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Exploring the microstructure, antibacterial, and antioxidant features of Boswellia serrate extract -loaded Alginate/Gelatin porous Scaffolds for medical application

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Abstract: This study developed a novel sponge-like hydrogel composed of sodium alginate and gelatin (ALG/GEL) for the incorporation of *Boswellia serrata* extract (BSE), a promising bioactive agent for tissue regeneration. The synthesized BSE-loaded ALG/GEL scaffolds using emulsion freeze drying method. The obtained emulsion samples were characterized by TEM and DLS. The BSE nanoparticles exhibited an average diameter ranging from 196 ± 52 nm to 368 ± 142 nm, dependent on the BSE content. The bioactivity of the scaffolds was confirmed through a DPPH radical scavenging assay. The pure BSE extract demonstrated significant dose-dependent antioxidant activity with an IC_{50} value of 72 µg/mL, confirming its inherent potency, though it was lower than the ascorbic acid positive control (IC_{50} = 17.9 µg/mL). Crucially, the BSE-loaded scaffolds retained this beneficial property in a loading-dependent manner. The 20%BSE-loaded scaffold (IC_{50} ≈ 78 µg/mL) was significantly more potent than the 10% loaded variant (IC_{50} ≈ 135 µg/mL), highlighting the efficacy of the loading process. the in vitro findings lead to the conclusion that the developed BSE-loaded ALG/GEL scaffolds are effective antimicrobial agents. These multifunctional properties make the developed scaffold a highly promising candidate for advanced tissue regeneration applications.

Keywords: Hydrogel sponges, Boswellia oleo-gum resin, single patch dressing, wound healing

1. Introduction

Because of their bioactive ingredients, which are synthesized internally and released externally to fight against infection and other or other pathogenic symptoms, medicinal plant recipes, also known as traditional medicines, have been used throughout human history to treat human and/or animal illness and to promote general healthcare [1, 2]. Nearly 80% of people worldwide are thought to receive their medical care through traditional methods, with 85% of those methods involving plants, according to estimates from the World Health Organisation (WHO) [3]. As a result, gathering and examining plant components offers a useful foundation for creating new, safe medicinal agents and advancing current studies on chronic illnesses. Furthermore, public health organisations fund evidence-based studies on the potential therapeutic applications of medicinal herbs in the diagnosis, prevention and treatment of numerous chronic diseases. Medicinal plants and their products that are rich with alkaloids, saponins, terpenes, essential oils, and polyphenols[4], have been confirmed to be effective against various conditions, including burns[5], diabetes[6], atherosclerosis[7], infections[8], and malignant tumors[9].

Natural products often contain high levels of antioxidants, such as flavonoids, polyphenols, and vitamins, which help neutralize reactive oxygen species (ROS) generated during the

wound healing process. Excessive ROS can lead to oxidative stress, damaging cellular components like lipids, proteins, and DNA, which in turn impairs the healing process [5]. By scavenging ROS, antioxidants protect cells and tissues, reduce oxidative damage, and promote a more favorable environment for tissue regeneration. *Boswellia* serrata, also known as frankincense or olibanum, is an aromatic-rich resin obtained from Boswellia trees (family Burseraceae) that usually grow in dry areas of India, Northern Africa and Middle East [10-12]. The *Boswellia serrata* contains 70% resin, 10% volatile oils and 30% polysaccharides gum. *Boswellia serrata* homemade preparations have long been used in folk medicine to treat numerous chronic inflammatory [13] and digestive conditions. [14], and even cancer [15].

