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Hydrogel beads bio-nanocomposite based on *Kappa-Carrageenan* and green synthesized silver nanoparticles for biomedical applications

Susan Azizi[a,*], Rosfarizan Mohamad[a,b,**], Raha Abdul Rahim[c], Reza Mohammadinejad[d], Arbakariya Bin Ariff[a,e]

[a] *Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[b] *Laboratory of Biopolymer and Derivatives, Institute of Tropical Forestry and Forest Products, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[c] *Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[d] *Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran*
[e] *Bioprocessing and Biomanufacturing Research Centre, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

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ABSTRACT

This paper describes the fabrication and characterization of bio-nanocomposite hydrogel beads based on *Kappa-Carrageenan* (*κ-Carrageenan*) and bio-synthesized silver nanoparticles (Ag-NPs). The silver nanoparticles were prepared in aqueous *Citrullus colocynthis* seed extract as both reducing and capping agent. Cross-linked *κ-Carrageenan*/Ag-NPs hydrogel beads were prepared using potassium chloride as the cross-linker. The hydrogel beads were characterized using XRD and FESEM. Moreover, swelling property of the hydrogel beads was investigated. The Ag release profile of the hydrogels was obtained by fitting the experimental data to power law equation. The direct visualization of the green synthesized Ag-NPs using TEM shows particle size in the range of 23 ± 2 nm. The bio-nanocomposite hydrogels showed lesser swelling behavior in comparison with pure *κ-Carrageenan* hydrogel. Regardless the slow Ag release, *κ-Carrageenan*/Ag-NPs presented good antibacterial activities against *Staphylococcus aureus*, *Methicilin Resistant Staphylococcus aurous, Peseudomonas aeruginosa* and *Escherichia coli* with maximum zones of inhibition 11 ± 2 mm. Cytotoxicity study showed that the bio-nanocomposite hydrogels with non-toxic effect of concentration below 1000 μg/mL have great pharmacological potential and a suitable level of safety for use in the biological systems.

1. Introduction

Hydrogels derivative from natural polysaccharides are high-water content polymeric materials which have a number of characteristics such as biodegradability, biocompatibility, stimuli-responsive characteristics and biological functions promising for biomedical applications such as tissue engineering, drug delivery and biosensor [1,2].

The *κ-Carrageenan* is a sulphated linear polysaccharide of D-galactose and 3,6-anhydro-D-galactose obtained by alkaline extraction from red algae. Because of their biocompatibility and high capacity to form hydrogels, *κ-Carrageenan* has been widely used in food and pharmaceutical industries [3]. The use of complexation between oppositely charged macromolecules to generate *κ-Carrageenan* beads can attract great attention as a drug-controlled release formulation because of the simplicity and gentleness of this manner and production of small size and uniform shapes in compared with the conventional hydrogels [4–7].

Owing to the greater biomedical relevance, there is an increasing attention to develop the antibacterial hydrogels [8]. Among the antimicrobial hydrogels, the antibacterial inorganic-based nanocomposite hydrogels are especially favorable to inhibit the bacterial growth, consequently making them attractive in the fields of biomedical and biotechnology [9]. Several types of inorganic

* Corresponding author.
** Corresponding author at: Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
E-mail addresses: azisusan@gmail.com (S. Azizi), farizan@upm.edu.my (R. Mohamad).

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Fig. 1. Photograph of dried *Citrullus colocynthis*.

nanoparticles with antimicrobial activities have come up such as titanium [10], zinc oxide [11], magnesium, gold [12] and silver (Ag) [13]. Among the above nanoparticles, silver has established to be favorable candidates for antimicrobial activity against a broad spectrum of pathogenic bacteria [14], as well antibiotic-resistant bacteria [15,16]. Sliver nanoparticles also well known for its antiseptic, anti-inflammatory and multilevel antimicrobial activities (multidrug resistance) [17,18] accompanied by low systemic toxicity [18]. Hence, many studies had been done on the development of silver nanocomposite hydrogels for biomedical applications during the last few years [19–22]. However, in most of these studies chemical methods have been applied to synthesize silver nanoparticles, which restrict the medical applications of hydrogel nanocomposites, due to toxicity of starting materials. Considering to the above concern, this study was undertaken to develop silver nanocomposite hydrogels with green chemistry to enhance their level of safety for use in biological systems.

Recently, plant-mediated biological synthesis of nanoparticles is gaining significance owing to its simplicity, eco-friendliness and extensive pharmaceutical effects [23]. Bio-constituents of different plant extracts were known as potential synthesizers and stabilizers of metal nanoparticles. In addition, the plant metabolites with medicinal effects have the potential to be attached on the surface of nanoparticles during the synthesis process which finally leads to the occurrence of subsequent varied surface effects during their medicinal applications [24].

