones**: These are hormones secreted as a consequence of injury. ACTH, cortisone and other glucocorticoids can have profound effects on wound healing. Cortisone and its derivatives can decrease the rate of protein reactions. Deficiency of insulin retards wound healing. Anabolic steroid e.g Nandrolene have minimal effect when used alone, but reverses tensile strength lowering the effect of corticosteroids.

3. **Autocoids**: Histamine is a known mediator of inflammation. Histamine accelerates the collagen formed in healing wounds. Histamine depletion retards wound healing and decreases tensile strength. Bradykinins and catecholamine also stimulate fibroblast growth. Prostaglandins, especially PGE1 has been found to increase adenylcyclase activity in T-lymphocytes finally resulting in stimulation of mitosis and post-injury cell proliferation.

1.2.7 **Wound repair monitoring and models**

Based on the pattern of healing we can draw some parameters such as wound contraction epithelization, granulation, wound strength and scar changes. Monitoring any one of the parameters can assess the process of wound healing. Physical, mechanical, biochemical, and histological features could do the monitoring of all these parameters. It is essential to employ more than one type of wound model to study all the parameters of wound healing.
**Physical attributes:** Wound has got physical attributes like size, shape, raw surface and scar changes. These can be studied in open wounds by measuring the total area, raw wound area and noting the physical changes like size, shape, color etc. By using simple techniques we can assess physically the wound contraction, period of epithelisation, scar size and shape and gross weight of the granulation tissue. Planimetric measurement of wound area differentiates the process of contraction from epithelialization. The wound margin is both stained with tattoo marks or a few scattered stitches are put around the margin during the operation and the area within the margin is estimated to assess the contraction. The same tracing taken on a graph paper of standard division gives wound area. Period of epithelialization can be noted when the wound completely gets covered with epithelial cells. The changes in the scar can easily be observed by repeated wound tracing. However, to observe changes in the collagen, electron microscopic studies are necessary.

Quantification of granulation tissue is one of the best methods to the healing process, this can be done by weighing the dry granuloma tissue formed around the foreign body (cotton pellet) placed in the dead space wounds. The tensile strength of this tissue also can be measured which gives additional information about collagen maturation.
Mechanical attributes

These mechanical attributes of the healing wound are tensile or breaking strength. Tensile strength is measured in terms of load applied per unit of cross-sectional area and expressed as pounds per square inch or kilogram per sq.cm or per sq.mm. The tensile strength of the wound can be estimated noting the load applied per unit area to disrupt the incised re-sutured wounds. Breaking strength is the force required to break the wound or tissue with no reference to its dimensions. The uniform application of force can be achieved by tensiometer, a specially designed instrument or by continuous and constant water flow technique, to one end of the healed wound and at a constant rate so that disruption of the wound occurs and the load required disrupting the wound is determined. Breaking strength varies from area to area, but the tensile strength remains constant. This difference is due to the variation in the thickness of the tissue.

In the dead space wound model, the granulation tissue is used to determine the tensile strength, which depends on the collagen maturation of the healed wound.

1.2.8 Drugs

Anti-inflammatory agents

NSAID’s whose principle effect is to diminish granulocytic inflammatory reaction would be expected to inhibit wound healing. Healing suppressing effects of ibuprofen and mefenamic acid were also reported. It was also reported that aspirin and phenylbutazone
decreases the skin tensile strength in rats. There are reports, which has shown that when zinc was complexed with NSAID’s, it abolished the wound healing suppressant activity of NSAID’s without compromising their analgesic or anti-inflammatory effects. Further the copper complexes of ibuprofen and mefenamic acid were known to have pro-healing actions.

**Anti-neoplastic agents**

All anti-cancer agents are anti-proliferative and affect the healing process. Cytotoxic drugs delay gain in wound tensile strength in wounded animals. Mechlorethamine hydrochloride decreases the tensile strength of wounds in rats. Cyclophosphamide is known to interfere with wound contraction, epithelization, tensile strength and granulation tissue. In the presence of 5-Flurouracil, the collagen synthesis is decreased but this effect is diminished by delaying the drug administration for 3 days. 5-Flurouracil has been shown to produce a reversible delay of fibroblast growth in sub conjuctival and scleral tissues.

**Corticosteroids**

They impair wound healing mainly by inhibition of collagen synthesis, granulation and mucopolysaccharide synthesis. Steroids inhibit wound healing when applied topically. Rats administered with cortisone have a 30 % diminution of dorsal skin wound strength at 7 days compared with control rats.
Retinoid

Retinoic acid therapy stimulates corneal endothelial wound healing, possibly by potentiating the effects of endogenous growth factor.

1.2.9 Wound models:

Incision Wounds:

This is a simple and more convenient wound model. In this model, the parameter assessed is the gain in tensile strength. A suitable length of full thickness skin wounds on the back of the animal has been described for the purpose of studying the influence of drugs on the strength of the wound. These wounds are to be closed with a suitable suture material and it is better to remove the suture 2-3 days prior to the tensile strength measurement. Generally tensiometer or constant water flow technique is used to measure the tensile strength. This model can also be used to study other parameters like collagen content and scar remodeling. Animals used in this model are albino rats and mice.

Dead space wound (Granulation studies)

Healthy granulation formed around the foreign body placed in the dead space wound subcutaneously can be obtained in this model. In the past cotton pellets were used as foreign bodies. But now a days, plastic (Polypropylene) tubes of suitable size and shape are used to harvest granulation tissues. This granulation tissue can be used for histological studies as well as for the estimation of total
UHP (Urinary Hydroxy Proline) of the collagen content. Rats are used in this model.

Excision wound:

In this model, excision wounds of different shape and size at different sites have been employed. 2x2 Sq.cm. full thickness excision skin wounds made on the depilated back of rat. Commonly three main parameters are assessed by this model viz., contraction, epithelisation and scar remodeling. The other parameters like collagen estimation, wound histology etc can also be studied by this model.

1.2.10 Herbal CAM for the treatment of Wound healing

Due to the side effects and the high cost of conventionally used wound healing agents, the patients are increasingly using Complementary and Alternative Medicine (CAM) modalities of treatment a system originated in India. Many Ayurvedic herbal plants have a very important role in the process of wound healing since they promote the repair mechanisms in natural way and the process may be monitored physically through the rate of contraction of wound.

Natural herbal remedies can effectively heal the wounds and offer alternative to synthetic wound healing agents. Studies show that plant drugs were used by traditional practitioners to treat the wound. Nevertheless on the basis of the results obtained from animal models of wounds, it is found that herbs like Rubia cordifolia Linn, Ocimum kilimandscharicum, Tephrosia purpurea Linn, Aloe vera
Linn., *Carica papaya* Linn., *Allium cepa* Linn., *Gymnema sylvestre* R.Br., *Adhatoda vasica* Linn., *Lawsonia inermis* Linn., *Moringa oleifera* Linn., *Curcuma longa* Linn., *Ocimum sanctum* Linn. and *Tridax procumbens* Linn. etc. had marked wound healing property. Similarly the plant chosen for the research was *Urena lobata* which was used traditionally by the practitioners and not explored scientifically. Early appearance and higher accumulation of mucopolysaccharides, steroidal glycosides has been stated as indicators of hastened repair as compared to that of others.
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