Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Fabrication of Antimicrobial Polymeric Films by Compression Molding of Peptide Assemblies and Polyethylene

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Materials and Methods

Materials

DOPA-Phe(4F)-Phe(4F)-OMe was obtained from GL Biochem (Shanghai) Ltd. with a purity>95%. LDPE, Ipethene 320 produced by Carmel Olefins Ltd with MFR of 2 gr/min, were obtained from Carmel Olefins Ltd. (Israel). Ethanol 200 proof was purchased from Gadot. *Escherichia coli* strain (ATCC 11303), *Escherichia coli* strain (ATCC 25922), and *Escherichia coli* bacteriophage T4 (ATCC 113030-B4) bacteria were purchased from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). Difco LB broth and Difco Nutrient Agar were purchased from Becton Dickinson (New Jersey, USA). lecithin, tween 80, tryptic soy broth (TSB), sucrose, sodium chloride, potassium phosphate dibasic, sodium nitrate, potassium chloride, magnesium sulfate heptahydrate, ferrous sulfate, chloramphenicol, and propidium Iodide were purchased from Merck (New Jersey, USA). Agarose was obtained from LifeGene (Israel). Triple distilled water (TDW) water was obtained by filtering distilled water through a Milli-Q water system (Millipore). *Pythium* sp. was initially isolated from infected potato tubers and kept on agar slants. *Fusarium oxysporum* was initially isolated from infected tomato plants. *Sclerotium rolfsii* isolated from infected peanut plants. SYTO 9 was purchased from Thermo Fisher Scientific.

Preparation of the peptide assemblies as a powder

The peptide was dissolved in ethanol (100 mg/ml) and then diluted in TDW (10 mg/ml). This solution was shaken at 150 rpm at room temperature for 0.5 h and frozen in liquid nitrogen. Afterward, the frozen sample was lyophilized in freeze-drier (Lyophilizer Heto Drywinner3 -Heto) under vacuum at -40 °C and 0.001 atmospheres until dry.

Preparation of the polymer films

A powder mixture (50 gr) of LDPE with powder of either the peptide assemblies (4 wt%) or peptide (5 and 10 wt%) was melted and compounded at 160 °C using Rheomix polylab system (Rheomix 600p, Thermo Haake, Germany) with a screw speed of 50 rpm for 10 minutes. Subsequently, films with a thickness of 100 μm were prepared by compression molding (P200E, Dr. Collin, Germany) at 160 °C, for about 10 seconds.

High-resolution scanning electron microscopy (HR-SEM)

SEM images were taken using a high-resolution scanning electron microscope Magellan TM400L (ThermoFisher, former FEI) at an acceleration voltage of 2 kV and a working distance of 4 mm. A sample of peptide solution (30 μL) was drop-casted on the glass surface and allowed to dry at room temperature. The polymer film was placed directly on a stub. Then, the surface was coated with

Iridium by sputtering coating (Quorum 150 TS). The SEM images were analyzed using ImageJ software.

Laser confocal scanning microscope (CLSM)

CLSM images were taken using FV-1200 confocal microscope (Olympus, Japan) and a 60X/1.42 oil immersion objective. Green fluorescence was observed with a 488 nm excitation filter and 500–540 nm emission filter.

Thermogravimetric analysis (TGA)

TGA measurements were performed using the TGA Q5000 system (TA instruments, USA) at a heating rate of 10 °C/min under a nitrogen atmosphere, starting from room temperature to 800 °C. The results were analyzed using Universal Analysis 200 version 4.5A build 4.5.0.5 software.

Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectra were recorded using a Nicolet 6700 FT-IR spectrometer with a deuterated triglycine sulfate (DTGS) detector (Thermo Fisher Scientific, MA, USA). