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In vitro studies on the wound dressing prepared using collagen and teak leaves (*Tectonagrandis)*

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ABSTRACT

The inappropriate caring of the burn wound may delay its healing, causing the area to become infected and subsequently resulting in chronic wounds. In this study, methanolic teak leaves extract (*Tectonagrandis)* has been incorporated into collagen sheet and the bio-composite has been evaluated for its physical, biological properties and also its *in-vitro* wound healing efficacy using 3T3 fibroblast cell line. This composite sheet is cost-effective, as collagen is being extracted from chrome shavings a by product of tanneries, in which teak extract is incorporated. The results showed that addition of teak leaves extract increased the water absorption capacity (184 %), tensile strength properties (1.11 N/mm^2) and also stability of composite sheet against lysozyme. The characteristic spectra of collagen is shown by IR spectrum of amide I, II and III absorption bands at 1663, 1555 and 1242 cm^{-1} respectively. The bio-composite sheets have exhibited anti-microbial properties and extract release studies show 80 % extract release in 4 hrs. MTT based proliferation assay shows that collagen–teak bio composite sheets are not cytotoxic to cells and also supports the growth and proliferation as indicated by increase in cell number with respect to time.

KEY WORDS*:* Wound dressing, collagen, teak leaves, biocomposites, *in vitro*, 3T3 fibroblast

INTRODUCTION

Wounds are physical injuries that result in an opening or break of skin. Healing process is initiated in response to an injury .Wound healing is a complex process that results in the contraction of wound and restoration of a functional and integrity of damaged tissue. It involves continuous cell-cell and cell-matrix interactions that allow the process to proceed in three overlapping phases: inflammation, cellular proliferation and remodelling[1]. The presence of free radicals and microbes on the wound surface affects the healing process. There is substantial evidence of the role of antioxidants acting against free radicals by scavenging them[2]. Healing of wounds particularly from burns and skin diseases are a great challenge[3]. The fabrication of bio sheet with antioxidant and antimicrobial properties enhances the wound healing activity. Any material that can interact with biological system to treat or regenerate any tissue of the body can be termed as a biomaterial[4].

Delayed wound repair represents a significant burden for the patient, those involved with treatment and those responsible for defraying the costs of that treatment. The development of interactive devices, that promote healing by correcting imbalances of the wound environment, may represent a more appropriate and cost effective alternative to these high technology therapies. The lack of new tissue deposition in non-healing wounds is thought to be the consequence of both impaired tissue synthesis and elevated tissue destruction. Reduced tissue generation may be the result of a variety of derangements including phenotypic abnormalities in synthetic cells[5,6] compromised intracellular signalling[7,8] and reduced cellular viability.

The ideal performance characteristics of materials designed to promote tissue generation within chronic wounds should include: (i) the ability to attract cells capable of synthesizing new tissue to the wound site, (ii) the ability to promote cell proliferation, (iii) the ability to offer a biocompatible and transient (bioresorbable) template for cellular migration and matrix deposition, (iv) the ability to limit excessive degradative proteinase activity, (v) the ability to absorb and neutralise free radicals and/or excess metal ions[9].

Dressings play an important role in the management of wounds. Wound dressing is a biomaterial of synthetic or biologic origin that is designed to recover the healthy physiological and structural properties of the wounded skin .In addition to this it should have mechanical properties compatible with the skin and improve the healing process by actively attracting cells to the wound area. During the wound healing process, the dressing protects the injury and contributes to the recovery of dermal and epidermal tissues. The main objective of wound dressing is to accelerate wound healing by preventing bacterial infection and quickening of tissue regeneration. An ideal wound

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dressing material should have the ability to absorb exudates and toxic components from the wound surface, ease of application, bioadhesiveness to wound surface, easily sterilizable, sufficient water vapour permeability, elasticity ,mechanical strength, biodegradability, flexibility and should also protect wound from bacterial penetration[10]. There are various wound dressing material available which can be classified as traditional dressings, biomaterial based dressings and artificial dressings[11]. Collagen or collagen based matrix materials are the most commonly used biomaterials in the skin, connective tissue and nerve tissue engineering[12] . This natural protein is involved in all the phases of wound healing. Collagen was extracted from bovine skin, tendon, bone, eye, chrome shavings etc[13,14,15]. These are prominent solid waste in tanning industry and the disposal of these chrome shavings has been identified as a serious problem from environmental point of view because of toxic chromium content. This chrome containing leather waste mainly consist of collagen and Cr (III) complexes which could be treated to give potential resources of collagen, protein and chromium[1]. Selecting collagen as base material offers several advantages which has well documented structural physical and chemical properties. Collagen exhibits biodegradability and superior biocompatibility. Moreover it has low antigencity, low inflammatory property and has ability to promote cell attachment and proliferation. In addition to its physiological properties as a natural extracellular matrix component, the stability and microstructure also plays a vital role in cell-cell and cell-matrix interaction[16]. The implanted collagen will be degraded through native enzymatic pathways without any toxic response[17]. Crosslinking of collagen adds up extra strength thereby forming a tight intricate fibrillar assembly in the structure and acts synergistically in the significant improvement of wound healing along with the extract[2]. During wound healing, collagen activates integrin, which initiate signalling cascades that produce matrix metalloproteinase which are enzymes that degrade the collagen matrix and enable keratinocyte or fibroblast migration. Collagen besides stimulating the cellular elements of healing such as granulocytes and macrophages, also provide a template for cellular attachment, migration and proliferation .Cells such as fibroblast and keratinocyte specifically recognize collagen substrates. The fibroblast is the cell type which is the most prevalent in dermis and is responsible for synthesizing and depositing collagen fibres in continuous networks that form the structural scaffold. During connective tissue repair, fibroblasts exhibit several different activities. At first, they migrate from adjacent tissues into the wound region. In the wound region, they proliferate and synthesize a collagen-rich extracellular matrix that fills the wound defect. Fibroblasts produce different growth factors that induce proliferation of keratinocytes in vivo (normal skin) and in vitro (artificial living skin)[18] . As fibroblasts are known to play a key role in the repair and remodelling of tissues in dermal wounds, the chemo kinetic and proliferative effects of biocomposite prepared on this cell lineage were investigated by in vitro assays. The incorporation of plant extracts have shown to improve the wound healing efficacy. In this study teak leaf extract has been incorporated into the collagen to form the biocomposite sheet. *Tectonagrandis* is commonly known as Indian teak, and belongs to family verabinaceae. It contains mainly carbohydrates, tannins and anthraquinone glycosides. It is used as anti- inflammatory agents and also used topically for the treatment of burns. It is mainly used for the injuries like burn, inflicted wound and skin ulcers. The extract applied topically or given orally has been reported to promote the breaking strength, wound contraction and collegenation[19]. It is also considered as a major constituent in many folklore medicines. Teak possesses active constituents like alkaloids, aminoacids, flavonoids, tannins, saponins, sterols and triterpinoids. They are known for their anti-inflammatory, anti-microbial, anti-oxidant, anti-tumor and anti-ulcer properties. Extracts from various parts of this plant has been used against bronchitis, hyperacidity, diabetes, leprosy, dysentery and for swellings[20]. It is expected that the addition of teak extract to biomaterials like collagen will synergistically enhance healing properties.

