Review of literature

WOUND HEALING PROCESS

Arey (1936) reported two types of wound healing in skin, primary and secondary repair. In primary repair wounds may heal by (a) simple epithelial regeneration without involvement of other tissues. (b) by direct union of parts and thus permit uncomplicated regeneration of epithelium and connective tissue. (c) Healing under a scab (consisting of exudates fluid, tissue fragments, etc.) which provisionally closes the wound. According to him the secondary repair occurs by replacement of degenerated tissue by connective tissue and cicatrisation is formed by binding up of granulation tissue.

Whipple (1943) described two periods in healing of wounds, a latent or lag period and an active period of fibroplasia. According to him the period of fibroplasia involved three processes viz. amoeboid movement, mitotic proliferation and maturation of cells engaged in fibroplasia and fusion of wound surface.

Forbes (1952) stated that primary healing occurs rapidly requiring minimal tissue, while secondary healing requires the formation of much fibrous tissue and formation of granulation tissue thus takes much time.
Clark (1993) discussed biology of wound repair and emphasized that the events involved in wound repair can be temporarily, albeit arbitrarily, grouped into inflammation, granulation tissue formation and tissue remodeling. He reviewed the chronology of dermal wound repair, i.e., inflammation, granulation tissue formation, and tissue remodeling.

Krisner and Eaglstein (1993) described the wound healing process, both the basic science and the clinical aspect of wound healing and described the difference between primary and secondary intention healing in both. They also discussed the systemic approach in dealing with patients with chronic wounds and presented their problem-oriented treatment programme based on the cause of chronic wound.

Waldrof and Fewkes (1995) discussed wound healing in detail and said it to be a dynamic biological process of repairing insult to integumentry system. They also stressed that there are also extrinsic factors that can be influenced by caretakers of the wound to enhance wound healing. They also voiced that modern science and technology are giving us new insights into wound healing and leading us to exciting new ways of influencing it, including the
topical use of growth factors, artificial skins, cultured epithelium with and without dermal components and electric stimulus.

Boriker et al. (1999) described three phases in the process of wound healing. First is the phase of inflammation marked with haemorrhage and cleaning of wounds, followed by phase of debridement where break down of necrotic material under the influence of enzymes occurs and then the phase of repair where there is fibroplasia, angiogenesis and epithelialization.

**COW URINE - A HEALING AGENT**

It is well documented in religious and scientific literatures that the cow urine consists of various useful metallic ions, minerals, enzymes, vitamins favouring the wound healing viz. Fe, Ca, Cu, Mn, Ag, I, Na, N, Pb, Au, NH₃, S, steroids, glucose, citric acid, acetate, carbolic acid, and vitamins viz. A, B, C, D and E along with waste nitrogenous products (Bapu, 2001). Sushruta the founder of Indian surgery has mentioned in his famous text “Sushruta Samhita” wound as vranah and urine for the treatment of wounds. The Aryans were well acquainted with the qualities and properties of cow’s urine in art of healing.

Matis and Wundt (1951) reported that vitamin C treated wound heals more quickly than penicillin.
O Dell et al. (1961) reported the role of copper in the formation of elastin in the process of healing.

Miller and Fullemr (1966) reported the role of copper in the metabolism of connective tissue and deficiency of this element resulted into defective formation of elastin, which frequently caused rupture of aneurysm in swine and rats, due to improper weaving of the collagenous framework.

Runnels et al. (1976) stated that in the deficiency of vitamin C, wound healing is impaired because of poor maturation of fibroblasts leading to lack of collagen fibres synthesis and poor sprouting activity of angioblasts to form new capillaries.

Spais et al. (1978) suggested that the iron contents in the area of wound were increased significantly in animals. The deficiency of certain vitamins either due to poor assimilation or excess excretion in the urine adversely affects the wound healing.

Jadon (1983) observed variations in copper content in the wounds that were treated with cartilage powder, amnion and tissue extract. No definite trend in copper content in various groups could be recorded; however, the concentration of this metallic ion was higher in treated wounds as compared to control.

