Curcumin coated 3D biocomposite scaffolds based on chitosan and cellulose for diabetic wound healing

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ABSTRACT

The objective of present work is to fabricate porous three-dimensional biocomposite scaffolds with interconnected pore networks and mechanical strength for wound healing. Variable concentrations of chitosan and methylcellulose hydrogels were blended in the presence of calcium cations to prepare scaffolds by freeze-drying method. Curcumin-aerosol was deposited over the scaffold surface to improve antimicrobial efficacy. Scaffold stability and curcumin interaction were evaluated by Differential Scanning Calorimeter, Thermal Gravimetric Analyzer and Fourier Transform Infrared Spectrophotometer. Scanning Electron Microscopy indicate multi-layered porosity, mesh-like structure and pore-size ranging from 50 to 500 μm. Erythrocyte interaction with chitosan and methylcellulose using Surface Plasmon Resonance assay in the presence of curcumin depicted high binding affinity of chitosan alone than curcumin. The antibacterial activity of SCF-4C against Escherichia coli and Staphylococcus aureus and the instant haemostasis in erythrocyte-agglutination assay by SCF-7 indicate good material properties for wound treatment. Bleeding time and wound healing efficacy conducted on Sprague Dawley rats depict minimum clotting time of SCF-4 (~32 ± 2 s) compared to SCF-4C (~45 ± 2 s), while highest ~85 ± 5 s was observed in curcumin alone. SCF-4C exhibit complete wound healing on day14 in diabetic animals. In-vivo studies confirmed that high concentration of chitosan in presence of curcumin enhances diabetic wound healing process.

1. Introduction

Biomaterials refer to the natural or synthetic materials that interact with or replace biological tissues, thereby, playing an integral role in medicine by facilitating healing and restoring functions in a disease [1,2]. More than three thousand products of wound dressings are commercially available; however, the availability and suitability of an efficient and enhanced wound repair system is still a challenge [3]. Constant efforts in developing a holistic approach to wound management have resulted in the development of sustainable and advanced dressings for diabetic wound healing, including ConvaMax® superabsorber [4], EpiCord® expandable placental allograft [5], Aquacel Ag® hydrofiber [6] etc. Diabetic wound is the most devastating complication in patients suffering from diabetes mellitus and is characterized by poor wound healing due to impaired vasculization, improper re-epithelialization and chronic inflammation; diabetic wounds develop more serious sustained infections in contrast to the non-diabetic wounds [7]. In medical practice, cellulose gauzes, absorbent/surgical cotton, and bandages are common wound dressings that are cost-effective but have limited benefits due to their dryness and lack of medication. Such dressings are unable to impart the moist and active environment required for wound healing, and they do not protect the wound from infection [8]. Therefore, wound dressings with improved antimicrobial properties would be of great utility. The structural stability and cellular regeneration ability of biomaterial-based scaffolds mimic native extracellular matrix tissue in functionality and the porous nature of these scaffolds provides the desired moist environment for wound healing [9]. It is critical to select the right material(s) for building perfect 3D biocomposite scaffolds that would aid in cell-cell adhesion, cellular growth and migration, and enhance vasculature and re-epithelialization process [10]. The scaffolds prepared from single component system may not be able to impart all the desired properties, however, blending of two or more biopolymers may compensate for the deficiencies of single-component systems and result in a binary composite system that possesses excellent desired characteristics.

Cellulose and chitosan are the most abundant natural materials with diverse complexity and composition. Chitosan is an extensively investigated material for wound healing applications due to its biodegradability, biocompatibility, and safety along with antimicrobial, anti-inflammatory...
and antioxidant properties [11]. It facilitates rapid wound healing by allowing 3-D growth and proliferation of cells and collagen deposition, while its polycationic structure makes it an effective antimicrobial candidate [12]. However, chitosan-based scaffolds have limited clinical applications due to weak mechanical strength. This drawback may be overcome by forming polymer blends having compatible physicochemical attributes [13]. The blending of hydrogel-based scaffold materials using methylcellulose and chitosan would be favourable due to their structural and functional similarities with the body tissues, biocompatibility, low immunogenicity, less toxicity, and easy availability [14]. Such hybrid systems may be endowed with enhanced antibacterial properties to curb infection at the chronic wound site [15]. Methyl cellulose is a cellulose derivative containing methyl groups attached to the hydroxyl residues of the linear cellulose backbone [16]. Methylcellulose biopolymer is hydrophilic, biodegradable and exhibit excellent film-making properties for wound dressing applications [17]. Methyl cellulose can form miscible blends with Chitosan and these are compatible with curcumin [18]. Curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dion) is an active component found in the creeping rootstalk of Curcuma longa [19]. It is a yellow hydrophobic polyphenolic compound that exhibits excellent therapeutic efficacy due to potent pharmacological properties such as antibacterial, antiviral, and anti-inflammatory activities, however, it suffers from limitations such as hydrophobicity and instability in the biological systems [20, 21].

In the present study, we have reported curcumin deposited 3D bio composite scaffolds based on chitosan and methylcellulose with enhanced antimicrobial and wound healing efficiency. The low mechanical strength of chitosan limits its uses as a haemostatic and wound-healing agent. Therefore, various concentrations of biopolymers were used to fabricate the optimized three-dimensional bio composite scaffolds. On the other hand, use of curcumin in scaffold based systems results in rapid wound closure with symmetric granulation tissue formation through fibroblast proliferation, collagen deposition, and quick re-epithelialization [47]. Consequently, chitosan and methylcellulose blends, in combination with bioactive curcumin, were prepared that bestowed the resulting bio composite scaffolds with improved mechanical, chemical, and anti-microbial properties desirable in the management of chronic diabetic wounds.

2. Materials and methods

Low viscosity chitosan (from shrimp shells), dimethyl sulfoxide (DMSO), streptozotocin and curcumin powder were purchased from Sigma-Aldrich, USA. Methylcellulose was procured from Central Drug House, Mumbai, India. Calcium chloride salt, sulfuric acid and, glacial acetic acid were procured from the commercial suppliers and utilized without further purification.

2.1. Preparation of chitosan/methylcellulose hydrogels

Chitosan (0.5%, 1.0%, 1.5% and 2.0% w/w) and methylcellulose (0.5%, 1.0%, 1.5% and 2.0% w/w) were dissolved separately in 1% w/v glacial acetic acid and 1% w/v sulfuric acid solution, respectively. Each reaction mixture was stirred overnight and poured into spherical-shaped biocomposite scaffolds. The petri dishes were placed at -35 °C for 6 h to allow pre-freezing followed by freeze drying using Labconco Freeze Dryer, Fort Scott, USA, separately in triplicates. Scaffold materials thus obtained (SCF-1 to SCF-13) were stored in the fridge till further use.