Teak leaves, one of its organic wastes, have been used traditionally as food packaging and medicine. It has been known that the phenolic compounds of Teak leaves, such as quercitine, gallic acid, ellagic acid, and tectoquinone, have great potency to be antioxidant agent. Phenolic compound is the major active compound of Teak. Some phenolic compounds, such as quercitin, gallic acid, rutin, and ellagic acid of Teak leaves have already reported as polar antioxidant agent[21]. In this study collagen is extracted from chrome shavings, and a biocomposite sheet is fabricated using collagen and teak extract and its characterisation was studied using FTIR, SEM and SDS PAGE and the physical, biological and *in-vitro* studies are also performed to analysis the material for the wound healing purpose.

METHODOLOGY

Isolation and preparation of Collagen : Teak leaf extract bio-composite sheet

Collagen was extracted from chrome shavings by acid digestion method[1].The chrome shavings was weighed and soaked in 5% NaOH overnight for 24hr incubation. Then the pH of the material was made neutral (pH 7) by thorough washing. Following this it was treated with Conc.H_2SO_4 and kept for 30 minutes incubation for de-chroming. After thorough washing ,the de-chromed material was soaked in 30 % hydrogen peroxide solution for bleaching which removes any impurities and washed The obtained fibrous material was then lyophilized and stored at 4^0C until use. Teak leaves were washed, cleaned ,shade dried and powdered .About 20 g of teak leaf powder was subjected to Soxhlet extraction using methanol(100 ml) as solvent. Extraction was done until powder free extracts were obtained and solvent was removed using rotary evaporator. The obtained extract was then freeze dried and stored at 4^0C until use. Collagen (Col) obtained was soaked in 0.5 M acetic acid overnight at a concentration of 100mg/ml for solubilisation. The solubilised collagen solution was then sonicated and filtered. Teak leaf extract (TLE) weighed at the required concentration was then added to 20 ml collagen solution. To this solution mixture, ethylene glycol was also added which

acts as a cross linker and aids in uniform sheet formation. The mixture was casted in petriplate and allowed to air dry. The 20ml of collagen is added to 100µg -1500 µg of teak extract concentration along with 2.5% - 10% of ethylene glycol.

Physical Characterization

Water absorption study

Water absorption studies of the bio-composite sheets was determined according to the method followed by[22]. For WAC study, sheets were cut in 2*2cm^2 size and initial weight of sheet was recorded. The sheet was then placed in 5ml water containing beaker and the amount of water taken up by sheet was found by weighing the weight of swollen sheets after time intervals of 1hr, 2hrs, 3hrs and 24 hrs. Each time the excess water was blotted before weight measurements were taken. The Percentage water absorption capacity at a given time was calculated from the formula:

% Water absorption capacity (W.A.C) = [(final weight – initial weight) / (initial weight)]*100

Tensile strength and % elongation at break measurement

Tensile strength (MPa) and percentage of elongation at break (%) properties of the prepared sheets was measured using a universal testing machine (INSTRON model 1405) at an extension rate of 5 mm/min.

SEM analysis

Surface morphology of the samples was visualized by scanningelectron microscope (SEM Model LEICA stereo scan 440).SEM analysis of the sheets can be done directly todetermine the surface morphology.

FTIR analysis

Fourier transform infra red (FTIR) spectral studies of the prepared collagen sheet was carried out was done using ATR method. The spectra was measured at a resolution of 4 cm^{-1}in the frequency range 4000–500 cm^{-1} using Nicolet 360 Fourier Transform Infrared (FTIR) Spectroscope.

SDS-PAGE

Collagen extracted from chrome shavings was subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis following the method of Laemmli, 1970[23]. Separating gel of 7.5 % and stacking gel of 5% was used. In lane 1, broad range protein marker supplied by BIO-RAD was loaded, lane 2: extracted collagen sample (20 µg) was loaded and in lane 3 extracted collagen sample (40 µg) was loaded and the gel was allowed to run at 50 V and subject to Coomassie blue staining.