Yurdzh (1988) reported that the copper contents of the wounds were increased during inflammatory condition in cattle.
because copper played a significant role in the process of wound healing. He further observed that during granulation tissue formation the concentration of iron was relatively higher in brown cattles.

Al Sadi (1976) observed that the post administration of vitamin A resulted into increased fibroblastic proliferation and formation of collagen in wound healing.

Banergee (1998) stated that vitamin C deficiency invariably causes delay in wound healing.

Katiyar (1999) reported that the copper helped in the maintenance of proper frame work of elastin and collagen.

Vegad (2000) quoted that deficiency of Vitamin C caused poor and delayed wound healing due to little conversion of proline to hydroxyproline and lysine to hydroxyllysine. Thus, deficiency resulted into impaired synthesis of normal collagen. Absence of hydroxyproline would result in failure to achieve fibril formation (fibrillogenesis) consequently little production of fibroblasts.

Sastry (2001) described that vitamin C deficiency resulted into poor sprouting of capillaries at the site of wound healing.

Gupta (2003) observed the systemic effect of cow urine along with its topical application on surgical wound in rats. Healing in the urine treated wounds was better as assessed
by macroscopical and histomorphochemical examination. The urine treated rats did not require any supportive therapy and showed early development of granulation tissue and regeneration of epidermis with fibroplasias. The presence of vertically arranged fibroblast with production of thin and better quality of collagen fibers and formation of thin band of connective tissue without apparent scar mark were characteristic features in the rats of urine treated group. However, the rats of control group revealed fibroplasias with horizontally arranged fibroblasts, presence of thick and coarse collagen fibers and formation of wide band of connective tissue with apparent scar mark in different stages of surgical wound healing.

Garg et al. (2004) reported the beneficial effects of cow urine on serum bio-chemical profile (total serum protein, glucose, calcium, and cholesterol) of laying birds. Its usefulness as antimicrobial agent, positive effect on body weight gain, hematological profile, immunomodulatory effects on both humoral and cellular immune responses, and healing of surgical wounds in experimental rats was studied.

Maheshwari et al. (2004) observed that the cow urine is having antiseptic properties in wound healing and that the healing
time is somewhat less in comparison to wounds in which antiseptic cream was applied. Administration of fresh cow urine orally showed added effect on wound healing by virtue of its antiseptic and immunologically modifying properties in dogs, the healing process was observed a bit faster than the group of dogs which were not given cow urine orally.

Yadav (2005) reported that the cow urine has antiseptic properties and healing time of wounds is somewhat less in comparison to wounds on which antiseptic cream applied. He also reported that administration of fresh cow urine orally has added effect on wound healing.

**ANTIMICROBIAL ACTIVITY OF COW’S URINE**

Pandya (2000) reported that cow urine contains carbolic acid and manganese, which produce effective antibacterial and pesticidal activity. Auram oxide (AUCH) of cow urine also has antibacterial and antitoxic properties. It also possesses antacid and blood purifier properties due to the presence of sodium and iron.

Bapu (2001) documented the effective antiseptic and antibacterial properties of cow’s urine along with other beneficial effects due to regular drinking. It was also postulated that the urine produces beneficial systemic effects due to increased functional activities of pancreas, intestine, kidneys and other visceral organs.
Cow’s urine also produces antihelminthic properties especially for children who developed pot-bellied condition due to severe parasitic infestation. In such cases drinking of fresh cow’s urine @ 10-25 ml in children and 40 ml in adults per day were found to be very effective.

Sharma (2001) stated that cow’s urine possesses excellent bactericidal activity due to presence of various organic and inorganic materials.

Panicker (2002) reported the presence of various chemicals in the distillate portion of cow’s urine (popularly known as ark) enhancing the activity of antibacterial and anticancerous drugs like rifampicin and taxol commonly used to treat tuberculosis and cancer in humans.

Achliya et al. (2004) exhibited antibacterial and antifungal activity of cow’s urine at different concentration and concluded that fractions of cow’s urine obtained by solvent extraction possess antimicrobial activity.
IMMUNOMODULATORY EFFECT OF COW’S URINE

Bapu (2001) reported that cow urine therapy increases the resistance against microbes of different diseases by increasing the number of leucocytes in blood.