2.2. Development of 3D chitosan/methylcellulose biocomposite scaffolds

Polymeric hydrogels were used for the synthesis of biocomposite scaffolds using freeze-drying method as described previously [9]. Briefly, the prefixed concentration of biopolymeric hydrogels based on chitosan and methylcellulose were mixed separately under constant magnetic stirring of 300 rpm overnight at room temperature as given in Table 1. Each reaction mixture was stirred overnight and poured into spherical polypropylene petri dishes of 35 mm diameter to achieve spherical-shaped biocomposite scaffolds. The petri dishes were placed at -35 °C for 6 h to allow pre-freezing followed by freeze-drying using Labconco Freeze Dryer, Fort Scott, USA, separately in triplicates. Scaffold materials thus obtained (SCF-1 to SCF-13) were stored in the fridge till further use.

2.3. Surface deposition of curcumin

Curcumin solution was prepared by dissolving 2% w/v of curcumin powder in DMSO and used for the surface deposition of the scaffolds materials. Briefly, 400 mg of curcumin powder was dissolved in 20 mL of DMSO at 37 °C by gentle agitation for 10 min to obtain a 2 % (w/v) curcumin solution, after which it was passed through 0.2 μ syringe filter. With the help of Holmarc’s Spray Pyrolysis equipment (Holmarc Mechatronics Model No: HO-TH-04C3), curcumin solution was deposited over the surface of each biocomposite scaffold material at a stream rate of 1 mL min⁻¹ and pneumatic force of 40 lb/in² for 5 min as given in Table 1. A thin layer of curcumin covered the scaffolds surface after which the coated materials were removed and kept inside oven at 60 °C for 24 h. Scaffold samples thus prepared (SCF-1C to SCF-7C) were stored at 4 °C for later usage.

2.4. Physicochemical and morphological characterization

2.4.1. Physical assessment

Curcumin surface deposited biocomposite scaffolds based on chitosan and methylcellulose were carefully inspected for the physical parameters such as color, texture and surface integrity. The dimensions of all scaffolds were measured with a 0–150 mm digital vernier micrometer and the data values were calculated as statistical mean (Table 1).

2.4.2. Water retention ability

The water retention ability (WRA) of the scaffolds, with and without curcumin deposition, was measured using modified gravimetry approach. Scaffolds were dried in oven, weighed and placed separately on circular Grade 1:11 film (medium flow filter paper). Phosphate buffered saline (pH 7.4) was added drop-wise at 25 °C till flooding commenced. The pre- and post-investigation weights of the scaffolds with filter paper were measured. WRA was evaluated using the general formula:

\[
WRA = \left( \frac{W_d - W_\text{w}}{W_\text{d}} \right) \times 100
\]

where \(W_d\) represents the weight of dried scaffold and \(W_\text{w}\) represents the weight of scaffold after saline uptake.

2.4.3. Thermal analysis by differential scanning calorimetry and thermogravimetry

Biocomposite scaffolds, with and without curcumin surface deposition, were evaluated for thermal stability using Differential Scanning Calorimeter (DSC 6000 Perkin Elmer, Massachusetts, USA). Precisely weighed scaffold samples (5 ± 0.30 mg) were secured separately in the aluminium pan. The heat flow across the samples was set at the rate of 20 °C min⁻¹ in the temperature range of –50 °C–300 °C under constant nitrogen supply to obtain the respective DSC curves. On the other hand, the pyrolytic pattern (weight variation with respect to temperature) of 3D biocomposite scaffolds was assessed by thermogravimetric analysis (TGA) using Thermogravimetric Analyzer (TGA 4000, PerkinElmer, Massachusetts, USA). Precisely weighed samples (5 ± 0.3 mg) were retained in the TGA pan placed over the furnace at a heating rate of 20 °C.
Table 1. Various concentration of biopolymers in scaffolds with physical properties.

| Scaffold Code | Chitosan, CS % (w/w) | Methyl Cellulose, MC % (w/v) | Ratio of CS:MC (v/v) | Physical properties of scaffolds |
|---------------|----------------------|-----------------------------|---------------------|---------------------------------|
| SCF-1         | 2                    | 2                           | 1:1                 | Homogeneous, spongy, good mechanical strength, rough surface, highly porous. |
| SCF-2         | 2                    | 2                           | 1:2                 | Homogeneous, highly porous, smooth surface, good mechanical strength, with elastic in nature. |
| SCF-3         | 2                    | 2                           | 1:3                 | Good mechanical strength, smooth surface, highly porous, no cracks, white. |
| SCF-4         | 2                    | 2                           | 3:1                 | Homogeneous, very good mechanical strength, smooth surface, highly porous |
| SCF-5         | 2                    | 2                           | 2:1                 | Homogeneous, very good mechanical strength, smooth surface, highly porous, Brittle in nature |
| SCF-6         | 2                    | 0                           | 1:0                 | Spongy in nature, highly porous, yellow in nature. |
| SCF-7         | 2                    | 1                           | 1:0                 | Homogeneous, very good mechanical strength, brittle in nature, highly porous, white. |
| SCF-8         | 1.5                  |                             |                     | Spongy in nature, highly porous, brittle surface, elastic, white |
| SCF-9         | 1                    |                             |                     | Homogenous, highly elastic, highly porous, white, Uneven surface. |
| SCF-10        | 0.5                  |                             |                     | Floppy in nature, white, highly porous, poor mechanical strength, elastic in nature, poor mechanical strength |
| SCF-11        | 1.5                  | 1.5                         | 1:1                 | Homogenous, poor mechanical strength, hard in nature, uneven surface, porous |
| SCF-12        | 1                    | 1                           | 1:1                 | Uneven surface, Spongy in nature, highly porous, white, poor mechanical strength |
| SCF-13        | 0.5                  | 0.5                         | 1:1                 | Uneven surface, Spongy in nature, Highly porous, White, Poor mechanical strength |
| SCF-1C        | 2                    | 2                           | 1:1                 | Homogenous, spongy, poor mechanical strength, rough surface, highly porous, pale yellow in colour |
| SCF-2C        | 2                    | 2                           | 1:2                 | Homogenous, highly porous, smooth surface, poor mechanical strength, bright yellow in colour. |
| SCF-3C        | 2                    | 2                           | 1:3                 | Good mechanical strength, rough surface, highly porous, creaks, yellow. |
| SCF-4C        | 2                    | 2                           | 3:1                 | Homogeneous, good mechanical strength, Smooth surface, highly porous, bright yellow in colour. |

