International Journal for Innovative Engineering and Management Research
PEER REVIEWED OPEN ACCESS INTERNATIONAL JOURNAL
www.ijiemr.org

A STUDY OF CURCUMIN-LOADED, ALGINATE-GELATIN COMPOSITE FIBERS FOR WOUND HEALING APPLICATIONS

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ABSTRACT

Wound healing is the body's multi-step reaction to injury (trauma) that leaves behind damaged or missing structures in the cellular milieu or tissue bed. Pathogens or foreign substances must be removed from the injured tissue location before the healing process can function properly. An aseptic or sterile dressing should be used to protect the wounded tissue that caused the wound and speed up the wound healing procedure, a complex biological and chemical process. Wound healing consists of the four aforementioned cascades or transition points (haemostasis, inflammation, proliferation, and remodeling phase). A topical anti-inflammatory medication may be useful in this situation.

KEYWORDS: Curcumin-Loaded, Alginate-Gelatin, Composite Fibers, Wound Healing Applications, chemical process.

INTRODUCTION

Polyphenols found in plants are beneficial not only because they reduce inflammation, but also because they are powerful antioxidants that protect cells from free radical damage (Pankongadisak et al. 2019 and Hewlings et al. 2017). The anti-inflammatory and free radical scavenging properties of curcumin, found in the rhizome of the turmeric plant, Curcuma longa, are crucial to the healing process. Barchitta et al., 2019, and Akbik et al., 2014). As an anti-oxidant, anti-inflammatory, antitumor, antimicrobial, anti-HIV and anti-cancer agent, and most notably for its anti bacterial action, it has great promise as a natural antibiotic (Varaprasad et al., 2020). Kulkarni et al. (2020) note that in addition to its unique medicinal capabilities, it is also a superb wound healing agent. This factor has helped to the development of suitable curcumin carriers that greatly enhance the stability and bioavailability of curcumin. (Fereydouni et al. 2019; Kaur et al. 2019).

For example, Fibrous structures, such as microfibers and nanofibers, are well-known as viable carriers for uses in drug delivery. Fibers are a desirable option for wound dressings because to their softness, flexibility, and unique and considerable elastic qualities. Those findings were published in 2018 Mutlu et al 2018. Fibrous mats made from curcumin/PVA have been produced by researchers who have studied wound healing; these mats have shown regulated drug release and antibacterial action against both gram-positive and gram-negative bacteria. As stated by (Mahmud et al., 2020). Wound healing activity was seen in rats treated with membranes containing Curcumin and chitosan-PVA, created by a different research team. As reported by (Abbas et al. 2019). Alginate and gelatin fibers were combined to create a composite polymer for use in medical applications in this study. Alginate has also been used as a wound dressing material because of its ability to speed up the healing process. In a recent study (Varaprasad et al., 2020, Afjoul et al. 2020). Gelatin has antimicrobial qualities and promotes the fast migration of regenerated cells, making it useful for wound healing applications. According to a group of researchers (Zahiri et al., 2020). In order to aid in tissue regeneration and wound healing, curcumin fibers were created. Ionic crosslinking, an inotropic gelation technique, was used to create the fibers. Due to the complexity of electrospinning, it was challenging to prepare alginate fibers by themselves. Alginate has limitations when employed alone since it does not form a mesh but rather a sheet, and because the crosslinking of dried alginate fibers with calcium ions results in a surface rather than a bulk crosslinking. The electrospinner's nozzle becomes clogged with curcumin over time (Bediako et al., 2020). Morphological, physical, chemical, and mechanical properties were also evaluated in the curcumin fibers that had been manufactured. The therapeutic activity was also studied in an in vivo setting using a full thickness wound model. Anomalous drug release was supplied by the microfibers, which showed molecular interaction outline with polymeric matrix (alginate and gelatin). According to the data, it improves the wound healing cascade compared to the commercially available formulation.

Characterization and identification of drug

Determination of melting point

Melting point of curcumin was determined by capillary method.

DSC

Differential scanning calorimetry with liquid nitrogen was used for the thermal analysis. Dry nitrogen gas was used to conduct the analysis. The instrument's heat flow and heat capacity were calibrated using high-purity indium. The crimped cover of the aluminum cell, which contained the sealed sample (2.4 to 4 mg), did its job. The sample was heated at a predetermined pace of 10 ^{o}C min^{-1} from 40 to 300 ^{o}C in air temperature.

