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Physical, Degradable and Antibacterial Characteristics Of Chitosan/ Polyethylene Glycol /Nano hydroxyapatite scaffolds

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Abstract

Tissue engineering has emerged as a promising alternative approach in the treatment of malfunctioning or lost organs. This method requires the use of a temporary scaffold to act as both a physical support system and an adhesive substrate for the transplanted cells, helping to direct the development of the new organs.Biodegradable scaffold system was prepared using Chitosan (CS) blended with another polymer polyethylene glycol (PEG) at different ratios (90%/10%, 80%/20%, 70%/30%) respectively, by casting method and the (70/30) percent ratio was the best one due to its ideal properties and great flexibility. Then, three different weight fractions of hydroxyapatite nanoparticles (HANPs) were added to the CS/PEG mixture to reinforce it (1, 2, and 3 wt%). These nanocomposites were evaluated using an FTIR spectrum, FE-SEM, AFM test, and biodegradable and antibacterial test. The results of FTIR indicated good interactions and created hydrogen bonding between the compounds of nanocomposites ،FE-SEM scans showed a well-integrated blend of CS and PEG with good dispersion and embedding of HANPs. AFM results shows the surface roughness of CS increases by adding 30%PEG, but the surface roughness decreases with increases the percent of nanoparticles HANPs. The degradation rate decreased with the addition of nanoHA. The antibacterial activity results show an increase as the concentrations of HANPs increase inhibition zone increases due to addition the Hydroxyapatite HANPs. although (70%:30%:3%)(CS/PEG/HANPs) respectively it was the best sample in terms of results and the most suitable sample to be a biocompatible scaffold

Keywords: Chitosan, Polyethylene glycol, Bone scaffold, Hydroxyapatite and Nanocomposite

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1. Introduction

The surgical repair of severe size bone abnormalities is still a clinical necessity [1,2]. Current autograft or allograft treatments have significant limitations, including donor site disease, transmission of infectious diseases, and foreign body rejection [3]. To circumvent these restrictions, a wide range of synthetic materials and solvent casting technologies for ceramics, glasses, metals, and polymers have been created [4]. Titanium dioxide [5], Bioglass 45S5 [6], and calcium phosphates such as hydroxyapatite (HANPs) are examples of bio ceramics and glasses created for bone healing [7].

Chitosan as a biomaterial has recently gained popularity due to biological features such as biocompatibility and biodegradability, antibacterial activity, mucoadhesivity, and wound healing [8]. Chitosan is a partly deacetylated derivative of chitin, one of nature's most prevalent polymers found in crab shells and fungus walls. It is made up of a random distribution of (1-4)-linked D-glucosamine (glucosamine) and N-acetyl-d-glucosamine (N-acetylglucosamine) structure units, which are structurally similar to glycosaminoglycan, which is an essential part of the bone structure and cell surface that affects the bioavailability and activity of various osteoclastic and osteogenic components [9, 10]. Chitin is nearly never completely deacetylated, and the chitosan chain still retains some groups of amides [10]. The molar fraction of glucosamine in chitosan made consisting of N-acetylglucosamine and glucosamine structural units is used for calculating the degree of deacetylation (DD,%) [11]. Chitosan's DD (degree of deacetylation) is divided into four categories: low (55–70%), intermediate (70–85%), high (85–95%), and ultrahigh (95–100%), the latter of which can be hard to accomplish [12].

PEG is extensively employed in polymer blends because of its molecular weight range, water solubility, low toxicity, chain flexibility, and biocompatibility. PEG is easily removed from the body and non-toxic [13]. HANPs is utilized as a substitute for bone because of its chemical likeness to actual bone. Bone primarily consists of a mineral phase (69 wt%), an organic matrix (22 weight percent), and water (9 weight percent) [14]. The main calcified tissue in animals, bone is an intricately crafted ceramic-organic bionanocomposit. The inorganic component of bone matrix is quite similar to HANPs, which has a typical formula of $Ca_{10}(OH)_2(PO4)_6$ [15].