Citrullus colocynthis (*C. colocynthis*) (Fig. 1) a member of the *Cucurbitaceae* family, is an important and extensively used plant in traditional medicine distributed throughout Asia. It has been reported to have numerous important biological properties such as antioxidant [25] anticancer [26] anti-inflammatory [27] and antimicrobial [28–30] activities. The seed contains phytochemicals such as glycosides, flavonoids, alkaloids, carbohydrates, fatty acids and essential oil [31] which are capable of reduction and stabilization with high biological activities that motivate the our interest to utilize it in the synthesis of Ag-NPs.

In this study, we have first demonstrated a simple one-step green process to synthesizing Ag-NPs using *C. colocynthis* seed extract as the both reducing and stabilizing agent. Secondly, we designed a high safety hydrogel based antimicrobial bio-nanocomposite using biosynthesized Ag-NPs as an antimicrobial agent and *κ-Carrageenan* as hydrogel matrix. The effect of the concentration of the as synthesized Ag-NPs on the morphology, swelling behavior, cytotoxicity and antibacterial activity was examined.

2. Materials and methods

2.1. Materials

κ-Carrageenan (300 000 g/mol Fluka Chemie), $AgNO_3$ (□99.98%, Merck), potassium chloride (KCl) (>99%, Sigma-Aldrich). Ripe fruits of *C. colocynthis* were collected from Koohzar district, Khuzestan, Iran in May–June 2015. The plant specimens were identified and authenticated by Department of Botany, Shahid Chamran University, Iran.

2.2. Preparation of C. colocynths seed extract

The seeds were removed from the fruits and dried at room temperature and then milled to fine powder using grinder. The material that passed through 80-mesh sieve was used for extraction purpose. Briefly, the fine material was extracted with ethanol for 8 h in a Soxhlet apparatus. The solvent was removed by rotary evaporation. The dried, crude concentrated extract then stored in a refrigerator (−4°C).

2.3. Biosynthesis of Ag-NPs

Silver nitrate (0.003 M) was dissolved in 100 mL of distilled water under magnetic stirring. After complete dissolution, 0.2 g of the dried *C. colocynths* seed extract was added to the above solution under continuous stirring at 45 °C and allowed to react over 1 h. The reducing of silver ions to silver nanoparticles was observed by the changing color of solution mixture from light yellow to dark brown. For the purification of Ag-NPs, the fully reduced solution was centrifuged at 8000 rpm for 15 min. The supernatant liquid was discarded and the residue was dispersed in Millipore water. The samples were centrifuged five times to remove any constituents that had been absorbed onto the surface of the Ag-NPs. The final product was dried at 60 °C overnight. The resulting dried sample preserved in air-tight bottles for further studies.

2.4. Determination of percentage yield (%Y) of Ag-NPs

Concentration of Ag^+ ions before and after addition of extract was measured using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) model Perkin Elmer 1000. Equation (1) was used to calculate percentage yield (%Y) using initial concentration (I_C) and final concentration (F_C) of Ag^+ ions:

$$\%Y = \frac{Ic - Fc}{Ic} \times 100\% \quad (1)$$

2.5. Preparation of κ-Carrageenan/Ag-NPs hydrogel beads

A series of nanocomposites were prepared by blending the biosynthesized Ag-NPs with the *κ-Carrageenan* matrix as follows. 2 gr of *κ-Carrageenan* was added to 80 mL of distilled water under magnetic stirring, at 80 °C. After complete dissolution, 0.0, 0.1, 0.3, 0.5, 0.7 and 1.0 mL were taken from stock solution of silver nanoparticles (1 mg/mL) and added to 9 mL of *κ-Carrageenan* solutions under magnetic stirring until homogeneous viscose solutions obtained. The bio-nanocomposite beads were formed by dropping the hydrogel bio-nanocomposite solutions from a needle with 2 mL internal diameter into 1 M of KCL solution at room temperature. After 30 min, the beads were separated and washed with distilled water, then dried under vacuum at room temperature. The prepared beads based on the amount of Ag-NPs were called *κ-Ca*/Ag0, *κ-Ca*/Ag1, *κ-Ca*/Ag2, *κ-Ca*/Ag3, *κ-Ca*/Ag4 and *κ-Ca*/Ag5 mean *κ-Carrageenan*/Ag-NPs bio-nanocomposite hydrogel beads,