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Evaluated the Antibacterial Activity and Cytotoxicity of Wound Dressing for PCL/Chitosan and PCL/ Hydroxyapatite Nanofiber

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Abstract

The development of novel methodologies for completely functioning skin tissue regeneration was necessary to improve the healing quality of damaged lesions. Electrospin nanofibrous scaffolds based on Poly (ε-caprolactone) modified with Chitosan and hydroxyapatite biomimetic nanofibrous scaffolds at 0.01w_t% and 0.4 percent were investigated in this research. A disc diffusion test was used to assess antibacterial activity against Escherichia coli (*E.coli*) and Staphylococcus aurous (*S. aureus*). The cytotoxicity of the MTT cell line was assessed using an extraction technique, and proliferation was detected.

Antibacterial studies have shown that PCL / Chitosan and PCL / HA nanofiber membranes containing 4% gentamicin on bacterial cultured agar plates significantly inhibit the growth of adjacent *E. coli* and *S.* epidermises. Bacteria are possible, indicating that antibacterial activity was classified as "strong" or "significant" against the two types of bacteria.Compared to pure PCL, cell viability results show higher activity and improved mineralization, allowing PCL nanofiber films as a matrix of cell proliferation with minimal damage to cells. Shows sex and can be used in biomedical applications.

Key words: Poly ε- Caprolactone, Cytotoxicity, Antibacterial,Chitosan, Hydroxyapatite.

Introduction

With the advent of new polymers and manufacturing processes, advanced wound dressings with customizable features and excellent mechanical and structural characteristics have become possible. Bio-based polymer materials have long been recommended for skin regeneration as practical skin substitutes, wound healing patches, and dressings for a variety of wounds. Electrospinning nanofibers that imitate the natural tissue matrix for wound healing are made from a variety of natural biopolymers (chitosan, cellulose, gelatin, collagen, hyaluronic acid, and so on). [1]. Synthetic bio-based polymers such as polyhydroxyalkanoate and polycaprolactone are widely used for electrospinning wound dressings. Coaxial, multijet, or mixed electrospinning of natural and bio-based synthetic polymers can be used to adjust the deterioration, mechanical, and/or morphological properties of wound dressings [2]. An acyclic polyester with

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outstanding elastic, biodegradable, and bio absorbable qualities is frequently used in medicine to meet the needs of dressings [3]. Electrospinning biopolymers to make nanofibers have several biological uses, including wound treatment, tissue engineering, and drug delivery [4]. High porosity, biodegradability, biocompatibility, non-cytotoxicity, and the surface area to volume ratio are extremely high are just some of the distinguishing properties of nanofibers. On the other hand, biopolymer nanofibers have poor mechanical properties and lack antibacterial activity, making them unsuitable for wound dressing and skin regeneration [5]. One of the most well-known uses is wound dressing. 11–13 Nanofibers play a crucial function in preventing bacterial development and illness [6].

The electrospinning method is a well-known method for producing polymer fibers by generating an electric field. A jet of the polymer has been injected via a charges needle, and the solvent in which the polymer is dissolved evaporates, allowing solid polymer fibers to be deposited on the collector. In a conclusion, the electrospinning system, as shown in Fig. 1, incorporates a high voltage source, a syringe pump, a syringe filled with the polymer solution, and a collector [7].

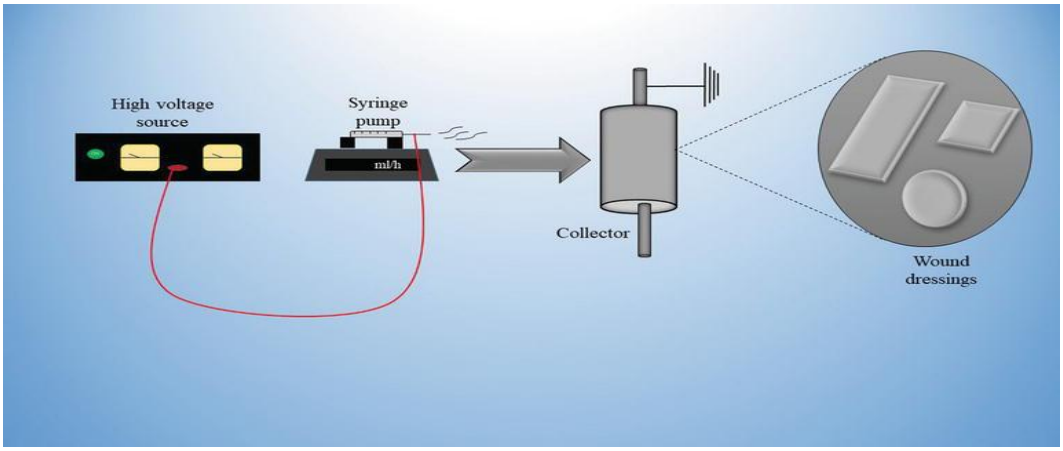

Fig (1): The electrospinning process.