Chauhan et al. (2001) studied the immunomodulatory effect of Kamdhenu ark (preparation of urine of cow) in mice by oral administration @ 1 ml/day in 10 mice along with water for 90 days. Blood was collected directly from heart at monthly interval and serum was separated for the study of immunoglobulin status. ELISA was carried out to estimate serum Ig G, IgM, and IgA level. Lymphoid cells were collected from the spleen of the mice and lymphocyte stimulation test was performed using mitogens (Con-A) for T-lymphocytes and lypopolysaccharides for B-lymphocytes (Chauhan, 1998). Mean O.D. was calculated for T and B-lymphocytes and results were compared for significance using ‘T’ test for humoral and cell mediated immune response. It was found that the humoral and cell mediated immune responses were significantly elevated as compared to control.

Kumar et al. (2002) compared the immunostimulatory effect of Kamdhenu Ark and Vasant Kusumakar in mice. Kamdhenu Ark
was found to have significantly higher immunostimulatory effect in comparison to Vasant Kusumakar. Increase in humoral immunity in mice was 3 times higher due to Kamdhenu Ark in comparison to Vasant Kusumakar. Similarly cell mediated immunity was increased by both but increase due to Kamdhenu Ark was 4 times more in comparison to Vasant Kusumakar.

Chauhan (2003) reported that according to old literatures cow’s urine possesses potent immunomodulating effect without having any side effect. The author further announced that humoral and cell mediated immunity were significantly increased up to 99.5% and 64.0%, respectively by the administration of cow’s urine due to increase in the phagocytic index of various cells to 104 % as a result of enhanced release of cytokines by lymphocyte and phagocytic cells.

Kumar (2004) reported that the cow urine exerts protective effect on lymphocytes of birds undergoing apoptosis and suggested the exploitation through trails for specific use of cow urine as an adjunct to vaccination. It enhances the activity of macrophages and reduces apoptosis in lymphocytes, thus is helpful in prevention and control of bacterial infection.
THERAPEUTIC USES OF COW’S URINE AND ITS VARIOUS PREPARATIONS

Goswami (1995) stated that the cow’s urine produces beneficial effect on hepatitis, constipation, piles, ascites, gastritis, obesity, dermatitis, ringworm, common cold, dropsy, ascariasis, arthritis, heart attack, increased cough, hyperchloestremia, headache etc.

Sharma (2001) reported that daily drinking of 20 ml cow’s urine cures anorexia, gastritis, hernia, hysteria, piles, diabetes, constipation, enteric diseases, jaundice, blood pressure, eye problems, common cold, fever, skin diseases, insomnia etc.

Dhingra and Mittal (2003) successfully cured the various acute and chronic diseases of human by the use of preparations of cow’s urine, which had failed to respond the allopathic treatment.

Kumar et al. (2004) reported the prevention of pathogenic effect of free radicals through cow urine therapy. These radicals cause damage to various tissues and attack enzyme, fat and proteins disrupting normal cell activities or cell membranes, producing a chain reaction of destructions leading to the aging process of persons. By regular use of cow urine one can get a charm of a youth as it prevents the free radicals formation.
Kumar et al. (2005) stated that cow urine can cure anything from skin diseases, kidney and liver ailments to obesity and heart ailments. He reported that U.S patent was granted to Indian scientist on the use of cow urine distillate as bioenhancer.

**BIOCHEMICAL CONSTITUENTS**

**TOTAL PROTEIN**

Stojic (1982) estimated physiological presence of total protein in 10 normal healthy bovine urine samples and found 1.01 g protein in 10.97 liters of urine, which was collected at 6 hours intervals. The mean value of protein in the different samples of urine was 0.064 g/l.

Wenga et al. (1982) studied the total loss of protein in the urine of 29 dogs, having no signs of renal diseases and reported that daily loss of protein in the healthy urine was less than 10 mg/kg body weight in 24 hours.

Center et al. (1985) reported the excretion rate of protein in the urine of 19 healthy dogs ranging from 0.2- 7.7 mg/kg with the mean value of 2.5 mg/kg/day.

