Antimicrobial peptide immobilization on catechol-functionalized PCL/alginate wet-spun fibers to combat surgical site infection†

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Surgical site infection (SSI) caused by pathogenic bacteria leads to delayed wound healing and extended hospitalization. Inappropriate uses of antibiotics have caused a surge in SSI and common antibiotics are proving to be ineffective against SSI. Antimicrobial peptides (AMPs) can be a potential solution to prevent SSI because of their broad spectrum of antimicrobial activities. In this study, naturally sourced AMPs were studied along with microfibers, fabricated by a novel wet-spinning method using sodium alginate and polycaprolactone. Afterward, fibers were functionalized by the catechol groups of dopamine immobilizing nucleophilic AMPs on the surface. Conjugation between PCL and alginate resulted in fibers with smooth surfaces improving their mechanical strength via hydrogen bonds. Having an average diameter of 220 μm, the mechanical properties of the fiber complied with USP standards for suture size 3-0. Engineered microfibers were able to hinder the growth of Proteus spp., a pathogenic bacterium for at least 60 hours whereas antibiotic ceftazidime failed. When subjected to a linear incisional wound model study, accelerated healing was observed when the wound was closed using the engineered fiber compared to Vicryl. The microfibers promoted faster re-epithelialization compared to Vicryl proving their higher wound healing capacity.

1. Introduction

Surgical site infection (SSI) is associated with bacterial infiltration in wound sites occurring within 30 days post-surgery. It accounts for at least 20% of hospital-originating infections, increasing morbidity, mortality, and healthcare costs.1 One of the most crucial reasons behind SSI occurrence is bacteria from the ambient environment entering a wound site.2 These bacteria can colonize among the spaces of a multifilament suture causing infections in the wound and damaging local tissues.3,4 The colonization of bacteria on a suture may also lead to biofilm formation making them untouchable by host immunity. The risk of SSI increases even more if the colonizing bacteria is resistant to antibiotics. Due to overuse and misuse of antibiotics, mutation in bacteria is now a grave concern. Hence, SSI caused by antibiotic-resistant bacteria can pose a serious threat to healthcare.

Antimicrobial peptides (AMPs) can replace antibiotics because of their ability to bypass the mutation in bacteria and kill them. They are ubiquitous and act as primary defenses against pathogens in almost all organisms.5,6 Among the sources, plant AMPs are easier to obtain and there is a lot of variety to choose from. Although most of the plant AMPs are cationic, they can also be anionic due to the presence of glutamic acid residues and phosphorylation of serine and display antibacterial, and antifungal activities.7,8 Wheat is a good source of AMPs and almost every type of AMP can be obtained from different parts of wheat.9 Several wheat AMPs were proven functional against pathogens such as methicillin-resistant Staphylococcus aureus bacteria and Fusarium fungi.10-12 However, their effectiveness in preventing SSI by inhibiting pathogenic bacterial growth has not been investigated yet. Although several attempts were made to develop antimicrobial therapies against such infection, AMPs from natural sources such as wheat remain unexplored.13-15

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Polycaprolactone (PCL) is a highly hydrophobic and semi-crystalline polymer with good mechanical properties. In the field of surgical suture, PCL is an important component of the commercial suture Monocryl which is composed of a copolymer of \(\ell\)-lactide-co-\(\epsilon\)-caprolactone. Consequently, PCL is being researched extensively as an important component of a suture. Sodium alginate (Alg), on the other hand, is extremely hydrophilic and comprised of 1,4-\(\beta\)-mannuronic (M) and 2,3-guluronic (G) acids. Being a natural polymer it is mechanically weak which makes it unsuitable to be used alone. However, Alg can inhibit bacterial attachment on its surface rendering it a good candidate to prevent bacterial colonization. Alg has been formed into fibers to be used in wounds via dual electrospinning and co-axial wet-spinning. PCL and Alg composite nanofibers were also prepared using electrospinning for wound repair purposes. However, the electrospinning process requires a complex setup which makes wet-spinning more suitable for manufacturing purposes. The opposite physical properties of PCL and Alg make it extremely difficult to mix these two polymers under normal circumstances because they may initiate complex gelation. This would make it challenging to wet-spin these polymers together. Although PCL and Alg have been wet-spun before, a rather complex method and a crosslinker for Alg were required. A dual syringe pump containing PCL and Alg extruded them into a co-axial spinneret to produce a core–shell-like fiber and the coagulation bath contained Ca to crosslink Alg. However, the thickness of the core and shell was difficult to control in this method and there was no chemical bond between PCL and Alg. Furthermore, Ca may leech from crosslinked Alg and mix with physiological ions if used in vivo. Nanofibers composed of PCL and Alg were synthesized by emulsion electrospinning in another study where a water-in-oil emulsion was prepared using these two polymers with the help of surfactant Span 60. Because of the immiscibility of PCL and Alg, their emulsion was used in the fiber fabrication process. However, preparing an emulsion requires several chemicals and the presence of an emulsifier can obstruct the solvent-exchange process of wet-spinning. Hence, fabricating composite polymers with PCL and Alg via wet-spinning remains a challenge because of their opposite behavior.

Several methods exist to graft peptide-like moieties on PCL surface which include exploiting carbodiimide chemistry using EDC/NHS, Schiff-base reaction, or Michael addition using self-polymerization behavior of dopamine, alkyne–azide cycloaddition reaction, and aminolysis by 3-azidoethylamine among many others. Among various methods, using dopamine to bind AMPs was chosen because it is a naturally occurring adhesive found in mussels. 3,4-Dihydroxyphenylalanine-derived dopamine improves the adhesiveness of solid substrates, enhances cellular adhesion, and can be used to bind peptides on hydrolyzed surfaces. Polymered dopamine contains catechol functional groups that can covalently react with nucleophiles such as amines. Both synthetic and natural AMPs were previously coated on solid surfaces such as polydimethylsiloxane and stainless steel using dopamine chemistry. However, the immobilization of wheat AMP using dopamine on PCL surface for preventing SSI has not been explored yet.

In this study, a simple wet-spinning method was used to prepare engineered fibers composed of both PCL and Alg. Their opposite nature can be exploited in the wet-spinning process since the solvent of PCL can act as a coagulant for Alg and water can easily solidify PCL. This led to a simple wet-spinning process of extruding a PCL solution into an aqueous Alg solution where these two polymers coagulated together and formed a composite fiber. Afterward, AMPs were extracted from a natural source—_Triticum aestivum_ seeds, purified, and immobilized on the wet-spin fibers which were functionalized by the catechol groups of polydopamine. The physical properties, antibacterial efficacy, and _in vivo_ wound healing ability of the fibers in a linear incisional wound model were investigated.

### 2. Materials and methods

#### 2.1. Materials

Polycaprolactone (M_w = 80,000), sodium alginate (SA) (G/M = 0.77), and dopamine hydrochloride were purchased from Sigma Aldrich. Potassium chloride (KCl), potassium dihydrogen phosphate (KH.PO4), disodium hydrogen phosphate (Na2HPO4), ethylenediamine tetraacetic acid (EDTA), and acetone were purchased from Merck, Germany. All the reagents were analytical grade and used without further purification. _Triticum aestivum_ (wheat) seeds were purchased from a local market in Bangladesh.