Table 1 (continued)

| Scaffold Code | Chitosan, CS % (w/w) | Methyl Cellulose, MC % (w/w) | Ratio of CS:MC (v/v) | Physical properties of scaffolds |
|---------------|----------------------|-----------------------------|---------------------|---------------------------------|
| SCF-5C        | 2                    | 2                           | 2:1                 | Homogeneous, very good mechanical strength, smooth surface, highly porous with crack, Brittle in nature |
| SCF-6C        | 2                    | 0                           | 1:0                 | Smooth surface, spongy in nature, good mechanical strength, highly porous, yellow colour. |
| SCF-7C        | 2                    |                             | 1:0                 | Homogeneous, poor mechanical strength, brittle in nature, highly porous, yellow colour. |

The mechanical strength of SCF-1, SCF-4, SCF-4C and SCF-7 (uniform size of diameter 30 mm x thickness 3.7 mm) was analyzed using uniaxial compression assay. The compression strength and compressive modulus were observed at a crosshead speed of 1.33 mm min⁻¹ equipped with Load Cell 3-ton Universal Testing Machine (UTM, International equipment, Mumbai, India).

2.4.4. Mechanical Strength Measurement.

2.4.5. Identification of chemical bonds by Fourier transform infrared spectroscopy

Table 2. Comparative thermogram of each scaffold

The molecular interactions between polymeric materials (with and without curcumin deposition), were evaluated using ‘Field Emission-Scanning Electron Microscopy (FE-SEM)’ (MIRA3 TESCAN, Brno, and Czechia). A tiny amount (5.0 ± 0.2 mm²) of each of the 3D biocomposite scaffolds were recorded for the systematic analysis of selected functional groups and bonding between the blended polymers using Spectrum Two spectrophotometer (PerkinElmer, Massachusetts, USA) in the transmittance mode.

2.4.6. Evaluation of microscopic structure

The surface appearance and topography of biocomposite scaffolds, with and without curcumin deposition, were evaluated using ‘Field Emission-Scanning Electron Microscopy (FE-SEM)’ (GE- Healthcare, Uppsala, Sweden). The association/dissociation assay was done using an in-house method developed in INMAS, DRDO, Delhi using biosensor chip CM-5 (carboxymethylated dextran coated gold surface) to allow covalent coupling of the functional groups of biomolecules with the sensor surface. The sensor chip consists of four flow cells for analysis [22]. After docking the sensor chip into the instrument, baseline stability was achieved by sequential interactive association and dissociation for 500 s and 600 s, respectively. Complete dissociation was followed by regeneration of the surface for 12 s. The amine coupling reaction with carboxymethyl groups present on the surface of CM-5 chip was done using NHS/EDS method. Injection of 10 mL of 115 mg mL⁻¹ of N-hydroxysuccinimide and 750 mg mL⁻¹ of

2.5. In-vitro evaluation of biological properties

2.5.1. Surface Plasmon Resonance Based Assay

The mechanical strength of scaffolds was measured using the mechanical strength of scaffold [23]. A steady flow rate of 20 mL min⁻¹. Comparative thermogram of each sample was recorded and analysed.

2.4.4. Mechanical Strength Measurement.

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2.4.5. Identification of chemical bonds by Fourier transform infrared spectroscopy

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) of chitosan, methylcellulose, curcumin and each of the 3D biocomposite scaffolds were recorded for the systematic analysis of selected functional groups and bonding between the blended polymers using Spectrum Two spectrophotometer (PerkinElmer, Massachusetts, USA) in the transmission mode.

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The surface appearance and topography of biocomposite scaffolds, with and without curcumin deposition, were evaluated using ‘Field Emission-Scanning Electron Microscopy (FE-SEM)’ (MIRA3 TESCAN, Brno, and Czechia). A tiny amount (5.0 ± 0.2 mm²) of each of the 3D biocomposite scaffold was dried for 8 h at 60 °C and fixed on a stub with double-sided carbon adhesive tape. Under reduced pressure conditions, a thin layer of gold particles was sputter-coated over the dried samples and images were recorded at 5 kV accelerating voltage.

2.5. In-vitro evaluation of biological properties

2.5.1. Surface Plasmon Resonance Based Assay

The molecular interactions between polymeric materials (with and without curcumin) and the erythrocytes were measured using highly sensitive ‘Biacore T-200 system’ (GE- Healthcare, Uppsala, Sweden). The association/dissociation assay was done using an in-house method developed in INMAS, DRDO, Delhi using biosensor chip CM-5 (carboxymethylated dextran coated gold surface) to allow covalent coupling of the functional groups of biomolecules with the sensor surface. The sensor chip consists of four flow cells for analysis [22]. After docking the sensor chip into the instrument, baseline stability was achieved by sequential interactive association and dissociation for 500 s and 600 s, respectively. Complete dissociation was followed by regeneration of the surface for 12 s. The amine coupling reaction with carboxymethyl groups present on the surface of CM-5 chip was done using NHS/EDS method. Injection of 10 mL of 115 mg mL⁻¹ of N-hydroxysuccinimide and 750 mg mL⁻¹ of

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N-ethyl-N-dimethylaminopropyl carbodiimide, in equivalent volumes, was used to activate the chip surface. An injection of ‘chitosan-methylcellulose mixture only’ and ‘chitosan-methylcellulose mixture with curcumin coating (SCF-4 and SCF-4C),’ was administered at constant flow rate of 0.5 mL min⁻¹ 5 mL aliquot (0.5 mg mL⁻¹) of SCF-4, SCF-4C (curcumin-coated) biocomposite scaffolds and the curcumin were immobilized on the surface of the flow cell number 2, 3 and 4, respectively. The untreated flow cell number 1 was chosen as control after being treated with 15 mL of ethanolamine at a flow rate of 0.5 mL min⁻¹ to block the extran surface. The four flow cells were all constructed in the same way and used for the scaffold interaction investigation at the same time [23]. After surface activation, the erythrocytes were isolated from Sprague Dawley rat blood using standard centrifugal sedimentation method and counted using eucount+3 (Medsecure Ozone, Biomedical Pvt. Ltd. India). Erythrocytes were suspended into phosphate buffered saline (pH 7.4) at a concentration of 5.0 \times 10⁶ cells/mL for 60 s and injected individually into all four flow cells at a flow rate of 3 μL min⁻¹ for 180 s. Four different dilution concentrations (10X, 20X, 30X and 40X) of erythrocytes were analyzed separately in each of the flow cells to evaluate concentration dependent binding pattern. Association was allowed till 2 min followed by 3 min of dissociation. The cycle of erythrocyte interaction was repeated thrice to achieve threshold binding. The response values were subtracted from blank interaction was repeated thrice to achieve threshold binding. The respective scaffolds. These bacteria frequently dominate the diabetic wound site. The antimicrobial susceptibility assay was performed by disc diffusion assay [26].