Extract release from Teak leaf extract incorporated collagen sheets (Col-TLE)

The sheets were cut in 2*2 cm^2 size and dry weight of sheets was recorded initially. The Col-TLE sheets loaded with 50 µg and 75 µg of extract was used for the study. The sheet was then placed in phosphate buffer solution (pH 7.4) to which continuous stirring was given. Every 30 minutes, amount of extract released into PBS solution was determined by O.D. measurements at 450 nm. The wavelength 450 nm corresponds to the wavelength at which teak leaf extract shows absorption maxima .This procedure was repeated till the extract released reached a saturated point. The amount of extract released at each time interval was calculated using the formula.

$$E=\frac{Qp}{Qt} *100$$

Where, E is the percentage extract release from Col-TLE, Qp the quantity of extract release and Qt is the quantity of teak extract added in the dressing, i.e., total amount of teak extract in the loaded collagen sheet.

Biological characterization

***In vitro* biodegradation studies using lysozyme on the biocomposite sheets**

Sheets were cut in 2*2 cm^2 size and dry weight of sheets was noted down. Collagen sheets as well as Col-TLE sheet was used for the study. Lysozyme is one of the major enzymes found in wound fluid. The sheets was placed in 10 ml 1X PBS solution containing lysozyme at a concentration of 1.6 µg/ml which corresponds to the amount present in human serum. Sheets placed in 10 ml PBS solution without enzyme was used as control which helps to eliminate the degradation by dissolution. Every day the dry weight of sheets put in lysozyme as well as control was taken, after blotting off excess water from surface. This was done for 21 days. For all the studies, the wound dressing prepared with collagen alone was treated as control sample. The *in-vitro* degradation was calculated as follows:

$$\text{Weight after degradation} = \frac{Wd-Wo}{Wo} * 100$$

% Weight loss = 100 - (weight after degradation %)

W_o denoted the initial weight of the scaffolds, W_d: the weight of the scaffolds at time t.

Bacterial culture and anti-microbial activity

Two bacterial strains were used-Gram positive *B. subtilis* and Gram negative *E.coli*. The culture was sub-cultured periodically and maintained on nutrient agar plates. The anti-microbial activity was done using disc diffusion method. In this method, sterile discs were placed on agar plates swabbed with culture (*E.coli* and *B.subtilis)*.The teak extracts were loaded on to these discs, placed on bacterial culture swabbed plates and was incubated at 37^{0}C for 24 hrs. Three concentrations of plant extract (200µg, 400 µg and 600 µg) were added to the disc.

Cell culture and viability studies

The 3T3 fibroblast cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM high glucose–glutamine) supplemented with fetal bovine serum (FBS, 10%, v/v) and penicillin/streptomycin

(10 U/ml) at 37°C under humidified atmosphere of 5% CO2–95% air in incubator. When the cells reached at least 80–90% confluency, they were trypsinized for passaging. The composite sheets of 10 mm size were UV sterilized prior to viability study and placed in 24 well plates (15 mm well diameter) .Sheets were also saturated with the serum free media for 24 hrs prior to cell seeding. The initial seeding density onto each composite sheet was 2×10^4 cells/composite sheet. Three 24 well plates were used –one each for 24 hr,48 hr and 72 hr incubation of cells on composite sheet. Collagen only group and cells alone in polystyrene wells were used as the controls.EGF (Epidermal growth factor) at a concentration of 2ng/ml was used as positive control for proliferation and Triton-x was used as positive control for cytotoxicity studies.

MTT Assay

The cytotoxicity and cell proliferation on composite sheets was evaluated with MTT (methylthiazolyldiphenyl-tetrazolium bromide, Sigma-Aldrich) assay. This method is based on reduction of tetrazolium salt by mitochondrial dehydrogenases to a dark blue formazan product [24]. Initially Triton-x treatment was given to the corresponding wells for half an hour. The media was then removed from all wells. About 100 µL MTT solutions (5 mg/mL in 1 X PBS) was added to each well and incubated for 4 h at 37°C in dark. After removing the MTT solution from the wells about 100µL dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals formed inside the cells giving a purple color. The absorbance was measured at 540 nm wavelength using ELISA reader. The extent to which MTT is reduced to a formazan product has been correlated with the cell viability after incubations of 24, 48 and 72 hrs.

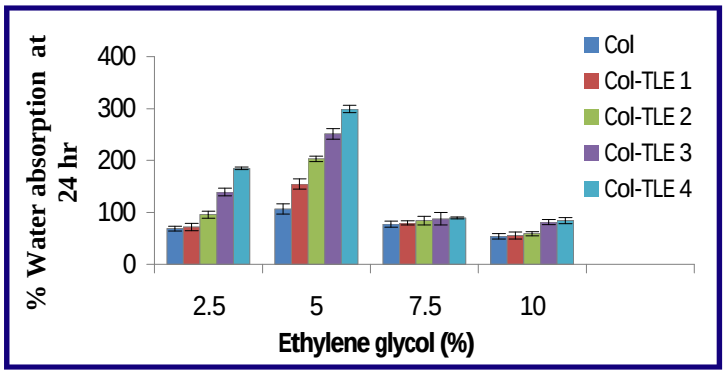
Migration assay-Scratch assay

The composite sheets were sterilized by exposure to UV radiation for 30 min. Migration assay for teak extract incorporated sheet was done using conditioned media. The conditioned media was obtained by incubating the sheets in 1 ml of DMEM in a CO_2 incubator at 37 ^{0}C for 24 hrs. The sheets were then removed and the conditioned media were obtained. The conditioned media were filtered to remove any degraded sheet.

