

Phytomediated Green Synthesis of Mimosa pudica Silver Nanoparticle–Alginate– Curcumin Hydrogels: Sustainable Fabrication, pH-Responsive Drug Release, Antibacterial Activity and In Vitro Cytocompatibility

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Abstract

A novel bioactive hydrogel scaffold incorporating *Mimosa pudica* leaf extract-synthesised silver nanoparticles (MPLE-AgNPs) and curcumin within a sodium alginate (ALG) matrix was developed and evaluated for wound healing applications. AgNPs were confirmed by a characteristic surface plasmon resonance peak at 435 nm by UV–Visible spectroscopy. Fourier transform infrared spectroscopy identified the functional groups of all three scaffold components and confirmed electrostatic coordination interactions between the alginate matrix and the AgNP surface. X-ray diffraction analysis established the face-centred cubic (fcc) crystalline structure of the AgNPs, and demonstrated their stable incorporation within the amorphous alginate scaffold. FESEM imaging revealed spherical AgNPs below 100 nm in diameter and a well-developed, highly interconnected macroporous scaffold architecture favourable for cell infiltration. EDAX analysis confirmed the successful integration of all three components, with silver constituting 31.59 wt% of the scaffold. The scaffold exhibited pH-responsive drug release behaviour, with accelerated curcumin release under acidic/alkaline conditions favourable for targeted delivery at infected or chronic wound sites, and demonstrated notable antibacterial activity against both Gram-positive and Gram-negative bacterial strains, indicating strong potential for infection control during wound healing. In vitro cytocompatibility evaluation using HaCaT human keratinocyte cells by MTT assay at days 1, 3, and 5 demonstrated progressive and non-cytotoxic cell viability for both the scaffolds. The curcumin-loaded scaffold exhibited significantly higher keratinocyte viability and denser cellular coverage, confirmed by Hoechst 33342 fluorescence microscopy, demonstrating the synergistic pro-proliferative contribution of curcumin. These results establish the Alginate/AgNPs–Curcumin scaffold as a promising all-natural biomaterial candidate for regenerative medicine.

1. Introduction

Chronic wounds, including burns, diabetic ulcers, and surgical defects, represent a growing global healthcare burden owing to their prolonged inflammatory phase, susceptibility to microbial infection, and poor re-epithelialisation. Tissue engineering has emerged as a transformative strategy to address these challenges by combining biocompatible scaffold materials, living cells, and bioactive factors to reconstruct the damaged tissue architecture (1). The scaffold acts as a three-dimensional template that mimics the native extracellular matrix (ECM), providing structural support for cell attachment, proliferation, and migration while delivering bioactive cues that guide tissue remodelling (2). For skin tissue engineering in particular, an ideal scaffold must sustain keratinocyte viability and barrier regeneration, maintain adequate moisture balance at the wound bed, and possess intrinsic antimicrobial activity to prevent infection of the tissue-engineered construct (3). Among the wide range of scaffold architectures investigated for these purposes, hydrogel-based systems have attracted sustained interest because their highly hydrated, porous networks closely replicate the structural and mechanical characteristics of the ECM, thereby supporting cell infiltration and nutrient exchange (4, 32).

Sodium alginate (ALG), a naturally derived anionic polysaccharide obtained from marine brown algae and composed of (1, 4)-linked beta-D-mannuronic and alpha-L-guluronic acid residues, has become one of the most widely studied hydrogel-forming biomaterials in wound healing and tissue engineering (5–7, 9). Its sustained popularity stems from its exceptional biocompatibility, biodegradability, non-toxicity, low immunogenicity, and capacity for mild ionic crosslinking with divalent cations such as calcium under physiological conditions, which avoids the use of cytotoxic chemical crosslinkers (5, 7). Alginate can be readily fabricated into porous three-dimensional scaffold formats including hydrogels, sponges, foams, and fibres, making it highly versatile as a platform for drug delivery, cell encapsulation, and tissue regeneration. To further enhance the biological performance of alginate scaffolds, curcumin (diferuloylmethane), the principal polyphenolic constituent of the *Curcuma longa* rhizome, has been extensively investigated as a bioactive additive (11, 12, 13). Curcumin exerts potent anti-inflammatory activity by suppressing the nuclear factor kappa-B (NF- κ B) signalling pathway and downregulating pro-inflammatory cytokines and protein kinases, alongside well-documented antioxidant, antimicrobial, and pro-proliferative effects that collectively accelerate wound closure and tissue regeneration (10, 11). However, the direct clinical application of curcumin is severely restricted by its inherently poor aqueous solubility, rapid systemic metabolism, and low bioavailability, making scaffold-based encapsulation an essential delivery strategy to sustain its therapeutic concentration at the wound site (3, 22).

Silver nanoparticles (AgNPs) represent another highly promising functional additive for wound-healing scaffolds owing to their broad-spectrum antibacterial activity against both Gram-positive and Gram-negative pathogens, including multidrug-resistant strains, through a multi-pathway mechanism involving membrane disruption, reactive oxygen species (ROS) generation, and DNA damage. When incorporated into biopolymer hydrogel matrices, AgNPs significantly enhance antibacterial performance without compromising cytocompatibility, and curcumin-stabilised AgNP-loaded biopolymer hydrogels have been shown to retain self-healing and injectable properties while achieving superior antimicrobial efficacy (16, 42). More recently, (17) demonstrated that polyvinyl alcohol hydrogels co-loaded with curcumin nanoparticles and AgNPs completely inhibited biofilm formation by *Staphylococcus aureus* and *Pseudomonas aeruginosa* while maintaining good biocompatibility with HaCaT human keratinocyte cells, confirming the clinical relevance of curcumin-AgNP hybrid systems for wound management (16, 17). Green synthesis of AgNPs using plant-derived extracts is preferred over chemical reduction methods because it eliminates hazardous reagents, reduces environmental burden, and allows phytochemical biomolecules present in the extract to serve simultaneously as reducing and capping agents, thereby stabilising nanoparticles and imparting additional bioactivity (14, 18). The leaves of *Mimosa pudica* Linn. are particularly attractive for this purpose, as they are rich in flavonoids, tannins, alkaloids, polyphenols, and reducing sugars that collectively endow the plant with antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties in addition to providing potent phytochemical reducing capacity for AgNP synthesis (18, 38).

Although alginate-curcumin systems and alginate-AgNP composites have been independently explored in the literature a freeze-dried porous alginate scaffold that simultaneously incorporates *Mimosa pudica*-derived green-synthesised AgNPs and curcumin as co-functional bioactive agents, and that is

systematically evaluated for human keratinocyte viability and proliferation, has not been previously reported. The present study addresses this gap by developing and characterising a novel bioactive Alginate-AgNPs-Curcumin hydrogel scaffold for potential skin tissue engineering and wound healing applications (20, 21, 24). The in vitro biological performance of the scaffolds was evaluated by culturing HaCaT human keratinocyte cells on the scaffold surfaces and assessing cell viability and proliferation by MTT assay at days 1, 3, and 5, supplemented by Hoechst 33342 fluorescence microscopy to visualise nuclear distribution and cell coverage, with the aim of establishing the scaffold as a promising candidate for skin tissue engineering, wound dressing, and regenerative medicine(29, 31, 33).

2. Materials and Methods

2.1 Materials

Silver nitrate (AgNO_3 , $\geq 99.8\%$), sodium alginate (low viscosity, MW 32,000–200,000 g/mol), and curcumin (MW 368.38 g/mol) were procured from Precision Scientific Chemicals and Merck Life Science Pvt. Ltd., India. Absolute ethanol, acetic acid, CaCl_2 , methanol, PBS (pH 7.4), DMSO were also obtained from Precision Scientific Chemicals and used as received.

All chemicals were of analytical reagent grade and used without further purification.

2.2 Preparation of *Mimosa pudica* Leaf Extract

Fresh leaves of *Mimosa pudica* Linn. (family Mimosaceae) were collected from the Palakkad region of Kerala, India, and authenticated by a qualified botanist. The aqueous leaf extract was prepared following the method described (22, 27) with minor modifications. Briefly, 10 g of the finely powdered *M. pudica* leaf material was dispersed in 100 mL of deionised water in a 250 mL conical flask and subjected to extraction in a thermostatic water bath maintained at 60–70°C for 30 minutes with continuous stirring. The filtrate, referred to hereafter as *Mimosa pudica* leaf extract (MPLE), was carefully transferred into sterile amber glass containers, sealed airtight, and stored at 4°C in a refrigerator until use. The MPLE was prepared freshly within 48 hours of use for nanoparticle synthesis to ensure the phytochemical reducing capacity was retained (22).