FTIR

Pure curcumin's infrared spectrum was examined utilizing the ATR-FTIR with the KBr disc method. Spectrum smoothing and baseline correlation methods are now in use. The research was conducted at wavelength of 4000–400 cm^{-1}. In a recent study

Validation of analytical method for curcumin

The validation of an analytical method for curcumin is a critical step in ensuring the reliability and accuracy of results obtained from the analysis. Analytical methods play a pivotal role in pharmaceutical, food, and research industries where curcumin is frequently employed. The validation process involves a comprehensive assessment of various parameters to demonstrate that the method is suitable for its intended purpose.

One crucial aspect of method validation is specificity, ensuring that the analytical method can accurately and selectively measure curcumin in the presence of potential interfering substances. This is typically achieved through the use of high-performance liquid chromatography (HPLC) or other appropriate techniques, coupled with detectors that specifically identify and quantify curcumin.

Precision is another key parameter that must be assessed during validation. This involves evaluating both repeatability (intra-day precision) and intermediate precision (inter-day precision) to ensure that the method consistently produces reliable results under different conditions. The precision of the method is typically expressed as the relative standard deviation (RSD) of repeated measurements.

Linearity

40 µg/ml Six batches of curcumin stock solution were made using the above solvent combination. Analyses were performed in a combination of phosphate buffer saline (pH 7.4) and ethanol (40:40 v/v), with concentrations ranging from 0.4 µg/ml to 5 µg/ml having been produced from the aforementioned stock solution.

Accuracy

Six different solvents were used to make curcumin stock solution (n=6). Absorbance values were measured after dilutions of the stock solution were made to concentrations ranging from 0.40 µg/ml to 5.00 µg/ml. Concentrations (experimental values) were compared with the real (nominal) concentrations using the absorbance values that were obtained. Within 14 % of the actual value is acceptable for the mean.

Precision

Spectrophotometric analysis was used to compare the effects of preparing a curcumin solution (40 µg/ml) in six different solvents. Within 14 % of the theoretical value is acceptable for the mean.

Limit of detection (LOD)

The lowest detectable concentration of an analyte in a sample is known as the limit of detection. The LOD was calculated using data from six separate samples. LOD=3.3 σ/S was used as the determining factor. In this case, σ was the standard deviation of the regressed lines' intercepts. The average slope (S) of the regressed lines was calculated.

Limit of quantification (LOQ)

It is the concentration below which relative standard deviation becomes a meaningful measure of accuracy. Inaccuracy, as evaluated by the percentage difference between the measured and real values, is less than 20 %, but still over 80 %. In order to establish LOQ, six samples were tested. The primary purpose of the measurement was for impurity analysis, and the value was found to be LOQ=10 σ/S.

In this case, σ was the standard deviation of the regressed lines' intercepts. Linear regression average slope was S.

Intra-assay precision and accuracy

The absorbance of six samples was monitored and studied over the course of three consecutive days, alongside standard calibration curves, to determine the precision and accuracy of the intra-assay.

Inter- assay precision and accuracy

By running a series of calibration and quality control samples (at three levels, evaluated twice) through six separate batches, we were able to calculate the precision and accuracy of the inter-assay comparison.

Preparation of curcumin loaded composite microfibers

Ionotropic gelation was used in conjunction with other gelation techniques to create Curcumin-loaded alginate-gelatin fibers. The fiber composition is displayed in Table 4.3, and it was achieved by preparing solutions of sodium alginate: gelatin (1:1) at different quantities. After that, we solubilized the polymeric solution at 500 rpm for 30 minutes to get it ready for use.

Gelatin was fully dissolved in the aforementioned polymeric solution by heating it to 50 degrees Celsius. After dissolving the medicine (curcumin, 40 mg) in acetone (4 ml), it was progressively added to the biopolymeric solution. The curcumin polymeric dispersion was then withdrawn into a beaker containing 1 wt% $CaCl_2$ (with a 22 gauge needle). The resulting fibers were washed in water and dried in the air. The aforementioned procedure was used to create drug-free alginate/gelatin fibers.

Physicochemical evaluation of curcumin loaded microfibers

Physical properties like degradation study, water absorption, mechanical characteristics, in vitro release studies, and in vivo animal studies were examined for the curcumin-loaded microfibers along with surface morphology (SEM) analysis, entrapmemt efficiency, physicochemical analysis using different techniques like FTIR, and computational technique (molecular simulations).

