Curcumin nanoparticles supported gelatin-collagen scaffold: Preparation, characterization, and in vitro study

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**Abstract**

It is possible to reveal the potential of water-insoluble drugs by increasing their solubility in water with some nanotechnology techniques. Nanosuspension technology can solve this problem by increasing the water solubility and as well as bioavailability of these drugs. The present work is pointed at the evaluation of nanosuspension of curcumin, a poorly water-soluble drug. The Curcumin nanoparticles (CNs) were prepared with ultrasonication method using dichloromethane as solvent and water as antisolvent and characterized via spectroscopic methods (UV-vis and FT-IR) and Scanning Electron Microscopy (SEM). Curcumin nanoparticles Biosfilms (CNs-BF) supported gelatin-collagen scaffold were prepared. Curcumin nanoparticles were obtained by nanosuspension technique. And then, to overcome the limited effects of curcumin such as solubility and bioavailability, nanoparticle films were prepared by incorporating it into the structure of biocompatible collagen-gelatin scaffolds. Curcumin is limited by some factors that limit its clinical applicability, such as low oral bioavailability, poor water solubility and rapid degradation. However, they can be applied clinically when they are included in the structure of biocompatible gelatin-collagen scaffolds.

1. Introduction

Nanotechnology, which was coined by Norio Taniguchi in 1974 [1], has attracted great attention in recent years due to its superior opportunities. In short, nanotechnology concerns with the conversion of larger molecules to nanometer size and it changes the physicochemical properties which are mainly solubility, absorption, bioavailability, efficacy and transporting system [2]. The medicinal effect of various drugs or natural compounds can be enhanced by their nanoparticles [3]. Nanosuspensions is a powerful approach to increase the solubility, biocompatibility, and stability of biomaterials [4]. Nanosuspensions are colloidal dispersions of nanoscale particles and consist of biomaterial with low water solubility [5]. The reformation of biomaterial particles to nanometer size causes an improved dissolution ratio because of expanded surface area. Two main techniques were reported for the preparation of nanosuspension called bottom-up technology and top-down technology [4,6]. In the case of top-down techniques, materials are fragmented into micro or nano-sized ranges using mechanical force, but these techniques require high energy. In the bottom-up techniques, the material is dissolved in a solvent and precipitated with a non-solvent. The bottom-up techniques are greener and more economical [7].

Most newly discovered drugs are insoluble in water and therefore have poor bioavailability [8]. The improvement of the dissolution rate and bioavailability of drugs having poor water solubility is one of the fundamental targets of drug development [9]. Various techniques have been evolved such as physical and chemical modifications. These modifications include size reduction, solubilization, solid dispersion, complexation, and salt formation. But these techniques have several disadvantages and limitations in practice such as poor stability and inability to obtain the desired particle size [10]. Thanks to nanotechnology, it is possible to overcome these limitations.

Danazol is the first example of the development of drug nanotechnology. Researchers reduced the particle size of the danazol, which has poor water solubility, to 169 nanometers and formed a nanosuspension, thus increasing the oral bioavailability of danazol [11]. Also drugs such...
as valsartan [12], metformin [13], tinidazole [14], and celecoxib [15] are formulated as nanosuspension. The efficacy of these drugs is increased in vitro or in vivo both utilizing nanosuspension.

Curcumin or diferuloylmethane [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a golden phenolic pigment extracted from turmeric, Curcuma longa, a traditional Asian spice, cosmetic, and folk remedy [16]. Over the last era, curcumin possesses potent antimicrobial [17], anti-inflammatory [18], antioxidant [19], and antitumor [20] properties. Also, curcumin is known to contribute to the healing of skin wounds [21]. However, these properties of curcumin are limited due to some factors such as low oral bioavailability, poor aqueous solubility, and restricting clinical applicability [22]. Despite the diverse biological activities of curcumin, clinical trials are still ongoing for different diseases such as colon and pancreatic cancers, and Alzheimer’s [23]. The main reason why curcumin has not been able to reveal its potential as a drug is its poor water solubility. Adewale et al. reported the protecting capability of curcumin against sodium nitrite-induced hepatotoxicity in Wistar rats [24]. Abolaji et al. investigated the effect of Cu\(^{2+}\) toxicity on wild-type flies (Drosophila melanogaster). They observed that when exposed to copper with curcumin, inhibited copper toxicity due to its metal chelation and antioxidative effect [25]. In a review, researchers mentioned the therapeutic applications of curcumin with different nanoformulations in neurological diseases [26].

Gelatin is a biopolymer that is developed by thermal denaturalization of collagen and it is obtained from animal skin and bones [27]. Gelatin consists of glycine, proline, and 4-hydroxy proline amino acids and it is translucent, colorless, and almost tasteless. Gelatin is capable of making a large number of hydrogen bonds and is held together by second interactions such as Van Der Waals forces. It is primarily used in the food, pharmaceutical, and cosmetic industries for a variety of purposes, its gelling properties are important and form a gel at temperatures below 35 °C [28,29]. Nanoparticles can be incorporated with gelatin to produce gelatin-based films and this cooperates to improve the mechanical and biological properties of composites [30]. Collagen is not a uniform substance, it is a family of proteins. It is a naturally occurring protein group found in animals, especially in the meat and connective tissues of mammals. Different types of collagen can obtain in large volumes from mammalian animals as well as marine organisms [31].