Kumar (2001) recorded the concentration of total protein in urine of healthy cows, buffaloes and goats as 0.1037 g/l, 0.1045g/l and 0.1011 g/l, respectively at an organized farm.
**UREA**

Gowenlock (1988) reported concentration of urea in human urine samples as 330 mmol/l, with total daily excretion of 550 mmol.

Kumar (2001) reported urea concentration in the urine of healthy cows, buffaloes and goats, which were 5.5418 mmol/l, 5.2990 mmol/l and 66.8477 mmol/l, respectively.

**CREATININE**

Singh et al. (1983) reported normal value of creatinine in urine of bovine was 4.07 mg/100 ml.

Kaneko et al. (1997) documented that the presence of creatinine in the urine of normal cows and goats were 15-20 mg/kg/day and 10 mg/kg/day, respectively.

Kumar (2001) reported creatinine values in the urine of healthy cows, buffaloes and goats, and found the creatinine level as 0.997 g/l, 0.885 g/l and 0.985 g/l, respectively.

**URIC ACID**

Gowenlock (1988) compared the uric acid values in the urine of two groups of humans fed on nucleoprotein free diet and nucleoprotein containing diet and observed that the uric acid values
ranged from 1.8 to 3.0 mmol/day and 3.5 to 4.2 mmol/day, respectively.

Kaneko et al. (1997) documented that the presence of uric acid in the urine of apparently healthy cows and goats, ranges from 1-2 and 2-5 mg/kg/day, respectively.

Kumar (2001) recorded the uric acid values in the urine of healthy cows, buffaloes and goats, as 135.028 mg/l, 127.690 mg/l and 60.356 mg/l, respectively.

**LACTATE**

Hawk et al. (1965) described 100-300 mg daily excretion of lactate in the urine of healthy human.

Kumar (2001) reported lactate values in the urine of healthy cows, buffaloes and goats, as 3.783 mmol/l, 3.231 mmol/l and 6.907 mmol/l, respectively.

**PHENOLS**

Hawk et al. (1965) described the presence of free volatile phenol and conjugated volatile phenol in the urine of human, which ranged from 0.2 to 0.4 mg/day and 20-70 mg/day, respectively.
Martin et al. (1983) reported the origin of simple phenols in the urine due to ruminal fermentation of phenolic compounds present in the diet.

Kumar (2001) observed the concentration of free volatile phenol in the urine of cows, buffaloes and goats, were 0.713 mg/dl, 0.543 mg/dl and 0.622 mg/dl, respectively.

**VITAMINS:**

Gowenlock (1988) reported the presence of several vitamins in the urine of human beings and are excreted in the urine @ 20-30 mg ascorbate, 50-500 µg thiamin and 0.5 to 0.8 mg riboflavin, per day.

Kumar (2001) reported the presence of ascorbate, riboflavin and thiamin concentration in the urine of cows, buffaloes and goats, as 216.408 mg/l, 174.845 mg/l and 11.464 mg/l, 0.6339 mg/l, 0.4959 mg/l, and 0.503 mg/l and 444.125 µg/l, 163.764 µg/l and 2831.505 µg/l, respectively.
ENZYMES:

Hawk et al. (1965) described the excretion rate of acid phosphatase (ACP) activity as 80 – 300 KA unit/ day in the urine of healthy human.

Habibabadi et al. (1996) analyzed the activity of lactate dehydrogenase (LDH) and alkaline phosphatase (AP) in the urine of 50 healthy female cross bred Holstein cattle in the age group of 6 M, 6-18 M, 19- 60M and > 60 M and observed that the value of enzymes were 5.943, 5.55, 5.80 and 5.61 IU/l and 1.50, 1.51, 1.48 and 1.47IU/l, respectively.

Kumar (2001) investigated the presence of various enzymes viz. lactate dehydroginase, alkaline phosphatase, acid phosphatase and amylase in the urine of cows, buffaloes and goats, and recorded the values as 21.780IU/l, 23.759 IU/l and 34.120 IU/l, 110.11, 141.23 and 182.72, 456.62, 262.22 and 202.18 KA units and 90.236, 56.722 and 46.760 street close units, respectively.