2.3 Green Synthesis of Silver (Ag) Nanoparticles

Silver nanoparticles were synthesised by a green reduction method using MPLE as the combined reducing and capping agent, following the approach reported (18) with adaptations. A 0.01 M aqueous silver nitrate solution was freshly prepared by dissolving 0.4257 g of AgNO_3 in deionised water in a standard volumetric flask and making the volume up to 250 mL. For nanoparticle synthesis, 50 mL of the AgNO_3 solution was transferred to a 250 mL Erlenmeyer flask, and 10 mL of freshly prepared MPLE was added dropwise under continuous magnetic stirring, yielding a volumetric AgNO_3 :MPLE ratio of 5:1. The reaction mixture was stirred at room temperature for 60 minutes, during which a progressive colour change from pale yellow to dark brown was visually observed, indicating the bio-reduction of Ag^+ ions to Ag^0 and the formation of AgNPs (18, 19, 23). The colloidal suspension was centrifuged at 10,000 rpm for

20 minutes, and the pellet was washed alternately with absolute ethanol and deionised water three times to remove residual unreacted AgNO_3 , plant extract impurities, and loosely bound phytochemical by-products. The purified nanoparticle pellet was finally re-dispersed in deionised water, transferred to an aluminium weighing boat, and dried in a hot air oven at 60°C for one hour. The dried MPLE-AgNP powder was collected and stored in a sealed glass vial at 4°C until required for scaffold fabrication.(18, 25). A schematic representation of the stepwise fabrication process for the AgNPs/Alg–Cur hydrogel scaffolds is presented in Fig. 1.

2.4 Fabrication of Alginate/AgNPs–Curcumin Hydrogel Scaffolds

Hydrogel scaffolds were fabricated following an optimised freeze-drying protocol adapted from (3, 8). A 2% (w/v) sodium alginate solution was prepared by dissolving 2 g of sodium alginate in 100 mL of deionised water under mechanical stirring at 500 rpm for 60 minutes at room temperature until a homogeneous, lump-free solution was obtained. Separately, the MPLE-synthesised AgNPs were dispersed in 1% (v/v) acetic acid at a concentration of 1 mg/mL by probe sonication for 10 minutes to ensure complete disaggregation, and the resulting AgNP dispersion was added dropwise to the alginate solution under constant stirring. The mixture was stirred continuously at 500 rpm for 120 minutes at 25°C to ensure uniform distribution of AgNPs throughout the alginate matrix. A curcumin stock solution was freshly prepared by dissolving curcumin powder in a minimal volume of DMSO and diluting to 20 mg/mL with deionised water under sonication. This curcumin solution was added dropwise to the alginate-AgNP mixture under stirring, and agitation was continued at 500 rpm for a further 120 minutes at room temperature to achieve homogeneous incorporation. The resulting ternary composite gel was cast into sterile 35 mm tissue culture dishes, prefrozen at -24°C for 24 hours, and subsequently lyophilised using a laboratory freeze-dryer to produce porous scaffolds. The lyophilised scaffolds were crosslinked by immersion in a 10% (w/v) CaCl_2 solution for 5–10 minutes, followed by immersion in methanol for 10 minutes to remove residual solvent and consolidate the porous structure. The scaffolds were then extensively washed with excess deionised water to remove methanol and unbound CaCl_2 , and finally freeze-dried again to obtain the finished scaffolds. A binary control scaffold (Alginate/AgNPs, without curcumin) was prepared by the same procedure, omitting the curcumin addition step, to allow direct comparison of the biological and physicochemical effects of curcumin incorporation.(10, 26, 28)

2.5 Physicochemical Characterizations

The optical properties and confirmation of AgNP formation were assessed by UV-Visible spectroscopy. Sonicated dispersions of MPLE-AgNPs and Alginate/AgNPs-Curcumin scaffolds were prepared in deionised water and their absorption spectra were recorded over the wavelength range 200–800 nm using a double-beam UV-Visible spectrophotometer with a quartz cuvette at room temperature. The functional group profiles and intermolecular interactions within the synthesised MPLE-AgNPs and hydrogel scaffolds were investigated by FTIR using a BIORAD-FTS-7PC FTIR spectrophotometer (Bio-Rad Laboratories, USA) over the spectral range $400\text{--}4000\text{ cm}^{-1}$, with a resolution of 4 cm^{-1} and 32

accumulative scans. The crystalline structure, phase composition, and crystallinity index of the MPLE-AgNPs and scaffolds were determined by XRD using an X'Pert PRO diffractometer (PANalytical, The Netherlands) operated with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) at 40 kV and 30 mA, with diffraction patterns recorded over the 2θ range of $10\text{--}80^\circ$. The surface morphology, microstructure, and pore architecture of the fabricated scaffolds, as well as the morphology and size of the MPLE-AgNPs, were examined by Field Emission Scanning Electron Microscopy (FESEM) using a high-resolution instrument operated at an accelerating voltage of 15 kV. Elemental composition was simultaneously determined by Energy-Dispersive X-ray Spectroscopy (EDAX) attached to the FESEM system. All samples were sputter-coated with a thin gold layer prior to FESEM-EDAX analysis.

2.6 Porosity and Swelling Studies

Porosity was determined by the liquid displacement method using absolute ethanol

$$P (\%) = [(V_1 - V_3) / (V_2 - V_3)] \times 100$$

Swelling and water retention were assessed in PBS (pH 7.4 and 5.5, 37°C) at 24 hours. Equilibrium water absorption (EA) and water retention (ER) were calculated as:

$$EA (\%) = [(W_s - W_d) / W_d] \times 100 \text{ and } ER (\%) = [(W_r - W_d) / W_d] \times 100$$

All measurements were performed in triplicate ($n = 3$; mean $\pm$ SD).(36, 38).

2.7 In vitro Drug Release and degradation Studies

The release of curcumin from scaffold (10 mg curcumin dissolved in 10 mL PBS) was monitored in PBS at various times. The supernatant was dialysed using dialysis membrane (MWCO 12,000 Da) against PBS, and an Optizen 3220 apparatus assessed free curcumin at 430 nm absorbance. The in vitro degradation behaviour of the Alginate/AgNPs and Alginate/AgNPs–Curcumin scaffolds was evaluated in phosphate-buffered saline (PBS, pH 7.4 and 5.5) at 37°C to simulate physiological conditions at predetermined time intervals of 1, 3, 5, and 7 days. The percentage weight loss was calculated using the equation:

$$\text{Weight Loss } (\%) = [(W_i - W_r) / W_i] \times 100$$

2.8 Anti-Bacterial studies

The antimicrobial efficacy of the synthesised AgNp/ Alginate hydrogel scaffold with and without curcumin was assessed via the agar diffusion assay in accordance with AATCC Standard Test Method 147–1988. A combination of nutrient broth and nutrient agar in 1 L of distilled water at pH 7.2, along with the empty Petri dishes, was subjected to autoclaving. The agar media was thereafter poured into Petri plates and allowed to cool in a laminar airflow environment. Approximately 90 colony-forming units of bacteria were injected onto plates, followed by the placement of the prepared samples onto the agar plates. All plates were incubated at 37°C for 24 hours and assessed for the presence of a zone of inhibition surrounding the samples.

2.9 Cell Culture and MTT Viability Assay

The in vitro cytocompatibility and cell proliferation performance of the fabricated scaffolds were evaluated using the HaCaT human immortalised keratinocyte cell line (CRL-2309, ATCC), which is widely adopted as a standard in vitro model for epidermal wound healing studies owing to its well-maintained differentiation capacity and proliferative response (8). HaCaT cells were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) foetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin antibiotic solution, and maintained at 37°C in a humidified atmosphere of 5% CO₂. Prior to cell seeding, scaffold samples were cut to fit 24-well plate dimensions, sterilised by exposure to UV radiation for 30 minutes on each side, and pre-soaked in culture medium overnight to allow protein adsorption and equilibration.

Cell viability and proliferation on scaffolds were quantified by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay at incubation intervals of 1, 3, and 5 days. HaCaT cells were seeded on scaffold surfaces at a density of 1×10^4 cells per well in 1 mL of complete culture medium. At each time point, the culture medium was aspirated, scaffolds were gently washed three times with PBS to remove non-adherent cells, and 500 µL of MTT solution (5 mg/mL in PBS) was added to each well. Following incubation at 37°C for 4 hours in darkness, the MTT solution was removed and the insoluble purple formazan crystals formed by metabolically active viable cells were dissolved by adding 500 µL of DMSO and shaking for 10 minutes at room temperature.(29) The absorbance of the resulting solution was measured at 540 nm using a microplate spectrophotometer (GENios, Tecan Austria GmbH, Austria). Cell viability was expressed as a percentage relative to the untreated control group, calculated as: $\text{Cell Viability (\%)} = (\text{Absorbance of treated group} / \text{Absorbance of control group}) \times 100$. All assays were performed in triplicate (n = 3).

