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Original Article

Design and characterization of natural extracts impregnated collagen based dermal films for wound healing

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ABSTRACT

Aim: The aim of this work is to design & characterize a collagen based dermal films containing natural extracts such as *Terminalia arjuna* and *Centella asiatica* having antioxidant properties for enhancing the quality of wound healing.

Methods: The collagen and cross-linked collagen films were formulated with different concentrations of the selected extracts. The formulated films were subjected to physical, biochemical and histopathological examinations.

Results: Folding endurance reports revealed that the films possessed the desired mechanical strength to withstand the stress observed during handling. Micro shrinkage temperature of the extract impregnated films was found to be above 68 °C indicating their hydrothermal stability. Wound healing studies on male Wistar rats revealed that the groups treated with 1.5% *T. arjuna* extract impregnated cross-linked collagen dermal films (1.5% TAEICCDF's), 15% extract impregnated collagen dermal films (1.5% TAEICDF's), 1.5% *C. asiatica* extract impregnated cross-linked collagen dermal films (1.5% CAEICCDF's), 1.5% *C. asiatica* extract impregnated collagen dermal films (1.5% CAEICDF's) possessed higher amount of hydroxyproline content compared to the marketed formulation – Neuskin™. The cross-linking of the CAEICDF's, TAEICDF's had a significant impact at $p > 0.05$ from student 't' test on wound healing compared to the non-cross-linked films. Further one-way classification ANOVA studies conducted for all the groups treated with natural extract impregnated films varied significantly ($P = 0.05$ from one-way ANOVA).

Conclusion: The histopathological studies indicated more production in the collagen content in groups treated with 1.5% TAEICCDF's, 1.5% TAEICDF's, 1.5% CAEICCDF's, 1.5% CAEICDF's which

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resulted in the epithelial group reduction. Further rise in fibroblasts in these treated groups suggested that the films are fit to productive approach in the improvement of dermal wound healing process.

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1. Introduction

Wound healing is a complex issue involving many sequential steps in the restoration of the skin to its normal state.[1] Wound healing process is delayed by the free radicals present in the wound. The free radicals act by causing lipid peroxidation, enzymatic degradation & DNA breakage. Moreover the exposure of the wound to the external environment worsens the phases of wound healing since it is prone to the microbial attack. There is a substantial evidence of the role of antioxidants acting against the free radicals by scavenging them.[2]

Natural extracts like *Centella asiatica*, *Terminalia arjuna* are renowned for their antioxidant properties.[3] They possess the active constituents such as quinones, iridoids, triterpenoid derivatives, coumarins, flavonoids and isoflavonoids which play an important role in wound healing.[4–6] The applicability of these extracts as such, is a point to be considered as inappropriate concentration may lead to subtherapeutic or undesired effects. At this juncture the need of a suitable delivery system for this extracts is inevitable. In this research an attempt was made to impregnate these extracts in a collagen matrix (a natural polymer) which acts as a base or platform by providing the physical support and ensuring the slow release of the extract.[12,14]

Further, collagen is interlinked with the wound healing property by the virtue of its nature. Other advantages of the usage of collagen include its biodegradability & biocompatibility.[7,8] Cross-linking of this polymer 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. Materials

Natural extracts like *C. asiatica*, *T. arjuna* natural extracts were procured from Chemiloids, India. Collagen was obtained from Shevoroy's Ltd India. 2,2[1] azo bisisobutyronitrile (AIBN) were purchased from Merck (India). All other chemicals used in this research activity were of analytical grade.

3. Methods

3.1. Development of various natural extract collagen based dermal films

Collagen was soaked in 0.05 M glacial acetic acid at 25 mg/ml concentration for 24 h at 4 °C. The obtained viscous solution was homogenized for 5 min, deaerated for 15 min by using sonicator and squeezed through a muslin cloth to get rid of undissolved solid traces if any (Note: for cross-linking 0.8 ml of 25% v/v glutaraldehyde solutions were added to the formulation at this stage).[7] Various solutions with different concentrations of *C. asiatica* and *T. arjuna* (Table 1) were prepared by dissolving them in 3 ml of alcohol. Each of the prepared solutions was mixed with 40 ml of the above cross-linked collagen solution separately. The obtained mixture was casted in petri plate (64 cm^2) having polyethylene membrane base and placed in incubator at 37 °C until dried. The scaffold thus obtained was sterilized under UV-radiation for a period of 18 h.

3.2. Thickness[9]

The thickness of the plain collagen, cross-linked collagen and different natural extracts (*C. asiatica* and *T. arjuna*) of varying concentration impregnated collagen based films was measured by using a screw gauge (LINKER-20 × 1/100 mm). The mean of 3 observations was calculated.

3.3. Folding Endurance[10]

Folding Endurance was measured manually for the prepared films. For this a strip of film (2 × 2 cm^2) was cut evenly and repeatedly folded at the same place until it broke. The number of times the film could be folded at the same place without breakage gave the exact value of folding endurance. The mean of 3 observations was calculated.

3.4. Equilibrium swelling ratio

The equilibrium swelling ratio (E_s) was measured by the conventional gravimetric method. The dry weight of different scaffolds was measured before immersing in 0.05 M phos-

Table 1 – Composition of the collagen and cross-linked scaffolds impregnated with various natural extracts.

Name of the extract	% Concentration of the extract used with reference to 1000 mg/40 ml collagen base	Volume of the alcohol consumed (ml)	Volume of (25% LR) glutaraldehyde used
Terminalia arjuna	1, 1.5, 2	3	0
Centella asiatica	1, 1.5, 2	3	0
Terminalia arjuna	1, 1.5, 2	3	0.8
Centella asiatica	1, 1.5, 2	3	0.8

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