Hydroxyapatite (HANPs) has high bioactivity, biocompatibility, and osteoconductivity, and it allows for easy attaching to human bone. Because of these great qualities, HANPs is a useful material for a variety of biomedical applications, such as bone tissue engineering, biological delivery systems, bioactive coatings, and so [14]

In this study, PEG was blended with chitosan to reduce the brittleness of chitosan and thus increase the bearing capacity of deformations and loads. Furthermore, HANPs was added to a blend as reinforcement, which is a part of the human bone structure, which increases biocompatibility and improves cell growth.

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2. Methodology

2.1. Materials

The powder of chitosan CS was procured from a central drug house, in India. polyethylene glycol (PEG) was procured from (Himedia, India). Nano hydroxyapatite HANPs obtained from N&R INDUSTRIES, INC, Average Particle Size =>20nm

2.2. Film formation:

1- Chitosan powder was dissolved in 2% acetic acid and mixed by magnetic stirring at 40 °C for 1hr.

2- PEG flakes that have been dissolved using distilled water by continuous stirring for 15 minutes at room temperature.

3-Three percent of chitosan and polyethylene glycol (PEG) (90%:10%,80%:20%,70%:30%) were blended, stirred at 25 □ C for half an hour, and cast into a Petri dish. It was left at room temperature for 72 hours for drying.

4- The(70%:30%) (CS: PEG) film was reinforced with (1,2 and 3) wt% HANps, the nanoparticles were dispersed in distilled water by an ultrasonic dispersion device for 15 min at a temperature of 40 °C. Then, it was poured gradually into the CS/PEG (70%:30%) blend. The blend was mixed for 30 min then left to dry for 72 hr.

3.Tests

3.1 Infrared Fourier transform spectrometer (FTIR) Test

This method is used to characterize complex blends using the FTIR analysis equipment Type (IR Affinity-1). Blend films were measured among 400 and 4000 cm^{-1} in transmission mechanism, either directly or by embedding the films in KBr pellets.

3.2 Filed Emission SEM (FESEM)

The Field Emission Scanning Electron Microscope (FESEM) image objects up to 500000 times more effectively than a standard scanning electron microscope (SEM) due to its significantly brighter electron source and thinner beam size. The capacity to conduct high-resolution imaging with relatively low accelerating voltages is a second feature of the FESEM. This improves the ability to observe very small surface details. This experiment was carried out in the labs of the Engineering Department at the University of Tehran**.**

3.3 In Vitro Degradation Test:

The samples are, immersed in small bottles containing 20 ml of a solution of PBS (Buffer Silene Phosphate) for nine days. The samples are taken out, washed with distilled water, then dried in ovens for 4 hours at a temperature of 40 □ C [15]. then the dissolution rate is measured using the following equation [16]:

$$(W1 - W2)/W1 \times 100\% \text{.....(1)}$$

Where W_1: the dry weight before degradation (g) and W_2 the dry weight after degradation (g) at the specified time.

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3.4. Anti-bacterial Test

Determination of antibacterial activity was achieved using the Agar Well Diffusion Method, which must be prepared the Muller Hinton agar in advance and the positive and negative bacteria were selected. In this test (Escherichia coli, staphylococcus aureus), several holes or gaps were made in the agar layer, then solution drops or a solid film were placed. The bacteria were distributed on the plate and placed in an incubator for 24 hours at a temperature of 37 °C. The antibacterial activity against E-Coli and S. aureus microorganism was assessed by measuring the diameter of the inhibition zone and recorded in mm [15].

3.5. Atomic Force Microscope (AFM) test

The thin film's surface morphology was f investigated using traditional tapping mode probes with constant magnitude (200 mV) AFM according to ASTM E 2865. The rotated tapping method with an etched silicone probe was used, with a frequency response of 55 kHz.

4.Results and Discussion

4.1 FTIR

FTIR aimed to detect the hydrogen bonding interaction between functional groups in small molecules and also in macromolecules. As they cause to shift in the absorbance bands or increased the intensity of absorbance bands, also create a broadening of the particular bands of functional groups, it indicates the behavior of these groups in polymer blend [17]. The FTIR spectrum of blend in the wave number (400-4000 cm-1) is shown in Figure (1) which exhibited many bands such as the bands at peak at 3419 cm^{-1} for O-H band ,while C=O at 1619 cm-1, and for Nanocomposite blends O-H peak shifted to 3425 cm^{-1} and CH_2 stretching was also shifted to 2893 cm^{-1} while C=O shifted to 1622 cm-1, Moreover, phosphate stretching PO4 vibration in HANPs at 1111 cm-1 The shift in band O-H suggested strong hydrogen bonding and excellent HA distribution in the matrix