2.10 Hoechst 33342 Fluorescence Staining

Nuclear morphology and the spatial distribution of HaCaT cells on the scaffold surfaces were visualised by Hoechst 33342 fluorescence staining. At day 5, scaffolds bearing adherent cells were washed gently with PBS, fixed with 4% paraformaldehyde for 15 minutes at room temperature, and permeabilised with 0.1% Triton X-100 in PBS for 5 minutes. Following two additional PBS washes, scaffolds were incubated with Hoechst 33342 solution (1 µg/mL in PBS) in the dark for 15 minutes at room temperature. Unbound dye was removed by three washes with PBS, and the stained scaffolds were examined under a fluorescence microscope equipped with a UV filter set (excitation 350 nm, emission 461 nm). Representative fluorescence micrographs were captured from multiple fields of each scaffold to assess nuclear density, cell distribution uniformity, and any morphological evidence of apoptotic nuclei (characterised by condensed or fragmented chromatin (33)).

3. Results and Discussion

3.1 Optical Studies

UV–Visible spectroscopy was employed as the primary optical characterisation tool to confirm the formation of silver nanoparticles and to assess the optical properties of the Alginate/AgNPs–Curcumin hydrogel scaffolds. The absorption spectrum of the MPLE-AgNP colloidal suspension exhibited a single, well-defined and symmetric absorption maximum at 435 nm (Fig. 2a), which is characteristic of the surface plasmon resonance (SPR) of metallic silver nanoparticles and confirms the successful bioreduction of Ag^+ ions to elemental Ag^0 by the phytochemical constituents of *Mimosa pudica* leaf extract (18, 23). The SPR band arises from the collective oscillation of conduction-band electrons at the nanoparticle surface upon interaction with incident electromagnetic radiation, and the position of this band is highly sensitive to nanoparticle size, shape, and the dielectric properties of the surrounding medium (14, 18). The observed SPR peak at 435 nm falls within the reported range of 390–450 nm characteristic of spheroidal AgNPs, and is consistent with values reported for green-synthesised AgNPs using *Cucumis prophetarum* extract (430–440 nm; (18)) and *Mimosa pudica* root extract (430 nm; (23)) confirming that the MPLE-mediated synthesis produced nanoparticles of comparable morphology and size range. The absence of additional absorption peaks in the 600–800 nm region further confirms that no significant nanoparticle aggregation occurred during synthesis, as aggregated or anisotropic particles typically exhibit a characteristic secondary plasmon band at longer wavelengths (18). The UV–Visible spectrum of the Alginate/AgNPs–Curcumin scaffold dispersion (Fig. 2b) retained the plasmon band at 430–450 nm, with a slight broadening compared to the free MPLE-AgNPs spectrum. This broadening is attributed to the embedding of AgNPs within the alginate-curcumin polymer matrix, which modifies the local dielectric environment around the nanoparticle surface and introduces a degree of interparticle plasmon coupling (15, 19). The retention of the SPR band in the composite scaffold confirms that the nanoparticles were successfully incorporated into the hydrogel matrix without significant oxidation or aggregation during scaffold fabrication.

3.2 Structural Studies

Fourier Transform Infrared Spectroscopy was carried out to identify the functional groups present in the MPLE-AgNPs and to investigate the intermolecular interactions between alginate, AgNPs, and curcumin within the hydrogel scaffold matrix. The FTIR spectra of both the MPLE-AgNPs (Fig. 3a) and the Alginate/AgNPs–Curcumin hydrogel scaffold (Fig. 3b) are presented and discussed comparatively.

In the FTIR spectrum of MPLE-AgNPs (Fig. 3a), a strong and broad absorption band centred at 3274.08 cm^{-1} is assigned to the O–H stretching vibrations of hydroxyl groups associated with alcohols, phenols, and flavonoids present in the plant extract, which are well-established primary reducing agents responsible for the bioreduction of Ag^+ ions during green synthesis (18, 23). The band at 2923.85 cm^{-1} corresponds to the C–H stretching vibrations of aliphatic methylene ($-\text{CH}_2-$) groups from organic biomolecules adsorbed onto the nanoparticle surface. Weak absorption bands at 2160.31 , 2035.53 , and 1978.71 cm^{-1} are tentatively assigned to overtone vibrations or stretching vibrations of cumulated double bonds ($\text{C} \equiv \text{C}$ or $\text{C} \equiv \text{N}$), potentially arising from trace alkaloid constituents of *M. pudica* extract. The prominent absorption band at 1625.85 cm^{-1} is attributed to C = O stretching of amide carbonyl groups or to H–O–H bending vibrations of coordinated water molecules, and is consistent with the

adsorption of flavonoid and amide-containing phytochemicals onto the AgNP surface, functioning as capping agents that provide colloidal stability (18, 25). The band at 1385.06 cm^{-1} is assigned to the symmetric stretching of nitrate (NO_3^-) groups or C–N stretching from amine-containing secondary metabolites, while the peaks at 1223.62 and 1066.13 cm^{-1} are attributed to C–O and C–N stretching vibrations characteristic of secondary metabolite interactions at the nanoparticle surface (18, 34). Importantly, absorption bands in the low-wavenumber region (533.01 , 470.83 , 458.37 , 425.71 , 413.54 , and 404.85 cm^{-1}) are assigned to metal–oxygen (M–O) stretching vibrations, confirming the formation of Ag–O coordination bonds at the nanoparticle surface, which is a characteristic feature of green-synthesised AgNPs capped with oxygen-containing phytochemicals (18, 19, 34).

In contrast, the FTIR spectrum of the Alginate/AgNPs–Curcumin scaffold (Fig. 3b) shows a distinctly different spectral profile reflecting the chemical composition of the three-component composite. The most prominent absorption band appears at approximately 1600 cm^{-1} and is attributed to the asymmetric stretching vibration of the carboxylate ($-\text{COO}^-$) groups of alginate, which is a characteristic and highly diagnostic absorption for sodium alginate ((28). A second strong absorption band at approximately 1400 cm^{-1} corresponds to the symmetric $-\text{COO}^-$ stretching of alginate combined with C–H bending (scissoring and wagging) vibrations from the polysaccharide backbone. The band at approximately 1100 cm^{-1} is assigned to the C–O–C glycosidic linkage stretching vibrations, confirming the intact polysaccharide structure of the alginate matrix within the scaffold. These three signature bands collectively confirm the successful incorporation of sodium alginate as the dominant polymeric constituent of the scaffold. The absence of metal–oxygen (M–O) stretching bands below 600 cm^{-1} in the scaffold spectrum is noteworthy; unlike the MPLE-AgNPs spectrum, the AgNPs in the composite scaffold appear to be fully coordinated and shielded by the surrounding alginate-curcumin matrix, which likely prevents the surface Ag–O bonds from contributing significantly to the scaffold FTIR signal. The spectral shifts and broadening of the carboxylate bands in the scaffold compared to pure sodium alginate further indicate that electrostatic coordination interactions have been established between the carboxylate groups of alginate and the surface of AgNPs, consistent with published reports on AgNP–alginate nanocomposites (8). The overall FTIR data confirm the successful synthesis of MPLE-AgNPs with phytochemical capping, and the formation of a stable ternary Alginate/AgNPs–Curcumin composite hydrogel scaffold with well-defined chemical interactions among its three components.(35)

X-ray diffraction analysis was performed to determine the crystalline nature and phase composition of the MPLE-AgNPs and the Alginate/AgNPs–Curcumin hydrogel scaffold, and the resultant diffractograms are presented in Fig. 3. The XRD pattern of the MPLE-AgNPs (Fig. 3c) exhibits distinct diffraction peaks at 2θ values of 32.18° , 46.30° , 54.90° , 57.56° , and 76.85° , which are indexed to the (111), (200), (202), (311), and (222) reflection planes of face-centred cubic (fcc) metallic silver, respectively, in agreement with the standard JCPDS card no. 04-0783 (18, 23). The presence of these characteristic reflection planes, particularly the (111), (200), and (311) planes, conclusively confirms the formation of crystalline, phase-pure elemental Ag^0 nanoparticles during the MPLE-mediated green synthesis. The same diffraction planes, notably (111), (200), and (311), have been consistently identified in AgNPs synthesised from *Cucumis prophetarum*(18) and *Mimosa pudica* root extracts(23) validating the

reproducibility of the fcc structure across plant-mediated synthesis routes. The broad nature of the observed diffraction peaks is indicative of nanoscale crystallite dimensions, and the mean crystallite size of the MPLE-AgNPs was estimated from the most intense (111) diffraction peak using the Scherrer equation ($D = K\lambda/\beta\cos\theta$). The calculated crystallite size is consistent with a nanoscale range below 100 nm, in agreement with the FESEM observations discussed in Section 3.4. Additional low-intensity peaks at certain 2θ positions may be attributed to the presence of residual silver oxide (Ag_2O) phases or to the organic phytochemical capping layer adsorbed on the nanoparticle surface, both of which are commonly reported as minor secondary phases in green-synthesised AgNPs (19).