Degradation study

Microfibers were placed on aluminum foil and submerged in phosphate buffer saline (pH 7.4) for decomposition analysis. Microfibers in their medium were cultured at 37 degrees Celsius for 20 days. The microfibers were processed for 20 days, cleaned, air-dried, and then baked for several hours to ensure they were completely dry.

Entrapment efficiency

Microfiber samples (10 cm in length) were weighed and a ternion used to calculate the quantity of curcumin contained inside them. For maximal drug extraction (24 hours), the samples were placed in a solvent system (phosphate buffer saline/ethanolic solvent) at a ratio of 40:40 v/v. After being broken down using a pestle and mortar, the fibers were added to a flask of hydro alcoholic solvent. More filtering and wavelength 421 nm analysis were applied to this combination. Standardized ethanolic solutions were used to plot the curve.

Water uptake

Phosphate buffered solution (pH 7.4) was used to study how much water a polymeric matrix of microfibers could absorb. Finally, after measuring the microfibers' starting weight, they were soaked in the aforementioned buffer solution (ten milliliters for twenty-four hours). After this soaking period ended, the strands were carefully plucked out using tweezers. The surface water was absorbed using filter paper to get the wet weight, also known as the final weight. The percentage of water consumed was determined using the following equation.

$$Wateruptake = [Initialweight - finalweight / Initialweight] \times 100 \qquad (1)$$

Mechanical properties

Tensile analyzers with predetermined loading cell capacities and testing times (4 mm/min) were used to evaluate the microfibers' mechanical properties. Pieces of fiber were cut to a length of 4 centimeters. The aforementioned experimentation was carried out in ambient conditions.Using the provided formulae, we found the values for the two intrinsic parameters, tensile strength and elongation at break.

Characterization of curcumin loaded microfibers

SEM

Curcumin-infused microfibers were fastened to a metal probe by double-sided tape (adhesive). Gold or palladium was used to coat the fibers, and the fibers' surfaces were interpreted using a 240x and 400x magnification and a 10 kV acceleration. Paint.net, a freeware raster graphics editing tool, was used to enhance the clarity, brightness, and contrast of the scanning electron microscope images that were retrieved.

FTIR

Both blank and drug-loaded composite microfibers underwent ATRFTIR spectroscopy analysis to evaluate molecular interactions between biopolymers; and the drug and biopolymers. Wavelengths between 4000 to 400 cm^{-1} were used for the research.

XRD

Curcumin, polymer (alginate), and curcumin-loaded fibers were examined for crystallinity using X-ray diffractometry. The research was conducted using an X-ray diffractometer, a powerful and well-developed instrument that measures the scattered intensity of an X-ray beam impinge on a sample as a function of the incident and scattered angles, polarizations, wavelengths, and energies. This technology not only reveals the chemical content of raw materials because to its adaptability and lack of damaging operating processes, but it also reveals their crystalline structure. Crushed microfibers were scanned in the 0 to 80 diffraction angle range using the following parameters: nickel-filtered source, Cu K-alpha radiation; voltage 34 kV; current 24 mA; scan speed 0.04 min^{-1}.

Molecular modelling

The energy reduction strategies in Molecular Mechanics were implemented utilizing computer tools for modeling and analysis. Alginates and gelatin both have saccharide units with naturally occurring bond angles that are comparable to one another. The MM+ Force Field technique was used to set up the models, and the models were then integrated to create the molecular complex. For energy minimization, the Polak-Ribiere gradient approach was used until an RMS derivative gradient of 0.001 kcal/mol (RMSD) was shown.

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In vitro dissolution studies

The drug release profile of drug-loaded composite microfibers in vitro was obtained by placing the microfibers in a buffer solution (pH 7.4 phosphate buffer solution) at 37 °C for 72 hours. The aforementioned buffer solution was added to a sealed container with 4 mg of curcumin, and then placed on an orbital shaker. Sink condition was maintained by isolating 3 ml aliquots at predetermined time intervals (0.4, 1, 2, 4, 8, 12, 24, 48, and 72 hours) before replenishing with new buffer solution. At a wavelength of 421 nm, the samples were analyzed. Kinetic models were fit to the release data, including the Zero order, First order, Higuchi, Hixon-crowell, and Korsmeyer-peppas models, to better understand the drug's release mechanism.