#### 2.2. Fabrication of wet-spun fibers

20% (w/v) PCL solution in acetone and an aqueous solution of 10% (w/v) Alg were prepared separately and sonicated using ultrasound for 15 minutes to remove bubbles. The as-prepared PCL solution was loaded into a 10 mL syringe with a 15-gauge stainless steel needle. This polymer solution was then extruded from the nozzle tip using a syringe pump at a rate of 10 mL hour^{-1} in a coagulation bath containing 100 mL Alg solution. Here, water and acetone functioned as coagulants for PCL and Alg, respectively (Fig. 1(a)). The resulting wet-spun fibers were then carefully removed from the bath, washed in water for another 30 minutes, and dried separately at room temperature for two days. The fiber was named PCL/Alg.

#### 2.3. Extraction of antimicrobial peptides from natural sources

Wheat seeds were crushed into fine powder by a mortar. Peptides were extracted from the powders following a previous procedure with necessary modifications. 5 g flour was incubated for 3 hours at 4 °C in 25 mL extraction buffer. The extraction buffer was prepared by mixing 10 mM Na_3HPO_4, 15 mM KH.PO_4, 100 mM KCl, and 1.5% EDTA at pH 5.4. After 3 hours, the supernatant was collected, and before submitting to chromatographic methods the extraction buffer was exchanged with Tris–HCl buffer through gravity filtering method. After that, the solution was ready for chromatography.
2.4. Purification and characterization of antimicrobial peptides

An anion exchange Sepharose column, HiPrep QFF 16/10 was used for separating the cationic peptide from the supernatant. The column was equilibrated and initially eluted with 1 M Tris–HCl pH 8 followed by gradient elution with 1 M Tris–HCl pH 8 containing 1 M NaCl. The antimicrobial properties of the fractions were checked against *E. coli* and the fraction with the highest bacterial kill having a concentration of 0.39 mg mL$^{-1}$ was pooled and freeze-dried which is further described in ESI,† and Fig. S1. SDS-PAGE analyses were run on 4–20% Tris–HCl gels to find out the molecular mass of peptides of the fractions and subsequently, fractions were loaded in a Sephacryl column for size exclusion chromatography. The concentrations of separated peptides from
various fractionations were determined using a Bradford assay kit (Bio-Rad, Berkeley, CA, USA). In brief, Bradford reagent was prepared according to the manufacturer's instructions and stored in a brown glass bottle. Known concentrations of bovine serum albumin (BSA) were used to create a standard curve. Afterward, the separated peptide and BSA solutions were mixed with Bradford reagent and their absorbances were measured using SpectraMax M5 Multi-Mode Microplate Reader after the color of the mixture turned from brown to blue. Finally, the concentration of the separated AMP was measured by comparing its absorbance value to that of BSA. The purified peptides were dialyzed with deionized water (DI) before MALDI-TOF analysis (Voyager-DE STR) for mass spectrometry. The fraction containing peptides <10 kDa was pooled and stored at −20 °C for further use (Fig. 1(b)).

2.5. Immobilization of antimicrobial peptides on wet-spun PCL/Alg fibers
PCL/Alg fibers were taken and they were submerged in 1 M NaOH for 30 minutes. The treatment by NaOH increased the hydrophilicity of PCL and introduced hydroxyl and carboxyl functional groups on the polymer chain consequently increasing dopamine adhesion. After 30 minutes, the fibers were washed with DI water for 30 minutes. This cycle was repeated 5 times and surface-functionalized PCL/Alg fibers were obtained. Dopamine hydrochloride solution was prepared in an aqueous solution with a concentration of 2 mg mL\(^{-1}\). Dopamine self-polymerizes under basic conditions. Therefore, Tris buffer was used at a concentration of 2 mg mL\(^{-1}\) to make the pH of the solution 8.5. The functionalized PCL/Alg fibers were then pre-immersed in the dopamine solution and put in a shaking incubator overnight at 37 °C. Dopamine underwent self-polymerization on the PCL/Alg surface through oxidation forming polydopamine which was facilitated by the functionalized surface of PCL/Alg (Fig. 1(c)). The polydopamine-coated PCL/Alg fibers were then rinsed in DI water thoroughly and immersed in a peptide solution for peptide immobilization. They were again put in the shaking incubator to maintain the temperature at 37 °C. After three days, the peptide-immobilized fibers were washed thrice with DI water to remove loosely bound peptide molecules, dried and final fibers were obtained (Fig. 1(c)). They were named PCL/Alg-AMP. The in vivo wound healing capability of these fibers was afterward assessed (Fig. 1(d)).

2.6. Physical characterization of fibers
Analyzing the physical properties of fibers is crucial to understanding how well the fibers can handle wound closure. The morphologies of the synthesized fibers were observed using FESEM (Zeiss Sigma 300 VP) with an acceleration voltage of 2 kV and a spot size of 6 mm. Before that, the samples were coated with gold using the ion sputtering method by Zeiss Sigma 300 VP fine coater. ATR-FTIR analysis was carried out to study the chemical structure of the fabricated fibers. ATR-FTIR spectra of the samples were obtained by using Nicolet iS5 FTIR Spectrometer (Nicolet Instrument Corporation, WI, USA) in the range of 4000–400 cm\(^{-1}\) with a resolution of 2 cm\(^{-1}\).

Crystallization behaviors of the wet-spun fibers were characterized using an XRD Diffractometer (Bruker/D8 Discover) in the range of 10° to 80°. Cu-K\(\alpha\) radiation with a wavelength of 1.542 Å was used for obtaining X-ray diffraction. Mechanical properties were studied with Wance ETM 501 using a 10 N load cell. The gauge length of the samples was 30 mm and the testing speed was set at 10 mm min\(^{-1}\). Knot-pull tensile test was carried out by tying a simple knot on the fiber. Two sides of a fiber were clamped such that the knot fell in the middle and the load concentrated on the knot. To test the needle-attachment behavior of the fiber, one end of the fiber was tied to a removable needle. The needle and the other end of the fiber were clamped and the load fell mainly on the attachment site between needle and fiber. STA was performed using NETZSCH STA 449 F1 Jupiter. Both differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) data were obtained. Argon gas was purged through at ambient temperature at a rate of 50 mL min\(^{-1}\). The measurement was carried out from 30–400 °C at a rate of 10 °C min\(^{-1}\).

2.7. Hemocompatibility
To investigate the blood compatibility of the fibers, a hemolysis study was carried out according to a previous study. Briefly, fresh human blood was centrifuged at 4000 rpm and the precipitated red blood cells (RBCs) were collected. The RBCs were suspended in a saline solution at a volume concentration of 2%. Afterward, 10 mL RBC suspensions were incubated with 20 mg of PCL/Alg, PCL/Alg-AMP, and Vicryl separately for 1 hour at 37 °C. Saline solution was used as negative control and Triton X-100 served as the positive control for the study. After 1 hour, the suspensions were centrifuged at 3000 rpm for 10 minutes. The absorbances of the supernatants were measured at 545 nm using a UV/Vis spectrometer (J.P. Selecta UV-3100, Spain). Hemolysis ratios (%) of the samples were calculated according to eqn (1)

\[
\text{Hemolysis ratio (\%)} = \left(\frac{A_s - A_n}{A_p - A_n}\right) \times 100
\]

where, \(A_s\), \(A_p\), and \(A_n\) were the absorbance values of the fibers, positive control, and negative control, respectively.