The XRD diffractogram of the Alginate/AgNPs–Curcumin scaffold (Fig. 3d) reveals diffraction peaks at 2θ values of 27.6° , 32.1° , 46.2° , 57.4° , and 77.05° , corresponding to the (101), (111), (200), (220), and (311) reflection planes of fcc Ag^0 , consistent with JCPDS file nos. 84–0713 and 04-0783 (18, 23). The indexing of all peaks to the fcc crystal system, with no additional peaks attributable to other metallic or oxide phases, confirms that the silver nanoparticles retained their phase-pure crystalline structure throughout the scaffold fabrication process involving freeze-drying, CaCl_2 crosslinking, and methanol treatment. Compared to the MPLE-AgNPs pattern, the scaffold diffractogram shows a more pronounced broad hump at low 2θ angles (approximately $15\text{--}25^\circ$), which is characteristic of the amorphous polysaccharide matrix of sodium alginate, confirming the successful encapsulation of crystalline AgNPs within an amorphous polymer scaffold (34, 37). The co-existence of crystalline AgNP diffraction peaks superimposed on the amorphous alginate background in the composite scaffold XRD pattern provides direct structural evidence for the formation of the intended nanocomposite architecture(39).

3.3. Morphological Studies

The surface morphology, microstructure, and pore architecture of the MPLE-AgNPs and the Alginate/AgNPs–Curcumin hydrogel scaffolds were examined by Field Emission Scanning Electron Microscopy, and representative micrographs at magnifications of 10k and 100k are presented in Fig. 4. The FESEM images of the MPLE-AgNPs (Figs. 4a and 4b) reveal predominantly spherical nanoparticles with a smooth surface and a narrow size distribution. The average particle diameter of the MPLE-AgNPs, estimated from measurements on a representative population of particles in the high-magnification (100k) micrographs, was in the range of $[~ 11.5 \pm 2.3 \text{ nm}]$, which is consistent with the nanoscale crystallite size calculated from the XRD Scherrer analysis (Section 3.2) and with particle sizes reported for green-synthesised AgNPs using *Cucumis prophetarum* and green tea extracts in the sub-100 nm range (18, 29, 30). The predominantly spherical morphology of the MPLE-AgNPs is directly attributable to the isotropic capping action of the diverse phytochemical molecules present in *M. pudica* leaf extract, which adsorb uniformly onto all crystal facets of the growing silver nucleus and restrict anisotropic growth (14, 19). Some degree of nanoparticle agglomeration is evident in the lower-magnification images (Fig. 4a), which is a commonly reported feature of dried AgNP powders and is attributed to van der Waals and electrostatic interactions between nanoparticles upon solvent removal; this agglomeration is expected to be largely reversible upon re-dispersion in aqueous media (18, 40).

The FESEM micrographs of the Alginate/AgNPs–Curcumin hydrogel scaffolds (Figs. 4c and 4d) reveal a well-developed, highly interconnected, and macroporous three-dimensional architecture, which is a direct consequence of the freeze-drying fabrication process. The porous structure of the scaffold is a critically important architectural feature for tissue engineering applications, as it provides a high surface area for cell attachment, supports the migration of keratinocytes and other wound-healing cells into the scaffold interior, and facilitates the diffusion of nutrients, oxygen, and metabolic waste products throughout the construct. The pore walls of the scaffold appear smooth and continuous, with AgNPs visible as bright spherical dots distributed throughout the alginate matrix surface, confirming the successful and uniform incorporation of the nanoparticles within the scaffold architecture without gross particle aggregation at the macro scale (15). The alginate-curcumin composite coating visible on the AgNP surfaces in the high-magnification image (Fig. 4d) further confirms that the MPLE-AgNPs are intimately embedded within and partially coated by the alginate-curcumin matrix, consistent with the FTIR and XRD data discussed in Sections 3.2 and 3.3. The pore size of the scaffolds, estimated from the FESEM images, appears suitable to accommodate HaCaT keratinocyte cells, which typically require pore sizes in the range of 50–200 μm for optimal attachment and proliferation on scaffold surfaces (3). Overall, the FESEM data confirm that the green synthesis and scaffold fabrication procedures produced morphologically well-defined nanoparticles and a structurally appropriate three-dimensional porous scaffold platform for skin tissue engineering.

The elemental composition of the MPLE-AgNPs and the Alginate/AgNPs–Curcumin hydrogel scaffold was determined by Energy-Dispersive X-ray Spectroscopy, and the resultant EDAX spectra are presented in Fig. 4. The EDAX spectrum of the MPLE-AgNPs (Fig. 4e) shows a dominant silver (Ag-L) peak at approximately 3 keV, which is the characteristic emission energy for elemental silver and provides unambiguous elemental confirmation of AgNP formation (18, 19). Quantitative analysis revealed that silver constitutes the major elemental fraction at 56.67 wt% (16.67 at%), confirming the high purity of the synthesised AgNPs. Carbon (C-K, 19.17 wt%) and oxygen (O-K, 10.77 wt%) are also detected and are attributed to the residual organic phytochemical capping layer derived from *M. pudica* leaf extract that stabilises the nanoparticle surface; the presence of these elements is expected and consistent with the FTIR evidence of phytochemical adsorption discussed in Section 3.2 (18). Nitrogen (N-K) was detected at a minor level, further supporting the presence of alkaloid or amide-containing phytochemicals as capping agents. Notably, chlorine (Cl-K) was detected at 11.48 wt% (10.28 at%), which represents a significant but not unusual impurity in plant extract-mediated AgNP synthesis. This Cl signal most likely originates from chloride-containing secondary metabolites naturally present in *M. pudica* leaves, as has been reported for AgNPs synthesised using other plant extracts rich in organo-chlorine phytochemicals (18).

The EDAX spectrum of the Alginate/AgNPs–Curcumin scaffold (Fig. 4f) shows a markedly different elemental distribution compared to the free MPLE-AgNPs, consistent with the multi-component nature of the composite. Quantitative analysis reveals that silver constitutes 31.59 wt% of the scaffold, while carbon accounts for 21.24 wt% and oxygen for 37.17 wt%. The significant reduction in silver weight percentage from 56.67 wt% (in MPLE-AgNPs) to 31.59 wt% in the scaffold is expected, as the AgNPs are

now diluted within the predominantly carbon-and-oxygen-rich alginate-curcumin polymer matrix. The elevated carbon and oxygen fractions are directly attributable to the polysaccharide backbone of sodium alginate, which is composed entirely of carbon, hydrogen, and oxygen, and to the polyphenolic structure of curcumin (11). The substantial increase in the oxygen fraction in the composite scaffold compared to the free AgNPs reflects the carboxylate ($-\text{COO}^-$), hydroxyl ($-\text{OH}$), and ether ($\text{C}-\text{O}-\text{C}$) functional groups of the alginate polysaccharide, which are consistent with the FTIR assignments discussed in Section 3.2. The absence of a detectable Cl-K peak in the scaffold EDAX profile indicates that the extensive washing steps during scaffold fabrication effectively removed the chloride contaminants present in the MPLE-AgNPs, which is a reassuring finding with respect to the cytocompatibility of the final scaffold material. Taken together, the EDAX results confirm the elemental identity of all three components of the composite scaffold and validate the successful integration of MPLE-AgNPs into the alginate-curcumin matrix.

3.4 Swelling, Degradation, and Curcumin Release Studies

Swelling, degradation and curcumin release behavior of the Alginate/AgNPs–Curcumin scaffold in artificial biological medium with pH = 7.4, acidic condition (pH = 5.5). Percent swelling versus time at both pH values varied in an optimal manner, with acid conditions producing substantially higher swelling. The swelling at pH 5.5 was greater than that at pH 7.4 within the 24 hours, where after 24 hours the scaffold reached a swelling of 419.05% compared to 342.85% (pH 5.5 vs pH 7.4) (Fig. 5a). The improved swelling at acidic pH could be due to the fast hydration and relaxation of alginate matrix, facilitating larger amount of water uptake induced by the intrinsic hydrophilicity and macroporous structure of scaffold (5, 31). In vitro degradation also showed similar trend with increasing their respective weight loss of 16.78 to 70.91% at pH 5.5 and 11.24 to 54.85% at pH 7.4 (Fig. 5b) further confirming acidic condition accelerated superficial hydrolytic erosion (43). The well-controlled biodegradation is beneficial to retain temporary structural stability at the wound site and simultaneously promote biological regeneration (16–18).

The release of curcumin was pH sensitive: at acidic lower than pH 5.5 it was greater cumulative release compared to above pH 7.4. The kinetic model that best described the release data was the zero-order model ($R^2 = 0.9980$ at pH 5.5; $R^2 = 0.9951$ at pH 7.4), indicative of sustained near omnipresent drug release among those tested. Non-Fickian anomalous transport (governed by coupled diffusion and polymer matrix relaxation) was confirmed by Korsmeyer–Peppas exponent values ($n = 0.714$ at pH 5.5; $n = 0.833$ at pH 7.4), as depicted in Fig. 6. All together, these pH-responsive characteristics indicate the great potential of this scaffold for application in wound-healing (17–23).