CONCLUSION

Composite microfibers will be created using the anti-inflammatory and wound-healing medication curcumin. The first pass metabolism it experiences shortens its half-life to about 3.3 hours. Using a UV/visible spectrophotometer, a reliable technique for analyzing curcumin was created. Maximum absorption of curcumin was observed at 421 nm in a phosphate buffer (pH 7.4) and ethanol (40:40, v/v) combination. This research showed that sodium alginate and gelatin may affect the physicochemical characteristics of blank composite microfibers. Crystal deposition, perhaps in the form of calcium chloride crystals, was seen by SEM to have occurred on the surface of fibers. The morphology of the fiber surface, on the other hand, was uniform and smooth indicating that the alginate and gelatin mix was very miscible and had a high degree of homogeneity. Sodium alginate and gelatin were also shown to interact via hydrogen bond formation using Fourier transform infrared spectroscopy. First order release kinetics was found for both gelatin and curcumin over a range of alginate concentrations. Anomaly or non-fickian release behavior is shown by the n value for all formulations. This number suggests that the medication was released from the composite microfibers by a process involving a coupling of diffusion and erosion control. Molecular simulations show that they are separated by an energy gap and a force of Vander Waals attraction. Maximum release with a prolonged duration of 72 hours at a large rate of release is achieved in the formulations of drug-loaded composite microfibers. Researcher found that drug-loaded composite microfibers contracted more than commercially available

formulations in an animal investigation. Because of their advantageous mechanical features, composite microfibers may provide a feasible strategy for developing controlled medication delivery.

REFERENCES

Augustine R., Rehman S.R.U., et al. (2020). Electrospun chitosan membranes containing bioactive and therapeutic agents for enhanced wound healing. International Journal of Biological Macromolecules, 156, 153-170.

Azimi B., Maleki H., et al. (2020). Bio-based electrospun fibers for wound healing. Journal of Functional Biomaterials, 11, 67-79.

Bagher Z., Ehterami A., et al. (2020). Wound healing with alginate/chitosan hydrogel containing hesperidin in rat model. Journal of Drug Delivery and Science Technology, 55, 101379.

Bakhsheshi-Rad H.R., Hadisi Z., et al. (2020). In vitro and in vivo evaluation of chitosanalginate/gentamicin wound dressing nanofibrous with high antibacterial performance. Polymer Testing, 82, 106298.

Bao P. Kodra A. et al. (2009). The role of vascular endothelial growth factor in wound healing. Journal of Surgical Research, 153, 347-358.

Barchitta M., Maugeri A., et al. (2019). Nutrition and wound healing, an overview focusing on the beneficial effects of curcumin. International Journal of Molecular Sciences, 20,1-14.

Batista M.P., Goncalves V.S., et al. (2020). Novel alginate-chitosan aerogel fibres for potential wound healing applications. International Journal of Biological Macromolecules, 156, 773-782.

Bayomi S.M., El-Kashef H.A., et al. (2015). Synthesis and biological evaluation of new curcumin analogues as antioxidant and antitumor agents, molecular modeling study. European Journal of Medicinal Chemistry, 101, 584-594.

Bediako J.K., Lin S., et al. (2020). Benignly-fabricated crosslinked polyethylenimine/calciumalginate fibers as high-performance adsorbents for effective recovery of gold. Journal of Cleaner Production, 252, 119389.

Behm B., Babilas P., et al. (2012). Cytokines, chemokines and growth factors in wound healing. Journal of the European Academy of Dermatology and Venereology, 26, 812-820.

Beyene R.T., Derryberry S.L., et al. (2020). The effect of comorbidities on wound healing. Surgical Clinics, 100, 695-705.

Bhardwaj N., Chouhan D., et al. (2017). Tissue engineered skin and wound healing: current strategies and future directions. Current pharmaceutical design, 23 , 3455-3482.

Bhatia M., Rajulapati S.B., et al. (2017). Synthesis and implication of novel poly (acrylic acid)/nanosorbent embedded hydrogel composite for lead ion removal. Scientific reports, 7, 1-16.

Biazar E., & Keshel, S.H. (2013). The healing effect of stem cells loaded in nanofibrous scaffolds on full thickness skin defects. Journal of biomedical nanotechnology, 9, 1471-1482.

Blanco P.A., Lopez R.A., et al. (2015). Characterization, release and antioxidant activity of curcumin-loaded amaranth-pullulan electrospun fibers. LWT-Food Sci Technol, 63, 1137-1144.

Boateng, J., & Catanzano O. (2015). Advanced therapeutic dressings for effective wound healing—a review. Journal of Pharmaceutical Sciences, 104, 3653-3680.