Table (1) FTIR Bands for Blend and Nanocomposite

Type of bands	Blend CS/ PEG	Blend with Nano 1% HANPs	Blend with Nano 2% HANPs	Blend with Nano 3 %HANPs
OH	3419	3417	3425	3425
C-H stretching	2885	2893	2893	2893
C=O Amid I	1619	1620	1622	1620
NH Amid II(CH_3)	1419	1442	1419	1435
P-O stretching	-----	1111	1111	1111

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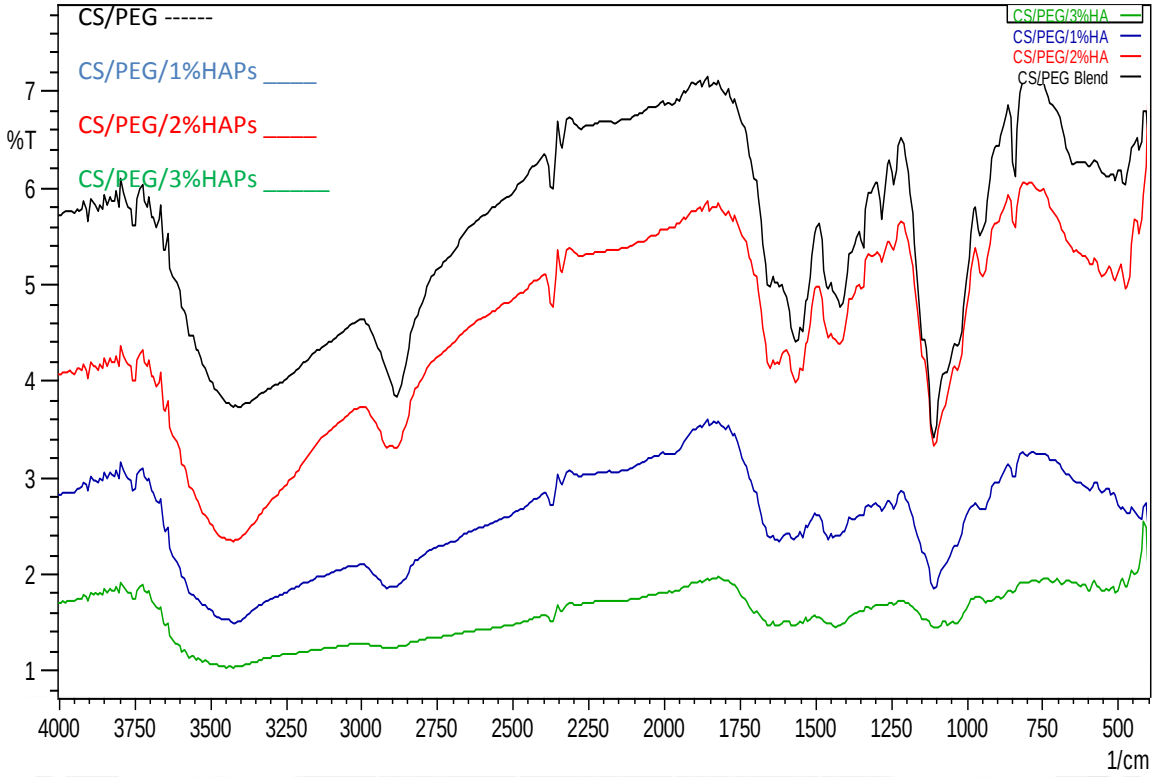

Fig.1 FTIR Spectra (CS/PEG) and (CS/PEG) with HANPs% Nano Composite

4.2 Morphology Test

A scanning electron microscope can provide information on the morphology and structure of the neat polymer and composite material. It is a device that show the distribution of reinforced phase, particularly for Nanomaterials [18].

Figure (2) shows a micrograph of the 70:30 CS/PEG blend at 100kx, and 200 kx magnifications that reveals a homogeneous structure and the absence of defect and voids. In addition, it contained some small holes and a rough surface as a result of the inclusion of PEG, which promotes the adherence and ingrowth of live cells since PEG changes the morphology of the surface and then develops the surface contact [19].

It found that Nano-HANPs was well distributed throughout the matrix (CS/PEG) and there are good interactions among CS/PEG/HANPs. Moreover, The uniform distribution of HANPs was produced from good dispersion of nanoparticles during mixing and dispersion by ultrasonic which led to good embedded "the HANPs within the matrix and then enhance the transfer of load from matrix to reinforced particles [18].

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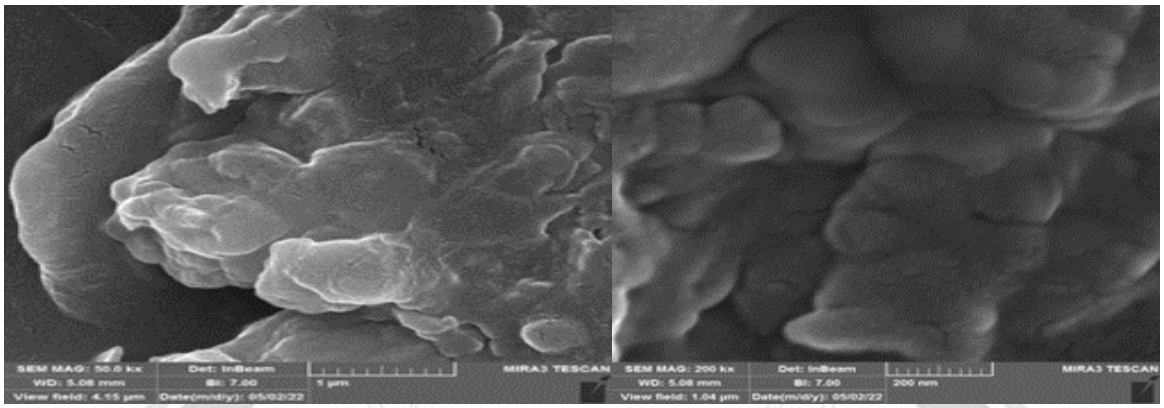


Fig.2 FE- SEM of Blend 0.7CS/0.3PEG at Magnification a-100kx b- 200kx

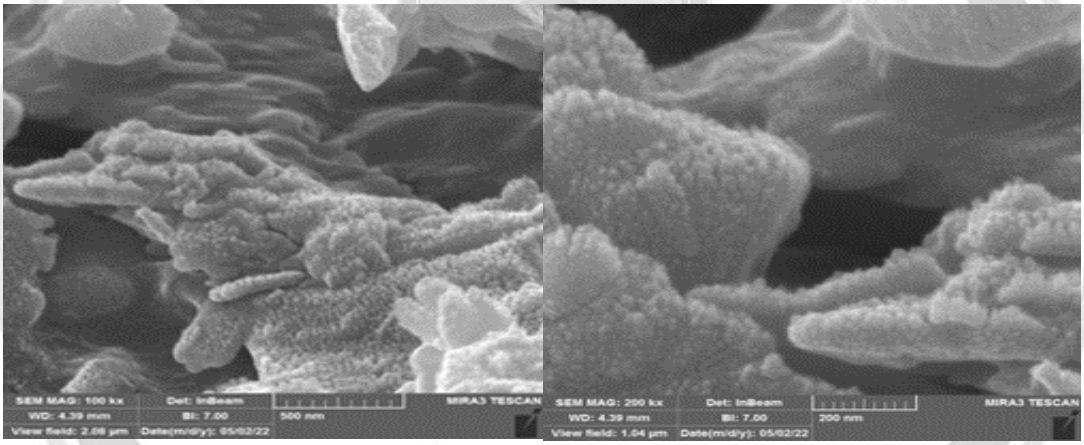


Fig.3 FE-SEM of Nanocomposite CS/PEG/3% HANPs at Magnification a-100kx b-200kx

4.3 AFM

To study the effect of adding polyethylene glycol and nanoparticles on the nature of the polymeric surface , a three-dimensional Topography images of surfaces of neat chitosan, (CS/PEG)blend and nanocomposites shown in the Figure (4), and the Table (2) also shows the parameters of roughness, The results indicated that Chitosan film has the roughness surface with average Sa(18.4nm) and Sq(24nm) and the surface roughness increased by adding 30%PEG and became (Sa:19nm and Sq(25)nm due to the role of PEG which used as plasticizer which causes to increase the roughness of CS[20].

When adding hydroxyapatite nanoparticles, the surface roughness Sa, Sq decreases with the increase in the percent of HANPs, Nanoparticles of HANPs dispersion between the polymeric chains and filled the voids which decreased the surface roughness [21]. Another parameter of roughness is the surface bearing index (Sbi) which is a function of the mechanical properties [22]

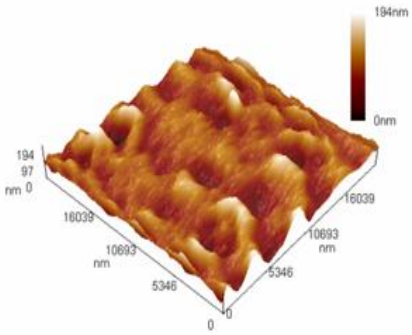
Surface roughness is an important factor in the adhesion of bacteria to surfaces. Increasing the roughness means increasing the adhesion of bacteria [23]. The Sa and Sq parameters shown in Table 2 noted that the roughness decreases with increasing addition ratios, and thus

the adhesion of bacterial cells to the wall of the scaffold will decrease, and antibacterial results confirmed it**.**

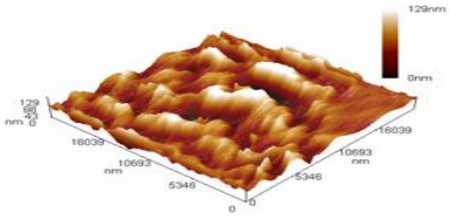
Table.2 AFM Parameters for Neat CS, CS/PEG as Nanocomposite as Function of HANPs

Sample	S_a (nm)	S_q (nm)	S_{bi}
CS	18.4	24	0.438
CS/PEG	19	25	0.454
CS/PEG/1%HANPs	17	23	0.495
CS/PEG/2%HANPs Nps	16	21	0.738
CS/PEG/3%HANps	14	18	0.867

A



B



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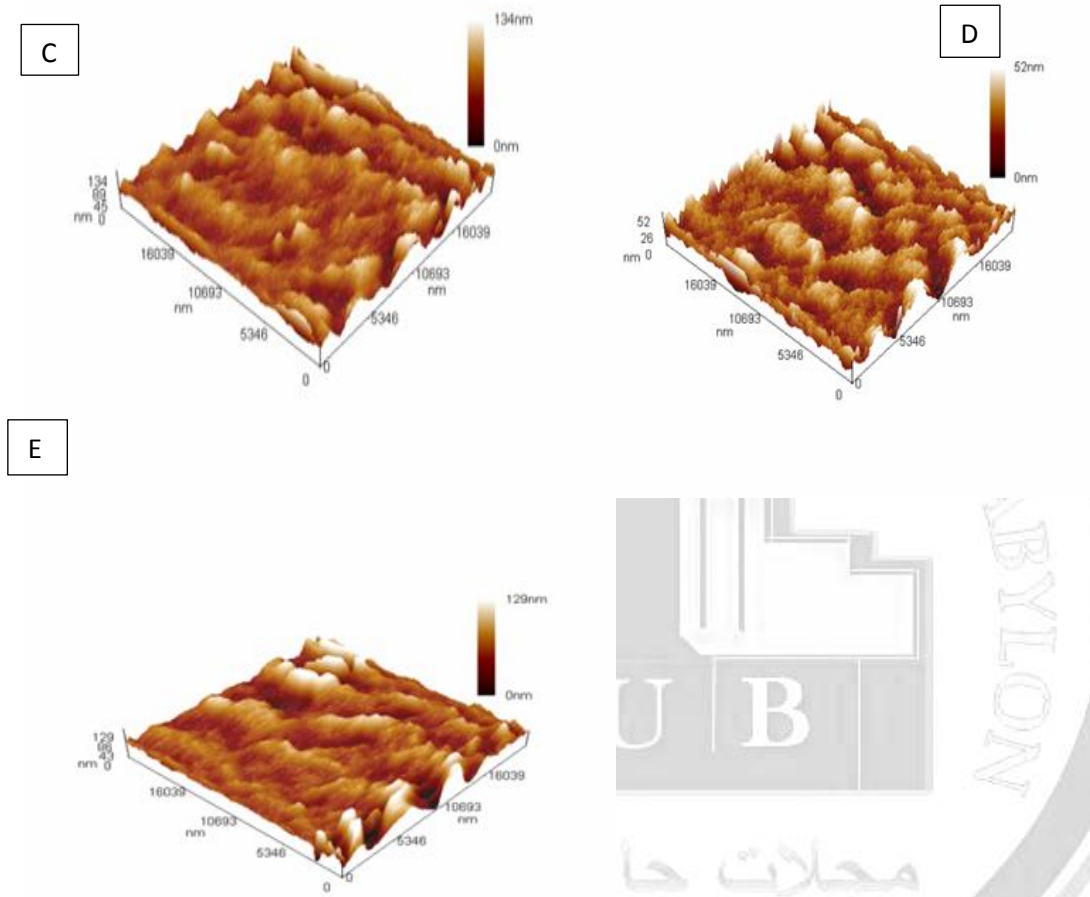

Fig.4 3D AFM Images of A- Neat CS B-CS/PEG Blend C-(70CS/30PEG)+1%HANPs D- (70CS30/PEG)+2%HANPs E-(70CS/30PEG)+3%HANPs

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4.4 Degradation

Degradation rate is the most important factor for degradable polymeric scaffold. When scaffold has an appropriate rate of degradation, it will encourage cells to grow and stimulate new tissue creation [24]. Neat CS, CS/PEG blend, nanocomposite film(CS/PEG/HANPs), It has been shown from the Figure (5) that the blend CS/PEG have the highest degradation rate. The addition of PEG increased the degradation potential for all given times, which can be clarified by a rise in the porosity of the CS/PEG as compared to CS due to the presence of PEG which is hydrophilic in nature [25].

The ionic interaction between the PBS solution and the functional groups of the chitosan in the composite was essential for the chitosan to degrade and eventually decrease the absorption of the solution. However, degradation rate of composite scaffolds is decreased with increased (HANPs)w.t% nanoparticles more than neat scaffolds. The decreasing in degradation rate of these scaffolds due to the higher chemical stability of nanoparticles and uniformly distributed throughout of scaffolds, this led to resisting the PBS solution seepage into the scaffold architecture. The increase in the concentration of nanoparticle in the nanocomposite scaffolds also decreased the degradation rate [25]. which can give suitable time to bone tissue to repair and cell to growth to complete the regeneration process [24].

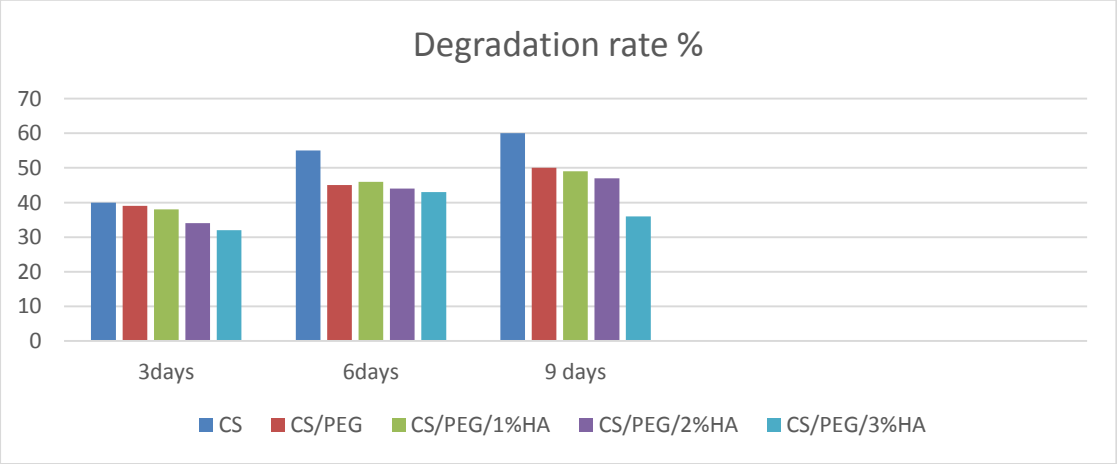

Fig.5 Degradation Rate for CS, CS/PEG Blend, Nanocomposite with HANPs wt% and Hybrid Nanocomposite

4.5 Antibacterial Test

The antibacterial test must be carried out on the bone scaffold to evaluate the ability of the prepared scaffold to resist bacterial infection. Figures 6 and 7 show the inhabitation zone against the E-Coli (Negative gram) and S.aureus (Positive gram). The neat Chitosan exhibited the highest inhibition zone because is an antibacterial polymer due to the positive charge of amino groups that don't interact with the membranes of bacteria [26].