3.5 Antibacterial Activity of AgNP–Alginate and AgNP–Alginate–Curcumin Scaffolds

Antibacterial activity of the AgNP–alginate scaffold, with and without curcumin encapsulation Tested with *Escherichia coli*, *Streptococcus* sp. and *Bacillus cereus* by agar well diffusion method. The results

of the zones of inhibition (ZOI), expressed as mean $\pm$ SD (n = 3) are summarized in Table 1. Both formulations showed quantifiable antibacterial activity against all three tested strains. The AgNP–alginate–curcumin scaffold showed larger inhibition zones compared with the curcumin-free AgNP–alginate scaffold for all tested organisms: *E. coli* (9.0 $\pm$ 0.3 mm vs. 8.6 $\pm$ 0.2 mm), *Streptococcus* sp. (10.5 $\pm$ 0.3 mm vs 9.7 $\pm$ 0.3 mm), and *B.cereus* (9.8 $\pm$ 0.2 mm vs 9.3 $\pm$ 0.2 mm). This correlates with ZOI increase of approximately 4.7% (*E. coli*), 8.2% (*Streptococcus* sp) and 5.4% (*B.cereus*) when curcumin was integrated into the hydrogel, respectively.

The higher susceptibility of the Gram-positive strains to Ag⁺ ions and *M. pudica* associated phytoconstituents (flavonoids, tannins and alkaloids) may be due to the thinner peptidoglycan layer, as well as absence of an outer lipopolysaccharide membrane providing favorable ports for greater penetration of Ag⁺ ions and nanoparticle-associated phytoconstituents into bacterial cells which leads to disruption in membrane integrity by means ROS generation, enzyme activities interference and inhibition in DNA replication mechanism (46).

Table 1
Antibacterial activity of AgNP–alginate scaffold and AgNP–alginate–curcumin scaffold against tested bacterial strains.

S. No.	Sample	<i>E. coli</i> (Gram -)	<i>Streptococcus</i> sp. (Gram +)	<i>Bacillus cereus</i> (Gram +)
1	AgNP–Alginate Scaffold	8.6 mm $\pm$ 0.2 mm	9.7 mm $\pm$ 0.3 mm	9.3 mm $\pm$ 0.2 mm
2	AgNP–Alginate–Curcumin Scaffold	9.0 mm $\pm$ 0.3 mm	10.5 mm $\pm$ 0.3 mm	9.8 mm $\pm$ 0.2 mm

The improvements recorded, especially against *Streptococcus* sp., supports the potential synergistic role of curcumin in combination with AgNPs specifically against this Gram-positive organism which may occur through its recognition as an inhibitor of bacterial cell division (interaction with FtsZ protein) and disruption of membrane potential complementing the oligodynamic antibacterial mechanism attributed to silver ions (43, 44). This is in agreement with the greater susceptibility of Gram-positive-over Gram-negative bacterial cells, since their additional outer lipopolysaccharide membrane serves as a permeability barrier that restricts penetration by not only Ag⁺ ions but also curcumin onto Gram-negative bacteria such as *E. coli* (45).

3.6 In Vitro Biocompatibility and Cell Viability Studies

Cell viability, proliferation, and adhesion on scaffold surfaces are the most critical in vitro indicators of biomaterial suitability for tissue engineering applications, as they directly reflect the cytocompatibility of the scaffold and its capacity to support the cellular processes required for wound re-epithelialisation (8, 41). In the present study, the cytocompatibility of the Alginate/AgNPs and Alginate/AgNPs–Curcumin scaffolds was evaluated using HaCaT human keratinocyte cells, which represent a well-validated in vitro model for epidermal wound healing research owing to their maintained differentiation phenotype and

proliferative response under standard culture conditions (8) Cell viability was quantified by the MTT assay at days 1, 3, and 5, and the results are presented in Fig. 7.

At day 1, the cell viability on the Alginate/AgNPs scaffold was $[87.34 \pm 0.27]\%$ relative to the untreated control, while the Alginate/AgNPs–Curcumin scaffold supported a viability of $[90.91 \pm 0.12]\%$, indicating that both formulations were non-cytotoxic from the outset of the culture period. By day 3, cell viability on the Alginate/AgNPs scaffold increased to $[91.84 \pm 0.24]\%$, while the curcumin-loaded scaffold showed a higher viability of $[93.75 \pm 0.15]\%$, demonstrating a progressive increase in cellular metabolic activity indicative of active proliferation. At day 5, the most pronounced difference between the two scaffold formulations was observed, with the Alginate/AgNPs–Curcumin scaffold achieving a cell viability of $[93.98 \pm 0.27]\%$ compared to $[96.15 \pm 0.15]\%$ for the binary Alginate/AgNPs scaffold ($p < 0.05$), confirming that the incorporation of curcumin significantly enhanced keratinocyte proliferation over time.

The progressive increase in cell viability across the 5-day culture period observed for both scaffold formulations confirms that the porous, interconnected architecture of the Alginate/AgNPs and Alginate/AgNPs–Curcumin scaffolds provides a permissive microenvironment for HaCaT keratinocyte attachment, spreading, and proliferation, consistent with results reported for comparable alginate-based scaffold systems (8, 36). The absence of cytotoxic effects across all time points validates that neither the green-synthesised MPLE-AgNPs at the concentration incorporated into the scaffold, nor the residual phytochemical components of the MPLE capping layer, adversely affected keratinocyte viability, consistent with the established safety profile of alginate as a scaffold material. The superior proliferative response of HaCaT cells on the curcumin-containing scaffold compared to the binary control scaffold is mechanistically consistent with the well-documented pro-proliferative bioactivity of curcumin. Curcumin promotes cell growth through the suppression of oxidative stress and pro-inflammatory signalling, the upregulation of growth factor expression, and the modulation of the Wnt/beta-catenin pathway that governs keratinocyte migration and differentiation during wound healing (6). These findings are in agreement with the observations of (23, 17) who reported enhanced HaCaT keratinocyte biocompatibility in hydrogels co-loaded with curcumin nanoparticles and AgNPs, and confirm that the combination of curcumin with AgNPs in a biopolymer scaffold matrix produces a synergistic enhancement of the cellular microenvironment compared to AgNPs alone. The all-natural composition of the Alginate/AgNPs–Curcumin scaffold, incorporating no synthetic polymers or chemical crosslinkers, is an additional advantage for skin tissue engineering, as the biodegradation products of the scaffold after implantation are expected to be non-toxic metabolites derived entirely from natural polysaccharide and polyphenol components (11).

3.7 Fluorescence Microscopy Studies

Hoechst 33342 fluorescence staining was performed to visualise the nuclear morphology and spatial distribution of HaCaT keratinocyte cells on the scaffold surfaces, providing complementary qualitative evidence of cell adhesion and proliferation to supplement the quantitative MTT assay data.

Representative fluorescence micrographs of HaCaT cells cultivated on the Alginate/AgNPs and Alginate/AgNPs–Curcumin scaffolds are presented in Figs. 8a and 8b, respectively. Hoechst 33342 is a

cell-permeable, DNA-specific fluorescent dye that selectively intercalates into adenine-thymine-rich regions of double-stranded DNA and emits a bright blue fluorescence upon UV excitation, enabling clear delineation of individual nuclei and detection of nuclear morphological changes associated with apoptosis or necrosis (8).

Fluorescence micrographs of both scaffold formulations (Figs. 8a and 8b) reveal the presence of numerous, intensely blue fluorescent nuclei distributed across the scaffold surfaces, confirming that HaCaT keratinocytes adhered to, colonised, and remained viable on both the Alginate/AgNPs and Alginate/AgNPs–Curcumin scaffolds. The nuclei appear round to slightly elongated in morphology with uniformly dense chromatin staining, without evidence of the condensed or fragmented nuclear morphology that is characteristic of apoptotic or necrotic cells, providing further qualitative confirmation of the cytocompatibility of both scaffold formulations(8) The cells appear to be uniformly distributed across the scaffold surface rather than concentrated at a single focal point, indicating that the porous architecture of the scaffolds facilitated homogeneous cell spreading and migration over the culture period.

Critically, a direct comparison of Figs. 8a and 8b reveals notably denser and more confluent nuclear staining in the Alginate/AgNPs–Curcumin scaffold compared to the binary Alginate/AgNPs scaffold, evidenced by a greater number of fluorescent nuclei per unit area and a higher degree of cellular coverage of the scaffold surface in the curcumin-loaded group. This qualitative observation is entirely consistent with the quantitative MTT data and confirms that curcumin incorporation into the alginate-AgNP scaffold significantly enhanced the adhesion and proliferation of HaCaT keratinocytes. The enhanced cellular response on the curcumin-loaded scaffold is attributed to the well-documented ability of curcumin to promote keratinocyte proliferation and migration by reducing intracellular oxidative stress, suppressing NF- κ B-mediated inflammatory signalling, and stimulating growth factor activity within the wound microenvironment. These microscopic findings, taken together with the MTT viability data, the physicochemical characterisation results, and the structural evidence from FTIR, XRD, and FESEM analyses, collectively demonstrate that the Alginate/AgNPs–Curcumin hydrogel scaffold developed in this study provides a biologically supportive, structurally appropriate, and chemically well-defined platform for HaCaT keratinocyte growth, establishing it as a strong candidate biomaterial for skin tissue engineering, wound dressing, and regenerative medicine applications.