This study aimed to enhance curcumin dissolution rate and thereby bioavailability by forming nanosuspension. Also, curcumin nanoparticles have been incorporated into the structure of biocompatible gelatin-collagen biofilm to overcome the limited effects of curcumin. Characterization of the obtained nanosuspension and biofilm was also performed.

2. Materials and methods

Reagents were acquired from Aldrich and Merck. The reagents were used as received from suppliers without further purification. Infrared spectra were collected on a Perkin Elmer Spectrometer 100 IR spectrometer using the KBr pellet technique between 450–4000 cm\(^{-1}\). UV–vis analyses were made with a Varian 100 Bio UV–vis Spectrophotometer. Scanning Electron Microscopy (SEM) analyses were collected on a Thermo Scientific Apreo S.

2.1. Preparation of curcumin nanoparticles (CNs)

Curcumin (100 mg, 0.27 mmol) was added to a beaker and dissolved in 20 mL dichloromethane. Boiling water (50 mL) was taken into a 100 mL flask, and 1 mL of the curcumin/dichloromethane solution was added into boiling water dropwise with a flow rate of 0.2 mL/min in 5 min under ultrasonic conditions. The ingredients were stirred at 800 rpm at RT for 20 min when a clear orange-colored solution was obtained.

2.2. Preparation of curcumin nanoparticles biofilms (CNs-BF)

The gelation (GL) and collagen (CL) was prepared according to the method of [32,33]. To form films, three different Petri dishes were prepared and a mixture containing collagen (2 mL) and gelatin (2 mL) were added to each. Three different volumes (1, 2, and 3 mL) of CNs were added to these mixtures. The last mixtures were spread on the bottom of the Petri dishes and ethylene glycol and water mixed in the ratio (1 mL:99 mL) was applied on their surfaces. As a result, nanoparticle-collagen-gelatin biofilms were obtained with volumes of 1 mL-2 mL-2 mL (CNs-BF I), 2 mL-2 mL-2 mL (CNs-BF II), 3 mL-2 mL-2 mL (CNs-BF III), respectively. The films were left to dry for 2 days.

2.3. Mechanical properties

Mechanical properties were assessed using three dumbbell shaped specimens of 4 mm wide and 10 mm length. Tensile strength (MPa) and elongation at break (%) were measured using Universal testing machine (INSTRON model 1405) at an extension rate of 5 mm/min. Water absorption (%) capacities of different RLCs prepared were determined according to Sekar et al. (2007) [34].

2.4. Cell culture

Human cell lines were purchased from ATCC (American type culture collection). HaCaT cell lines were maintained in RPMI supplemented with 10 % fetal bovine serum and 1% l-glutamine at 37 °C, 5 % CO\(_2\) in a humidified incubator. When the cells were confluent, they were routinely sub-cultured using 0.25 % trypsin–ethylenediaminetetraacetic acid (EDTA) solution.

2.5. Application of CNs-BF

A stock solution of CNs-BF was prepared at a concentration of 10 mM in the cell culture medium. Then, 100 μL of HaCaT cells was added to the solution, and cells were incubated for 48 h in 37 °C and 5% CO\(_2\).

2.6. Cell viability analyses with MTT assay

The cell viability was determined the tetrazolium reduction assay. The stock solution of 3-(4,5- dimethylthiazol-2-yl)-2,5- diphenyltetrazoliumbromide (MTT) was prepared in PBS. Cells were cultured for 24 h. HaCaT cells were added on CNs-BF III instead well plates. The culture medium from all wells was discarded and MTT solution was added and then incubated for 1 and 2 days at 37 °C. MTT solvent (DMSO) was added to each well and then shaken for 15 min. Absorbance was measured at 570 nm with a 690 nm reference filter.

2.7. Statistical analysis

The results are presented as mean ± standard deviation (SD) for the three individual experiments (n = 3). ANOVA (Analysis of variance) and Duncan’s multiple range analysis were done to determine the significant differences among the different groups. P values of p < 0.05 were considered significant.

3. Results and discussions

The use of curcumin as a nanoparticle has promoted the basic difficulties of curcumin such as low solubility, instability, and weak bioavailability [35]. To use these advantages, we prepared a nanosuspension of curcumin which has good water solubility. The formed nanosuspension was clear and no sedimentation on shaking. Also, we obtained a film that has a gelatin-collagen scaffold from curcumin nanoparticles. Rashed et al. developed curcumin nanoparticles/hydrogel composite and displayed faster reformation of
diabetic skin wounds [36].

