Research article

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Production of Tetracycline Hydrochloride-Collagen-Doped Chitosan Nanofiber Scaffolds And Investigation of Their Antibacterial Properties

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ABSTRACT

The study aims to develop a noble biomaterial that can accelerate the healing process without the risk of infection by loading tetracycline hydrochloride and collagen hemostatic agent into the chitosan tissue scaffold. After the trials, a good tissue scaffold was obtained from chitosan and PVA by electro-spinning. To increase the hemostatic features of this biomaterial, a 10% (by weight) collagen hemostatic agent was added to the PVA chitosan blend. After the amount of collagen hemostatic agent in the chitosan tissue scaffold was set, various amounts of tetracycline were added and 5 different biomaterials were developed to augment the antibacterial and wound healing properties. Antibiotic concentration in the biomaterial was IV 10% in the first, 15% in the second, 20% in the third, 25% in the fourth, and 30% in the fifth sample. Finally, the effects of the obtained biomaterials on the nosocomial bacteria (gram-positive: Staphylococcus Aureus, gram-negative: Pseudomonas Aeruginosa) were analyzed with in-vitro tests at Kahramanmaras Sutcu Imam University, School of Medicine Department of Microbiology laboratories. As a result of the examination, it was examined how much the biomaterial should be and how effective it was against bacterial growth on the first, third, and fifth days. It is thought that the biomaterial material will be very effective in emergencies and surgical procedures.

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Chitosan, collagen, hemostatic agent, biomaterials, scaffold

Introduction

Piterresi et al. posted in 2012 the characterization and manufacturing of electrospun fibers of the proposed --poly(N-2-hydroxyethyl)-DL-aspartame-graft-polylactic acid (PHEA-g-PLA) copolymer for the cap potential to close by the release of ibuprofen. Before the electrospinning technique, a physically certain medicated solution with PHEA-g-PLA and/or a chemically certain medicated solution with PHEA-g-PLA was modified into organized. The synthesis of PHEA-g-PLA copolymer become done with the usage of an answer of 1. eighty g of PHEA in 36 ml of anhydrous DMSO and 1.7 ml of DEA as a catalyst. The reaction becomes mounted

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below the Argon fuel line for twenty-4 hours at 40 0C.PHEA-gPLA-IBU medicated copolymer answer; 117,15 mg of IBU become dissolved in 1. five ml of anhydrous DMC and saved for a half-hour at -14 0C withinside the presence of 7 mg of DMAP and 117,15 mg of DCC. Then, the IBU solution becomes modified and added dropwise to a 500 mg PHEA-g-PLA solution in 4. five ml of DCM. This chemical synthesis reaction took place at -14 0C for 1 hour, and then it become at room temperature for 3 hours. The nanofibers of these samples, on the alternative hand, have been created with the useful resource of electrospinning after obtaining the medicated copolymer solution with the physical combination of five% IBU and PHEA-g-PLA polymer at the most useful ratios in the solvent combination of acetone and N, N-dimethylformamide. Dulbekko phosphate buffer (DBSO) answer become used for launch testing. A piece of decreased nanofiber withinside the buffer answer modified and dissolved, and a sample is modified and become taken at sure time durations. These samples were examined with the useful resource of the UV-vis technique. Images of drug-loaded nanofiber structures were inquisitive about the useful resource of SEM [1].

Nanofiber ground was modified into received for the primary time with the usage of an electrospinning approach from a combination of the aqueous answer of poly (vinyl alcohol) polymer, which has extremely good nanofiber formation ability and mechanical houses of propolis extract, which can not be drawn alone. They received linear nanofibers that could provide the drug release mechanism. In their experiments, they truly located that the nanofiber ground modified form in the SEM image of the nanofiber surfaces they received at a 5 percent PVA solution and 10 cm walking distance. They claimed that as PVA attention improved, so did the linear fiber form, and the connection between walking distance and fiber form has become at the least proportional. The maximum helix shape became located within the nanofiber ground image of the solution prepared with 3 percent propolis extract and 11 percent PVA. As a result of scanning electron microscope pix, the pleasant fiber structures were received within the pix of nanofiber surfaces produced at a fifteen cm walking distance in an electrospinning tool with the resource of the use of getting prepared propolis extract solutions at 7% and 9% PVA concentrations at 60 0C. It has been determined that the factors decided within the natural wound flora are formed in vitro and are effective in competition with the S. Aureus microorganism, one of the gram-first-rate microorganisms that reason the most wound formation on human skin. They concluded that due to the fact the bacterial boom surrounding the affected character's wound flora may be removed or reduced with the usage of the composite

plaster, the affected character will do away with the thing results of the drug due to systematic or oral antibiotic intake and will avoid the price of antibiotics withinside the route of the treatment [11].

In taking a study with the resource of Taepabioon et al. In 2006, they efficiently prepared the fibers with the resource of electrospinning the usage of the drug-loaded biodegradable polymer PVA (polyvinyl alcohol) and examined the usability of these fibers as a drug transport system. It was modified right into a 10% PVA (polyvinylalcohol) solution with the resource of dissolving sufficient PVA (polyvinylalcohol) powder in distilled water at eighty C for 3 hours. As a drug model, 4 tremendous non-steroidal anti-inflammatory tablets with water solubility residences: sodium salicylate (SS) (water-soluble), diclofenac sodium (DS) (slightly water-soluble), Naproxen (NAP), and Indomethacin (IND) (every water-soluble and insoluble) had been used. These tablets have been combined one after the alternative with 10% PVA solutions at 20% and 10% ratios. Drug-loaded nanofibers of prepared answers had been acquired through the use of the electrospinning technique, and nuclear magnetic resonance (NMR) became used to make clear the chemical form of drug-loaded PVA fibers [12].

Yu et al., in their, examination posted in 2012, investigated the release houses of nonsteroidal anti-inflammatory drug active substance KET (ketoprofen), which they used as a drug model, from CA (cellulose acetate) polymer fibers. Eleven mg of CA and more than one g of KET, one hundred ml of DMAc (dimethylacetamide), acetone, and ethanol (4:1:1 ratio) were dissolved within the solvent. Then, KET-loaded CA fibers had been prepared with the useful resource of an electrospinning approach. The houses of nanofibers in conjunction with morphology, crystalline shape, form, length, and composition were evaluated using FESEM (problem emission scanning electron microscopy). a hundred mg of fiber samples derived from nanofibers changed into taken and dissolved in 600 ml of ninety-percentage physiological saline (PS) at 321 C. The Fickian diffusion mechanism of KET, which is amorphous and dispersed in six CA-based drug-loaded fibers, changed into used to research a non-stop drug release profile for one hundred forty-four hours. The oscillation 38 changed into theoretically examined with the useful resource of the use of the UV-vis approach at the samples taken over time, and on the give up of this appearance, they decided that the oscillation kinetics modified into 0th order [13].

In their have a look at in 2005, Zeng et al. investigated the usability of drug-loaded PLLA (Poly (L-lactide)) nanofibers in controlled drug delivery systems. Paclitaxel, doxorubicin, and

doxorubicin hydrochloride were used as drug models. PLLA (Poly (L-lactide)) polymer solution changed into prepared in a 2:1 chloroform-acetone solvent combination at the attention of 3.9 wt%. 15.5% paclitaxel, 1.6% doxorubicin hydrochloride, and 1.6% doxorubicin drugs were added to the prepared PLLA (Poly (L-lactide)) polymer answers. The morphology of the prepared drug-loaded nanofibers and the images of the drug-loaded cloth were examined with the resource of SEM (scanning electron microscopy). In the in vitro release of the paclitaxel drug model, it changed into studied in 25 ml of 0.05 mol/L Tris-HCl buffer solution (pH = 8.6). In addition, the release of drug-loaded PLLA (Poly (L-lactide)) polymer form was modified whilst studied with proteinase K (0.01 mg/ml). For the in vitro release of doxorubicin hydrochloride, it is 3.0 x 10-3 mg/ml prepared in a proteinase K enzyme buffer solution. The drug-loaded fibers have been dissolved withinside the enzyme solution, samples have been taken at sure time durations, and UVvis analyses have been carried out. Similarly, an in vitro test of Doxorubicin changed into performed. The studies executed are, briefly, The person releases of these three first-rate drugs from drug-loaded PLLA nanofibers were investigated, every with the presence of proteinase K enzyme and without the usage of proteinase K enzyme. As a result, they discovered that Paclitaxel changed into not released withinside the absence of the proteinase K enzyme, however, Doxorubicin and Doxorubicin Hydrochloride have been released. The Proteinase K enzyme degraded PLLA nanofibers and released drugs. In addition, they proved from kinetic studies that the release of Paclitaxel and Doxorubicin drugs is of the 0th order [14].