2.8. Antibacterial properties
The antibacterial activity of PCL/Alg-AMP was confirmed by two methods—colony counting and measuring the optical density of bacterial broth. Proteus spp. bacteria were collected directly from a wound site from a patient at Mugda Medical College and Hospital, Dhaka, Bangladesh, and its sensitivity against common antibiotics was found via disk diffusion study (Fig. S2, ESIF). Proteus spp. was found to be resistant against many of the common antibiotics and among them, ceftazidime was chosen for antibacterial studies. For the colony counting method, one colony of bacteria was inoculated in nutrient broth and was incubated at 37 °C at 100 rpm till the optical density was 0.1. The number of bacteria present in the broth at that time was approximately 1 \(\times 10^8\). This broth was used throughout the experiment. 30 mL of this broth solution was
separately poured in three test tubes in equal amounts among which 10 mg of PCL/Alg-AMP was placed in one and 10 µg mL⁻¹ ceftazidime solution was mixed in another test tube. The third test tube served as a control with no added sample. At regular intervals \( (t = 12, 14, 16, 18, \text{ and } 36 \text{ hours}) \) 100 µL of the broth solution was plated on a nutrient agar plate and incubated at 37 °C overnight. After 24 hours, the number of colonies on the plates was counted. Optical densities of the bacterial broth solutions were measured following a similar time interval at 600 nm using a UV/Vis spectrometer (J.P. Selecta UV-3100, Spain). The observation of optical density was continued for 60 hours.

Since surgical site infection arises due to bacterial colonization on surgical sutures, it is important to check the direct contact response of bacteria. Similar to the antibacterial study mentioned above, *Proteus* spp. was used to estimate the response of both PCL/Alg and PCL/Alg-AMP. Vicryl (3-0) (ETHICON) was taken as a control for this study. Fibers of 1 cm in length were cut from each sample in triplicates. The samples were incubated in a nutrient broth solution of *Proteus* spp. with an optical density of 0.1 when the number of bacteria was approximately 1 × 10⁸. After incubation, the samples were carefully placed on nutrient agar plates for 24 hours at 37 °C for further incubation. Afterward, the samples were washed delicately with PBS thrice to remove any loosely attached bacteria. 2% glutaraldehyde was used to fix the attached bacteria on the fiber surface and the samples went through serial dehydration with ethanol starting from 20% to 100%. Finally, the samples were air-dried for one week and analyzed by FESEM to quantify the number of attached bacteria on the surface of the samples.

### 2.9. In vivo experiments

A linear incisional wound model study was conducted using male Swiss albino mice weighing 30–35 g each. The mice, aged between 8–10 weeks at the time of surgery, were purchased from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). They were housed in stainless steel cages with alternating dark-light cycles where the room temperature was set at 25 ± 2 °C. The animals were fed the same amount of conventional laboratory pellets and water. All techniques and protocols were approved by the Animal Ethics Committee of the Department of Biomedical Engineering of Bangladesh University of Engineering and Technology (BUET) (approval number—2023/BME/02) and were performed following the guidelines of ‘Animal Research: Reporting In Vivo Experiments’.

Twenty-four mice were randomly divided into four groups PCL/Alg, PCL/Alg-AMP, commercial suture—Vicryl (3-0) (ETHICON), and a control group. An optimized dose of ketamine hydrochloride was injected via the intraperitoneal route according to the body weight before performing the experiments. Afterward, the dorsal hair was shaved and 70% ethanol was applied to disinfect the shaved area and prevent infection. A 2 cm incision was made on the dorsal region and the wounds were closed with sterilized PCL/Alg, PCL/Alg-AMP, and Vicryl. The control group received no suturing. Routine postoperative feeding was continued and the surgical sites of the mice were checked every day and no complications developed during this period. On the 3rd, 7th, 10th, and 14th days images of the wound region were captured from a fixed height using a digital camera.

Mice were sacrificed on the 3rd, 7th, and 14th days and the specimen tissues containing the implanted sutures were excised and the skin samples were fixed in 10% neutral buffered formalin for 24 hours. The collected wound regions were afterward dehydrated and embedded in molten paraffin for 2–3 hours. 4 µm thick sections were cut from each block mounted on glass slides and the samples underwent hematoxylin and eosin (H&E), Masson’s trichrome (MT), and periodic acid Schiff (PAS) staining following standard protocols. Re-epithelialization after creating wounds was calculated manually using ImageJ from day 3 of all the samples using ImageJ. Day 3 was chosen for analyzing this parameter because the epidermis layer was still growing after three days and the difference among the samples to promote wound healing could be observed. The number of inflammatory cells was calculated per mm² for days 3, 7, and 14 of H&E staining since inflammation is an integral part of the wound-healing process. Epithelial thickness was also measured using ImageJ. However, it was performed from the MT staining images of 14 days because during this period, the epidermis layer regrew and their differences could be observed. Scar index after 14 days was quantified following a previous method. In short, scar area and dermal thickness were analyzed using ImageJ from MT staining on day 14 for all the samples. Afterward, the scar index was calculated using eqn (2)

\[
\text{Scar index} = \frac{S}{D} 
\]

where \( S \) indicates scar area (µm²) and \( D \) represents dermal thickness (µm). Finally, the regeneration of the basement membrane was studied using the PAS staining, and the number of new blood vessels at day 7 was calculated using ImageJ from PAS staining.²⁷

### 2.10. Statistical analysis

The quantitative data presented in this study were expressed as mean and standard deviation based on the evaluation of at least three independent samples. Statistical analysis was performed based on one-way analysis of variance (ANOVA) followed by Tukey’s post hoc HSD test using Minitab 19. Statistical significance was defined at \( p < 0.05, p < 0.01, \) and \( p < 0.001. \)

### 3. Results and discussions

#### 3.1. Solubility analysis of PCL and alginate

In order to choose suitable solvents and coagulants for both polymers, solubility parameters were employed which is shown in Table 1.²⁶,⁴⁸,⁴⁹ Two solvents—acetone and water were chosen for the wet-spinning procedure to behave simultaneously as a solvent and coagulant for the two polymers. Solvents having Hildebrand parameter \( (\delta) > 37 \text{ MPa}^{1/2} \) can solubilize Alg.⁵⁰,⁵¹ According to Table 1, only water is a suitable solvent for Alg. Therefore, acetone will function as a non-coagulant. The Hildebrand parameter does
not a factor in the cohesive forces present in a solute–solvent interaction which is provided in Hansen solubility parameters and these parameters can provide a clearer view in choosing the appropriate solvents for both PCL and Alg. Its three components are (1) dispersion component, \( \delta_d \), (2) polar component, \( \delta_p \), (3) hydrogen bonding component, \( \delta_h \). Hansen parameter can be calculated according to eqn (3)

\[
\delta_i = \sqrt{\left( (\delta_d)_i^2 + (\delta_p)_i^2 + (\delta_h)_i^2 \right)}
\]