Chitosan, a deacetylated form of chitin, is derived from the exoskeletons of insects and crustaceans, as well as the cell walls of fungi; and chitosan, the second most abundant natural polymer, has been widely used in many biological applications due to its bioactivity, degradability, cellular binding capability, wound healing acceleration, hemostatic properties, and anti-bacterial/fungal properties [8, 9].

Hydroxyapatite (HAP: Ca10(PO4)6(H)2) is a bio ceramic that gets its name from the fact that it looks like a mineral found in mammalian bone[10]. It is biocompatible, bioactive, osteoconductive, chemically and thermally stable as a result. Due to its strong inclination to fracture as a ceramic, however, it has poor tensile strength. Furthermore, it

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exhibits structural resilience, allowing a diverse variety of ions to be absorbed into its lattice while maintaining crystal stability [11].

Experimental work

Preparation of PCL nanofibres

By dissolving an adequate quantity of PCL in DMF: $CHCl_3$, a solution with different quantities of chitosan and hydroxyapatite (0.01, 0.4 wt. %) was created (50:50 weight ratio). Magnetic stirrers were used to mix the solutions for 4 hours at 50 °C until the PCL was completely dissolved. 4% wt. chitosan and hydroxyapatite were first dispersed in DMF using a homogenizer for 30 minutes before being concentrated on PCL solutions. The polymer combinations were agitated for at least 24 hours at R.T. before being loaded into a syringe with a needle (inner diameter of 0.8 mm). A high-voltage power supply was used to conduct electrospinning. The nanofibres were collected on a grounded 15 cm piece of aluminum foil.

Fig (2):The nanofiber sample

Antibacterial activity

The zone of inhibition approach was used to study the antibacterial activity of PCL, PCL/chitosan, and PCL/hydroxyapatite nanofibrous scaffolds. Gram-negative bacteria *(E. coli*) and gram-positive bacteria (*Staphylococcus aureus*) were utilized as model microorganisms, and nanofibrous scaffolds were cut onto circular discs (1.4 cm in diameter) and served as the positive control. A nutrient agar plate was infected with 1 ml of a bacterial solution containing roughly 108 CFU/ml of each bacterium using the spread plate technique. Scaffolds were carefully put on inoculation plates and incubated for 24 hours at 37 degrees Celsius. The clear region that developed surrounding each scaffold was used to define the zones of inhibition.

Cytotoxicity measurement

Cell cytotoxicity was evaluated using the MTT technique on PCL, PCL/chitosan, and PCL/hydroxyapatite nanofibrous scaffolds. Nanofibrous scaffolds were sectioned into circular discs (1.6 cm in diameter) and sterilized by washing three times with PBS

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containing 1% penicillin/streptomycin after the growth medium was sucked off. The wells are then filled with 180 liters of fresh medium and 20 mL of MTT reagent (5 mg/mL). The fibroblasts were cultivated for another 4 hours at 37°C. After the medium was taken out, the wells were filled with 200 ml of dimethyl sulfide (DMSO).

Results and discussion

Electrospun nanofibers are essential to avoid bacterial colonization of the wound surface and associated infections, which can lead to bacterial biofilms and delay healing.The results showed that various antibacterial activities were obtained against both bacteria, as shown by Table 1. & Fig (3) The most effective antimicrobials, Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative), were used to test the antibacterial activity of the nanofibre formulations, with results showing that the chitosan and hydroxyapatite inhibited bacterial growth, implying that a better antibacterial effect can promote wound healing more effectively. That agrees with Ramazan A. et.al. [1].