Material and Methods

It is of incredible significance to save blood loss in wounds due to accidents, emergencies, diverse diseases, and surgical interventions and to heal wounds speedy without the threat of infection [1]. Although bleeding might also additionally appear easy in such cases, prevention of blood loss is of essential significance. Also, one of the largest risks for open or closed wounds is infection. Antibiotics have to end up crucial safety in opposition to the threat of infection [1]. In this study, Tetracycline Hydrochloride antibiotic and Collagen anti-bleeding agent became loaded onto the tissue scaffold received with the aid of using blending Polyvinyl alcohol (PVA) and Chitosan. Thus, it's far aimed to achieve a biomaterial that hastens the recovery technique of wounds without the threat of infection. For this, first of all, a tissue scaffold became received from Polyvinyl alcohol (PVA) and Chitosan with the aid of using the electrospinning method. Collagen astringent and Tetracycline Hydrochloride had been delivered to the tissue scaffold

on the charges stated withinside the content. Codes had been given to the samples received. SEM pictures of the samples are given in Figure 1(a) above [1].

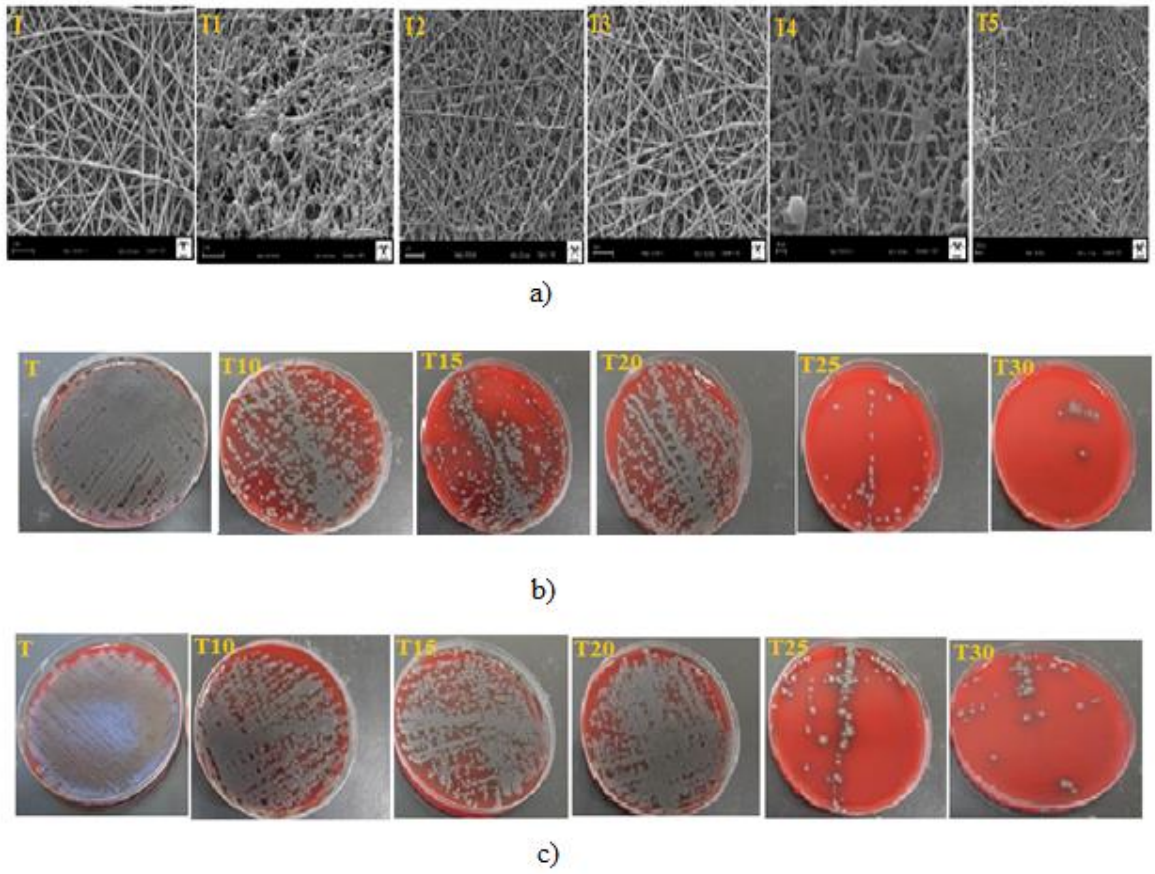

Fig 1 a) Sem images of 5 prepared samples, b) 3rd-day growth results of 5 prepared samples against staphylococcus aureus bacteria, c) 3rd-day growth results of 5 prepared samples against pseudomonas aeruginosa bacteria

The results of the received biomaterials at the medical institution micro organism Staphylococcus Aureus (gram +) and Pseudomonas Aeruginosa (gram-) have been tested with the aid of using in-vitro check withinside the Microbiology Laboratory. Bacterial boom becomes measured in synthetic surroundings breaking away the residing thing. First of all, biomaterials have been sterilized with the aid of using the recent method. At the give up of the first day, third day, and fifth day of the organized biomaterials, bacterial boom becomes found in sheep blood agars. Thus, colony counts on sheep blood agar have been counted for every sample. As a result, we cautiously tested the in-vitro check consequences of biomaterials and supplied the received data. Figure 1 (b) suggests the efficacy of 5 samples in opposition to the S. aureus microorganism. Figure 1 (c) suggests the effectiveness of 5 samples in opposition to

the P. aeruginosa microorganism. The consequences will make considerable contributions to the literature.

Material

Of the substances for use withinside the research: PVA (polyvinyl alcohol) Acn Chemical Mad.İnş tex. conf. Singing. ve Tic.A.Ş. Chitosan became obtained from Acros Organics. Acetic acid and different chemical compounds have been bought from Sigma Aldrich. Tetracycline hydrochloride and physiological saline have been furnished with the aid of using Mustafa Nevzat İlaç san. A collagen hemostatic agent became received from the Pahacel hemostat. P. aeruginosa and S. aureus microorganisms have been received from the microbiology laboratory of the Faculty of Medicine, Kahramanmaraş Sutcu Imam University. The media used for bacterial cultivation (sheep blood agars) have been received from Salubris A.Ş. In addition, the microbiology laboratory of Kahramanmaras Sutcu Imam University became used for the flame burner, tube spores, and bacterial germ cells used in the course of the research to decide antibacterial properties.

Methods

In the study, solutions to chitosan in several bureaucracies were first prepared and drawn via electrospinning, and nanofibers were received from PVA-supported chitosan. After obtaining nanofibers from chitosan, the acetic acid answer became become a prepared option to acquire the samples, and all parameters have been tried to be saved consistently for each sample.

For the education of biomaterials, one-of-a-type solutions had been prepared after which blended. The first solution weighs 10 g. As the solvent for the answer, 2% acetic acid became used. PVA and chitosan have been dissolved in a 2% acetic acid answer in a magnetic stirrer for twenty-4 hours at room temperature. Second, for 2 hours at room temperature, hemostatic collagen matrix and antibiotic tetracycline hydrochloride have been dissolved in saline (with the aid of using weight) in a magnetic stirrer. The solutions were blended. In all prepared solutions for nanofiber spinning, the total percentage of additives (PVA, chitosan, collagen, and tetracycline hydrochloride) withinside the solution is normally 10%. Furthermore, an normal saline answer of 3.5 g became used withinside the education of the second solution, and the number of solids withinside the solution became saved constantly. When making ready the physiological saline answer, the quantity of soluble salt and the NaCl content material of the answer have been taken into account, and 3.5 g of physiological saline answer have become

used after a few experiments. Finally, the samples were assigned codes for each prepared solution, which can be listed in Table 1 together with their respective ratios.