2.5.7. Whole blood clotting

Blood clotting analysis was done using borosilicate micro-capillaries (n = 36, Premier Industrial Corporation, Delhi, India). Six different groups of micro-capillaries were formed, each consisting of six capillaries (n = 6), and the inside wall of each capillary was surface-coated using a 1 mL syringe injection. Group 1 capillaries were left uncoated and used as control, while Group 2, 3, 4, 5, and 6 capillaries were coated with 2% w/w solution of SCF-1, SCF-3, SCF-4, SCF-4C and the curcumin respectively [27].

2.5.8. Bleeding time determination

SCF-1, SCF-2, SCF-3, SCF-4, SCF-5, SCF-6, and SCF-7 bio-composite scaffolds, along with their curcumin-coated counterparts, were used for the study. To test the bleeding time of the bio-composite scaffolds, ninety Sprague Dawley rats were confined into cages and separated into fifteen groups (n = 6 each). The common circular filter paper was taken as control. Prior to the procedure, experimental animals were anesthetized using intraperitoneal ketamine-hydrochloride injection (20 mg kg⁻¹ body weight). The coated glass capillary tubes were inserted medially into the retro-orbital vein of the rats. The capillary action of the tubes allowed blood to fill till it reached the terminal end. The chronometer was started as soon as the blood made first contact with the glass capillary tube. The capillaries packed with blood were broken into small bits after every 20 s and blood clotting time was measured as the time-point where a fine thread of clotted blood could be witnessed.

2.5.9. Streptozotocin (STZ)-Induced diabetic rat model

The study employed adult male Sprague Dawley rats weighing 250–350 g. The animals were housed in a controlled environment with...
temperature $25 \pm 2\, ^\circ\text{C}$, humidity $55 \pm 10\%$, 12 h light and dark cycle and supplied with feed and water free choice. After an overnight fasting, the animals ($n = 12$) were injected intraperitoneally with a dose of 50 mg kg$^{-1}$ streptozotocin (STZ). The blood glucose level of animals was measured after three days of STZ injection using glucometer (Accu-Chek Advantage, Roche Diagnostics GmbH, Germany). For the next seven days, animals with elevated blood glucose levels ($\geq 300$ mg/dL) were kept under observation and only those animals, which demonstrated consistency in elevated blood glucose levels were selected for further studies.

2.5.10. Evaluation of wound size

A total of thirty-six male Sprague Dawley rats ($n = 12$ diabetes-induced and $n = 24$ normal) were chosen at random and placed into six groups ($n = 6$ each). Diabetes-induced rats in Groups 1 and 6 were found to possess blood glucose levels more than 300 mg/dL$^{-1}$. Prior to the procedure, all animals were administered with a 20 mg kg$^{-1}$ intra-muscular dose of ketamine and xylazine injection as general anesthesia. The animal dorsal surface was shaved, and the underlying skin was cleaned with 70% ethanol. The punch-biopsy procedure was used to create a full thickness excisional wound of eight millimeters on the dorsal thoraco-lumbar region. Group 1 (diabetes-induced) and Group 2 (normal) animals were taken as control and did not receive any treatment. Group 3 and 4 animals were treated with SCF-1 and SCF-4, respectively, while both Group 5 and 6 animals were treated with SCF-4C biocomposite scaffolds. Excision wound area of each group was elected with respective 3D biocomposite scaffolds and fixed with sutures. The scaffold materials were replaced every alternate day till complete healing was achieved. A digital vernier caliper was used to measure the wound contraction. To compute the percent wound area reduction, the area of the wound on day 0 was considered to be 99.99 percent. The following equation was used to calculate the percentage reduction of wound area.

$$\text{Percent Wound contraction} = \text{Wound area healed}/\text{Total wound area} \times 100(3)$$

2.6. Evaluation of biological safety

2.6.1. Acute dermal irritation study

The acute dermal irritation study was performed in accordance with the Revised Organization for Economic Cooperation and Development (OECD) guidelines 404 and the wound site was examined for erythema and oedema symptoms if any, for up to 24 h [28]. Healthy and faultless skinned experimental animals were housed under standard environmental conditions with free access to fresh water and feed. Prior to the treatment, body hairs were shaved from the dorsal surface and the skin was disinfected using 70% ethanol. SCF-1, SCF-4 and SCF-4C scaffolds were applied on the skin surface and a patch test was performed on each of the six animals, encompassing a 3 cm$^2$ zone on the dorsal body surface.

2.6.2. Histopathology

The rats were sacrificed in accordance with the ‘AVMA guidelines for euthanasia of animals’ [29]. Autopsy samples were excised from the wound site of rat skin by punch biopsy on days 0, 7 and 14, and fixed in 10% formalin solution for histopathology investigation. For dehydration, the collected samples were rinsed with tap water and treated with repeated dilutions of alcohol. The samples were rinsed in xylene and fixed in paraffin for 24 h at 65°C in a hot air oven. The histopathological examination of the skin tissues of animals applied with SCF-1, SCF-4, and SCF-4C biocomposite scaffolds, were observed using Olympus BX 60 microscope.

2.6.3. Data analysis

One-way analysis of variance (ANOVA) was used to analyze the data and communicated as mean $\pm$ standard deviation. All experiments were done in triplicates. The measurable importance of the difference between the results was assessed using an ‘unpaired t-test’.

3. Results and discussion

3.1. Preparation of chitosan/methylcellulose hydrogels

Porous and biodegradable scaffold materials were successfully developed using a combination of natural polymers, chitosan and methylcellulose, endowed with a surface coating of antibacterial curcumin. The porous structure of methylcellulose permits infusion and carries extremely fine 3D network having reversible hydrogen bonding, in addition to unique structural, physico-chemical, mechanical, and biological properties [30, 31]. On the other hand, chitosan polymer derived from naturally occurring chitin possesses unique poly-cationic, antimicrobial and chelating activity along with diverse stimulating/inhibiting activities that promote wound healing [32]. The polymeric hydrogels synthesized using 0.5%, 1.0%, 1.5% and 2% chitosan were homogenous, translucent, yellowish in color and moderately viscous. The viscosity of the polymeric hydrogels was observed to rise as the concentration of chitosan increased. 0.5% chitosan hydrogel was almost transparent and homogeneous, while 2% hydrogel was homogeneous, translucent and yellowish with moderate viscosity. Similarly, polymeric hydrogels synthesized using 0.5%, 1.0%, 1.5% and 2% methyl cellulose were homogenous and transparent and exhibited an increase in viscosity with increase in the concentration of polymer due to increase in the intermolecular hydrogen bonding as given in Table 1.