The inhibition zone was decreased when adding PEG (blend CS/PEG) because the PEG doesn't have antibacterial properties so, it reduces the inhibition zone [28,29]. Moreover, the inhibition of the blend decreased with the addition of nanoparticles hydroxyapatite due to

some agglomerations of HANPs during the preparation process of the film which affects the antibacterial activity of HANPs. However, when increased the percentage of HANPs, the inhibition zone increases due to the Hydroxyapatite Nanoparticles are antibacterial particles that deactivate the bacterial grovel and spores so that the efficiency of bactericidal became higher [30].

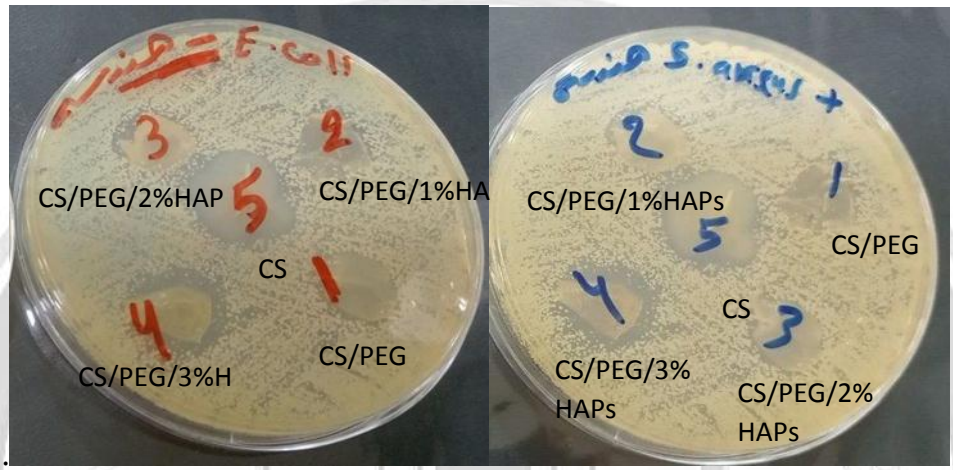

Fig.6 Inhibition Zone Diameter of Neat CS, CS/PEG Blend, and Polymer Blend Nano Composites for E. coli and Staphylococcus Colony.