Table 1 Sample codes and rates

Biomaterial Code	Additive ratios in biomaterials		Collagen and Tetracycline Hydrochloride added to Chitosan	
	PVA (%)	Chitosan + Collagen + Tetracycline Hydrochloride (%)	Collagen (%)	Tetracycline hydrochloride (%)
T5	60	40	0	0
T10	60	40	10	10
T15	60	40	10	15
T20	60	40	10	20
T25	60	40	10	25
T30	60	40	10	30

Results

Scanning electron analysis results

During the tries to create a splendid nanofiber from chitosan, it turned into observed that the super ground turned acquired with the useful resource of PVA. The PVA-supported chitosan solution became a 2% acetic acid solution. The ground turned first fashioned from the chitosan-PVA answer thru electrospinning, after which the T manipulator code turned into indicated. Then, the 10% hemostatic collagen matrix and T10 sample with 10% tetracycline hydrochloride, T15 sample with 15% tetracycline hydrochloride, T20 sample with 20% tetracycline hydrochloride, T25 sample with 25% tetracycline hydrochloride, and T30 sample with 30% tetracycline hydrochloride have been done inside the prepared PVA-chitosan solution with the useful resource of the electrospinning method. SEMS Photographs had been taken to represent six surfaces, including a manipulator and 5 samples.

EM snapshots were acquired to symbolize the T-coded controlled ground prepared with the useful resource of the use of the electrospinning technique from a PVA-brought chitosan solution in 2% acetic acid (Figure 2). By studying the SEM pictures of the prepared T-coded controlled ground, it changed into decided that the morphological form had come to be easy and uniform, and non-forestall fiber formations have been placed at the ground.

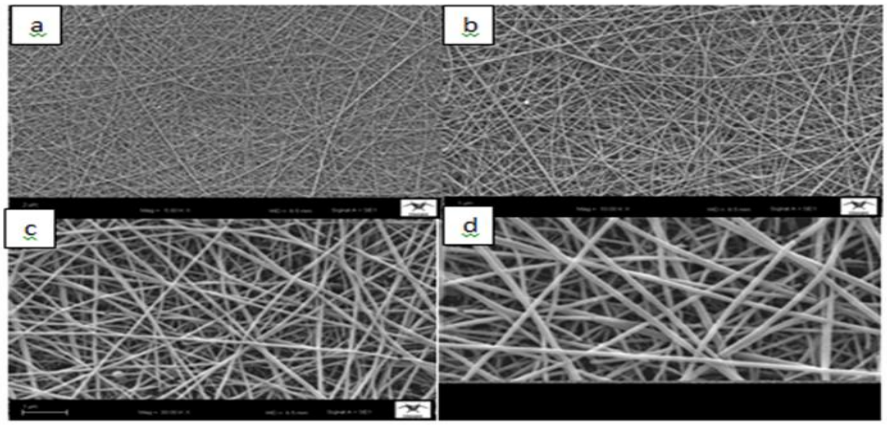

Fig 2 Sem İmages of T sample at different magnification ratios **a.** 5.00 kx magnification **b.** 10.00 kx Magnification **c.** 30.00 kx Magnification **d.** 50.00kx magnification

Due to the polycationic nature of chitosan, this is due to amine agencies in acidic solutions, it's miles more difficult to accumulate a fiber form with the useful resource of the electrospinning technique. The polycationic person of chitosan excessively will increase the ground tension of the solution and effects withinside the formation of droplets at the collection electrode all through the electrospinning process [15, 17, 18].

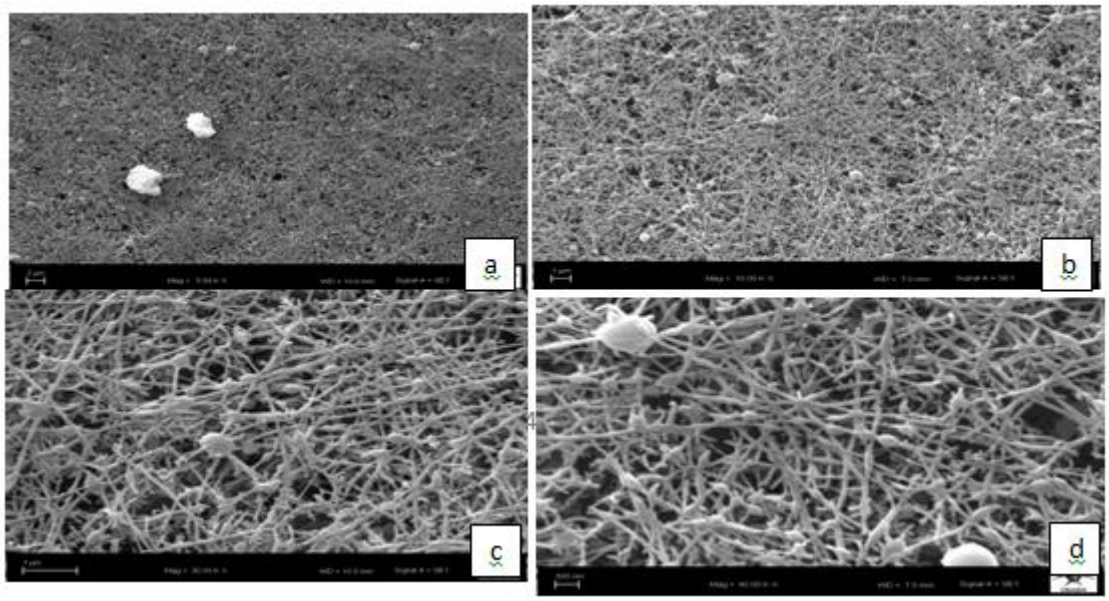

Fig 3 Sem images of t10 sample at different magnification ratios a. 5.00 kx magnification b. 10.00 kx magnification c. 30.00 kx magnification d. 40.00 kx magnification

SEM photos were acquired to symbolize the encoded T10 ground prepared thru electrospinning from a PVA-delivered chitosan solution in 2% acetic acid and a hemostatic collagen and tetracycline hydrochloride antibiotic solution in physiological saline (Figure 3). By analyzing

the SEM pictures of the prepared encoded T10 ground, it may be visible that the morphological form is not smooth. It can be seen that the fiber form is not formed. However, a beaded fiber form is formed, and similarly to the immoderate bead defects, the charged tablets make the fiber formation difficult.

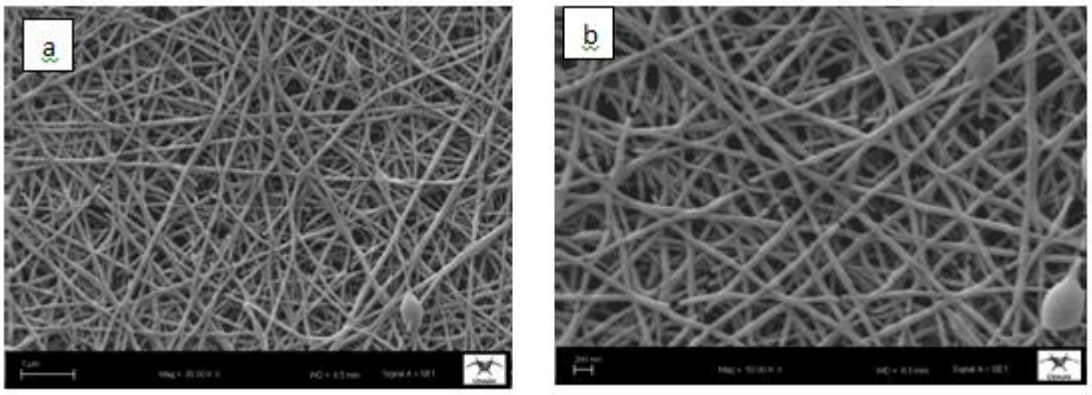

Fig 4 Sem images of t15 sample at different magnification ratios a. 30.00 kx magnification b. 50.00 kx magnification

SEM snapshots were received to symbolize the T15-encoded ground with a 15% tetracycline hydrochloride antibiotic (Figure 4). Examining the SEM snapshots of the produced encoded T15 ground, it may be visible that the morphological form is smoother than that of the T10 sample. It can be seen that the fiber form is formed, however, the pilling defect, which isn't uncommon in the electrospinning system, occurs due to the brought drugs, although very small.