The curcumin nanoparticles were identified via UV–vis spectroscopy, Scanning Electron Microscopy (SEM), and FT-IR (Fig. 1). For UV–vis analyses, 1 mL of nanosuspension solution was diluted to 2 mL with deionized water. The spectral data of the final solution were examined for curcumin nanoparticles from 200 to 600 nm at 25 °C. CNs showed an absorption peak at 260 and 400 nm with the absorbance values of 1.8 A and 1.1 A, respectively (Fig. 1a). The CNs have severe functional groups and double-bonds. The curcumin compound, featuring an extended π electron system, provides a remarkable effect on the physical properties of the nanoparticles. The absorption spectra of the CNs indicated the conjugation of the CNs with transitions like π→π*.

The surface morphology of the curcumin nanoparticles was unveiled by SEM (Fig. 1b). To prepare the CNs for SEM analysis, one drop of CNs was dropped on the aluminum foil and kept in the oven for overnight. The SEM result showed that the nanoparticles had a smooth surface. The IR spectrum of CNs showed a band, approximately 3500 cm$^{-1}$, which was assumed to be indicative of the νO-H vibrations. Furthermore, the vibrations appearing in the range of 1640 cm$^{-1}$ were associated with the alkene νC=C peaks (Fig. 1c). In the FT-IR spectrum of CNs, the stretching of the carbonyl group did not exist. Besides curcumin has resonance, the nanoparticle formation process could proceed through this functional group.

The CNs-BF III was characterized via Scanning Electron Microscopy (SEM) and FT-IR (Fig. 2). In the IR spectrum of CNs-BF III, the peak observed at region of 3300 cm$^{-1}$ corresponds to νN–H stretching. Successively, aromatic νC–H stretching vibration frequencies were observed at 2800 cm$^{-1}$. The surface morphology of CNs-BF III (SEM) revealed a fibrous structure, which is a characteristic feature of collagen, gelatin, and curcumin (Fig. 2b).

Mechanical strength of CNs-BF I, CNs-BF II and CNs-BF III were characterized by incorporation of CNs with three different concentrations (0.1 %, 0.2 %, and 0.3 %) from Table 1. It is regularly acknowledged that the broad surface to volume proportion of the nanoscale incorporations performs a vital role in increasing the mechanical property of the biomaterials [37]. The development of a nanostructured
network of finely dispersed particles, which firmly attaches to the biopolymer is presumably responsible for the reinforcement [38].

In vitro analysis of curcumin nanoparticle-collagen-gelatin CNs-BF III was assessed using MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (Fig. 3a). CNs-BF III was used in in vitro studies because its mechanical properties were slightly better. The biocompatibility of the human keratinocyte cell line (HaCaT) for CNs-BF on the day 1 and 2. Fig. 3b morphology of viability of HaCaT cells on films using fluorescence microscopy. The great biocompatibility and porous nature of the CNs-BF, as well as the equal distribution of collagen, gelatin, and CNs to which cells grow well clarify this result. Senthil et al. [39] proclaimed that the dispersion microstructure of carbon nanoparticles presents an essential role in the reinforcement of nano biocomposites. With the CNs-BF, the cells could preserve their HaCaT cell shape, according to the results. This shows that the CNs-BF, invented are cell-friendly and biocompatible. The positive surface area of collagen and gelatin helps it to effectively assist cell development [40]. Curcumin nanoparticles are natural substances that have a wide range of pharmacological effects and are used in medicine. It helps in the control of free radicals, inflammatory cells, and microbial growth [41].

Basically the development of collagen scaffold is a trend in study involving the treatment of skin wounds. Curcumin nano-based scaffold prepared from natural collagen and gelatin blends were studied as potential tissue regeneration materials.

In order to synthesize biocompatible CNs, important characteristics such as nanoparticle size and shape were considered for reinforcement materials in our study. The CNs-BF exhibited good mechanical, physico-chemical and ensuring cell growth without using any external growth factor. Low solubility and high hydrophobic stability based curcumin nanoparticles used for the biomedical application [26]. Curcumin nanoparticles with low solubility and high hydrophobic stability are employed in biomedical applications, and it is well recognized that effective nanocarrier-based delivery function can improve therapeutic compounds stability as well as its potential to penetrate biological barriers location of the body and achieve the target area [42].

4. Conclusions

Nanoprecipitation is the method that produces stable nanosuspension and can increase the dissolution rate and as well as bioavailability. Curcumin is a poorly water-soluble biomaterial. The bioavailability of biomaterials that are not soluble in water and organic solvents can be increased by the nanosuspension technique. Poor bioavailability problem of hydrophobic drugs are solved by nanosuspension. In this study, CNs were obtained by nanosuspension and collagen-gelatin supported curcumin nanoparticle films were prepared. The CNs were characterized by UV–vis, FT-IR, and SEM, and the CNs-BF structure was illuminated by FT-IR and SEM analyses. CNs-BF has exhibited biocompatibility on the HaCaT cells line and good mechanical properties. These films into suitable for tissue regeneration application.

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Conflict of interest

The authors declare no conflict of interest.
Serdar Batıkan Kavukçu: Methodology, Writing - original draft.
Sinem Çakır: Visualization. Ashlan Karara: Software. Hayati Türkmen: Formal analysis. Senthil Rethinam: Supervision, Methodology, Validation.

Declaration of Competing Interest

The authors report no declarations of interest.

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