Alginates (ALG) is a linear polysaccharide of d-mannuronic acid linked by β-(1-4) and l-guluronic acid linked by α-(1-4) units that is found in a wide variety of brown seaweed species. Alginate-based materials have been widely utilized in wound dressing applications because of their potential for high bioactivity, excellent biocompatibility, and lack of toxicity [16, 17]. In addition, gelatin-based biomaterials has been proven to act as biodegradable scaffolding materials for stimulating different cellular activity including cell migration, growth and differentiation, thus exhibiting optimal features for the advance of wound healing process [18, 19]. Gelatin-based dressings act as porous materials. Gelatin (GEL) comprises mainly of glycine (27-35%), proline (10-8%), and hydroxyproline (20-24%) residues and is similar to collagen, resembling the native extracellular matrix. The recent trend in fabricating functional bioactive materials is directed to incorporate therapeutic agents such as metal ions, micronutrients or other bioactive substances to fight against bacterial infection [20, 21]. To the best of our knowledge, this study presents the first attempt to investigate the loading of *Boswellia serrate* (BS) gum resin into ALG/GEL scaffolds

2. Materials and methods

Materials

Sodium alginate (ALG), Gelatin (GEL) and 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)-hydrazyl (DPPH)were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). The *B. serrata* was purchased from the local market. Other materials used were of analytical grade.

Preparation of BSE-loaded Alginate/Gelatin porous Scaffolds by using emulsion freeze drying method

The *Boswellia serrate* (BS) gum resin was grinded and sieved using a 250 μm sieve. The obtained finely powdered resin (50 g) were added to 200 ml of 96% methanol/water solution (50/50v/v). The mixture was then kept under slow string at 40 °C for 24 h in the dark condition before filtration using Whatman Grade 50 Filter Paper. The methanol aqueous solution was evaporated under reduced pressure at 40° C. The obtained Oleo-gum-resin (BSE) was collected and stored in dark at 4°C. The solvent evaporation method was used to load the extracted BSE resin into ALG/GEL matrix. In brief, the extracted BSE resin (0.1 or 0.2 g) was dissolved in 5 ml chloroform at room temperature. In parallel, 100 ml solution of 10% (w/v) ALG /GEL (1:1wt.%) was prepared by adding the desired amounts to distilled water for 30 min at 40 °C until obtaining a homogeneous solution. Afterward, the BSE chloroform solution was added dropwise to ALG/GEL solution at room temperature. The mixture was subject to high mixing speed using a high-shear homogenizer at 5000 rpm for 5 min, followed by magnetic stirring at 300 rpm for 4 h. The obtained BSE@ ALG/GEL milky emulsions were frozen at −40 °C for 24 h. Finally, the frozen samples were dried in a freeze-drier (LyoLab 3000, Heto) for 48 h to remove the aqueous phase and to produce the sponge-like materials. The dried scaffolds were kept at 4 °C in dark until further characterization.

Morphological observation and hydrodynamic diameter

The size and shape of BSE-NPs were observed by Transmission Electron Microscope ((JEOLH-7650, Hitachi High-Technologies Corp., Tokyo, Japan). The mean particle size, size distribution, and zeta potential for BSE-NPs were examined by the dynamic light scattering device (Malvern Zetasizer Nano ZS Instruments Ltd, UK). In addition, the AlG/Gel scaffolds were coated with gold and observed

with field emission scanning electron microscope (FE-SEM) (Jeol JXA 840, Japan).

Antioxidant activity: DPPH free radical scavenging activity

The antiradical activities of pure BSE and BSE-loaded scaffolds were determined by DPPH radical scavenging assay[22]. For the DPPH analysis, 4 mL blank solution (1 mL of 0.5 mM DPPH solution diluted with 3 mL methanol) was used as a negative control (A_blank). Each sample of different concentrations (10 μg/mL, 20 μg/mL, 40 μg/mL, 80 μg/mL and 160 μg/mL in methanol) was taken, and mixed with 1 mL of DPPH reagent for the analysis. Similarly, each concentration of standard ascorbic acid (AA) was treated with DPPH reagent (1 mL) to use as positive control. After 30 min of incubation in dark, the color is changed from dark violet to bright yellow, and absorbance is recorded at 517 nm, and the data are generated in triplicate (Table 4). The antioxidant activity is calculated using Eq. (1).

% Inhibition = [(A_blank - A_sample) / A_blank] × 100 (1)

Where: A_blank is the average absorbance of the negative control and A_sample is the average absorbance of your test solution.