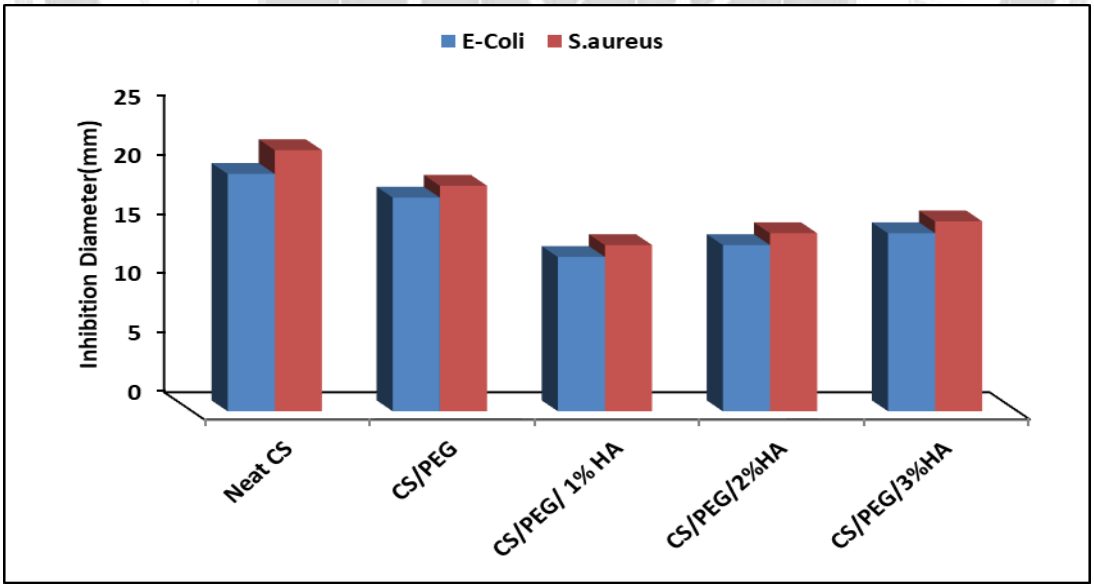

Fig.7 Inhibition Diameter of Neat CS, CS/PEG Blend, and Nanocomposites as a Function of HANPs-Nanoparticles Content in Composite

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CONCLUSIONS

FTIR results show that there's a good interaction (hydrogen bonding) between the components and there is no chemical bond between the components of the blend. SEM outcomes found that Nano-HANPs was uniformly distributed throughout the matrix, the results of degradation rate show that the addition of PEG improves the degradation of biofilms due to PEG increase the porosity and hydrophilic of the CS/PEG blend. While the degradation rate decreased with addition the Nano HANPs, which can give suitable time to bone tissue to repair and cell to growth to complete the regeneration process. AFM results showed the surface roughness of CS increased by adding PEG, HANPs. In antibacterial test the inhibition zone increases due to addition the Hydroxyapatite HANPs.

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الخصائص الفيزيائية وقابلية التحلل والمقاومة للبكتيريا

لدعامات الشيتوزان / البولي إيثيلين جلايكول / نانو هيدروكسيباتيت

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[1,2] قسم البوليمرات والصناعات البتروكيماوية

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الخلاصة:

قد برزت هندسة الأنسجة كنهج بديل واعد في علاج الأعضاء المعطوبة أو المفقودة. هناك حاجة إلى دعامه مؤقتة لتكون بمثابة ركيزة لاصقة للخلايا المزروعة ودعم مادي لتوجيه تكوين الأعضاء الجديدة. تم تحضير نظام دعامه جديد قابل للتحلل باستخدام الشيتوزان(CS) الذي يعد بوليمر طبيعي ممزوج مع بوليمر آخر بولي إيثيلين جلايكول (PEG)بنسب مختلفة (٩٠%/١٠%، ٨٠%/٢٠%، ٧٠%/٣٠%) على التوالي بطريقة الصب وكانت النسبة (٧٠/٣٠) بالمئة هي الأفضل نظراً لامتلاكها خواص مثاليه و لمرونتها الأكبر. بعد ذلك، تمت إضافة ثلاثة أجزاء وزنية مختلفة من جسيمات الهيدروكسيباتيت النانوية (HANPs) إلى خليط CS/PEG لتعزيزه (١، ٢، و٣ بالمائة). تم فحص هذه المركبات النانوية باستخدام طيفFTIR ، واختبارFE-SEM ، وAFM، واختبار التحلل ومقاومه للبكتيريا. أشارت نتائج FTIR إلى تفاعلات جيدة وتكوين رابطة هيدروجينية بين المركبات النانوية، وأظهر مسح FE-SEM مزيجًا متكاملًا جيدًا من CS و PEGمع تشتت جيد وتوزيع متجانس للمادة النانوية داخل الخليط. أظهرت نتائج AFM أن خشونة السطح لـــ CS تزداد بإضافة PEG 30% wt ، لكن خشونة السطح تقل بزيادة نسبة الجسيمات النانوية HANPs، ويزداد النشاط المقاوم للبكتريا مع زيادة نسب الهيدروكسيابتيت وأيضا أوضحت النتائج انخفاض معدل التحلل مع إضافة الجسيمات النانوية HANPs .أظهرت نتائج النشاط المقاوم للبكتيريا زيادة مع زيادة تراكيز HAPs.على الرغم من أن (٧٠%:٣٠%:٣%)(CS/PEG/HANPs)على التوالي كانت أفضل عينة من حيث النتائج والعينة الأكثر ملاءمة لتكون دعامه متوافقة حيويا.

الكلمات الدالة: الشيتوزان ,بولي اثليين كلايكول , هيدروكسيباتيت,جسيمات نانويه,دعامة العظم

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