MINERALS

Singh et al. (1983) estimated the level of calcium and inorganic phosphorus in the urine of healthy bovine as 10.54 ±3.07 mg/dl and 0.86 ±0.13 mg/dl, respectively.
Gowenlock (1988) reported that the regular excretion of phosphorus in the normal urine of human being was 32 mmol/l.

Ivanov et al. (1990) determined calcium in 9 healthy cows at 2\textsuperscript{nd} and 5\textsuperscript{th} month of lactation during spring and winter seasons which were 4.7 and 6.8 mmol/l and 2.4 and 2.6 mmol/l, respectively.

Kaneko et al. (1997) documented that the concentration of calcium in the urine of cattle and goats were 0.1- 0.4 and 1.0 mg/kg, respectively.

Kumar (2001) analyzed the urine of cows, buffaloes and goats and found that the concentration of calcium was 5.735, 7.086 and 7.497 mmol/l, respectively. The author further estimated excretion rate of phosphorus in the urine of cows, buffaloes and goats which were 0.4805, 0.4934 and 0.9355 mmol/l, respectively.

Bhadauria (2002) reported that cow urine contains all the beneficial elements in it, hence it is natural and universal medicine that fulfils the deficiency of elements and reduces the increased element in the body and it is the quality of urine, which helps in curing even the most incurable diseases. Cow urine contains 95% water, 2.5%
urea and the remaining 2.5% mixture of minerals, salt, hormones and enzymes. It contains iron, salts, carbonic acid, potassium, nitrogen, ammonia, manganese, sulphur, phosphates, urea, uric acid, amino acids, enzyme, cytokines, lactose etc..

Parihar et al. (2004) compared the mineral profile in urine of crossbred, Sahiwal and Non-descript cattle. He observed that on an average Non-descript cattle showed maximum concentration of different mineral elements like Zinc, Potassium and Calcium. But iron was lowest in non-descript as compared to Sahiwal and Crossbred. Concentration of Calcium was minimum in Sahiwal. However, Crossbred cattle showed minimum concentration of Zinc, and Potassium in their urine.

Chauhan (2005) mentioned that the cow urine has natural disinfectant and antiseptic qualities. He reported that the cow urine contains 24 types of salt as well as iron, calcium, phosphorus, carbonic acid, potash and lactose. For diabetics, test has shown that it controls the sugar level and aids their fitness.
CONTENTS OF COW URINE AS WOUND HEALING AGENT

Holder and Mackay (1937) used urea for the treatment of wounds clinically on human patients and reported that application of gross quantities of urea crystals or of strong to saturated aqueous urea solutions to infected wounds definitely hasten healing and were frequently efficacious when other therapeutic agents were in effective. According to him urea application is cheap and does not irritate the surrounding normal tissue and practically obliterates all odour arising from an infected wound.

Raghvan (1964) applied urea-sulphanilamide powder 1:4 on many cases of wounds as dressing in all species of animals. The wounds healed up depending upon their size in a period ranging from 7 to 10 days.

Raghvan et al. (1977) used powdered furea bolus containing nitrofuralzone and urea in proportion of 0.06 gm and 6 gm as topical dressing for punctured wounds, fistula, horn injuries, injuries caused by dog bites, wounds of foot
pad and complications of foot and mouth disease lesion in cattle and buffaloes.

Levine (1986) reported that Vitamin C is needed to make collagen (connective tissue) that strengthens skin, muscles, and blood vessels and to ensure proper wound healing. Severe injury appears to increase vitamin C requirements.

Srinivasan (1990) mentioned that urea acts as lymphagogue and antiseptic when applied topically on wounds.

Sandstead (1994) reported that zinc is a component of many enzymes, including some that are needed to repair wounds. Even a mild deficiency of zinc can interfere with optimal recovery from everyday tissue damage as well as from more serious trauma.