Antibacterial Activity

The antimicrobial activity of the synthesized samples was evaluated against five distinct microorganisms, encompassing gram-negative bacteria such as Gram-positive bacterium (*Staphylococcus aureus*) Gram-negative bacterium (*Pseudomonas aeruginosa*), unicellular fungi (*Candida albicans*) and multicellular fungi (*Aspergillus fumagitus*). Antibacterial efficacy was assessed following the methodology outlined by Perez et al. [23]. Müller-Hinton agar was melted, inoculated with 100 μL of a microbial suspension (1.0×10^8 CFU/mL), and subsequently poured into sterilized Petri dishes. For the disc diffusion assay, 5 mm paper discs were saturated with BSE-loaded ALG/Gel emulsions. Polymexin B was used as the positive control for bacterial strains, while amphotericin B was used as the standard antifungal agent. After allowing the discs to air dry, the samples were placed on the surface of the inoculated agar plates. Bacterial strains were cultured on nutrient agar at 37 °C for 24 hours, while fungal strains were incubated on malt extract agar (MEA) plates for 3 days at 28 ± 2 °C. The inhibition zone diameters were measured in millimeters (mm) to evaluate antibacterial activity. Each experiment was performed in triplicate.

3. Results and Discussion

The formulated emulsions were analyzed using Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM), as shown in Figure 1 and table 1. DLS revealed that the hydrodynamic diameter of the 10BSE-loaded ALG/GEL nanoparticles was 196 ± 52 nm (PDI = 0.24), while the 20BSE-loaded ALG/GEL nanoparticles were larger, at 368 ± 142 nm (PDI = 0.41). TEM micrographs (Figure 1c and d) confirmed the spherical morphology of the nanoparticles for both formulations. The particle sizes observed by TEM were in good agreement with the hydrodynamic diameters obtained from DLS. However, the TEM size (177.9 nm) and DLS size (196nm) of sample 10BSE-loaded ALG/GEL sample are in reasonable agreement. The slight increase in the DLS size is expected and confirms the presence of a hydrated polymer layer around the nanoparticle core. The TEM size (401.5 nm) and DLS size (368 nm) for20BSE-loaded ALG/GEL are in reasonable agreement. The DLS size is slightly smaller, which could be due to the increase of PDI (0.41), which is high and indicates a polydisperse sample. The DLS size SD is also very large (±142 nm).

Table 1 compares the size of BSE-loaded ALG/GEL emulsions, made with two different concentrations of BSE (10 and 20wt %), as measured by TEM and DLS.

Sample name	**TEM Particles Mean Size (nm)**	**PDI**	**DLS size** distribution **± SD (nm)**
10BSE-ALG/GEL	177.9± 72.3	0.24	196±52.16
20BSE-ALG/GEL	401.5±39	0.41	368±142.6

SEM analysis highlighted that all samples interconnected porous structure surfaces (Figure 2). The ALG/GEL cross-section revealed an irregular porous structure with different pore size distribution ranging from

few microns up to 50 µm (Figure 1(a)). Although, similar porous structure morphology was also observed for 10BSE- laoded ALG/GEL and 20BSE-laoded ALG/GEL scaffolds. The addition of BSE resin resulted in a homogenous pore volume and smaller average pore size around few microns, which could be due to the evaporation of chloroform during preparation process and elimination of water during freeze drying step.