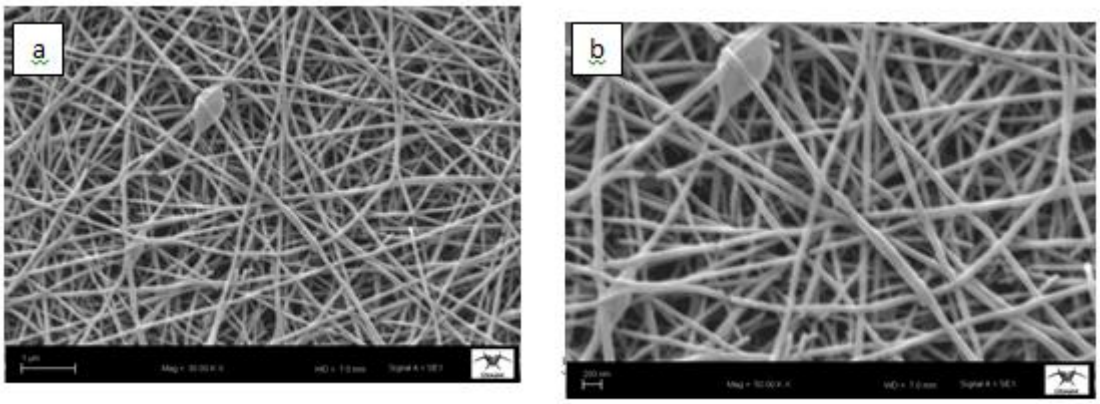

Fig 5 Sem images of t20 sample at different magnification ratios a. 30.00 kx magnification b. 40.00 kx magnification

SEM photographs were taken of the solution obtained via mixing PVA-introduced chitosan solution prepared in 2% acetic acid solvent with hemostatic collagen and tetracycline hydrochloride antibiotic solution prepared in physiological saline to indicate the T20-encoded

ground normal via the electrospinning method (Figure 5). By reading the SEM photos of the prepared T20 encoded ground, it's miles now seen that the morphological form is smoother than that of the T10 sample, but not smoother than that of the T15 sample. It can be seen that the fiber form is normal, however, the pilling defect, which is commonplace in the electrospinning system, takes location from region to region due to the introduced drugs.

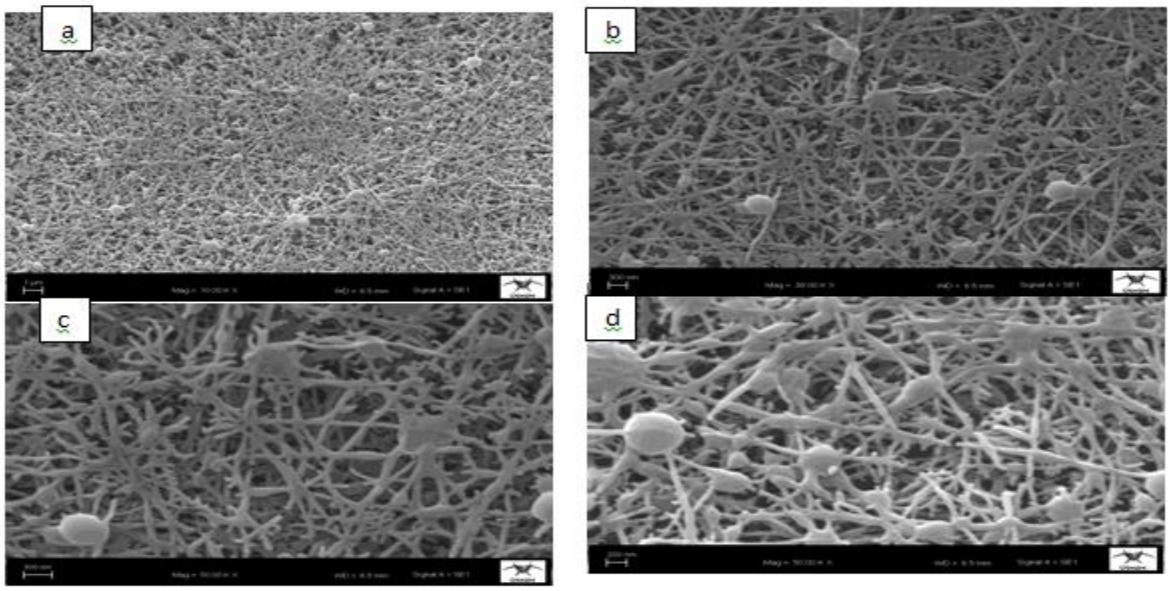

Fig 6 sem images of t25 sample at different magnification ratios a. 10.00 kx magnification b. 30.00 kx magnification c. 40.00 kx magnification d. 50.00 kx magnification

SEMS To constitute the encoded T25 surface, photos were acquired via way of means of using the electrospinning approach thru approach of mixing the PVA-added chitosan solution prepared in 2% acetic acid with hemostatic collagen and tetracycline hydrochloride antibiotic solutions prepared in saline (Figure 6). By examining the SEM photos of the prepared encoded T25 surface, it may be visible that the morphological form is not smooth. It can be seen that the fiber form is not formed; instead, the formation of reticular structures and bead defects is high, and the increase in the amount of charged pills complicates the fiber formation.

SEM pictures had been acquired to represent the encoded T30 ground prepared through a way of electrospinning from a PVA-delivered chitosan solution prepared in 2% acetic acid and a saline-prepared hemostatic collagen and tetracycline hydrochloride antibiotic solution (Figure 7).

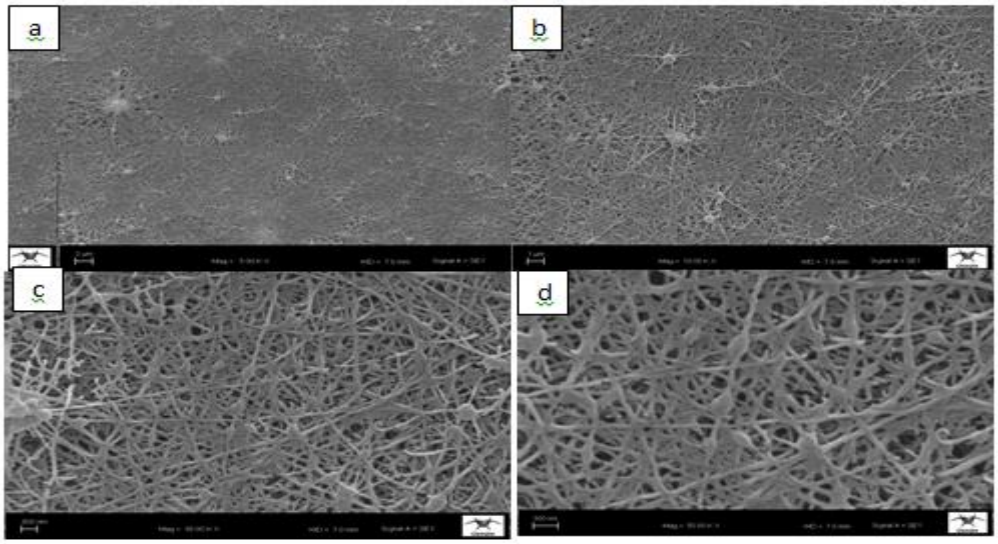

Fig 7 Sem images of t30 sample at different magnification ratios a. 50.00 kx magnification b. 10.00 kx magnification c. 30.00 kx magnification d. 50.00 kx magnification

By studying the SEM pictures of the prepared T30-encoded ground, it's miles viable to look that the morphological form isn't always smooth. It can be seen that the fiber form isn't always absolutely formed; instead, many net-like structures and bead defects are formed, and the charged pills make the fiber formation difficult. Table 2 shows the fiber and bead diameters are in keeping with the consequences of the assessment SEM.