3.2. Development of 3D chitosan/methylcellulose biocomposite scaffolds

Varying ratios of biopolymeric hydrogels were used for the fabrication of thirteen different combinations of 3D biocomposite scaffolds as given in Table 1. Biocomposite scaffolds synthesised using ‘methyl cellulose alone’ (SCF-6, SCF-8, SCF-9 and SCF-10) were highly porous, bright white in colour, spongy and floppy with poor mechanical strength. These scaffolds were very light-weight due to low polymeric density and broke into powdery pieces on application of slight pressure. Similarly, the scaffolds SCF-11, SCF-12 and SCF-13 synthesised using 0.5%–1.5% polymer concentration were light-weight, highly porous, possessed uneven heterogeneous surface with spongy texture and poor mechanical strength. Cracks were visible on the scaffold surface on application of slight mechanical pressure, and therefore, the above scaffolds were found unsuitable for the wound healing application. SCF-2 and SCF-3 were homogeneous, highly porous with good mechanical strength and smooth surface. These scaffolds were fragile and broke into small pieces on application of slight pressure probably due to higher concentration of methyl cellulose and lesser intermolecular hydrogen bonding. SCF-5 showed homogeneous, highly porous surface with optimal mechanical strength, however, after 24 h of drying, cracks emerged on the surface. On the other hand, SCF-1, SCF-4 and SCF-7 showed optimal mechanical strength, homogeneous smooth surface with satisfactory texture, uniformity and stability and were therefore, found to be suitable for further studies related to wound healing.

3.3. Surface deposition of curcumin on biocomposite scaffolds

Biological performance of biocomposite scaffolds was improved using surface deposition of curcumin considering its unique properties such as wound healing, anti-oxidant, anti-inflammatory, and anti-bacterial activities [33, 34]. Spray pyrolysis is one of the most widely used method for the surface coating due to its low cost, ease of handling, and provision to form uniform thin film deposit over the surface. Curcumin aerosol was deposited over the surface of SCF-1, SCF-4 and SCF-7 biocomposite scaffolds using spray pyrolysis method. The coated scaffolds were coded as SCF-1C, SCF-4C and SCF-7C and showed an improvement in the mechanical properties during physical assessment. Curcumin-coated scaffolds were amber colored with improved smooth surface texture. However, slight shrinkage was found at the surface of curcumin-coated scaffolds, which was most likely owing to an increase in moisture content.
3.4. Water retention ability

Absorption of liquid exudate and maintenance of moist environment at the wound-site plays a pivotal role in wound healing and this may be confirmed experimentally by the measurement of water retention ability [35]. An ideal wound healing system must have superior fluid retention ability. Hydrophilic nature of chitosan and methyl cellulose and their capability to maintain a 3D structure resulted in enhanced swelling capabilities of the biocomposite scaffolds. The water retention ability (WRA) of seven different types of 3D biocomposite scaffolds (SCF-1, SCF-2, SCF-3, SCF-4, SCF-4C, SCF-6 and SCF-7) was evaluated as given in Figure 1. An increase in methyl cellulose concentration resulted in decrease in the degree of water retention as observed in SCF-1 to SCF-3. SCF-6 showed the lowest WRA of ~900, while the highest ~5400 was noticed in case of SCF-7 scaffolds. Biocomposite scaffold SCF-4 showed WRA of ~5000, which reduced to ~4800 in case of SCF-4C probably due to moisture retention and surface pore shrinkage after curcumin deposition. P-value of <0.001 was observed in all scaffolds.

3.5. Thermal analysis by differential scanning calorimetry and thermogravimetry

The DSC thermogram of methylcellulose, chitosan, and curcumin exhibited initial endothermic peaks at 71.90 °C, 50.98 °C and 183.78 °C, respectively, as shown in Figure 2. Single and sharp endothermic peak of curcumin emerged at 183.78 °C depicting the melting point of curcumin, while the endothermic peaks of methyl cellulose and chitosan may be attributed to the loss of water often termed as dehydration temperature, and the interaction between the hydrophilic moieties due to hydrophilicity of the polymer systems [36, 42, 43]. SCF-1, SCF-4, SCF-4C and SCF-7 showed primary endothermic peaks at 70.60 °C, 70.02 °C, 64.58 °C and 73.72 °C respectively, which confirms the retention of moisture content in the scaffold samples due to hydrophilicity and is in good agreement with water retention ability measurement. The melting point of curcumin was observed at ~ 172.6 °C as previously reported, while the same melting peak was not observed in case of SCF-4C probably due to very less amount of curcumin used in the coating [44]. Another substantial peak in the SCF-1 and SCF-7 thermogram was observed at 162.61 °C and 240.54 °C, respectively that indicates degradation due to dehydration and depolymerization. SCF-4 and SCF-4C showed the endothermic peak at 140.68 °C, while notable difference was observed in the peak intensity after curcumin coating, which confirmed the aerosol deposition of curcumin solution. Peak-shift in case of SCF-4 may be attributed to the fusion of crystalline regions of methylcellulose. In particular, the thermal and molecular stability of the polymeric components used to fabricate the biocomposite scaffolds was established using DSC.