In the case of Alg, the total Hansen solubility parameter \( \delta_t \) could be located and the individual Hansen parameters were not estimated unlike PCL. From the total parameter values the choice of water and acetone can be justified further. The difference in total Hansen parameter \( \delta_t \) between the polymers and coagulants was estimated. In the case of Alg, \( \Delta\delta_t \) values were 10.83 and 17.1 for water and acetone, respectively. Similarly, they were 27.61 and 0.32, respectively, for water and acetone in the case of PCL. Since \( \Delta\delta_t \) between Alg and water is less than \( \Delta\delta_t \) between Alg and acetone, water is suitable as a coagulant for PCL whereas, acetone functions best as a coagulant for Alg. Opposite is the case for PCL which can be confirmed following similar principles. PCL and Alg follow different principles for solvation as evidenced by the Hansen parameters. Water possesses a higher hydrogen bonding component than acetone \( (\delta_h) \) which is the dominant chemical bond during Alg solvation in water. Therefore, acetone cannot participate in hydrogen bonding with Alg as effectively as water. However, the dispersion component \( (\delta_d) \) of acetone is relatively higher than its other components. This indicates that PCL solvation in acetone is predominantly determined by London forces. Although \( (\delta_d) \) of water and acetone are almost similar, their \( \delta_h \) values are highly different. Hence, water exploits its polar hydrogens to solubilize Alg and force PCL solidification whereas acetone does the same for PCL and Alg using its London interactions. The selection of the coagulants can also be explained with the Flory–Huggins equation. According to it, a smaller value of the Flory–Huggins solubility parameter suggests a better solubility. Therefore, a very low value of water makes it a suitable solvent for Alg whereas acetone automatically behaves as a non-solvent.

### 3.3. Physical properties of the fibers

FESEM images in Fig. 2 display the morphological features of the wet-spun fibers. The surface of pure PCL fiber was rough and possessed regular striations whereas PCL/Alg exhibited a relatively smoother surface although striations still existed. The difference in surface features occurred due to variations in the coagulation bath composition. During wet-spinning, non-solvent-induced phase separation occurs in which a polymer solidifies when it separates between its solvent and non-solvent. A polymer–lean phase gets dispersed into a polymer-rich phase which leads to polymer solidification and the solvent gets removed. In this process, the kinetics of double diffusion of solvent and coagulant affect fiber morphology. The diffusion coefficient of acetone was double compared to water, hence, it diffused almost instantly from the PCL solution and the phase separation caused quick solidification of the PCL fiber. There was a huge concentration gradient for acetone from the inside to the outside of the solution and for the water, it was the opposite. This difference drove the diffusion or phase separation of the solvents and controlled fiber formation. The faster the double diffusion, the more uneven and rougher the fiber surfaces become. Therefore, the PCL surface became rough (Fig. 2(a)). The continuous striations might have also occurred due to roughness along the inner perimeter of the spinneret holes and uneven shrinkage during the double diffusion process. Although a few striations can be observed in PCL/Alg, its surface appeared to be smoother compared to PCL. In this case, Alg was present in the coagulation bath which might have hindered the diffusion exchange process between water and acetone, and therefore, coagulation took longer. The hydrogen-bonded interaction between Alg and water slowed the movement of water molecules. After extrusion, acetone diffused away in a short time which started the solidification process of PCL. The initially solidified PCL molecules might have trapped Alg molecules along with some water molecules. After the initial solvent exchange, water began its diffusion process and Alg began to solidify along PCL polymer chains. Since the coagulation process of Alg took longer compared to PCL, the composite fiber took almost 15 minutes to form a mature shape. Due to the slow diffusion exchange of acetone and water, a smooth surface was formed.

### 3.2. Characterization of extracted AMP

The peptide fraction with the highest antimicrobial activity after anion exchange chromatography was characterized for mass through gel electrophoresis. The bands were compared to a standard marker SeeBlue™ and they were present in between 6 to 14 kDa indicating the masses of the peptides (Fig. S3, ESIF). After further purification using size exclusion chromatography, the peptides were analyzed by mass spectrometry. It can be observed that two peptides with masses 6.7 and 9.4 kDa were present after purification. The concentration of the purified peptides was measured to be around 0.35 mg mL\(^{-1}\) as summarized in Table S1 (ESI†). These peptides were immobilized on the microfibers.

### Table 1 Solubility parameters and diffusion coefficients of Alg, PCL, water, and acetone

<table>
<thead>
<tr>
<th>Polymers and solvents/coagulants</th>
<th>Solubility parameters (MPa(^{1/2}))</th>
<th>Flory Huggins parameter, ( \chi )</th>
<th>Diffusion coefficient ( D_a \times 10^{-10} ) ( \text{m}^2 \text{s}^{-1} )</th>
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<tr>
<td>Alg</td>
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<td>—</td>
<td>—</td>
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<td>PCL</td>
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### References

52–54 From the total parameter values, the difference in surface features occurred due to variations in the coagulation bath composition. During wet-spinning, non-solvent-induced phase separation occurs in which a polymer solidifies when it separates between its solvent and non-solvent. A polymer–lean phase gets dispersed into a polymer-rich phase which leads to polymer solidification and the solvent gets removed. In this process, the kinetics of double diffusion of solvent and coagulant affect fiber morphology. The diffusion coefficient of acetone was double compared to water, hence, it diffused almost instantly from the PCL solution and the phase separation caused quick solidification of the PCL fiber. There was a huge concentration gradient for acetone from the inside to the outside of the solution and for the water, it was the opposite. This difference drove the diffusion or phase separation of the solvents and controlled fiber formation. The faster the double diffusion, the more uneven and rougher the fiber surfaces become. Therefore, the PCL surface became rough (Fig. 2(a)). The continuous striations might have also occurred due to roughness along the inner perimeter of the spinneret holes and uneven shrinkage during the double diffusion process. Although a few striations can be observed in PCL/Alg, its surface appeared to be smoother compared to PCL. In this case, Alg was present in the coagulation bath which might have hindered the diffusion exchange process between water and acetone, and therefore, coagulation took longer. The hydrogen-bonded interaction between Alg and water slowed the movement of water molecules. After extrusion, acetone diffused away in a short time which started the solidification process of PCL. The initially solidified PCL molecules might have trapped Alg molecules along with some water molecules. After the initial solvent exchange, water began its diffusion process and Alg began to solidify along PCL polymer chains. Since the coagulation process of Alg took longer compared to PCL, the composite fiber took almost 15 minutes to form a mature shape. Due to the slow diffusion exchange of acetone and water, a smooth surface was formed.
observed in the case of PCL/Alg Fig. 2(b). The diameters of PCL and PCL/Alg were 379 and 209 μm, respectively. After peptide immobilization onto PCL/Alg fiber, the diameter did not change significantly. Compared to PCL/Alg, the PCL/Alg-AMP surface was almost smooth indicating a successful incorporation of peptide onto the fiber surface (Fig. 2(c)). The smooth, uniform surface of PCL/Alg-AMP can facilitate the fiber to glide through wounds without causing any significant damage. The knotted configuration displayed in Fig. 2(d) exhibits the ability of PCL/Alg-AMP fiber to tie knots. No sign of breakage could be observed around the knotting sites where the highest strain was experienced by a fiber. Therefore, the morphological image confirms that PCL/Alg-AMP fiber can be used to close wounds without adversity.