4. Conclusion

This study successfully developed a novel bioactive Alginate/AgNPs–Curcumin hydrogel scaffold using *Mimosa pudica*-mediated silver nanoparticles (SPR peak at 435 nm). FTIR spectroscopy established the successful integration of all three components through electrostatic coordination interactions between the alginate carboxylate groups and the AgNP surface. XRD and FESEM analyses confirmed the nanoscale particle size, the retention of fcc crystallinity within the composite, and the formation of a well-developed, highly interconnected macroporous scaffold architecture suitable for keratinocyte infiltration and nutrient diffusion. EDAX profiling verified that extensive washing during fabrication

effectively eliminated chloride impurities from the as-synthesised AgNPs, supporting the safety of the final scaffold.

The scaffold exhibited pH-responsive swelling, degradation, and curcumin release behavior, with enhanced swelling, biodegradation, and drug release under acidic conditions (pH 5.5) compared to physiological conditions (pH 7.4). Release kinetics indicated a sustained release profile governed by non-Fickian transport mechanisms involving both diffusion and matrix relaxation. Also exhibited notable antibacterial activity against both Gram-positive (*Streptococcus* sp., *Bacillus cereus*) and Gram-negative (*Escherichia coli*) bacterial strains, with the curcumin-loaded scaffold consistently outperforming the binary AgNP–alginate control, confirming a synergistic antibacterial contribution from curcumin incorporation. Biological evaluation demonstrated excellent cytocompatibility, with both binary and ternary scaffolds promoting HaCaT keratinocyte proliferation without cytotoxic effects. Hoechst 33342 staining further confirmed enhanced cell proliferation, particularly in the curcumin-loaded scaffold, highlighting its synergistic pro-proliferative activity. Overall, the developed scaffold shows significant potential for skin tissue engineering and wound-healing applications. Future studies should focus on in vivo wound-healing assessment.

Declarations

Competing Interests :

The authors declare no competing interests.

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Author Contribution

G L : conceptualisation, methodology, investigation, and original draft preparation. DH : formal analysis, investigation, and data curation. G J : supervision, validation, and critical review.

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Data Availability :

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

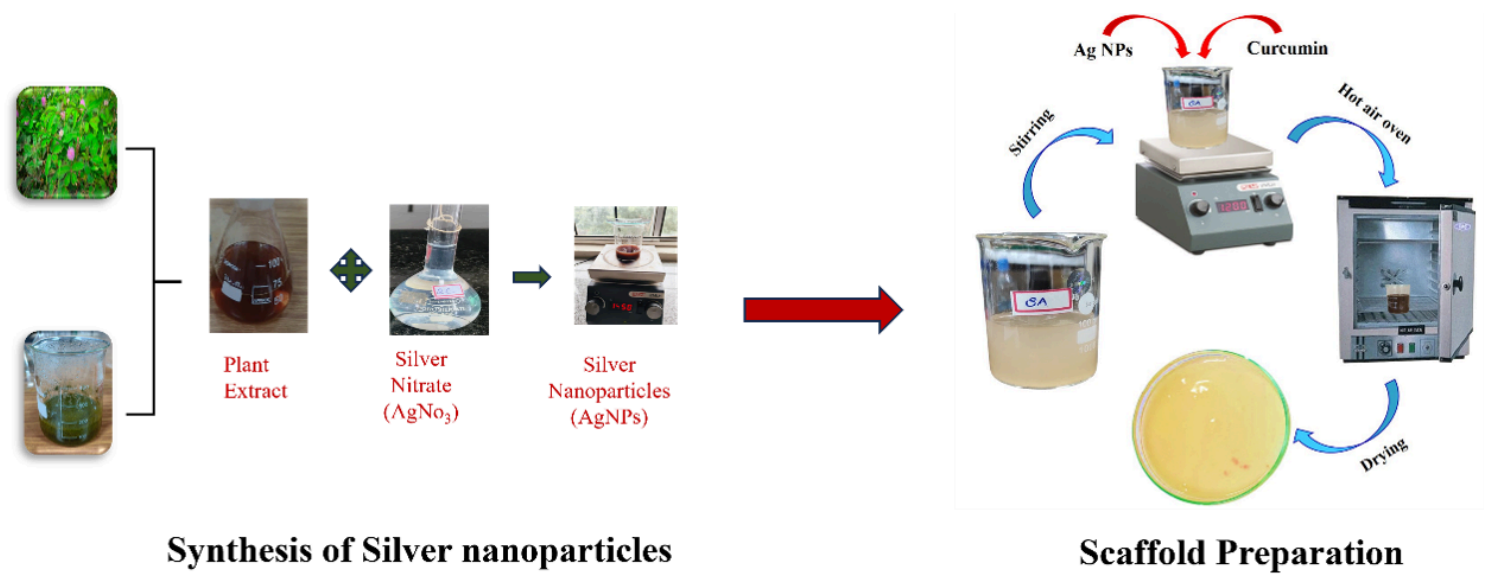


Figure 1

Schematic procedure of AgNPs / Alg – Cur hydrogel scaffolds fabrication

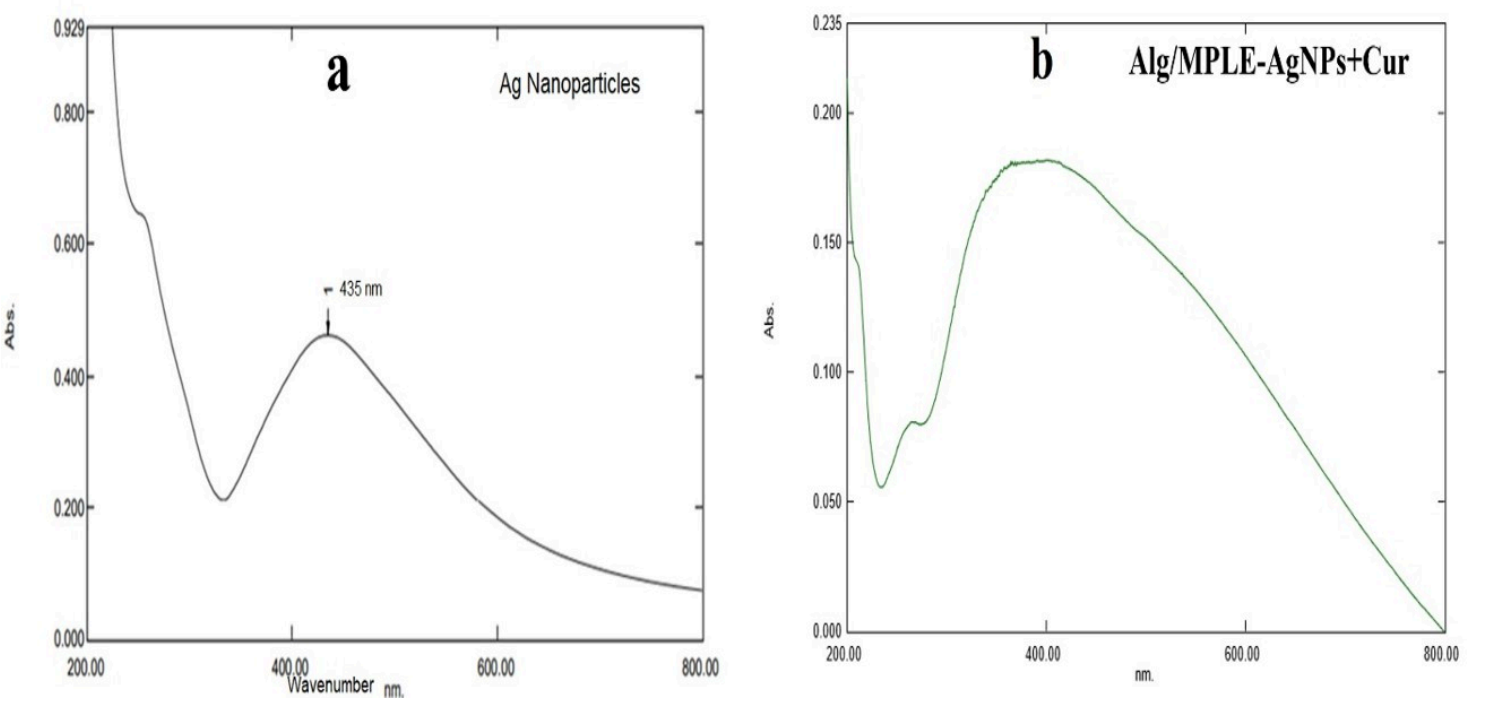


Figure 2

UV–Visible spectra of MPLE-AgNPs (a) and Alginate/AgNPs - Curcumin hydrogel scaffolds (b)