On the other hand, thermogravimetric measurement confirmed the thermal and oxidative stability, kinetics, and shelf life of the scaffold materials. TGA of 3D biocomposite scaffolds in comparison to bio-polymers and curcumin was evaluated to observe weight variation in the temperature range of 50–800 °C as shown in Figure 3. TGA thermogram depict an initial mass-lose stage corresponding to loss of water coupled to the polymeric hydroxyl groups. Maximum weight loss of 9.89% was observed in case of chitosan, while minimum (1.91%) was observed in case of curcumin. The weight loss of 4.2% in case of methylcellulose may be ascribed to the physically bound water corresponding to reduced number of hydroxyl groups available to interact with the water molecules in the respective samples [37]. In case of SCF-1, SCF-4, SCF-4C and SCF-7, the biocomposite scaffolds showed weight reduction of 12.02%, 7.25%, 15.4% and 5.74% respectively, which is due to moisture loss and change in the shape of macromolecules in the internal molecular disposition because of internal crosslink infractions in the range of 80 °C–150 °C. Additionally, these results confirm higher moisture retention by SCF-4C, as predicted in the water retention ability study also. Another major weight reduction of chitosan, methylcellulose and curcumin with weight loss of 72.48%, 54.28% and 85.67% respectively, occurred in the range of 250–450 °C. On the other hand, secondary major degradation of SCF-1, SCF-4, SCF-4C and SCF-7 scaffolds with weight loss of 67.19%, 56.64%, 58.66 and 34.07% respectively, occurred in the range of 250–450 °C. Thermal disintegration of biopolymeric groups, pyrolysis of polysaccharides, and breakage of glycosidic linkages followed by breakdown are all possible causes of weight loss at this stage [38]. Increase in the thermal stability with increase in the concentration of chitosan in the biocomposite scaffolds suggests conformational changes in the side alkyl chains of the polymer and this increase in crosslinking caused an elevation in the thermal stability.

3.6. Mechanical strength measurement

Highest and lowest compression modulus of 39.25 MPa and 12.24 MPa were observed in case of SCF-7 and SCF-1 respectively, while in case of coated scaffolds, the SCF-4 and SCF-4C depicted compression modulus of 16.83 MPa and 19.61 MPa, respectively [45]. The compression strength test of biocomposite scaffolds suggested little or no particular change with coating probably due to highly porous structure. Increase in the compression modulus of SCF-4C in comparison to SCF-4 confirmed the increase in mechanical strength of the scaffold after curcumin coating. Higher mechanical strength of SCF-7 also established that

![Figure 1](image-url)  
*Figure 1. Water holding capacity (WHC) of various biocomposite scaffolds (SCF-1, SCF-2, SCF-4, SCF-4C, SCF-6 and SCF-7). Statistical analysis by ANOVA and unpaired student t-test, p value <0.0005.*
Figure 2. Differential scanning calorimetry (DSC) thermograms of chitosan powder, methyl cellulose, SCF-1, SCF-4, SCF-4C and SCF-7.

Figure 3. Thermal gravimetric analysis (TGA) curves of chitosan powder, methyl cellulose, SCF-1, SCF-4, SCF-4C and SCF-7.

Figure 4. Fourier transform infrared spectra of chitosan powder, methyl cellulose, SCF-1, SCF-4, SCF-4C and SCF-7 scaffolds.
interaction occurred between the chitosan molecules in presence of calcium ions \[46\].

3.7. Identification of chemical bonds by Fourier transform infrared spectroscopy

Biocomposite scaffolds (with and without curcumin coating) were evaluated for the chemical integrity using ATR-FTIR as shown in Figure 4. Chitosan, methylcellulose and curcumin spectra demonstrated chemical integration in the biocomposite scaffolds in the respective samples. Spectra of SCF-1 and SCF-7 biocomposite scaffolds showed absorption band at \(~3254\) cm\(^{-1}\) for O–H intermolecular stretching with higher band intensity in comparison to chitosan, probably due to the presence of some moisture representing additional hydroxyl groups. Increased peak intensity of C=O at \(~1610\) cm\(^{-1}\) in case of SCF-7 compared to SCF-1 was also observed due to higher molar ratio of chitosan in case of SCF-7. On the other hand, SCF-4 showed almost similar spectra as of SCF-7 due to high molar ratio of chitosan compared to the methylcellulose, while in case of SCF-4C, due to overlapping of curcumin with significant C=O stretching vibration of the skeleton, the higher intensity peak shifted to \(~1580\) cm\(^{-1}\) in the shape of a broad band. Further, SCF-4C showed a broad band at \(~3300\) cm\(^{-1}\) due to hydroxyl groups of methylcellulose and retention of the moisture content during process of curcumin surface deposition. FTIR spectra of SCF-1, SCF-4, SCF-4C and SCF-7 biocomposite scaffolds revealed intactness of all the characteristic peaks of biopolymers and confirmed their stability before and after curcumin coating.

3.8. Evaluation of microscopic structure

FE-SEM micrographs of biocomposite scaffolds (Figure 5) demonstrated the 3D structure, multilayer porosity and mesh-like structure with surface coating. SCF-1 showed \(~50\) μm groove-like surface morphology with median pore-size values ranging from 100 to 200 μm along with particulate deposition over the surface of SCF-1C. Cross-sectional view corresponding to SCF-4 showed three-dimensional uniformity with the pore size of 50–100 μm and presence of consistent grooves over the surface, while uniform surface coating was observed over the surface of SCF-4C with almost no cracks after drying. Highly regularized and homogenous grooves were observed in case of SCF-7 compared to multiple cracks and biopolymeric pilling over the surface in case of SCF-7C scaffolds probably due to higher density, crosslinking and brittle nature. As shown in Figure 5, SCF-10 scaffolds showed massive porosity (\(~200–500\) μm) with interconnecting fibril-like structures having \(~3\) μm diameter, which also confirmed the spongy and flabby nature of the scaffold probably due to less biopolymeric density. Surface morphology of SCF-12 showed uniform groove like structure with \(~100–150\) μm size and fissures over the surface. Surface morphology analysis by FE-SEM confirmed the surface uniformity, multilayer porosity, texture variation with polymeric density/surface coating and stability of the prepared 3D biocomposite scaffolds.

3.9. In-vitro evaluation of biological properties

3.9.1. Surface Plasmon Resonance Based Assay.

The association/dissociation interaction between erythrocytes and biocomposite scaffolds SCF-4, SCF-4C and curcumin were studied using ‘surface plasmon resonance (SPR) assay’, as given in Figure 6. Interaction analysis between rat erythrocytes with curcumin showed the least response unit of only \(~3.5\) RU independent of the erythrocyte concentration. It was also observed that SCF-4C biocomposite scaffold showed the response unit of \(~5\) RU only, which increased to \(~6\) RU in case of SCF-4 scaffolds. While in case of control (FC-1 flow cell), highest

Figure 5. Scanning electron microscopy indicating microstructure of scaffolds (SCF-1, SCF-4, SCF-7 and SCF-10) and particulate deposition over the surface on SCF-1C, SCF-4C, SCF-7C and SCF-10C
response unit ~10 RU was observed. It may be probably due to non-availability of surface activated groups with curcumin and methylcellulose surface inhibiting the binding of the erythrocytes with the surface. On the other hand, erythrocyte concentration independent interaction analysis also suggested probable blockage in the interaction. A higher response unit in case of control flow cell suggests that erythrocytes have