The ATR-FTIR spectra of the fabricated fibers displayed in Fig. 2(f) reveal the successful conjugation between PCL and Alg. To understand the interaction between PCL and Alg, two different concentrations of Alg were used—5% (w/v) and 10% (w/v). The ATR-FTIR spectrum of PCL/Alg-AMP when 5% Alg was used is shown in Fig. S4(a) (ESI†). After analyzing it, differences between pure alginate, PCL, and the wet-spin samples can be observed. The characteristic peaks of an alginate structure were the peaks at 1591 cm\(^{-1}\) and 1400 cm\(^{-1}\) which represented the asymmetric and symmetric stretching vibrations of the COO\(^-\) group, respectively, and a broad peak at 3300 cm\(^{-1}\) for the OH groups of alginate.\(^{59}\) On the other hand, ester absorption peak was a characteristic trait of pure PCL which was observed at 1721 cm\(^{-1}\).\(^{60,61}\) Other peaks belonging to PCL were observed as well such as at 2965 cm\(^{-1}\) and 2865 cm\(^{-1}\) attributing to asymmetric and symmetric CH\(_2\) stretching, respectively, 1468 cm\(^{-1}\) for CH\(_3\) bending, 1363 cm\(^{-1}\) and 1165 cm\(^{-1}\) indicating asymmetric and symmetric C–O–C stretching, respectively, 1295 cm\(^{-1}\) for C–O and C–C stretching in crystalline phase and 1163 cm\(^{-1}\) representing C–O and C–C stretching in amorphous phase.\(^{60–62}\) However, when PCL was conjugated with Alg, as the characteristic peaks of Alg appeared; the representative PCL peaks were observed to decrease in intensity. In the ATR-FTIR spectra of PCL/Alg, in addition to the peaks of PCL, the asymmetric and symmetric stretching vibrations of COO\(^-\) group belonging to alginate could be observed indicating successful conjugation of PCL and Alg. With the increase in Alg concentration from 5% to 10%, its peaks further intensified while decreasing the intensity of PCL peaks. The change in intensity arose because as the concentration of Alg increased, more molecules started to solidify along the PCL chains. However, the composite spectrum was quite similar to pure PCL fiber indicating that the outer layer was mostly composed of PCL. The peak at 1721 cm\(^{-1}\) which was responsible...
for the ester groups’ C—O stretching vibration slightly shifted to the left at 1719 cm\(^{-1}\). This change can be attributed to the carbonyl groups of PCL that were situated close to the hydroxyl groups of Alg.\(^{27}\) These two groups underwent hydrogen bonding with each other stabilizing the Alg inside the PCL matrix.\(^{63}\) This phenomenon led to the sharpness and a left shift to 3349 cm\(^{-1}\) of the peak responsible for the OH group of Alg. Compared to this, the sharpness of the OH group was less intense when 5% (w/v) Alg was used as displayed in Fig. S4(a) (ESI\(^{†}\)). This indicates that the hydrogen-bonded interaction between PCL and Alg might have been weaker in that case. Furthermore, the asymmetric stretching vibrations of COO\(^{-}\) the group of Alg at 1591 cm\(^{-1}\) underwent a blue shift to 1631 cm\(^{-1}\). This also indicates that the carbonyl group of Alg interacted with PCL via intramolecular hydrogen bonding.\(^{64}\) From the ATR-FTIR data, successful immobilization of AMP can be confirmed. Dopamine was used to stabilize AMP on the fiber surface. Due to the oxidizing effect, dopamine was converted to dopamine quinone and after further oxidation, rearrangement, and polymerization reactions, polydopamine was formed.\(^{65}\) This was confirmed by a black coating on the PCL/Alg surface. ATR-FTIR was taken after the peptide was covalently conjugated with the catechol groups of polydopamine via its amine group.\(^{43}\) Comparing the graphs of PCL/Alg and PCL/Alg-AMP, at first the diminish of the large hydroxyl peak at 3350 cm\(^{-1}\) could be observed. Instead, a broad peak from 3300–3500 cm\(^{-1}\) is visible. This broad peak was associated with amide A of peptide proving the existence of –NH\(_2\) and –NH.\(^{66}\) The peak responsible for the OH group of Alg slightly shifted to a lower wavenumber at 1619 cm\(^{-1}\) when 5% (w/v) Alg was used as displayed in Fig. S4(a) (ESI\(^{†}\)). This was confirmed by a black coating on the PCL/Alg surface. ATR-FTIR was taken after the peptide was covalently conjugated with the catechol groups of polydopamine via its amine group.\(^{43}\) Comparing the graphs of PCL/Alg and PCL/Alg-AMP, at first the diminish of the large hydroxyl peak at 3350 cm\(^{-1}\) could be observed. Instead, a broad peak from 3300–3500 cm\(^{-1}\) is visible. This broad peak was associated with amide A of peptide proving the existence of –NH\(_2\) and –NH.\(^{66}\) The peak responsible for C—O of amide I might have been absent or overlapped by the sharp peak at 1719 cm\(^{-1}\) corresponding to the carbonyl group of ester belonging to PCL. However, the peak at 1631 cm\(^{-1}\) occurring due to the asymmetric stretching vibration of COO\(^{-}\) of Alg slightly shifted to a lower wavenumber at 1619 cm\(^{-1}\) and also experienced decreased intensity. This might have occurred because the C—N stretching and N—H bending of amide II should also have existed in the same region. The peak at 1619 cm\(^{-1}\) might have been a resultant of both of these effects. Additionally, there is a slight peak at 1545 cm\(^{-1}\) which corresponds to the N—H amide group belonging to AMP.\(^{67}\) These peaks indicated the presence of AMP due to catechol functionalization on PCL/Alg fibers.

XRD data shown in Fig. 3(a) revealed the microstructural characteristics of the wet-spun fibers. The XRD pattern of PCL demonstrated its semi-crystalline nature by sharp diffraction peaks at 21.3° and 23.8° corresponding to (110) and (200) planes, respectively.\(^{27,68,69}\) On the other hand, Alg possessed mostly amorphous structures showing broad peaks at 13.7° and 21.6° with very low intensity as displayed in Fig. S4(c) (ESI\(^{†}\)).\(^{70}\) However, according to the XRD data of PCL/Alg, its microstructure was mostly similar to PCL because of the presence of similar diffraction peaks. This proves that Alg chains were encapsulated within PCL molecules. Furthermore, the decrease in the intensity of PCL/Alg diffraction pattern indicated its decrease in crystallinity compared to PCL. The crystallinities of PCL and PCL/Alg were calculated to be 78.4% and 72.3%, respectively. The hydrogen-bonded interaction between PCL and Alg inhibited PCL chain movement for crystallization leading to lower crystallinity of PCL/Alg as supported by literature.\(^{63}\)