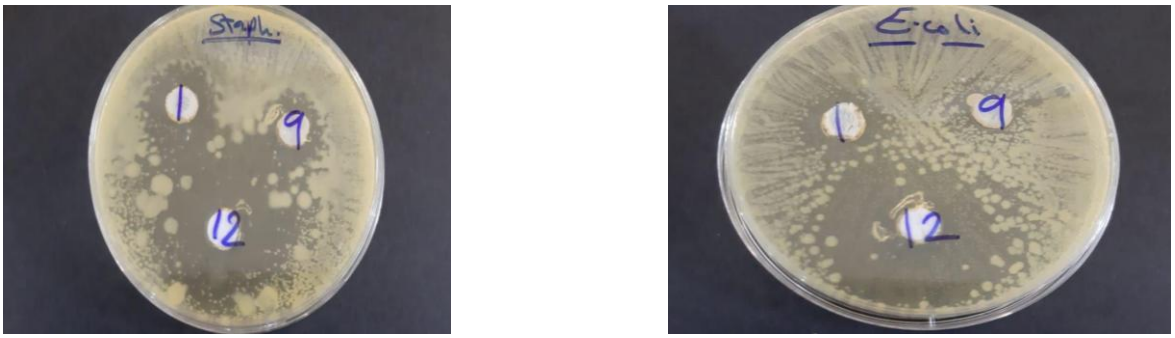

Fig (3): antibacterial activity of E.coli and Staphylococcu

Inhibition zone (mm) for *Staphylococcus*	Inhibition zone(mm) for *E.coli*	Sample
20	16	PCL
22	18	PCL / Chitosan
38	35	PCL/HA

Table (1): Inhibition zone for E.coli and Staphylococcus bacteria's

Cytotoxicity Test:

The wound dressing should be biocompatible, meaning that it should be able to perform its function without interfering with biological processes like cell adhesion and proliferation that occur during wound healing.

The MTT method was used to test the toxicity and biocompatibility of PCL nanofibers, PCL/Chitosan, and PCL/HA (Fig. 4), which shows vitro cytotoxicity cultures at 24, 48, and 72 hours of incubation, respectively. As seen in the graph at the end of the

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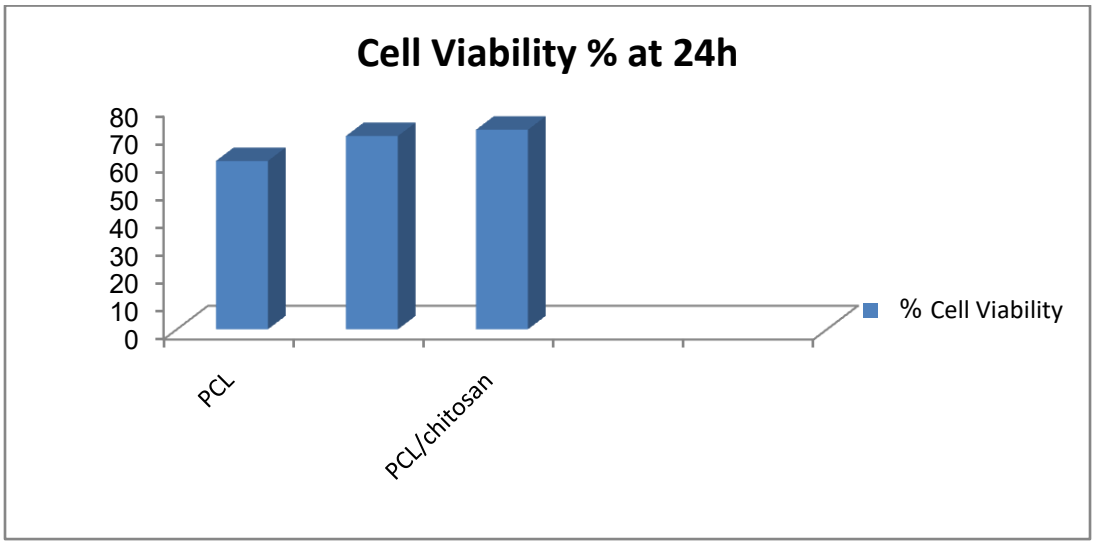
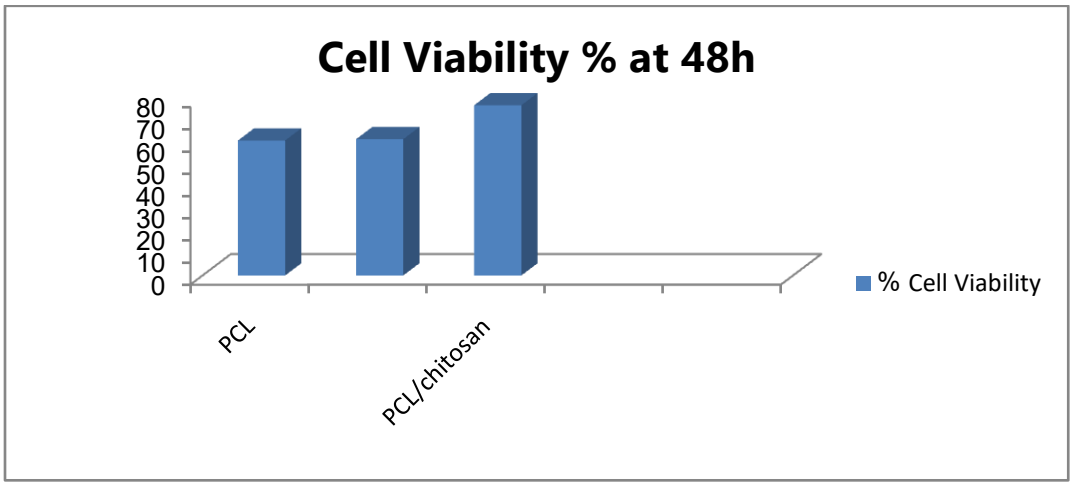
third day, the growth rate on the PCL/Chitosan and PCL/HA scaffolds is faster than on the PCL scaffold. The results revealed PCL/HA nanofiber scaffolds. The grave lens exhibits no cytotoxicity on fibroblast cells. Also, if the percentage of measured cell viability is less than 60%, then the resulting nanofiber has toxic properties and kills living cells.