Cell migration of fibroblasts were assessed by the ability of the cells to migrate into acellular area (area without cells). Fibroblast cells (2×10^4 cells) were seeded into each well of a 24-well plate and incubated with complete medium at 37 ^{0}C and 5% CO_2. After attaining confluency, the cells were starved in serum free DMEM medium for 1 hr. The fibroblast cells were scrapped horizontally with a P100 pipette tip and wells were viewed using optical microscope at 10 x magnification. Then the cells were treated with 10 µl extract treatments. The different treatments given were 1) conditioned media prepared from collagen, 2) conditioned media prepared from collagen containing10 µg teak extract 3) conditioned media prepared from collagen containing 100 µg teak extract 4)Teak extract alone at 10 µg and 100 µg concentration. Cells alone in the polystene wells were used as the control and EGF (Epidermal growth factor) at a concentration of 2ng/ml was used as positive control for migration and incubated at 37 ºC. Photographs were taken at 0 hr, 6 hr,12 hr and 24 hrs and distance travelled by the cells at the acellular front was determined. To determine the migration of fibroblast cells, the images were analyzed using Image J software. Percentage of the closed area was measured and compared with the value obtained before various treatments.

Statistical analysis: Results are presented as mean ± S.D. (n = 3). ANOVA (Analysis of variance) and student's t-test were done to determine the significant differences among the groups. The observed differences were statistically significant when $p < 0.05$.

RESULTS



Col. : Collagen sheet

Col-TLE 1 : Col incorporated with 5 µg/ml extract Col-TLE 2 : Col incorporated with 25 µg/mlextract

Col-TLE 3 : Col incorporated with 50 µg/ml extract Col-TLE 4 :Col incorporated with 75 µg/ml extract