Table 2. Fiber and bead diameters

Sample code	**Fiber diameter**		**Bead diameter**	
	Cover. Diameter (nm)	Standard deflection	Cover. Diameter (nm)	Standard deflection
T5	106,35	10,9172	None	None
T10	100,95	11,9361	217,20	79,4366
T15	74,4	12,5974	170,05	58,0816
T20	84,4	18,9999	234,00	99,7988
T25	94,05	19,0616	301,00	107,9645
T30	98,85	12,7455	182,00	79,8569

Colony count

Here, sheep blood agars are referred to as medium. The sheep blood agars are within the Petri dish. At the top of the incubation period, the samples are placed within the prepared tubes using sterile gloves, a flame burner, and a metal loop for colony counting in Petri dishes. It is based

on the principle that living cells form a colony, and with the resource of counting one's colonies, each living cell office work a colony, and the huge style of living cells in the fabric is calculated. For this purpose, a tremendous amount of the fabric to be remembered is taken and located into the lifestyle medium. The huge style of feasible cells in the fabric is calculated with the resource of counting the colonies in the Petri dish required for colony formation. In addition, consistent with the necessities of the microbiology laboratory, the problem is made consistent with the ground area of the Petri dish. For example, if the microorganisms have grown at the complete ground of the Petri dish, they will remember as 100 colonies. On the occasion that they've grown on half of the ground, they will remember as 50 colonies. On the occasion that they've grown in a ratio of 1 to 4, they will remember as 25 colonies. As for the productivity of the sample, a multiplication ratio of 1:4 approaches 25 colonies, and if the growth within the manipulating sample is 100, it approaches 75% of the microorganisms have been killed. However, to attain whole success, the multiplication rate must be between five and 10, i.e., 90–95% of the killed microorganisms. However, due to the fact vain cells cannot multiply and form colonies, best-stayed cells are counted in this method (Guerguen et al., 1990).

Results of bacteria cultivation in vitro

In this section, bacterial cultivation becomes become a success to investigate the effect of the biomaterial we prepared on Gramme-first-rate bacteria (S. aureus) and Gramme-bad bacteria (P. aeruginosa) inside the in vitro experiments we conducted [16, 19]. The information obtained from the seeding is given below. A manipulated planting become converted right into a one-of-a-type planting and photographed together.

As the result of planting the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline hydrochloride with S. Aureus bacteria solution with the useful resource of the usage of seek on the priority and manipulating agars at the 1st day of sowing, the give up result image of T10 Sample (Figure 8. a); even as 100 colonies had been customary on the pinnacle of factors cultivation, 90 colonies had been customary within the subject, and it changed into located that the biomaterial modified into effective at 10%. The give-up result of sowing the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline Hydrochloride with P. Aeruginosa microorganisms is: While 100 colonies had been shaped on the pinnacle of the priority, 100 colonies had been shaped within the subject, and it's miles now not effective.

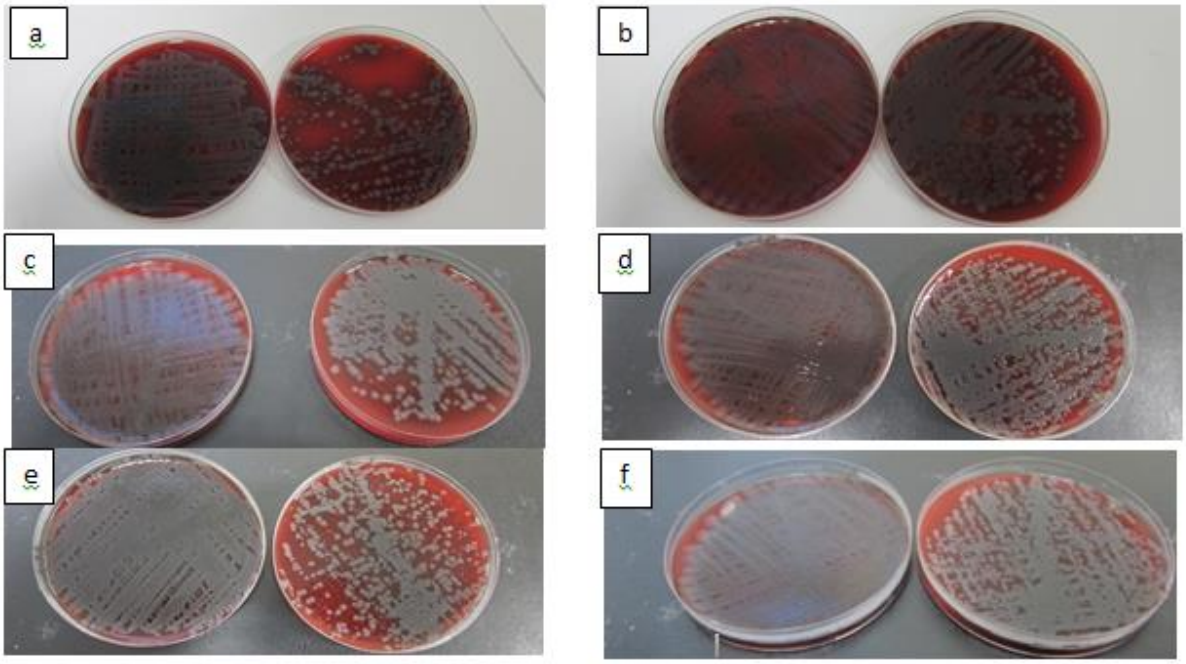

Fig 8 T10 sample **a.** image of control and subject (s. aureus) after cultivation 1st-day **b**. image of control and subject (p.aeruginosa) occurred after cultivation 1st day **c.** image of control and subject (s. aureus) occurred after cultivation 3rd-day **d.** image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day **e.** control and subject images (s. aureus) occurred after planting on day 5 **f.** image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

Planting the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline Hydrochloride with S. Aureus bacteria solution usage of looking at the trouble and manipulating agars led to 90 colonies being shaped inside the trouble, indicating that the biomaterial had very little electricity at 10%. As the result of planting the biomaterial containing 80% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with P.Aeruginosa bacteria solution with the useful resource of the usage of looking on the priority and manipulating agars at the 0.33 day of sowing, the give up result image of T10 Sample (Figure 8.d) suggests that even as 100 colonies had been customary inside the manipulative cultivation, 100 colonies had been customary inside the subject and it's miles now seen that the biomaterial has modified into now not effective. With the useful resource of the usage of seek on the priority and manipulation agars, they give up the result of sowing the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline hydrochloride with S. Aureus bacteria changed into While 100 colonies had been customary inside the control cultivation, 100 colonies had been customary inside the subject, and it's miles now seen that the biomaterial is now not effective. As the result of planting the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline

hydrochloride with P. Aeruginosa bacteria solution with the useful resource of the usage of looking on the priority and manipulating agars on the 5th day of sowing, give up result image of T10 Sample (Figure 8. f); While 100 colonies had been customary inside the manipulative cultivation, 100 colonies had been customary inside the subject, and it's miles now seen that the biomaterial is now not effective.