![Surface plasmon resonance analysis of erythrocyte interaction with scaffolds and curcumin.](image)

**Figure 6.** Surface plasmon resonance analysis of erythrocyte interaction with scaffolds and curcumin.

| Samples          | *K+ | **K- | SCF-1 | SCF-1C | SCF-4 | SCF-4C | SCF-7 | SCF-7C |
|------------------|-----|------|-------|--------|-------|--------|-------|--------|
| Hemagglutination index | ++++ | - | ++ | + | +++ | ++ | - | +++ |

*Human erythrocyte in different scaffolds*

*Positive control: RBC & agglutinating serum  
Negative control: RBC & saline solution*

![Hemagglutination assay](image)

**Figure 7.** (a) Hemagglutination assay of erythrocytes in different scaffold solutions (b) Comparative analysis of blood sorption between varying ratio of methyl cellulose and chitosan coated with curcumin.
better interaction with the uncoated surface in comparison to the flow cells immobilized with chitosan, cellulose or curcumin probably owing to the active site blockage or molecular hindrance. Decrease in response unit in case of SCF-4C in comparison to SCF-4 confirmed that curcumin inhibits erythrocyte binding with the coated scaffolds probably due to the hydrophobic nature of curcumin molecules resulting in reduced interaction with the erythrocytes. On the other hand, SPR analysis also confirmed saturation of erythrocyte binding at very low concentrations resulting in erythrocyte concentration independent interaction after further increase in the concentration of erythrocytes.

3.9.2. Evaluation of hemagglutination activity
Stavitsky scale was used to calculate the hemagglutination index of SCF-1, SCF-1C, SCF-4, SCF-4C, SCF-7 and SCF-7C biocomposite scaffolds, as illustrated in Figure 7a. Agglutination of erythrocytes was visible in the samples incubated with SCF-1, SCF-1C, SCF-4, SCF-4C and SCF-7C with hemagglutination index of (++), (+), (+++), (+) and (+++), respectively. SCF-7C and SCF-4 showed complete hemagglutination index, whereas SCF-7 displayed immediate hemagglutination most likely owing to the presence of active moieties on the chitosan surface that interact with the erythrocytes. Biocomposite scaffolds containing both methylcellulose and curcumin showed delayed hemagglutination probably due to the lack of fewer surface active moieties, as also observed in SPR analysis.

3.9.3. Blood absorption
SCF-4C showed maximum blood absorption of ~700%, while lowest ~400% was observed in case of SCF-7, probably due to the surface hemostasis in case of SCF-7, which prevent the blood to enter inside the scaffold lumen. In contrast, SCF-1C, SCF-4, and SCF-7C scaffolds exhibited ~650%, 580% and 620% of blood absorption, respectively, as shown in Figure 7b. The study confirmed that methylcellulose and curcumin play negative role in hemostasis, while pose a positive impact on the moisture retention, which is highly crucial for wound healing. P-value of <0.0001 was observed in each scaffold.

### Table 2. ‘Blood clotting time’ evaluation of scaffold materials (with reference to control, a ‘p’-value of <0.0001 was found to be extremely significant).

| Details of coated materials | Time taken to complete clotting (sec) | ‘p’-value wrt control |
|----------------------------|--------------------------------------|----------------------|
| Control                    | 90 ± 5                               | -                    |
| 2% Curcumin                | 85 ± 5                               | <0.0001              |
| SCF-1                      | 49 ± 2                               | <0.0001              |
| SCF-3                      | 52 ± 4                               | <0.0001              |
| SCF-4                      | 32 ± 2                               | <0.0001              |
| SCF-4C                     | 45 ± 2                               | <0.0001              |

3.9.4. Antimicrobial Activity
The antimicrobial assay was performed using disk diffusion method (Figure 8). Curcumin, SCF-1, SCF-4 and SCF-4C showed high antimicrobial activity by means of inhibition zones of 16 ± 0.2 mm, 12 ± 3 mm, 14 ± 0.4 mm and 15 ± 3 mm against E. coli, respectively. Mean values of inhibition zones of 17 ± 0.4 mm, 15 ± 3 mm, 15 ± 0.4 mm and 16 ± 6 mm against S. aureus were observed in case of curcumin, SCF-1, SCF-4 and SCF-4C, respectively. As reported previously, the possible mode of action of curcumin in E. coli is due to cell membrane lysis, while bactericidal activity against S. aureus may be ascribed to the diffusion of curcumin through the cell wall leading to disruption of the cellular organelles and finally leading to apoptosis [39]. SCF-1 and SCF-4 also showed antimicrobial activity probably because of the surface amine groups of chitosan. Antimicrobial analysis of the scaffolds after curcumin coating confirmed an increase in the antimicrobial activity. The findings suggest that the biocomposite scaffolds surface deposited with curcumin have potential to be used as antibacterial wound dressing.

### Table 3. Analysis of ‘bleeding time’ of the composite scaffolds.

| Group (Whatman’s filter paper) | Time taken for complete haemostasis (sec) | ‘p’-value wrt control | Amount of blood absorbed (mg) | ‘p’-value wrt control |
|-------------------------------|------------------------------------------|----------------------|-------------------------------|----------------------|
| Control                       | 216 ± 1                                  | -                    | 430 ± 1                       | -                    |
| SCF-1C                        | 155 ± 2                                 | <0.0001              | 39 ± 2                        | <0.0001              |
| SCF-2C                        | 142 ± 4                                 | <0.0001              | 38 ± 1                        | <0.0001              |
| SCF-3C                        | 157 ± 2                                 | <0.0001              | 28 ± 2                        | <0.0001              |
| SCF-4C                        | 143 ± 4                                 | <0.0001              | 32 ± 2                        | <0.0001              |
| SCF-5C                        | 154 ± 4                                 | <0.0001              | 29 ± 2                        | <0.0001              |
| SCF-6C                        | 160 ± 2                                 | <0.0001              | 29 ± 2                        | <0.0001              |
| SCF-7C                        | 130 ± 4                                 | <0.0001              | 53 ± 1                        | <0.0001              |
| SCF-1                         | 161 ± 1                                 | <0.0001              | 56 ± 1                        | <0.0001              |
| SCF-2                         | 140 ± 2                                 | <0.0001              | 35 ± 2                        | <0.0001              |
| SCF-3                         | 145 ± 3                                 | <0.0001              | 40 ± 2                        | <0.0001              |
| SCF-4                         | 125 ± 2                                 | <0.0001              | 24 ± 1                        | <0.0001              |
| SCF-5                         | 135 ± 1                                 | <0.0001              | 69 ± 1                        | <0.0001              |
| SCF-6                         | 160 ± 1                                 | <0.0001              | 41 ± 2                        | <0.0001              |
| SCF-7                         | 134 ± 1                                 | <0.0001              | 67 ± 1                        | <0.0001              |
3.10. In-vivo wound healing assay

3.10.1. Hemostatic efficacy of chitosan/methylcellulose matrix

Surface deposition of SCF-1, SCF-4, SCF-4C and SCF-7 was used to assess the hemostatic efficacy in terms of blood clotting time using the 'capillary test method'. Chitosan, methylcellulose and curcumin, in-combination and alone, were evaluated.