Fig.1: WAC of Col. and Col-TLE prepared using varying EG amount

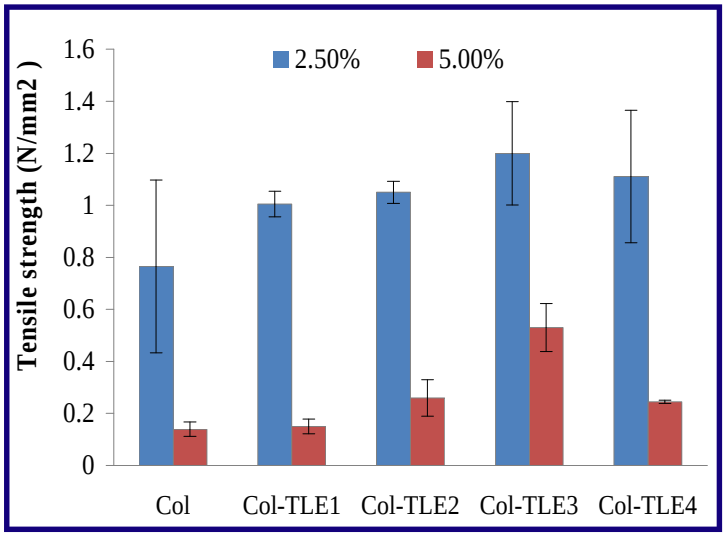


Fig.2: Tensile strength of C & Col-TLE prepared using 2.5 % and 5.0 % EG

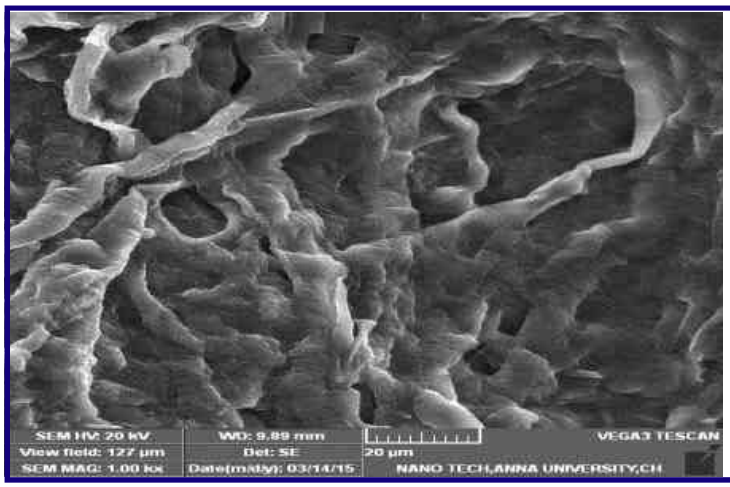


Fig. 3 a : SEM image of Collagen

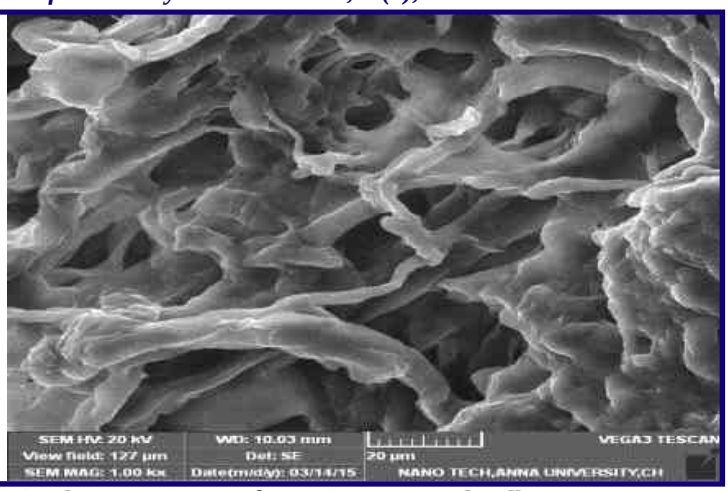


Fig. 3b : SEM image of TLE incorporated Collagen

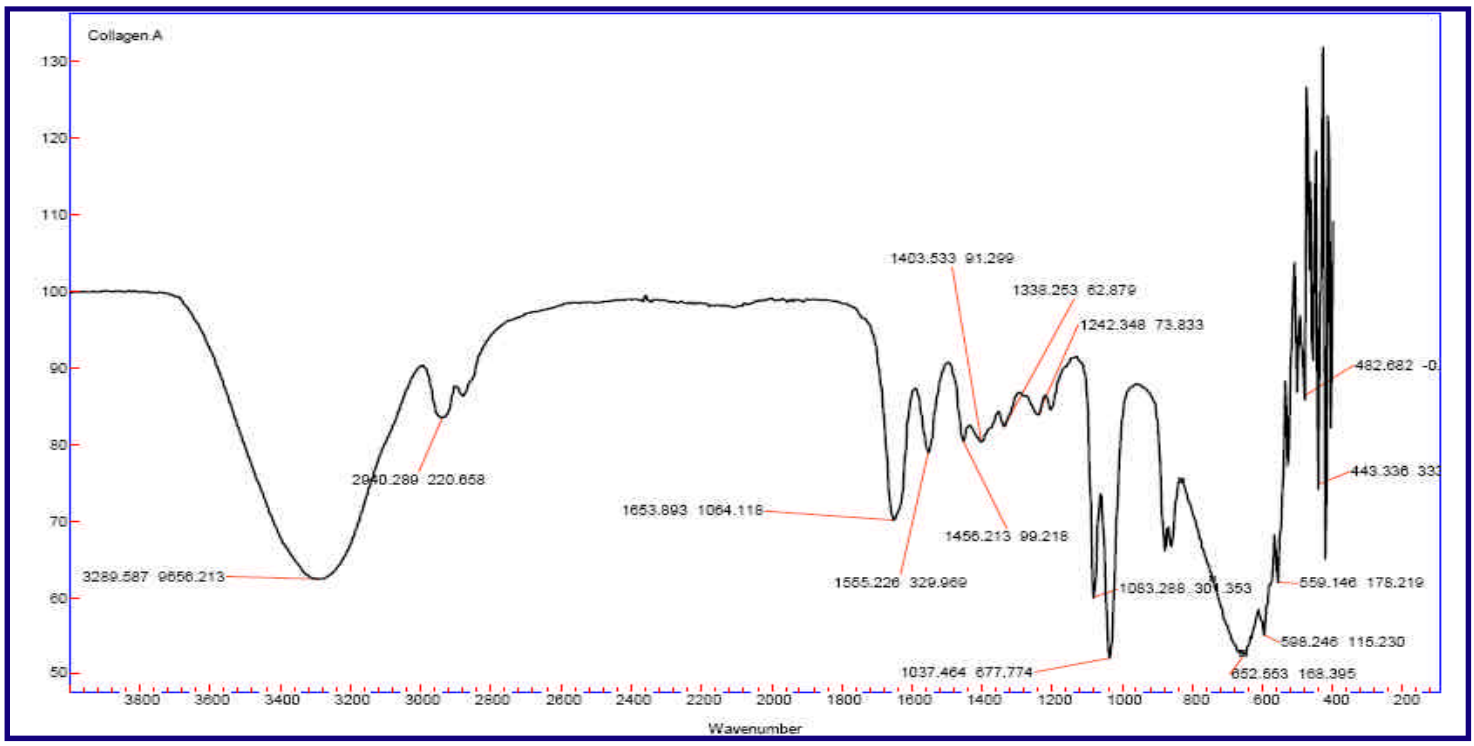


Fig.4 : FTIR spectra of collagen and teak extract

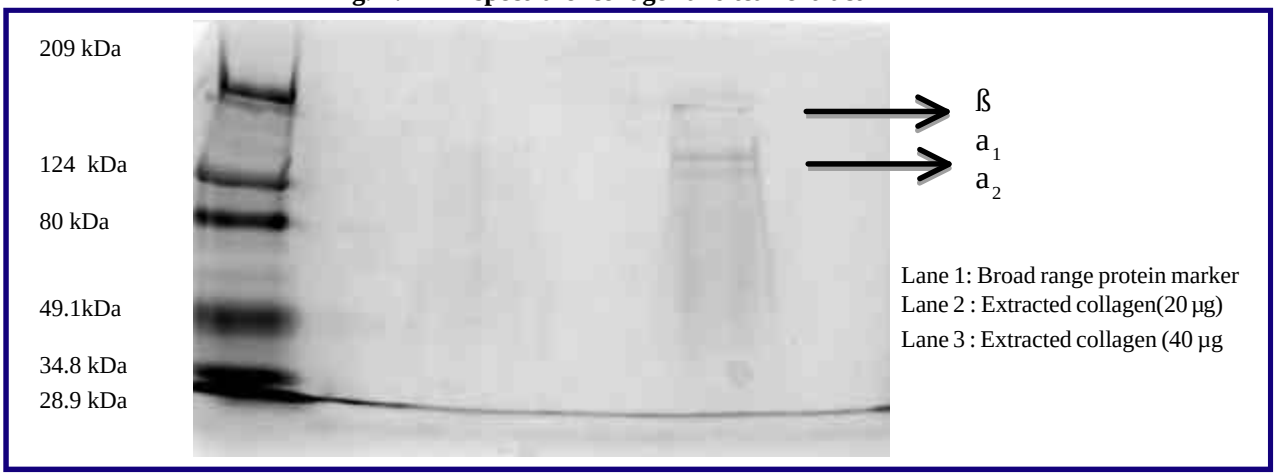


Fig. 5 : SDS-PAGE pattern of protein marker (lane 1) and extracted collagen (lane 2 & 3)

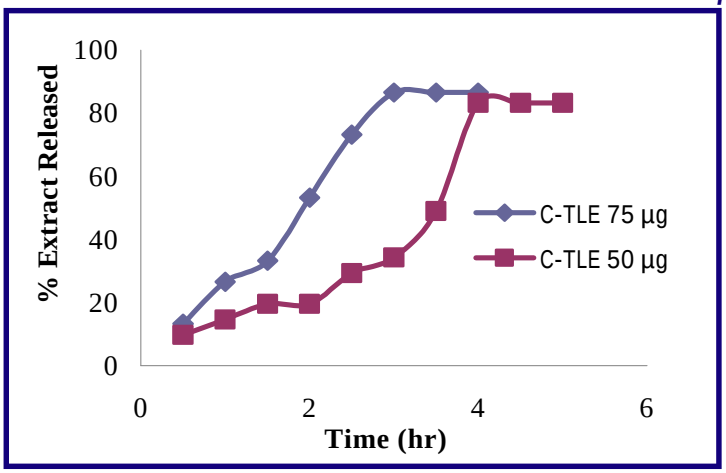


Fig .6: Extract release from TLE incorporated sheets

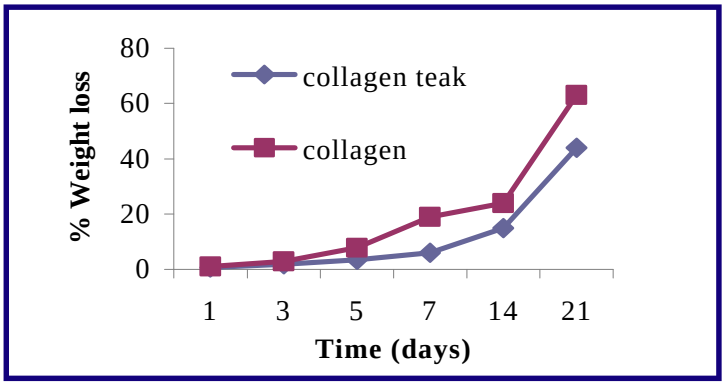
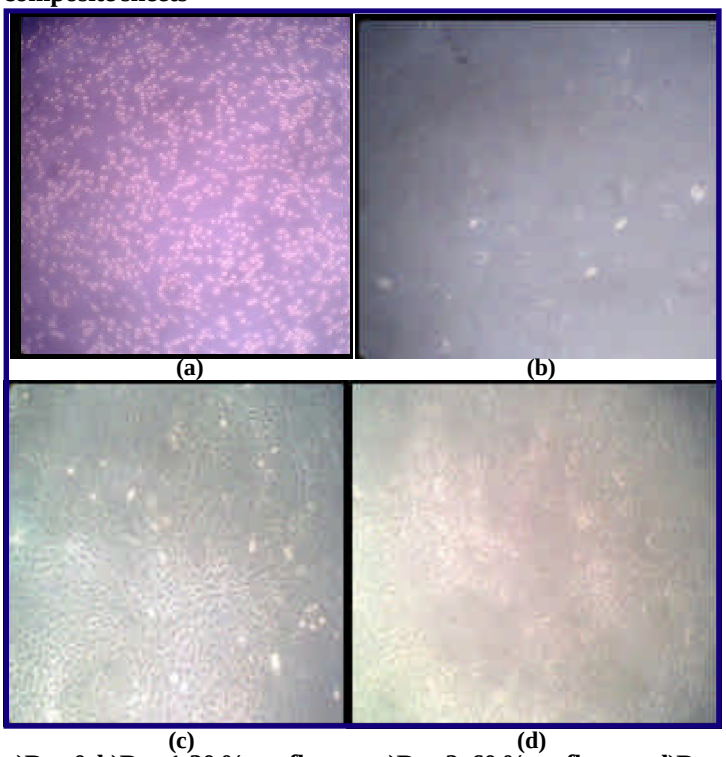


Fig. 7 *: In vitro* biodegradation studies using lysozyme on the bio-composite sheets



a)Day 0, b)Day 1:30 % confluency, c)Day 2: 60 %confluency, d)Day 4 : 80 % confluency

Fig. 8: Culturing of 3T3 cell line

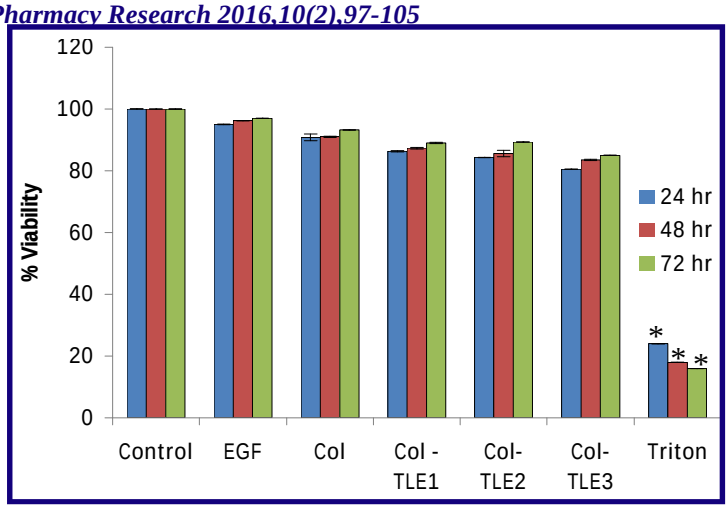


Fig. 9 : Cell viability and proliferation of fibroblast cells on C and C-TLE sheets (MTT assay) Cells alone in Polystyrene wells used as control ;* represents $p<0.05$ compared with control