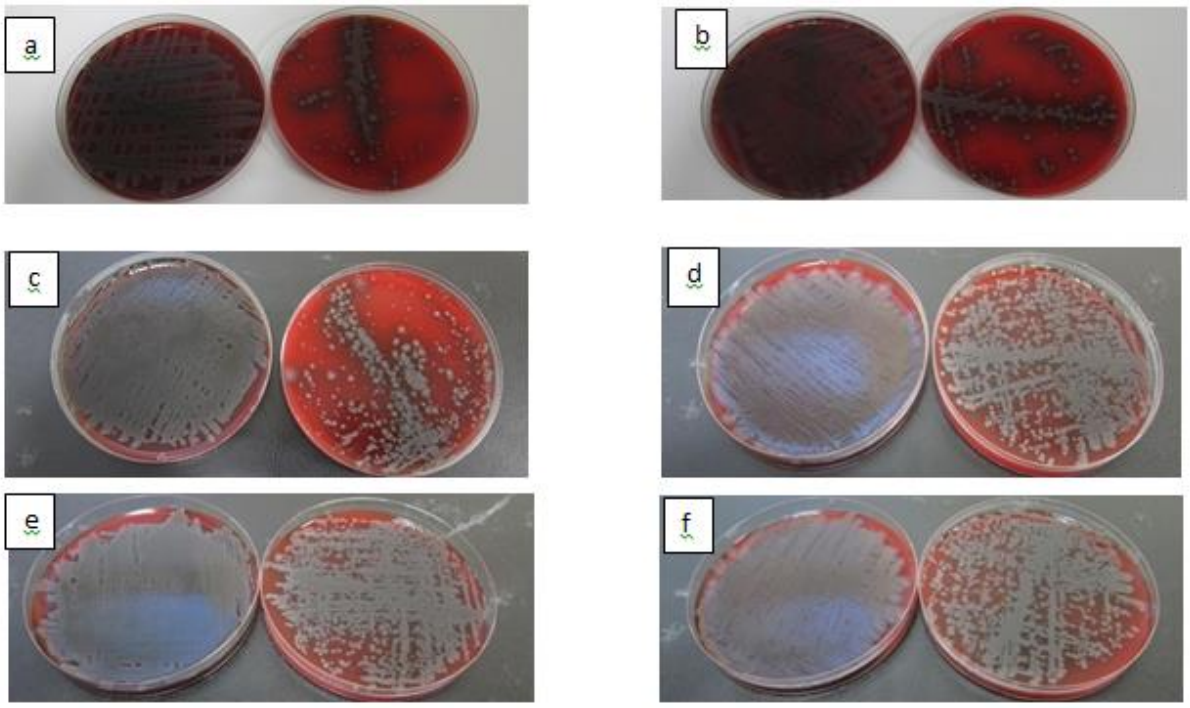

Fig 9 T15 sample **a**. image of control and subject (s. aureus) after cultivation 1st-day **b**. image of control and subject (p.aeruginosa) occurred after cultivation 1st day **c**. image of control and subject (s. aureus) occurred after cultivation 3rd-day **d.** image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day **e.** control and subject images (s. aureus) occurred after planting on day 5 **f.** image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

Sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with S. Aureus bacteria solution thru way of looking at the issue and handling agars on the primary day sowing quit result picture graph of T15 Sample (Figure 9. a), 100 colonies have been shaped in the viable planting, 40 colonies have been shaped inside the issue, and it changed into determined that the biomaterial has become effective. In the picture graph of T15 Sample 1st day sowing quit result (Figure 9. b), thru way of looking at the issue and handling agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with P. Aeruginosa bacteria changed into; While

100 colonies were normal in the viable planting, 60 colonies were normal inside the issue, and it changed into located that the biomaterial has become 40% effective. The 0.33 day of sowing quit result picture graph of T15 Sample (Figure 9. c), thru way of looking at the issue and handling agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with S. Aureus bacteria solution: While 100 colonies were normal in the viable planting, 80 colonies were normal inside the issue, and it has become seen that the biomaterial had emerged as very little effective at 20%. In the picture graph of the T15 Sample 0.33 day sowing quit result (Figure 9.d), thru way of looking at the issue and handling agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with P. Aeruginosa bacteria changed into; While 100 colonies were normal in the manipulate cultivation, 100 colonies were normal inside the issue, and it has become seen that the biomaterial had now emerged as now not effective. The T15 Sample, on the 5th day of sowing, quit result picture graph (Figure 9. e), thru way of looking on the issue and handling agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with S. Aureus bacteria solution: While 100 colonies were normal in the manipulate cultivation, 100 colonies were normal inside the issue, and it has become seen that the biomaterial had now emerged as now not effective. T15 Sample 5th day sowing quit result picture graph (Figure 9. f), looking at the issue and manipulate agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution; While 100 colonies were normal withinside the manipulate planting, 100 colonies were normal withinside the issue and it emerges as seen that the biomaterial emerges as now not effective.

As a result of the cultivation of the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with S. Aureus bacteria solution through looking at the hassle and handling agars on a primary day, sowing cease result image of T20 Sample (Figure 10. a), whilst 100 colonies have been shaped inside the controlled planting, 60 colonies have been shaped inside the hassle, and it changed into observed that the biomaterial has become 40% effective. As the result of sowing the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution through the method of trying to find the hassle and handling agars inside the 1st-day sowing cease result image of T20 Sample (Figure 10. b), whilst 100 colonies were formed inside the controlled planting, 90

colonies were formed inside the hassle and it has come to be observed that the biomaterial turns into effective at 10%.

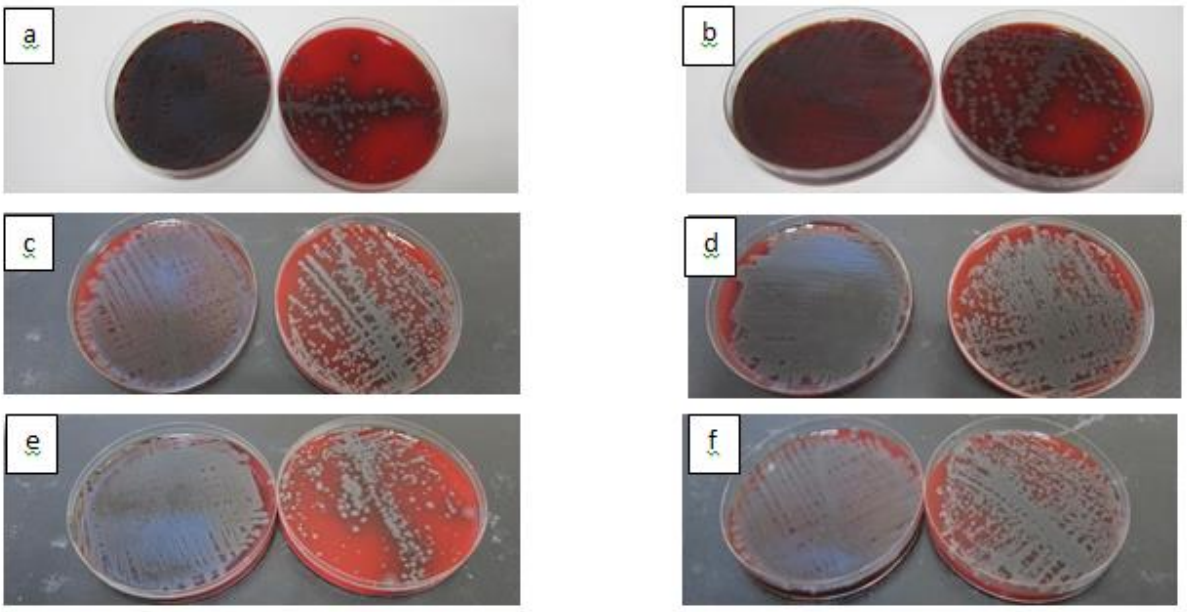

Fig 10 T20 sample **a.** image of control and subject (s. aureus) after cultivation 1st-day **b.** image of control and subject (p.aeruginosa) occurred after cultivation 1st day **c.** image of control and subject (s. aureus) occurred after cultivation 3rd-day **d.** image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day **e**. control and subject images (s. aureus) occurred after planting on day 5 **f.** image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

In the T20 Sample 0.33 day sowing cease result image (Figure 10. c), through the method of trying to find the hassle and handling agars, the cease result of sowing the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with S. Aureus microorganisms is: While 100 colonies were formed inside the controlled planting, 80 colonies were formed inside the hassle, and it has come to be seen that the biomaterial turns into very little strength at 20%. In the T20 Sample 0.33 day sowing cease result image (Figure 10.d), through the method of trying to find the hassle and handling agars, the cease result of sowing the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with P. Aeruginosa microorganisms is: While 100 colonies were formed inside the controlled cultivation, 100 colonies were formed inside the hassle, and it has come to be seen that the biomaterial has come to be now not effective Eighty colonies have been shaped inside the hassle due to the cultivation of the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with S. Aureus bacteria solution, through the method of looking at the hassle and handling agars at the 5th day of sowing, and it changed into observed that the

biomaterial turns into effective at 20%. In the image of the T20 Sample 5th day sowing cease result (Figure 10. f), through the method of trying to find the hassle and handling agars, the cease result of sowing the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution, whilst 100 colonies were formed inside the controlled cultivation, 90 colonies were formed inside the hassle, and it's miles now seen that the biomaterial has come to be now not effective.