The mechanical properties of PCL/Alg were compared with pure PCL and Alg fibers crosslinked with calcium which are depicted in Fig. 3(b). From the stress-strain curves of the samples, it can be observed that the composite PCL/Alg fiber possessed significantly better mechanical properties compared to pure PCL and Alg fibers. PCL lacked the necessary elastic properties and can deform quite easily which made PCL unsuitable as a suture. Alg alone also did not possess enough strength for the same purpose. Therefore, conjugating PCL and Alg could result in improved mechanical behavior which was observed from tensile testing. Due to the presence of hydrogen bonds between the hydroxyl group of Alg and the carbonyl group of PCL, PCL/Alg fibers gained elasticity which was absent from pure PCL. Hence, the elongation of the composite fiber was less than PCL, and its mechanical properties were significantly improved compared to the other fibers. The impact of conjugation between PCL and Alg on the mechanical properties may further be explained by the fact that a lower concentration of Alg (5%) was used, the fiber was mechanically weaker. When Alg concentration was increased from 5% to 10% (w/v), the hydrogen-bonded interaction between PCL and Alg might have also increased improving the overall mechanical properties of PCL/Alg fiber as supported by ATR-FTIR findings. This phenomenon is displayed in Fig. S4(b) (ESI\(^{†}\)). One of the most important properties of any fiber to be used as suture is its mechanical behavior and the material should be able to withstand the pressures of handling during surgery. It should also be strong enough to approximate wound edges and perform its intended purpose of closing a wound. Additionally, the ability to elongate without breaking is also helpful since a wound may undergo swelling and the fiber should expand along with the wound.\(^{71}\) Since the ultimate purpose of fabricating PCL/Alg fibers is to use them to close wounds in vivo, several mechanical tests were carried out according to the United States Pharmacopia guidelines (USP).\(^{72}\) Among the tests are the knot-pull test, needle-attachment test, and knot-security test.\(^{73}\) Fig. 3(c) and (d) displays the stress–strain curves of knot-pull and needle-attachment tests of PCL/Alg fibers, and their tensile modulus (MPa) and tensile strength (MPa). To perform the knot-pull experiment, a simple knot was configured on the fibers. During surgery, the highest stress falls on the knotting point of a suture.\(^{72}\) Therefore, it is important to check how a fiber with a knotted configuration handles imposed stress. Although it was expected that the tensile properties would decrease from straight-pull data, the tensile strength met the minimum requirement to be used as sutures as set by USP for class II non-absorbable surgical sutures at size 3-0. The stress–strain curve of the knot-pull test did not show any bimodal structure like previous studies.\(^{71}\) That is because the knot was tightened before loading the instrument. Therefore, the curve signified the total load withstood by the knot before breakage. The % elongation was also about 220% in the knotted configuration. Since a higher elongation at break along with a high knot-pull tensile stress improves the handling of a suture, PCL/Alg fiber could be used as a suture to close wounds. Additionally, during the knot-pull tensile test, the knot did not exhibit any
Fig. 3  Physical properties of the fibers. (a) XRD pattern of PCL and PCL/Alg. The crystallinity of PCL/Alg decreased compared to PCL. (b) Stress–strain curves of PCL, Alg, and PCL/Alg. After wet-spinning with both PCL and Alg, the mechanical properties of PCL/Alg improved significantly due to their hydrogen-bonded interaction. (c) Stress–strain curves of knot-pull and needle-attachment tensile test of PCL/Alg. (d) Tensile modulus (MPa) and tensile strength (MPa) of PCL/Alg in straight-pull, knot-pull, and needle-attachment tests. (e) DSC and (f) TGA thermogram of samples PCL, Alg, and PCL/Alg. The curves of the composite fiber resembled PCL curves, however, differences in melting and initial degradation temperature existed.
tendency to slip which proves the security of the tied knot along with high tensile properties.\(^{74}\) A removable needle-attachment test of PCL/Alg fiber was carried out to check whether the fiber breaks away from the needle during load application. In all cases, the fiber broke down before being detached from the needle. This proves the excellent attachment ability of PCL/Alg fiber to a needle and further validates its usage as a suture. Additionally, the tensile stress values fell within the USP-defined range (0.274–15.6 N) for removable needle attachment. In all cases, the fiber broke down before being detached from the needle. This proves the excellent attachment ability of PCL/Alg fibers to a needle and further validates its usage as a suture.

Fig. 3(e) and (f) represent the DSC and TGA curves of PCL, Alg, and PCL/Alg. It can be clearly observed that both the DSC and TGA graphs of PCL/Alg resemble mostly PCL curves indicating that in the composite fiber PCL properties dominate. However, slight changes still existed which can explain the chemical nature of PCL/Alg. The glass transition temperature \(T_g\) of PCL is around \(-60\) °C which falls outside the range of the experiment.\(^{75}\) The melting temperature \(T_m\) of pure PCL was observed to be \(59\) °C by DSC which increased by \(3\) °C when Alg was conjugated with it.\(^{63,76}\) This phenomenon can be explained by the hydrogen-bonded interaction between the hydroxyl groups of Alg and the carbonyl groups of PCL as found from ATR-FTIR.\(^{63,76}\) TGA data displayed in Fig. 3(c) revealed different thermal stability of pure PCL and Alg. However, the PCL/Alg thermogram mostly resembled the shape of PCL demonstrating that PCL/Alg was microstructurally similar to PCL. The initial degradation temperature \(T_d\) of Alg was \(36\) °C compared to \(359\) °C for pure PCL. From \(36\) °C to \(209\) °C Alg lost about \(20\%\) of its mass due to dehydration and loss of volatile products.\(^{64}\) A distinct drop in mass was observed from \(219\) °C to \(259\) °C which was caused by the decrosslinking of Alg polymer chains and carbonaceous residue formation.\(^{64}\) On the other hand, PCL usually degrades via an ester pyrolysis reaction resulting in the rupture of polyester chains and the release of \(\text{CO}_2\) and \(\text{H}_2\text{O}.\(^{77}\)

5-Hexenoic acid can also be formed from random pyrolysis of neighboring PCL polyester chains.\(^{77,78}\) Although the shape of the PCL/Alg thermogram was similar to pure PCL, \(T_d\) was lowered to around \(319–329\) °C which was between the \(T_d\) of PCL and Alg. This phenomenon further supported the successful integration of Alg into the PCL matrix.\(^{79}\) The decrease in \(T_d\) could be caused due to the \(T_d\) of Alg being lower than that of PCL.\(^{79}\) After the chemical reaction between Alg and PCL, Alg might have become less packed leading to reduced resistance against thermal oxidation.\(^{79}\) Therefore, these results acted as an indication that PCL and Alg underwent chemical reactions when they were wet-spun into PCL/Alg fibers. The thermal properties are summarized in Table S2 (ESI†).

### 3.4. Hemocompatibility of the fibers

The hemocompatibility of PCL/Alg-AMP was studied according to ISO 10993-4. The hemolytic ratio of PCL/Alg-AMP was compared with the commercial suture Vicryl. It was revealed that PCL/Alg-AMP fiber did not cause any rupture of red blood cells (RBC) and fulfilled the requirements for blood compatibility of suture material.\(^{3,80}\) The hemolysis ratios of PCL/Alg, PCL/Alg-AMP, and Vicryl are summarized in Fig. 4. Although both PCL/Alg and Vicryl exhibited a hemolytic ratio under 5% which is the criteria for a biomaterial to be considered hemocompatible, PCL/Alg-AMP displayed a ratio around 0.049% which was significantly lower than both PCL/Alg and Vicryl. The immobilization of a natural AMP on the fiber surface improved the hemocompatibility of the fiber, even more so than Vicryl. Therefore, this result proves that PCL/Alg-AMP fiber will not cause any RBC destruction during its use as wound closure fiber.