Vohringer (1994) reported that the urea increases the rate of healing in animals when applied locally. Application resulted in increase in lymph flow together with hyperaemia, the wound became clean more rapidly and granulations appeared and grew quickly. It was suggested that the wound should be cleaned with 10% solution of urea and the material then applied as powder.
Udasi et al. (1996) used urea (fertilizer grade) in the form of 8%, 12%, and 18% w/v aqueous solutions for wound dressing. The higher concentration of 18% hindered the healing process, whereas 8% or 12% solutions accelerated the wound healing. He also observed the proliferation of fibroblasts and endothelial cells with development of young capillaries, but with no evidence of collagen deposition on 5th day while using 8% and 12% urea solution as a topical medicament. On the 10th day, the healing tissue revealed abundant vascularity of granular tissues and marked proliferation of fibroblasts with scanty collagen deposition. However on 15th day there was abundant collagen deposition with less vascularity of fibrous tissue.

Rucker et al. (1998) reported that copper is a required cofactor for the enzyme lysyl oxidase, which plays a role in the cross-linking (and strengthening) of connective tissue.

Douglas and Mackay et al. (2003) reported that healing of wounds, whether from accidental injury or surgical intervention involves the activity of an intricate network of blood cells, tissue types, cytokines, and growth
factors. This results in increased cellular activity, which causes an intensified metabolic demand for nutrients. 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 tissue antioxidants. Adequate dietary protein is absolutely essential for proper wound healing, and tissue level of amino acid, arginine and glutamine may influence wound repair and immune function.

**WOUND CONTRACTION STUDY**

It has been demonstrated in several mammalian species that the successful healing of full thickness skin deficits relies on two mechanisms, epithelialization and wound contraction. (Billingham and Reynolds, 1952; Abercrombie *et al.*, 1954 and Watts *et al.*, 1958).

Carrel (1910) suggested that the wound contraction is the result of proliferative changes occurring within the granulation tissue.
Burrow (1924) believed that wound contraction results from changes occurring within the surrounding epidermis, causing skin to be pushed across rather than pulled across the area of deficit.

Grillo et al. (1958) suggested “the picture frame theory” and stated that removal of central area of granulation tissue does not materially effect subsequent wound contraction.

Walton and Neal (1972) observed healing of 2cm square full thickness cutaneous wounds infected on the flanks and lower limbs of Welsh ponies and found different pattern of wound contraction in two areas. The rate of healing observed in limb lesions was approximately half than the flank lesions and stallete scars were formed in the flank region whereas square scars were produced on the limb.

Jadon et al. (1985) studied the effect of cartilage powder, amnion and tissue extract in open wound healing in buffaloes and observed that the rate of wound contraction was significantly greater in all the treated wounds as compared to control group treated with normal saline at respective intervals.

Peacock (1986) stated that the wound contraction results in reduction in size of wound by inward (centripetal) movement of
surrounding skin and this process was due to contraction of myofibroblast in the granulation tissue.

Alice et al. (1987) observed the effect of non-adherent dressing materials on the healing of open wounds in dogs and concluded that regardless of the dressing used, all wounds contract rapidly from day 0 to 14, followed by slower contraction from day 14 to 21.

Varshney and Verma (1990) reported enhanced wound contraction rate of Himax treated wound as compared to normal saline treated wounds, at respective time interval while observing percent wound contraction in the clinical wounds ranging from 3-9 cm in diameter and approximately of the same depth in cattle and buffaloes.

COLLAGEN

Following skin injury, the epidermis heals rapidly by regeneration. However, the dermis is not capable of regeneration and its repair leads to scar tissue formation which is different from dermis in that it is relatively simple, undifferentiated structure consisting of parallel arrays of collagen bundles. Its formation, rise to maximum level and importance in mobility of epithelial cells in wounds have been documented.
Carrel (1930) reported that the depression of connective tissue formation in the wound is due to systemic factors inhibiting fibroblastic proliferation.

Meyer (1947) reported that the fibroblasts secret a mixture of hyaluronic acid, chondroitin sulphate and soluble collagen in tissue space, which influence the precipitation of soluble collagen as insoluble fibres.

Edwards and Dunphy (1958) observed early appearance of precollagen, which served as a precursor and substrate for formation of collagen fibres.