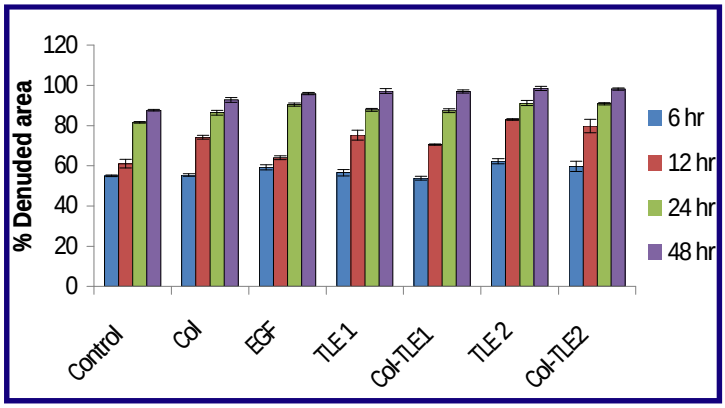


Fig. 10 : Migration of fibroblast cells into artificial scratch area expressed

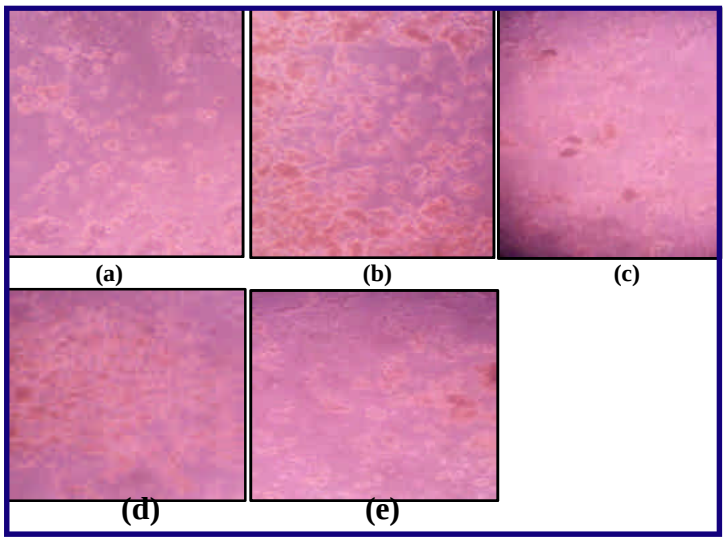


Fig .11 :Migration of fibroblast cells at 24 hr(Left to right) respectively of a)Control b)EGF treated well c)Collagen treated well d)TLE -1 treated well e)Col-TLE 3 treated well

DISCUSSION

A white fibrous material (collagen) was obtained after the extraction

procedure from chrome shavings. From 150 g of chrome shavings about 30 g of collagen was obtained. Teak leaf extraction was done using methanol as a solvent. Methanol extracts of teak has been reported to show significant wound healing activity compared to other non-polar extracts[20] . Moreover, methanol extracts of teak leaf also have been found to show fairly good anti-microbial activity against Gram positive and negative species. The active constituents like phenolic compounds, tannins and flavonoids were present in the extract, contribute to the anti-oxidant and anti-microbial property [21]. From the Soxhlet extraction of teak leaf, a yield of 12.1 % was obtained. It was observed, that when EG was added to collagen solution in the range of 2.5 to 10%, and the sheet formation was uniform and continuous, whereas, the collagen sheet formation was found to be discontinuous with the increase in the amount of EG (15-20 %).

The water absorption capacity and tensile strength of the sheets were evaluated. Water absorption capacity of a biomaterial plays a major role in absorption of wound exudates, which helps to keep the wound surface dry preventing bacterial infection and also provide moist environment[16]. It also depends on the microstructure and hydrophilic property of the material. .It was observed that water absorption capacity decreased in sheets prepared using more than 5 % EG compared to those prepared with 2.5 % and 5% EG. At 24 hrs, WAC of collagen sheet prepared with 2.5 % EG was 68.9 % which showed a significant increase in WAC up to 184.9% when 75 µg of teak extract was added to it and similarly significant increase was also observed for sheets prepared with 5% EG. (Fig.1). Water absorption capacity of the sheets increased with increase in time and it also increased with incorporation of teak extract which is due to its hydrophilic nature.

The mechanical properties like tensile strength are an important property for a wound dressing material. If the material fabricated is brittle or cannot be handled properly, it cannot be suggested to be used as a wound dressing material. Ethylene glycol was used in all the samples to increase the flexibility of the biomaterial. With increase in the amount of teak extract content in the collagen, the tensile strength was found to be increasing (Fig.2). Tensile strength of the composite sheets depends on their microstructure, pore size and thickening of the walls to pass the stress and bear the load[25]. From the WAC study and tensile strength measurements and % elongation at break, it can be concluded that 2.5 % of EG is the optimal volume for sheet preparation.