3.10.2. Whole blood clotting

The least clotting time of ~32 ± 2 s was shown by SCF-4, while SCF-4C group animals showed ~45 ± 2 s as the blood clotting time as shown in Table 2. Animal group exposed to the curcumin-coated microneedles showed average clotting time of ~85 ± 5 s in comparison to the SCF-1 and SCF-3, with 49 ± 2 s and 52 ± 4 s, respectively, while the control group depicted a clotting time of 90 ± 5 s on average (in comparison to the control, all scaffolds had 'p'-value of <0.0001). Presence of curcumin and methylcellulose inhibits the thrombin activity, thereby pose an anticoagulant effect; the same is confirmed in the SPR analysis and haemostasis study also, while chitosan promotes blood coagulation and hence the blood clotting time [40, 41]. Minimum clotting time, in case of SCF-4, predominately suggests that chitosan enhances the blood clotting and curcumin inhibits the same.

3.10.3. Bleeding time analysis

Bleeding time of SCF-1, SCF-2, SCF-3, SCF-4, SCF-5, SCF-6, and SCF-7 biocomposite scaffolds in contrast to a plain circular filter paper as a control are indicated in Table 3. Notable difference in the bleeding time was not observable in case of uncoated scaffolds, while minimum bleeding time of ~125 ± 2 s was noticed in case of SCF-4 and highest in SCF-1 (161 ± 1 s). Similarly, curcumin-coated biocomposite scaffold SCF-6C showed highest bleeding time of 160 ± 2 s and lowest 130 ± 4 s was observed in SCF-7C. SCF-4 showed lesser blood absorption of ~24 ± 1 mg, which may be due to the haemostatic efficacy of the biocomposite scaffolds. Highest amount of blood ~69 ± 1 mg was absorbed by SCF-5. In comparison to the control, all scaffolds had 'p'-value of <0.0001.

3.10.4. Evaluation of wound size

Biocomposite scaffolds SCF-1C, SCF-4 and SCF-4C were evaluated for wound healing efficacy on diabetic and normal rats as given in Figure 9. It was observed that SCF-4 and SCF-4C showed complete wound contraction by day 13, probably because of similar biopolymeric composition. In fact, SCF-4C also depicted almost complete wound healing by day 14 in the diabetic animals. In case of SCF-1C, wound contraction of ~80 % was observed on day 14 probably due to lower chitosan concentration. On day 14, control group animals showed the lowest wound contraction of 63% and 75% for diabetic and normal group animals, respectively. SCF-4C showed almost similar trend in the wound healing efficacy in diabetic and normal group animals in comparison to SCF-1C, which suggests that higher concentration of chitosan in presence of curcumin greatly enhances the wound healing efficiency of the scaffold material. Bleeding time analysis also confirmed faster hemostatic action in the scaffolds containing higher chitosan ratios. As reported earlier, chitosan has higher hemostatic capability, stability and moisture retention ability that result in faster wound healing [41]. Antimicrobial, anti-oxidative, and anti-inflammatory properties of chitosan further.

![Figure 9. Rate of wound contraction in excision animal wound model (normal and diabetic wound model); p-value < 0.0001.](image1)

![Figure 10. Histopathological analysis at different time intervals.](image2)
improved the SCF-4 outcome for hemostatic and wound healing potential. In comparison to the control, all scaffolds had a ‘p’-value of <0.0001.

3.11. Evaluation of biological safety

3.11.1. Acute dermal irritation study

The likely health hazards after dermal application of composite scaffolds SCF-1C, SCF-4 and SCF-4C (tested on diabetic and normal animal groups) was evaluated. The irritancy potential of each sample was examined following exposure period and response of animals was recorded. There were no observed symptoms of edema, erythema, or irritation/sensitization. No significant changes in feeding, water intake, or behavior were observed in any of the animals in the study. The study suggests that fabricated scaffold materials do not cause any irritation, sensitization, or toxic effects following application on the skin.

3.11.2. Histopathology

Hematoxylin and eosin-stained skin tissue sections of diabetic control, normal control, SCF-1C, SCF-4 and SCF-4C biocomposite scaffolds treated groups showed improvement in the skin tissue regeneration on days 0, 7 and 14, as shown in Figure 10. Till day 14, both control group animals revealed a line of demarcation as well as a thicker epidermis with substantial collagen deposition. On days 7 and 14, SCF-1C, SCF-4 and SCF-4C scaffolds (on diabetic and normal animal groups) showed signs of early recovery and rehabilitation, while in comparison to SCF-1C group animals, SCF-4C treated groups showed greater recovery in terms of skin extremities with systematized collagen and visible epidermal thickening.

4. Conclusions

The developed three-dimensional biocomposite scaffolds based on chitosan and methylcellulose coated with curcumin demonstrated enhanced wound healing (in diabetic and normal animals) in comparison to the single layer scaffolds. Blood interaction analysis of chitosan and methylcellulose confirmed their association with erythrocytes, while curcumin showed almost negligible interaction with the erythrocytes. According to the in-vivo efficacy evaluation in terms of bleeding time analysis and wound healing studies, it may be confirmed that the composite scaffolds (with and without curcumin coating) have a substantial function in wound healing following topical application. The microstructural and biodegradable, anti-microbial and anti-inflammatory properties of the 3D biocomposite scaffolds based on chitosan and methylcellulose have great potential to promote faster hemostatic action and healing of the diabetic wound.

Declarations

Author contribution statement

Megha Gupta: Conceived and designed the experiments; Performed the experiments.
Arpit Sharma: Analyzed and interpreted the data.
Chandra Shekhar Benival: Contributed reagents, materials, analysis tools or data.
Priyanka Tyagi: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest’s statement

The authors declare the following conflict of interests: Arpit Sharma and Chandra Shekhar Benival reports administrative support, equipment, drugs, or supplies, and writing assistance were provided by DRDO Institute of Nuclear Medicine and Allied Sciences.

Additional information

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