Grillo et al. (1958) reported a rise to the maximum level in total collagen content of wound tissue on day-8, followed by a fall to maximum by day- 20 of healing.

Porter and Pappas (1959) reported that the fibroblasts play an important role in the formation of collagen fibres.

Russell Ross et al. (1961) studied the regular sequence encountered during the healing of skin wounds by methods of light and electron microscopy. Collagen fibrils were first seen at 3 days extracellularly near the cell surfaces. They appear at the later times in two populations of sizes. With increasing wound age the fibroblasts retain their morphology and the wounds decrease in
cellularity concomitantly with the formation of increasing amounts of collagen.

Levenson et al. (1965) observed an increase in collagen content of wound tissue in 6-7 weeks of healing in rats. Microscopically, they further observed a progressive change in the number, caliber and density of collagen fibres upto one year of time. They opined that elastin play no definite role in wound healing.

Udupa (1969) suggested that to achieve good mobility of epithelial cells, firm base formed by collagenous material of dermis or fibrinous material are needed.

Udupa (1973) observed that the collagen content of healing tissue increases rapidly upto 12 days after injury and then gradually for about a month.

De Hann et al. (1974) reported that the severity of acute inflammatory changes and depression of connective tissue formation in wounds are due to collagenolytic effect of cellular bacterial enzymes.

Rein et al. (1976) and Raju et al.(1977) separately reported that hypoxia or anoxia in tissues, due to consumption of oxygen by
organisms, results in acidosis which in turn diminishes connective tissue formation.

Ghani et al. (1981) reported a significant rise in the level of tissue hydroxyproline and collagen on 12\textsuperscript{th} day in Himax treated wounds in calves.

Repesh et al. (1982) described the distribution of fibronectin and its association with reticulin fibres (type III collagen) and hyaluronic acid in shallow rabbit wounds. Early granulation tissue formation was apparent just below the epidermis in 5 day wounds. Fibronectin was observed in the matrix surrounding individual fibroblasts and codistributed with reticulin fibres and hyaluronic acid in both 5 and 8 day wounds. Granulation tissue of 8 day wounds stained intensely for fibronectin and extended to a greater depth in the reticular dermis. Dense fibrillar networks of fibronectin and fibroblasts were aligned parallel to the epidermis, giving the granulation tissue a highly structured and organized appearance. Fibroblasts contained fibronectin and were surrounded by less fibronectin at the wound periphery than within the granulation tissue.

Jadon and Kumar (1984) observed elevated collagen and hydroxyproline levels in cartilage treated wounds as compared to
control wounds and the values were maximum on 15\textsuperscript{th} day in healing wounds of buffaloes.

Pierce \textit{et al} (1988) studied the histological evaluation of growth factor-treated wounds and found the accelerated healing of wounds with a striking inflammatory cell infiltrate early after wounding, markedly increased formation of granulation tissue by 4-day, and increased fibrosis by 14- day in comparison to control.

Srivastava (1996) observed significant increase of tissue collagen and hydroxyproline from 7\textsuperscript{th} to 28\textsuperscript{th} days. However, these values were significantly higher in treated wounds as compared to control one at corresponding intervals.

Ansari \textit{et al.} (1997) reported gradual increase in the concentration of the collagen and hydroxyproline from 3\textsuperscript{rd} to 25\textsuperscript{th} day. They further observed gradual increase in tissue elastin from 3\textsuperscript{rd} to 15\textsuperscript{th} day and thereafter, elastin contents decreased.

Ashcroft (1999) reported that the estrogen treatment increases the extent of wound healing in both males and females with a decrease in wound size at day 7, increased collagen levels at both days 7 and 80, and increased day 7 fibronectin levels. In addition, estrogen enhanced the strength of day 80 wounds.
HEALING PATTERN WITH SOME UNCONVENTIONAL AGENTS:

Here, the pattern of wound healing described in various literature in terms of general appearance, appearance of granulation tissue, wound contraction, inflammatory changes, fibroplasias, epithelialization and formation of collagen, elastin and reticulin have been reviewed.

Jadon et al. (1985) observed that the granulation tissue become evident as early as 4th day in tissue extract treated wound and 5th to 6th day in amnion and cartilage powder treated wounds. The cavity of wound had nearly filled up by 10th day.