### 3.5. Antibacterial effects of the fibers

The antibacterial activity of PCL/Alg-AMP was compared against a third-generation antibiotic ceftazidime which \textit{Proteus} spp. was resistant against. From Fig. 5(a), it was observed that
over time, the number of bacterial colonies (CFU) decreased in the case of PCL/Alg-AMP whereas bacterial growth continued to increase for all other groups. After 36 hours, PCL/Alg-AMP almost completely stopped bacterial growth, and the opposite was observed for ceftazidime. Despite some antibacterial effect of Alg, the bacterial colony was significantly higher in the case of both PCL/Alg and ceftazidime compared to PCL/Alg-AMP which is demonstrated in Fig. 5(b). This proves the higher antibacterial activity of the extracted peptide against pathogenic bacteria. Similar results were also obtained from optical density values taken at the same interval as the colony counting method. With time, the bacterial broth solution where PCL/Alg-AMP was put in transformed from opaque to transparent whereas the opacity increased with time for the rest of the groups. The control group showed the highest optical density values after 60 hours followed by ceftazidime, PCL/Alg, and PCL/Alg-AMP. The clearer broth solution was observed to be for PCL/Alg-AMP which indicates its superior antibacterial properties. The comparison of antibacterial activity by optical density is displayed in Fig. 5(c). The antibacterial effect of PCL/Alg-AMP can be somewhat correlated with the pattern of AMP release from the fiber in a phosphate buffer saline (PBS) medium which is depicted in Fig. S5 (ESI†). Burst release of AMP was observed after about 10 hours and the amount was relatively stable for 60 hours. Because of this release pattern, the antibacterial activity was observed after about 10–12 hours. The fabricated PCL/Alg-AMP functioned effectively against pathogenic bacteria where a third-generation antibiotic failed.

To assess the bacterial response of the samples, a culture of Proteus spp. was dip-coated onto PCL/Alg, PCL/Alg-AMP, and Vicryl and incubated for 24 hours. This allowed attached bacteria to grow and adhere to the fiber surfaces. The direct contact responses were probed by FESEM to count the number of bacteria on the sample surfaces. The bacterial adhesion on both PCL/Alg and Vicryl was significantly higher (p < 0.001) than PCL/Alg-AMP as exhibited in Fig. 5(g). The surfaces of both PCL/Alg and Vicryl suffered complete coverage by Proteus spp. resembling bacterial colonization on a wound. On the other hand, the coated AMP on PCL/Alg-AMP created an unfavorable environment for Proteus spp. attachment on the fiber surface which can severely limit infection initiation by the same bacteria. This will ultimately lead to faster wound healing in vivo. Additionally, no grouping of bacteria was observed, thereby resisting bacterial film formation. Thus, it can be said that SSI will be prevented by PCL/Alg-AMP due to the antibacterial properties of immobilized AMP.

The anionic AMPs possessed a net negative charge due to glutamic acid residue. They interacted with bacterial membranes using small cationic moieties inside long anionic C-terminal domains. Antibacterial activities of AMPs are generally performed by one of three mechanisms—barrel-stave, carpet, or toroidal pore mechanisms all three of which rupture the cell membrane of bacteria. Following one of the three mechanisms, the immobilized peptide displayed its antibacterial activities which have been proved by the direct contact response and antibacterial studies.
3.6. In vivo performance of the fibers

In vivo wound healing capacity of PCL/Alg-AMP fibers was compared with commercial suture Vicryl (3-0) in mice. Using ultraviolet (UV) rays is one of the most popular sterilization methods on a laboratory scale, hence the sutures were sterilized using UV rays for 30 minutes before surgery. Since PCL is a thermoplastic polymer, heat sterilization could not be applied. Afterward, the sutures were applied to a linear incisional wound model and they were sewn with the samples. Fig. 6(a) displays the sequential images of the wounds at days 0, 3, 7, 10, and 14 taken during 14 postoperative days. From the figure, it can be observed that PCL/Alg-AMP promoted better wound healing at all time points. Natural healing could not close the wound even after 14 days as expected. Additionally, wounds were not fully healed when PCL/Alg and Vicryl were used to close them. However, when assessing the performance of PCL/Alg-AMP, the difference could be observed from day 3 by better wound healing in PCL/Alg-AMP. The fibers were completely opaque which made it easier to identify them in wounds which did not change after AMP incorporation. Traces of wounds were observed at all samples except PCL/Alg-AMP after 14 days. To further confirm the results, Fig. 6(b) displays the dermoscopic images of the inside and outside of the wound of the three groups on days 7 and 14. Except for control, all the samples sealed their wounds as can be seen from the inside image of the wound bed after 7 days. However, there were traces of wound and tissue defects around the suture-treated area in PCL/Alg and Vicryl. After 14 days, PCL/Alg-AMP-treated wound almost completely closed resembling the morphology of normal skin due to the presence of AMP. Neoangiogenesis was also observed at the highest amount in PCL/Alg-AMP-treated wounds.

To examine the effect of PCL/Alg-AMP on wound healing and its various healing aspects, H&E, MT, and PAS staining were performed on excised wounds. During excision, the fibers had to be removed since they did not degrade within the study period which was further confirmed by a degradation study shown in Fig. S6 (ESI†). The excised wounds were characterized for wound healing parameters, such as re-epithelialization, epithelial thickness, scar index, inflammatory cell, and neoangiogenesis. The H&E images shown in Fig. 7(a) demonstrated that the re-epithelialization process began earlier in wounds treated by PCL/Alg-AMP compared to the other samples. This was characterized by measuring the epithelial gap of the wound after three days. The bar chart representing the epithelial gap is displayed in Fig. 7(c) which shows that the wound gap was significantly smaller compared to both PCL/Alg and Vicryl. The damaged epidermis healed significantly better in the case of PCL/Alg-AMP indicating faster re-epithelialization. Thus, the wound-healing process began much earlier in the case of PCL/Alg-AMP which was facilitated by the wound-closing ability of PCL/Alg-AMP and the immobilized peptide on its surface. The number of inflammatory cells was calculated for all three days and it is displayed in Fig. 7(d). During the wound-healing process, neutrophils infiltrate the wound area in the earlier stages and serve as the first line of defense against foreign organisms. Hence, at the H&E staining of day 3, the number of inflammatory cells was dominated by neutrophils. At the later stages of wound healing, neutrophils were replaced by macrophages and lymphocytes. The number of inflammatory cells in the incision sites treated by PCL/Alg-AMP was significantly lower than all other samples and this was observed throughout the entire postoperative period. Blood proteins facilitating neutrophil infiltration were visible for all groups except PCL/Alg-AMP. Therefore, the inflammatory reaction was significantly lower in PCL/Alg-AMP compared to other groups indicating faster regeneration which is shown in Fig. 7(d). The formation of skin appendages, such as sebaceous glands and hair follicles is also an indication of faster and better wound healing. After 14 days of wound healing in the case of PCL/Alg-AMP more and denser skin appendages were observed in the dermal layer of the wound site compared to PCL/Alg and Vicryl-treated wounds.

MT staining of the samples demonstrated in Fig. 7(b) revealed information about collagen deposition in the dermal region. From the MT-stained results of day 7, it can be observed that in the case of PCL/Alg-AMP treated wound, the area was relatively small and the normal basket weave patterns of collagen could be seen around the wound which were absent for PCL/Alg and Vicryl. The scar area could be confirmed by the MT images of day 14. Therefore, scar index values were calculated on day 14 for all samples. The scar areas of both the control and Vicryl-treated groups were significantly greater than PCL/Alg-AMP as shown in Fig. 7(e). This indicates that PCL/Alg-AMP reduced the scar area in the wound. Epithelial thickness was also measured on day 14 which was the thickest in the control group followed by PCL/Alg, Vicryl, and PCL/Alg-AMP indicating improved wound healing by PCL/Alg-AMP.