According to the ISO 10993-5 criteria for evaluating the in vitro cytotoxicity of medical devices, biomaterials can be categorized as non-cytotoxic and biocompatible if their cell survival is greater than 70%. As a result, it is feasible to infer that the electrospun nanofibrous meshes created are safe and may be used as wound dressings without causing toxicity, as well as that both of these scaffolds were cell-free.





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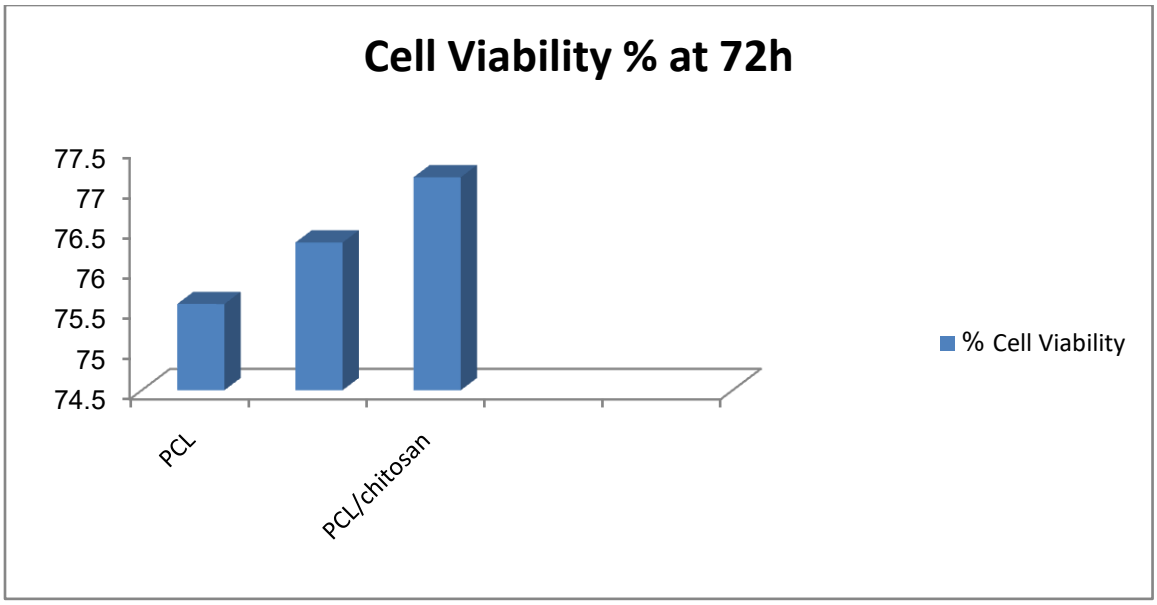

Fig (4): Cytotoxicity results of PCL, PCL/Chitosan, PCL/HA nanofiber.

Conclusion

A novel wound dressing containing chitosan and hydroxyapatite that has antibacterial action against gram-positive and gram-negative bacteria. Using the electrospinning method, the prepared PCL, PCL/Chitosan, and PL/HA nanofibres showed improved antibacterial properties.

The cytotoxicity measurement was carried out on PCL, PCL/Chitosan, and PL/HA nanofibers. Because the addition of chitosan and hydroxyapatite in PCL nanofibers provides a higher percentage of cell viability than PCL, it is suitable for use as a wound dressing.

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