Bhargava et al. (1986) evaluated the histomorphological and histochemical pattern of wound healing following topical application of Adhatoda vasica ointment. They observed early fibroblastic proliferation, less infiltrative changes and epithelialization of newly formed connective tissues in the treated wounds as compared to control one. In histochemical study, they observed more collagen, elastin and mucopolysaccharide in treated wounds than the control, while insignificant changes were noticed in the extent of formation of reticulin fibres.
Bhargava et al. (1988) noticed that the *Annona squamosa* treated wounds appeared pinkish to white on 3rd day. The granulation tissue appeared on 4th day on the surface of wounds. At 10th day, the process of granulation was more intense. The epithelialization and wound contraction were more distinct in treated wounds than control. Treated wounds healed completely in 21 days. Significant increase in collagen was observed from 3rd to 30th day interval and it was higher in treated wounds. The gradual increase in the elastin content was observed from 3rd to 30th day interval in the control and treated wounds.

Zama et al. (1988) reported that on 14th day, the wounds treated with the ointment of *Adhatoda vasica* and pancreatic tissue extract showed better organization of fibroblastic proliferation and bundles of mature connective tissue than control wounds. The whole surface of the wounds was covered with regenerating epithelium in the wounds treated with *Adhatoda vasica* ointment.

Dyson et al. (1992) made a comparison of the effect of moist and dry conditions on the process of angiogenesis during dermal repair and obtained a result that showed that the wounds maintained in a moist environment revascularised at a greater rate than those maintained in a dry environment and in general, moist
wound showed a more rapid decline towards uninjured skin levels of vascularization than dry wounds.

Sedlari et al. (1992) while investigating the healing of deep skin wound using a collagen sponge as a dressing material in the animal concluded that not only the healing time was shortened but also the quality of wound repair by dressing the wound with collagen sponge was enhanced.

Heggers et al. (1996) while investigating the beneficial effects of aloe on wound healing in an excisional wound model noted that aloe appeared to expedite wound contraction and neutralizes the wound retardant effect seen in the topical mafenide acetate alone, consequently, improving the collagen matrix and enhancing the breaking strength.

Ansari et al. (1997) observed formation of granulation tissues after 5\textsuperscript{th} to 6\textsuperscript{th} day following topical application of Charmil\textsuperscript{®} on the wound.

Malkonadaiaih et al. (1997) noted that amnion and gelatin treated wounds had well formed granulation tissues of high vascularity on day-6. On day-15, these treated wounds showed the
covering of the granulation tissues with stratified squamous epithelium and wavy collagen fibers.

Varshney et al. (1997) noticed mild inflammatory cellular reaction on day-7 in saliva treated wounds. In the treated wounds, epithelialization started on day-14 and extensive growth of granulation tissues was noticed at this period. On day-21, these wounds depicted collagen condensed into a thick fibrous layer.

Oryan and Zaker (1998) while studying the effect of topical application of honey on cutaneous wound healing in rabbits concluded that on applying honey topically on cutaneous wounds, it accelerates the healing process and appeared to have an important property that made it ideal as dressing for cutaneous wound.

Marget et al. (1999) performed a comparative study on the effect of povidone iodine on wound healing in control, diabetic and steroid depressed rats and came to conclusion that, normal and diabetic groups were comparable concerning collagen formation whereas steroid group showed significant retardation in healing time and epithelialization and collagen formation showed that povidone iodine did not overcome the steroid effect.
Kundu et al (2005) studied the potential efficacy of fresh turmeric (*Curcuma longa*) paste to heal wounds was tested in a preclinical study in an animal model. He compared turmeric paste with honey as a topical medicament against a control on experimentally created full-thickness circular wounds in 18 rabbits (*Oryctolagus cuniculus*). Wound healing was assessed on the basis of physical, histomorphological, and histochemical parameters on treatment days 0, 3, 7, and 14. He measured tensile strength on day 14 of treatment and observed that the wound healing was statistically significantly faster (P < .01) in both the treatment groups as compared to the control group.