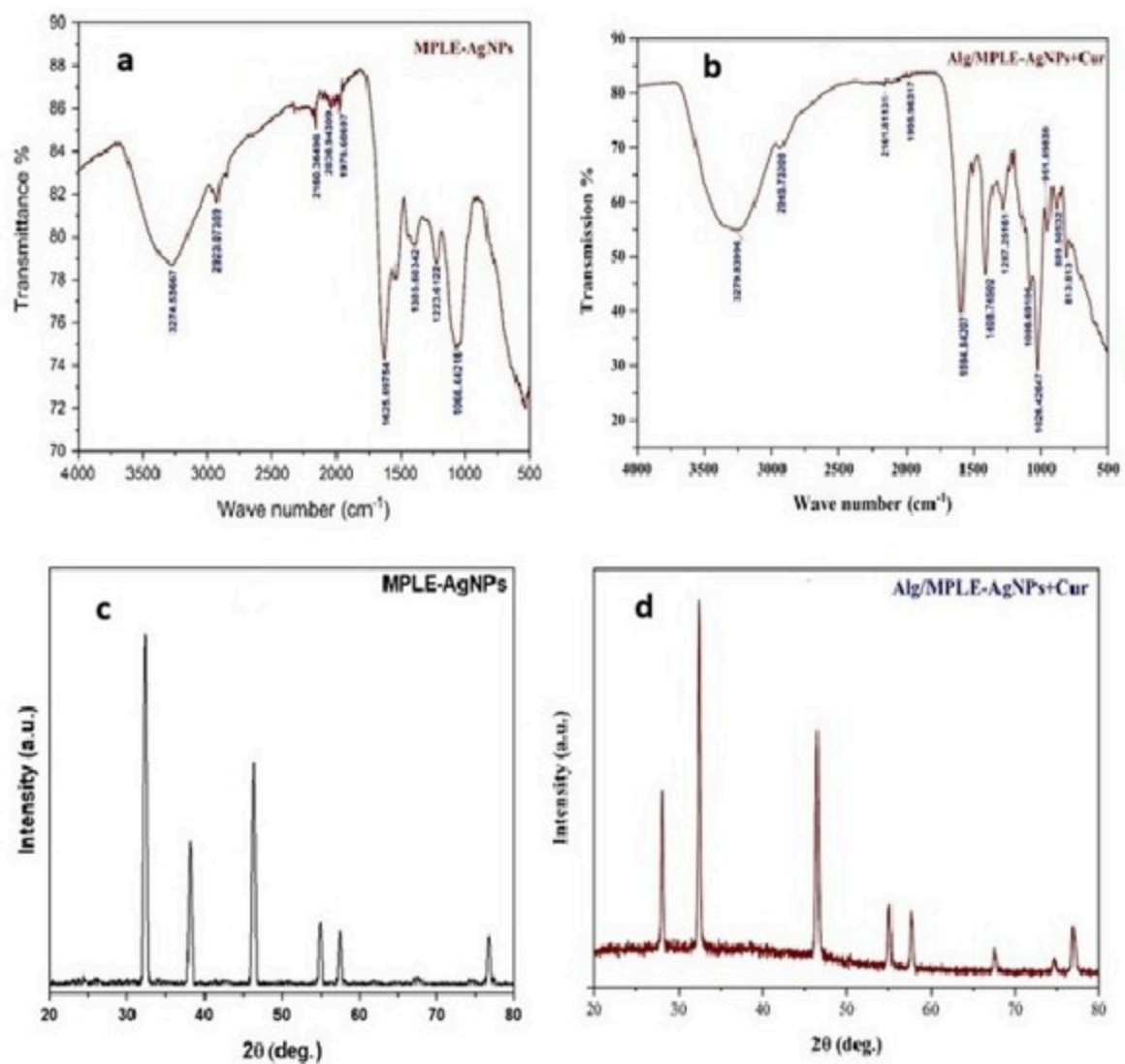


Figure 3

FTIR and XRD of MPLE-AgNPs (a, c) and Alginate/AgNPs-Curcumin hydrogel scaffolds (b, d).

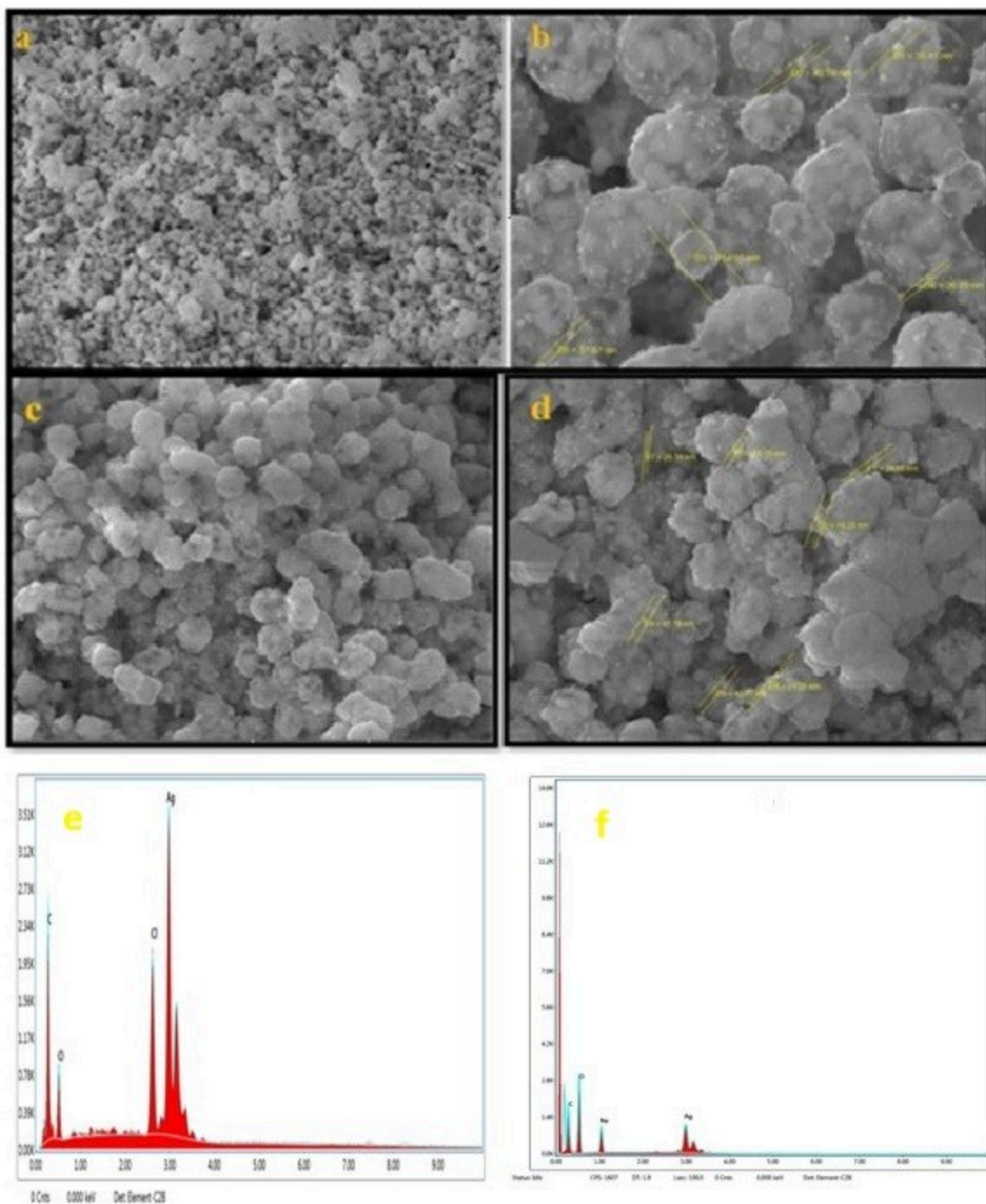


Figure 4

FESEM and EDX images of synthesized MPLE-AgNPs with magnification of (a) 10k, (b) 100 k and Alginate/AgNPs - Curcumin hydrogel scaffolds (c) 10k , (d) 100 k and EDX images (e and f).

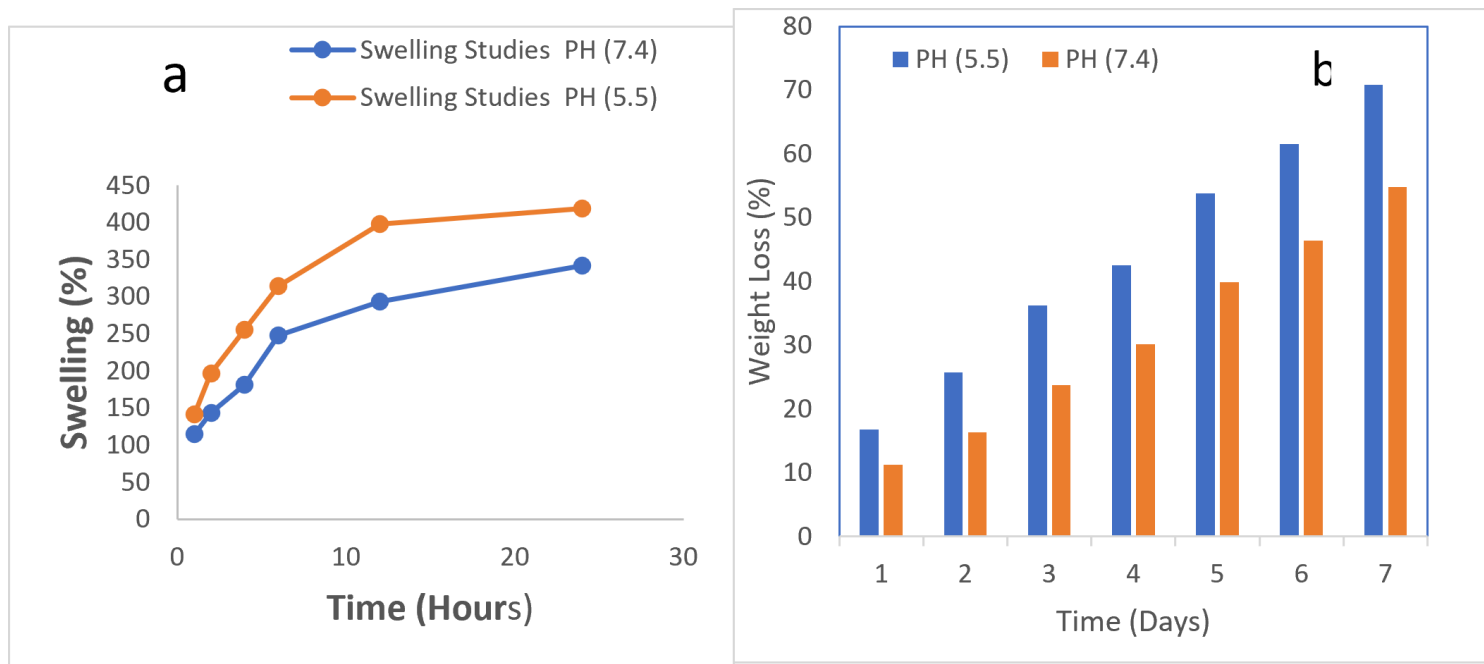


Figure 5

Swelling degree of hydrogel samples (a) and Invitro degradability (b) in different pH

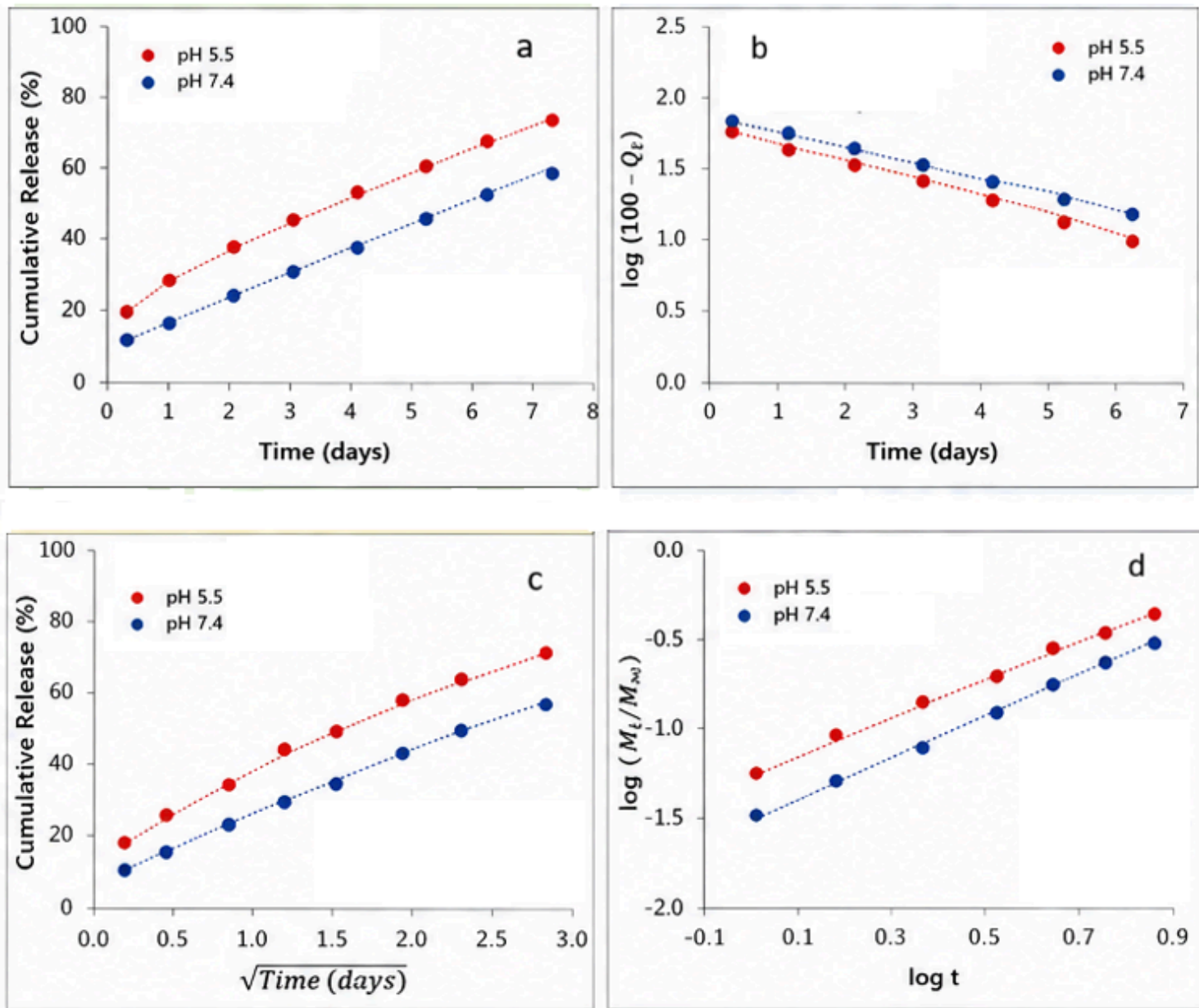


Figure 6

Drug release kinetics fitted to Zero-Order(a), First-Order(b), Higuchi(c), and Korsmeyer-Peppas models(d) at pH 5.5 and pH 7.4.



Figure 7

The antibacterial activity against several Gram-positive and Gram-negative bacteria strains.

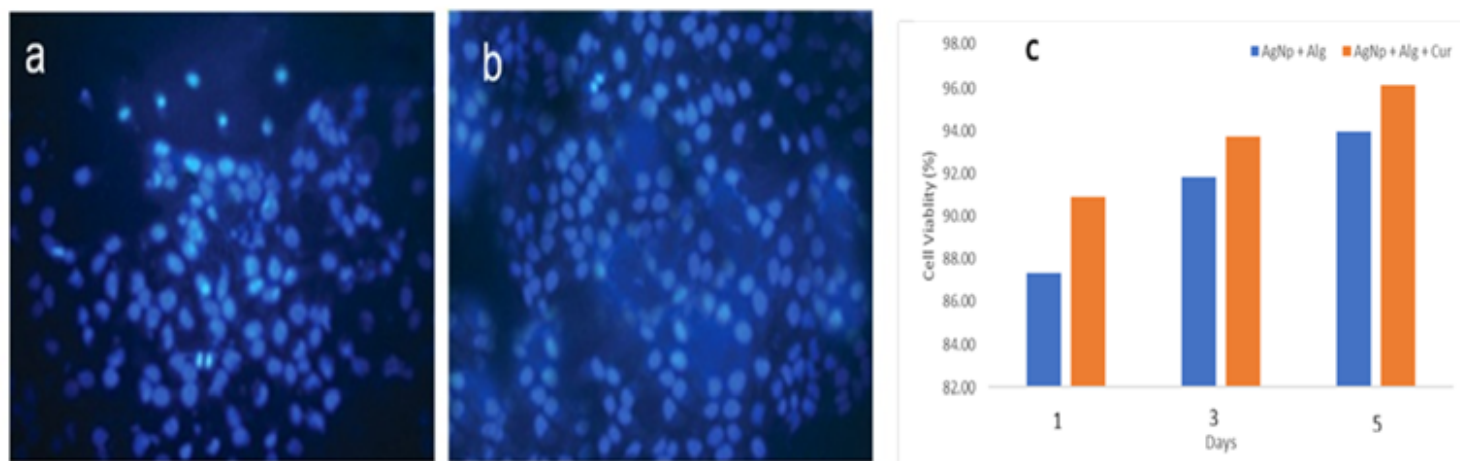


Figure 8

Fluorescent micrographs of hydrogel scaffolds stained with Hoechst 33342: (a) Alginate/AgNPs and (b) Alginate/AgNPs-Curcumin. (c) Proliferation of HaCaT cells on both scaffolds, assessed by MTT assay.