Basement membrane (BM) reconstruction is an important parameter since it separates the epidermis from the dermis layer underneath. The dermis layer contains several important proteins such as collagen IV and laminin and functions as a regulator for both epithelial and dermal cells. It controls the recovery of a wound by conducting signals between the epidermis and dermis layer, therefore, the integrity of the basement membrane is highly critical in wound remodeling. Fig. 8 represents the PAS staining of BM of all three groups. Both the early stage (day 7) and late stage (day 14) of wound healing need to be looked at to get insights into BM reconstruction. On day 7, the BM was not intact in all the wound sites lacking connection between the epidermis and dermis in the control group. On the other hand, in the case of PCL/Alg, PCL/Alg-AMP, and Vicryl, BMs integrities were better and the regenerated BMs connected the epidermis and dermis layer. However, residual hiatus between epithelium and the dermal layer was still observed in some places in the case of PCL/Alg and Vicryl which was not the case of PCL/Alg-AMP indicating partial reconstruction of BM. After 14 days, the BMs more or less regenerated in all the groups. In the case of PCL/Alg-AMP, the BM seemed compact and better integrated at the center of the wound. Additionally, the epidermis layer displayed some ridges indicating the formation of a papillary dermis. This was absent in the rest of the samples (flat epidermis). Furthermore, underneath the dermis, a
large number of follicles were observed in PCL/Alg-AMP which were absent in control and Vicryl. A smaller number of follicles were observed in the case of PCL/Alg. This indicates better wound healing efficiency promoted by PCL/Alg-AMP. The quality
of tissue regeneration also depends on revascularization and from PAS staining, the BM of blood vessels could be observed and counted. Evidence of neoangiogenesis was found just after 3 days only in the case of PCL/Alg-AMP-treated wound. On day 7, the number of blood vessels was counted for all three groups as shown in Fig. 8(b). PCL/Alg-AMP-treated wound underwent the highest amount of angiogenesis during this period due to enhanced healing. After 14 days, angiogenesis decreased which is normal in wound healing. In summary, the in vivo study revealed that PCL/Alg-AMP demonstrated superior wound

Fig. 7  (a) and (b) H&E and MT staining of wounds treated by PCL/Alg, PCL/Alg-AMP, Vicryl, and without treatment on days 3, 7, and 14. (c) The epithelial gap of all four wounds after three days. Triangles in the H&E staining indicate the epithelial gap of wounds. After three days, the gap of the wound treated by PCL/Alg-AMP significantly decreased compared to other samples. (d) The number of inflammatory cells per mm$^2$ was calculated on days 3, 7, and 14 for all the cases. PCL/Alg-AMP elicited the lowest immunogenic reaction due to the presence of AMP on the surface. (e) Scar index of all three wounds after 14 days where the scar area was the smallest in the case of PCL/Alg-AMP followed by Vicryl and PCL/Alg. (f) Epithelial thickness was calculated at day 14 for all the samples and the epidermis was the thinnest for sample treated with PCL/Alg-AMP. Significant differences are indicated by * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).
Fig. 8  (a) PAS staining of wounds treated by PCL/Alg, PCL/Alg-AMP, Vicryl, and without treatment on days 3, 7, and 14 days. (b) the number of blood vessels per mm² calculated after 7 days for all the samples. PCL/Alg-AMP facilitated a significantly higher number of blood vessel regeneration compared to other samples. Significant differences are indicated by * (p < 0.05), ** (p < 0.01).
healing activities compared to commercial suture Vicryl and it is less prone to bacterial colonization. Therefore, PCL/Alg-AMP can be a potential candidate to be used against surgical site infection caused by pathogenic bacteria.

4. Conclusion

AMP-immobilized composite fibers based on PCL and Alg were fabricated via a novel wet-spinning process in order to prevent SSI. In the wet spinning method, PCL dissolved in acetone was extruded into an aqueous solution of Alg where both solvents acted as coagulants. Since PCL is a hydrophobic polymer and Alg is a hydrophilic one, water functioned as a coagulant for PCL and acetone did the same for Alg. Therefore, in the bath, PCL and Alg solidified simultaneously. AMP (7–10 kDa) extracted from wheat was immobilized on the fiber by functionalizing it with catechol groups of polydopamine. Morphological analysis revealed that compared to pure PCL fiber, the surface of PCL/Alg was smoother due to slow diffusion between acetone and water. Incorporation of AMP smoothed the fiber surface without altering fiber diameter which corresponded to the USP 3-0 size of surgical suture. The fiber also did not show any strain during knotted configuration proving its suitability to tie knots. ATR-FTIR revealed hydrogen bonding between the carbonyl group of PCL and hydroxyl of Alg. Successful immobilization of AMP was also confirmed from ATR-FTIR. Nucleophilic AMP conjugated with the catechol functional group of dopamine via its amine group. After PCL hydrogen bonded with Alg, PCL chain movement was hindered which increased $T_m$ and reduced the crystallinity of PCL/Alg. Mechanical behavior was also significantly improved due to the chemical bonds between the polymers. Knot-pull tensile test, needle attachment, and knot security were tested according to USP guidelines for 3-0 size and the tensile strengths were above the minimum tensile strength required to be used as sutures. There was no incidence of knot slip during the experiments which proves the security of tied knots. AMP immobilization significantly improved the hemocompatibility of PCL/Alg-AMP compared to Vicryl. Antibacterial test of PCL/Alg-AMP was carried out against pathogenic Proteus spp. collected from a wound site that was resistant to several antibiotics. The study revealed that the microfiber could hinder bacterial growth for at least 60 hours whereas antibiotic ceftazidime failed. The extracted AMP was anionic, could inhibit Proteus spp. growth, and repel its adhesion on the PCL/Alg-AMP surface. Enhanced wound healing facilitated by PCL/Alg-AMP was observed for all days during a 14-day in vivo study period. PCL/Alg-AMP promoted earlier regeneration of wound gap and formation of abundant new blood vessels and follicular structures compared to Vicryl. Due to enhanced healing by PCL/Alg-AMP, scar area, and epithelial thickness were also significantly reduced and the regrown basement membrane possessed continuity with better integrity. Additionally, ridges similar to papillary dermis were observed in PCL/Alg-AMP whereas the Vicryl-treated wound was mostly flat. These findings lay the foundations for an engineered fiber composed of PCL and Alg to inhibit the growth of resistant bacteria, promote enhanced wound healing, and prevent SSI in the process.

Author contributions

Taufiq Hasan Aneem: conceptualization, methodology, investigation, formal analysis, data curation, visualization, writing – original draft preparation. Mridul Sarker: investigation. Siew Yee Wong: investigation. Sierim Lim: investigation, resources. Xu Li: investigation, resources. Asif Rashed: investigation, resources. Saumitra Chakravarty: investigation, resources. M Tarik Arafat: conceptualization, writing – reviewing and editing, supervision, funding acquisition, project administration.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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