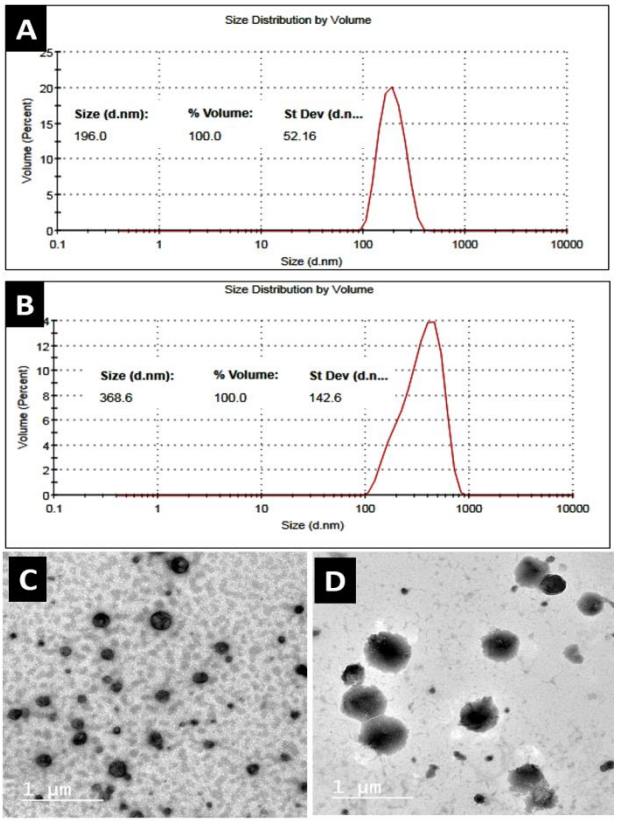

Figure 1: DLS and TEM analysis of the10BSE-loaded ALG/GEL and 20 BSE-loaded ALG/GEL emulsions.

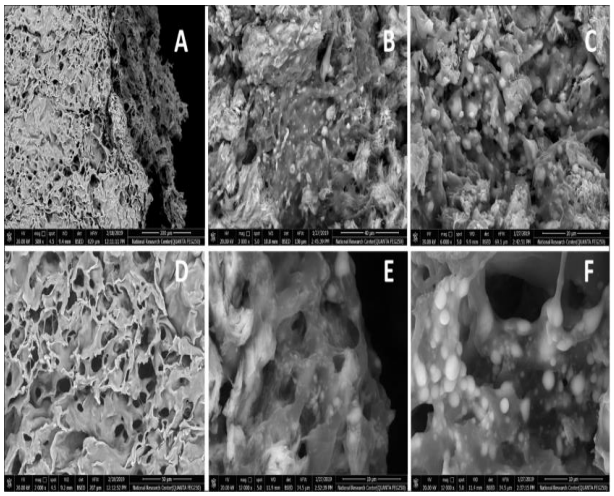

Figure 2: SEM images of (a, d) ALG/GEL, (c, e) 10BSE-laoded ALG/GEL and (c,f) 20BSE-laoded ALG/GEL samples.

The antioxidant activity (DPPH assay)

The antioxidant test (DPPH assay) demonstrates that both the pure BSE and BSE-loaded scaffolds possess significant free radical scavenging activity in a concentration-dependent response, as presented in Table 2. The pure BSE is exceptionally potent. At 160 µg/mL, it inhibits 79.26% of free radicals, which is statistically very close to the 82.09% inhibition achieved by the standard AA (a well-known potent antioxidant). The scaffold with a higher loading (20BSE-laodedALG/GEL) performs better (69.7% inhibition) than the one with lower loading (10BSE-laoded ALG/GEL) (57.7% inhibition). The observed decrease in activity for the scaffold samples compared to the pure BSE is attributed to the slow release of the active compounds from the ALG/GEL gel matrix. The DPPH assay is a quick test, and if the antioxidants are trapped inside a scaffold, they cannot react with the free radicals as immediately as the pure, freely dissolved compounds (BSE and AA) can. The BSE sample demonstrated significant dose-dependent DPPH radical scavenging activity, with an IC_{50} value of 72 µg/mL, compared to the positive control, ascorbic acid (IC_{50} = 17.9 µg/mL). On the other hand, the 20%BSE loaded scaffold (IC_{50} ≈ 78 µg/mL) is significantly more potent than the 10% loaded scaffold (IC_{50} ≈ 135 µg/mL).