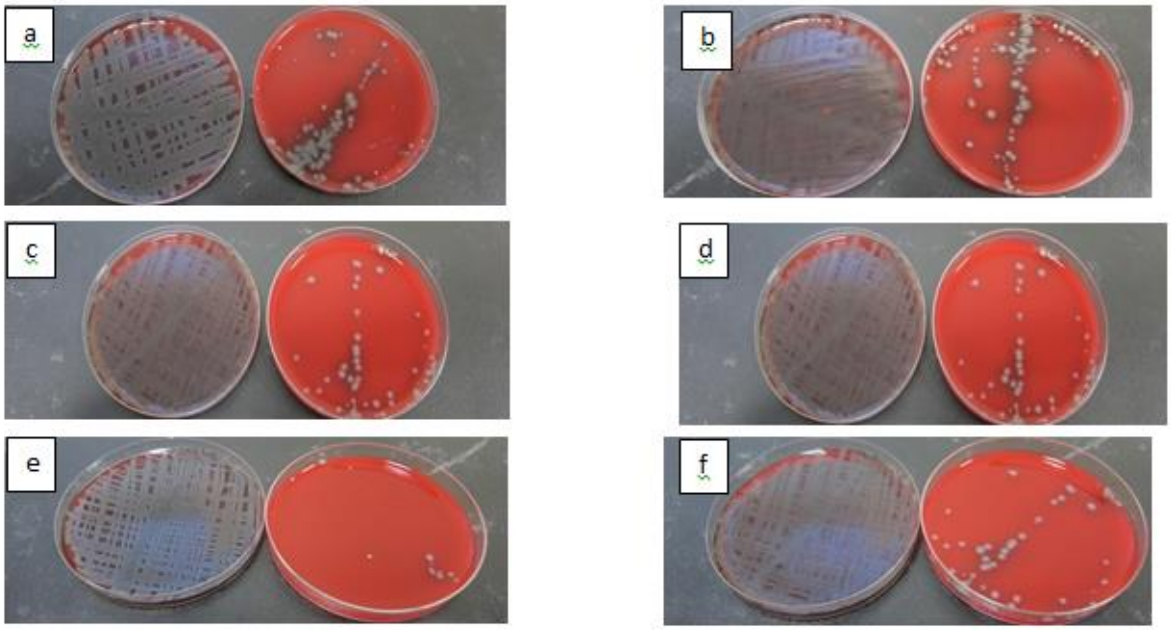

Fig 11 T25 sample **a**. image of control and subject (s. aureus) after cultivation 1st-day **b.** image of control and subject (p.aeruginosa) occurred after cultivation 1st day **c.** image of control and subject (s. aureus) occurred after cultivation 3rd-day **d**. image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day **e.** control and subject images (s. aureus) occurred after planting on day 5 **f.** image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

The final results of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with S. Aureus bacteria turned into as follows: even as one hundred colonies have been fashioned withinside the manage planting, 30 colonies have been fashioned withinside the venture, and it turned into determined that the biomaterial turned into 70% effective. With the useful resource of the usage of seek at the venture and manipulating agars, they give up the result of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with P. Aeruginosa bacteria turned into one hundred colonies have been formed withinside the manipulating planting, 30 colonies have been formed withinside the venture, and it turned into positioned that the biomaterial modified into 70% effective. As the result of sowing the biomaterial containing 65% chitosan, 10% collagen, and

25% Tetracycline Hydrochloride with S. Aureus bacteria solution with the useful resource of the usage of looking at the venture and manipulating agars at the 0.33 day of sowing, the give up result image of the T25 Sample (Figure 11. c) confirmed that even as one hundred colonies have been formed withinside the manipulating planting, 10 colonies have been formed withinside the venture, and it turned into positioned that the biomaterial has become 90 %effective. The T25 Sample on the 0.33 day of sowing give up the resulting image (Figure 11.d), looking at the venture and manipulating agars, they give up the result of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution; While one hundred colonies have been fashioned withinside the manipulating planting, 25 colonies have been fashioned withinside the venture, and it turned into determined that the biomaterial has become 75% effect. As a result of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with S. Aureus bacteria solution, with the useful resource of the usage of looking at the venture and manipulating agars at the 5th day of sowing, the give up result image of the T25 Sample (Figure 11. e) confirmed that even as one hundred colonies have been formed withinside the manipulating planting, 10 colonies have been formed withinside the venture and it turned into positioned that the biomaterial modified into 90% effective. The 5th day of sowing ended with an image (Figure 11. f) attempting to find the venture and manipulating agars. The give-up result of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with P. Aeruginosa bacteria turned into While one hundred colonies have been formed withinside the manage planting, 20 colonies have been formed withinside the venture, and it turned into positioned that the biomaterial modified into 80% effective.

The cultivation of the biomaterial containing 60% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with S. Aureus bacteria solution through a way of looking on the priority and handling agars inside the first-day sowing gives up result photograph of T30 Sample (Figure 12. a), at the same time as 100 colonies had been shaped inside the control planting, 10 colonies had been shaped inside the concern, and it changed into observed that the biomaterial changed into 90% efficient.

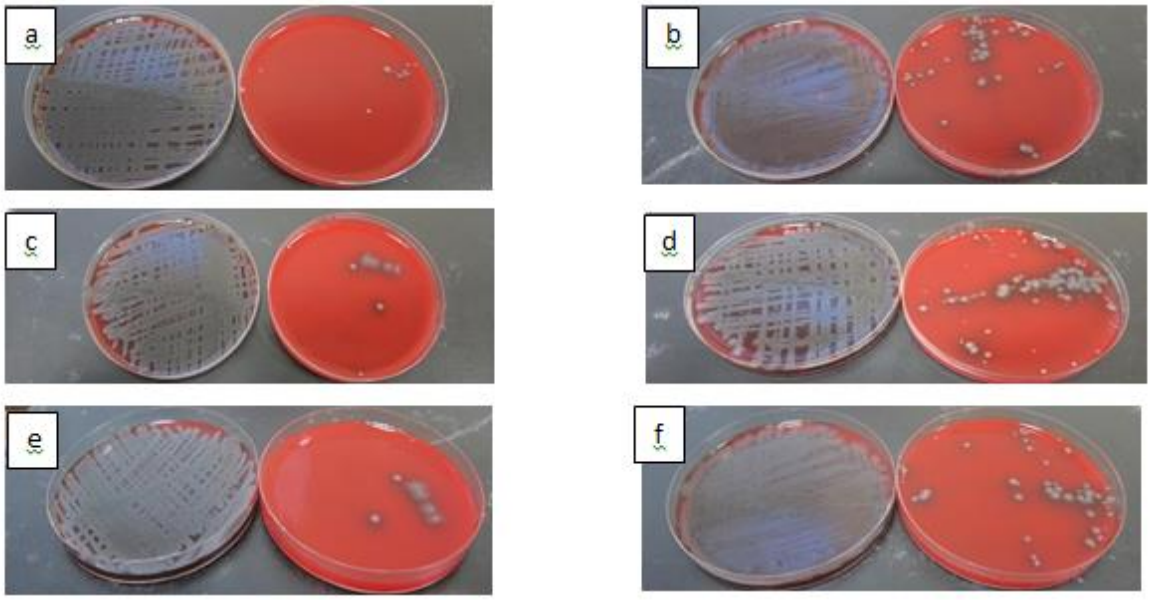

Fig 12 T25 sample a. image of control and subject (s. aureus) after cultivation 1st-day b. image of control and subject (p.aeruginosa) occurred after cultivation 1st day c. image of control and subject (s. aureus) occurred after cultivation 3rd-day d. image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day e. control and subject images (s. aureus) occurred after planting on day 5 f. image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

In the photograph of the T30 Sample 1st day sowing give-up result (Figure 12. b), through the way of looking at the priority and handling agars, the give-up result of sowing the biomaterial containing 60% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with P. Aeruginosa bacteria changed into; While 100 colonies were formed in the controlled planting, 25 colonies were formed in the concern, and it changed into positioned that the biomaterial modified into 75% effective. After sowing the biomaterial containing 60% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with S. Aureus bacteria solution at the 1/3 day of sowing, the give-up result photograph of the T30 Sample (Figure 12. c) found out that at the same time as 100 colonies had been shaped inside the achievable planting, ten colonies had been shaped inside the concern, and it changed into observed that the biomaterial modified into 90% effective. The 1/3 day sowing give-up result photograph of the T30 Sample (Figure 12.d), acquired by looking at the priority and handling agars, the give-up result of sowing the biomaterial containing 60% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution; at the same time as 100 colonies had been shaped inside the manipulate planting, 30 colonies had been shaped inside the concern, and it changed into observed that the biomaterial has become 70% effective. The cultivation of the biomaterial

containing 60% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with S. Aureus bacteria solution, through a way of looking at the priority and handling agars at the 5th day of sowing, found out that at the same time as 100 colonies had been shaped inside the control planting, ten colonies had been shaped inside the concern, and it changed into observed that the biomaterial In the photograph of the T30 Sample 5th day sowing give up the result (Figure 12. f), through the way of looking on the priority and control agars, they give up the result of sowing the biomaterial containing 60% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution, at the same time as 100 colonies were formed inside the control planting, 25 colonies were formed inside the concern, and it changed into positioned that the biomaterial modified into 75% effective.