Pore size and surface area of the sheets are widely recognized as important parameters for the materials used in tissue engineering. In addition to this pore shape, pore wall inter connectivity between pores are also been important for cell seeding, migration, mass transport, growth, gene expression and new tissue formation in three dimension[26]. Surface morphology of the C and C-TLE sheet are shown in Fig. 3a and 3b respectively and it shows a porous nature and fibrillar structure. There is slight thickening of pore walls observed on addition of extract and the pores might have formed due to the intertwining of collagen fibres and extract. The porous nature of the collagen sheet helps in absorbing wound fluids, keeps the wound dry and also helps in oxygen supply to the wound[27].

The FTIR spectra of collagen sheet samples are shown in Fig.4. The IR spectrum of has shown amide I, II and III absorption bands at 1663, 1555 and 1242 cm^{-1} respectively, which are the characteristic spectra of collagen confirms that the extracted fibrous material from chrome shavings is collagen[28]. The electrophoresis pattern obtained on SDS-PAGE displayed one ß band close to 200kDa and two different α-chains (α1 and α2) close to 124 k Da (Fig.5) . This pattern resembles that of collagen which indicates the unwinding polypeptide chains of the triple helical structure of collagen[29]. From the extract release studies (Fig.6), it was found that the extract release from C-TLE sheet increases up to 4hrs (80% extract release in 4 hrs) in both 75µg and 50 µg teak extract incorporated sheets which shows that the bio-material supports controlled extract release that would help in improving the efficiency of the wound healing process. The extract release from 50µg teak extract incorporated sheets shows a more sustained and prolonged release of extract which is ideal for a wound dressing material.

From the degradation studies (Fig.7), it was found that the C-TLE sheets possess controlled degradation rate (45 % degraded in 21 days), whereas about 65 % of collagen sheet was degraded by lysozyme in 21 days. Therefore the incorporation of extract improves the stability of the prepared sheet. A scaffold having biodegradation time for about 25 days can be suggested for wound healing[30].

The TLE incorporated collagen sheet shows antimicrobial activity against *E.coli* and *B.subtilis* (Fig.8). The zone of inhibition could not be detected at concentrations lower than 25 µg of teak extract but at higher concentrations (25 µg, 50µg ,75 µg)clear visible zones were observed against both *E.coli* and *B.subtilis*. The teak plant extract has been incorporated into collagen sheet, where the antimicrobial agents might be released slowly to the surface of the wound and for longer period of time. The direct application of antibacterial substances has very limited benefits, because the active materials are neutralized on contact with the wound or diffused rapidly[16].

The percentage cell viability was calculated by MTT assay (Fig. 9) which depends on the mitochondrial activity of viable cells and rep-

resents parameters for their metabolic activity. (cells alone on polystyrene wells) all the cells grown on the polystyrene wells (control well) as well as on composite sheets showed normal morphology of fibroblast cells. Along with control the cells grown on different composite sheets also supported cell growth and no observable cell death or inhibition of growth was noticed in treated wells. More than 80% cell viability was seen in Collagen sheet and Col-TLE 1 & Col-TLE2 treated cells compared with the Col-TLE 3 treated wells.

Usually up to 20% reduction in cell viability are accepted *in vivo*, in this study the treated wells also exhibits 20 % or less decrease in fibroblasts cell viability which shows that the prepared composite sheets supports the growth and proliferation of cells indicated by the increase in cell number with time[31]. It can be considered that the prepared sheets exhibit good biocompatibility profile for dermal applications. Researchers have reported that biomaterial made up of collagen attracts many cell types, mainly fibroblast which plays a major role in wound healing by producing extracellular matrix, it also been found that production of collagen was increased when fibroblast were bound to collagen implants[32,33] .

Migration assay results shows that teak leaf extract increases the number of cells migrating to the artificial scratch created and the presence of collagen does not interfere significantly with this rate of migration of fibroblast cells (Fig.12). The highest rate of migration was observed in wells treated with 100 µg teak leaf extract followed by wells treated with Col-TLE 2 conditioned media. The graphical data (Fig.11) also supports this as significant difference in the migration rate was obtained only in wells treated with 100 µg teak leaf extract followed by wells treated with Col-TLE 2 conditioned media.

CONCLUSION

Collagen was isolated from chrome shavings, which is a by product of tanning industry. FTIR spectra and the band pattern obtained on SDS-PAGE confirm that obtained material is collagen. Incorporation of teak extract improves the WAC. Based on the water absorption study and tensile strength measurement, the optimum concentration of EG chosen for sheet formation is 2.5%. The TLE incorporated collagen sheet shows antimicrobial activity and it was found that the release of extract from C-TLE sheet increases up to 4hrs (80% extract release in 4 hrs).The C-TLE sheets possess controlled degradation rate(45 % degraded in 21 days).A scaffold having biodegradation time for about 25 days can be suggested for wound healing [30]. MTT based proliferation assay shows that collagen –teak bio composite sheets are not cytotoxic to cells and also supports the growth and proliferation as indicated by increase in cell number with respect to time. From the migration assay, it can be concluded that incorporation of teak extract improves the migration rate of fibroblast cells. Collagen matrix acts as support matrix for release of extracts in a controlled manner and also helps in cell adhesion and proliferation. The phenolic compounds like tannin and flavonoid content present in teak leaf have antimicrobial and anti-oxidant properties, contributes to the increase in the rate of wound healing. The fabricated sheet with combined properties of collagen and teak leaf extract may reduce the epithelisation period. Further, from the *in-vivo* studies, the Col-TLE sheet may be used as a low cost effective wound dressing material particularly for burn wound healing.

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