Table 2. DPPH% free radical scavenging activity of the pure BSE and BSE-loaded scaffolds

Concentration (µg/mL)	BSE	10BS-LG/GEL	20BS-ALG/GEL	AA
10	2.23±0.19	0.9±0.41	1.1±0.32	22.64±1.83
20	5.91±1.57	3.32±0.92	4.05±0.70	44.08±1.91
40	23.38±5.41	19.44±1.81	26.21±2.43	57.79±2.49
80	45.61±10.12	33.1±19.51	51.1±7.54	74.15±4.13
160	79.26±13.12	57.7±12.41	69.7±1.20	82.09±3.88
IC_{50}	72.4 µg/mL	135 µg/mL	78 µg/mL	29 µg/mL

Antimicrobial activity

The antimicrobial efficacy of BSE and the scaffolds loaded with two different

concentrations of BSE (10BSE@ALG/GEL and 20BSE@ALG/GEL) was evaluated against two bacteria and two fungi using the agar well diffusion assay. The results measured by the inhibition zone in millimeters, are summarized in Table 3 and figure 3. Standard antibiotics were used as positive controls for comparison. The data indicates that the antimicrobial activity is concentration-dependent. The formulation with the higher loading of BSE (20BSE@ALG/GEL) showed a noticeable increase in the inhibition zones against all tested microorganisms compared to the emulsion with lower loading (10BSE@ALG/GEL). Notably, the 20BSE@ALG/GEL coating exhibited antimicrobial activity comparable to the standard antibiotic Polymyxin B against the bacterial strains S. aureus and E. coli. While the activity against the fungal strains C. albicans and A. niger was significant, it was slightly lower than that of the potent antifungal standard Amphotericin B.

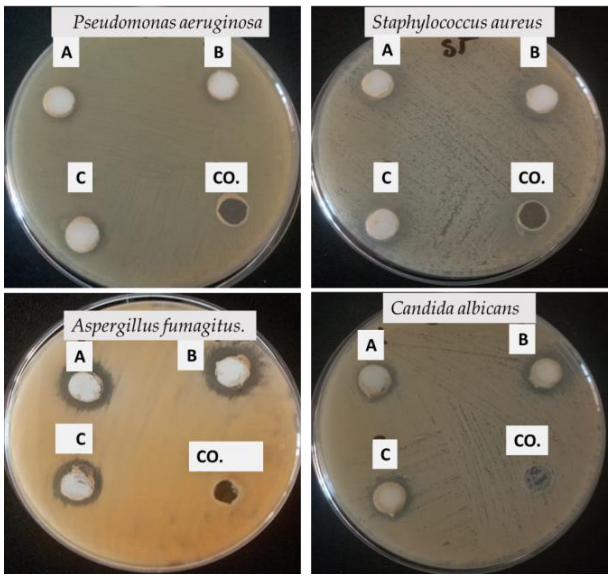

Figure 3. shows the antimicrobial activity of the obtained BSE and BSE-loaded scaffolds (**A**) 10BSE@ALG/GEL, (**B**) 20BSE@ALG/GEL and (**C**) Pure BSE extract.

Conclusion

A novel bioactive scaffolds were developed using ALG and GEL, and BSE extract. The lyophilized scaffolds exhibit an interconnected porous structure with average pore diameters 50µm. In addition, the antioxidant and antibacterial features of BSE loaded scaffolds were confirmed by in vitro DPPH and disc diffusion assays. The results demonstrate that loading BSE into the ALG/GEL matrix successfully creates an active scaffolds with broad-spectrum efficacy against both bacteria and fungi. The increased activity of the loaded scaffolds compared to the pure BSE extract suggests a potential synergistic effect or enhanced delivery mechanism facilitated by the nanoemulsion formulation.

Table 3. Inhibition Zone (mm)of the potent as-synthesized emulsions

Sample code	Inhibition Zone (mm)			
	Staphylococcus aureus	***E. coli***	***Candida albicans***	***Aspergillus niger***
BSE	3	1	3	4
10BSE@ALG/GEL	2	1	2	1
20BSE@ALG/GEL	3	2	3	3
Polymexin B	3	2	-	-
Amphotericin B	-	-	5	4

* Polymexin B was used as standard antibacterial agents; while Amphotericin B was used as standard antifungal agents at 20 µg/mL,

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