The efficacy chances obtained due to sowing on S. Aureus (Gram +) and P. Aeruginosa (Gram-) microorganisms of entire of 6 particular biomaterials produced together with the manipulating sample at the 1st day, 0.33 day, and 5th day, respectively, are given in Table three below.

In addition, the overall performance values obtained due to the primary day, 1/3 day, and 5th-day sowing of the produced biomaterials on S. Aureus (Gram +) and P. Aeruginosa (Gram-) microorganisms had been graphically drawn and people graphs had been established in Figure thirteen and Figure 14, respectively.

Table 3. Effective percentage of bacteria on biomaterial

Activity rates against S. Aureus (Gram +) bacteria (%)				**Activity rates against P. aeruginosa (Gram -) bacteria (%)**			
Sample Code	**First-day cultivation**	**Third-day cultivation**	**Fifth-day cultivation**	**Sample Code**	**First-day cultivation**	**Third-day cultivation**	**Fifth-day cultivation**
T5	0	0	0	T5	0	0	0
T10	10	10	0	T10	0	0	0
T15	60	20	0	T15	40	0	0
T20	40	20	20	T20	10	0	0
T25	70	90	90	T25	70	75	80
T30	90	90	90	T30	75	70	75

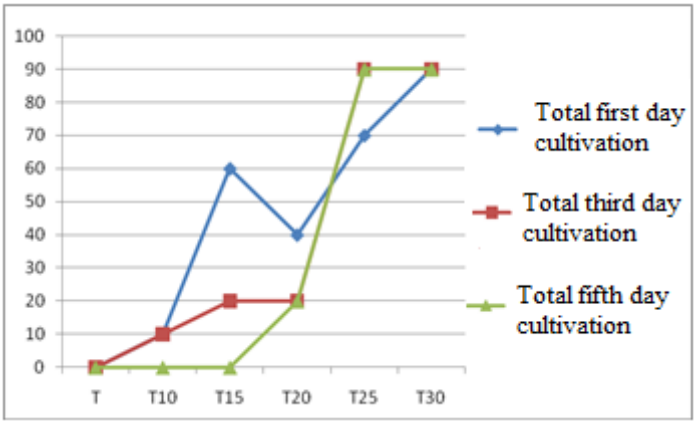

Fig 13 Efficacy of s. aureus (gram+) bacteria on biomaterial

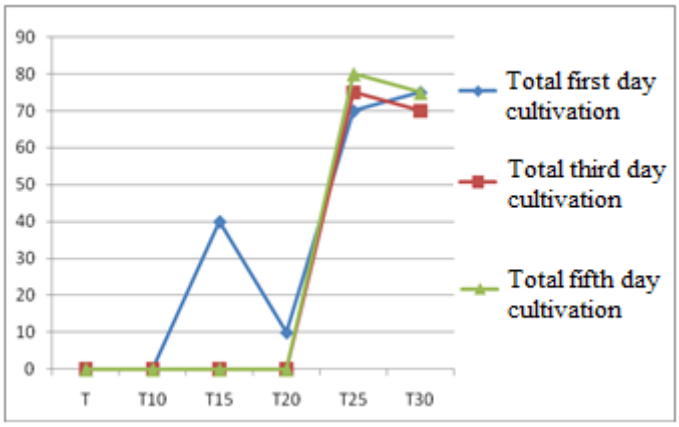

Fig 14 Efficacy of P. aeruginosa (Gram -) bacteria on biomaterial

Conclusion and Discussion

Tissue ground has become to be had thru the use of a mixture of polyvinyl alcohol (PVA) help polymer and Chitosan. Through the electrospinning technique, nanofiber biomaterials were obtained together with Collagen Hemostatic Matrix and Tetracycline hydrochloride antibiotic at precise rates into this biomaterial. To study the form of the obtained biomaterials, SEM analyses were performed and the results were visible. To study the overall performance of the biomaterial in vitro, the colonies common thru culturing microorganisms were counted.

To use the prepared samples as biomaterials, in vitro exams had been performed in the laboratory environment. The effectiveness of the prepared solutions on grammatical excessive pleasant and bad microorganisms changed into an investigation. Due to sowing on sheep blood agar with 0.5 McFarland bacterial solutions prepared with physiological saline, it changed into discovered that it has become effective at particular rates on gram excessive pleasant (S. aureus)

and gram bad microorganisms (P. aeruginosa). Biomaterials had been located to be extra effective in competition to gram-excessive pleasant (S. aureus) microorganisms than gram-bad (P. aeruginosa) microorganisms.

At the identical time, T15 and T20 coded samples had been slightly powerful towards gram excessive quality (S. Aureus) microorganisms, however enough has now not been found.

In particular, T25 and T30 coded samples with an immoderate tetracycline hydrochloride ratio are proven to be quite effective in competition with gram-excessive quality (S. aureus) microorganisms. However, the samples are not as effective as gram-excessive quality (S. Aureus) microorganisms in competition with gram-poor (P. aeruginosa) microorganisms. In addition, it become determined that the T25 coded sample has become extensively effective in competition to gram-excessive quality (S. Aureus) microorganisms, and there was no growth in charge of 90%, in particular on the 0.33 and 5th day of sowing. It become determined that the T30 coded sample has become extensively effective in competition to gram-excessive quality (S. Aureus) microorganisms and there was no growth in charge of 90%, in particular on the 1st day, 0.33 day, and 5th day of sowing.

It's been observed that the parabolic-delivered biomaterial is strong in competition to bacterial boom in some conditions, however, cannot prevent bacterial boom. However, T25 and T30 biomaterials had been found to have an inhibitory effect. In addition, at the identical time, because the biomaterial produced in Arkan's examination can be used for Band-Aids or open wounds, the biomaterials produced in this examination are biomaterials that can be used for each open wound and closed wounds.

In the continuation of this study, it's miles greater to bear in mind to deliver a biomaterial from honestly acquired biomaterials to relieve the affected man or woman for the duration of surgical remedy and provide comfortable use for surgeons. These developments, especially inside the health area and bio textile, provide essential opportunities to carry out that research.

As a result of the cultivation of gram-excessive quality (S. Aureus) and gram-poor (P. Aeruginosa) microorganisms via in-vitro tests, it changed into located that the nice biomaterial has become effective in competition with gram-excessive quality (S. Aureus) microorganisms of T25 and T30 samples. In the studies, it changed located that T25 biomaterial supplied 90% success in the competition to a boom at the 1/3 and 5th day of bacterial sowing. In addition, studies have verified that T30 biomaterial gives 90% success in the competition to a boom in 1st day, 1/3 day, and 5th-day bacterial sowing.

As a result, the T25 sample accomplished success at the 1/3 day and showed that it is a biomaterial that can be applied in open or closed wounds and not used a danger of infection. It is the concept that nanofiber structures are suitable and can be used as a superb nano and biodegradable biomaterial that can be used as a possibility in several fields of study.

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Data Availability statement
The authors confirm that data supporting the findings of this study are not available in the article.

Compliance with ethical standards

Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards

Authors' contributions
İbrahim ALTUN conceived and designed the study. All authors performed the experiments and contributed to the preparation of the manuscript.

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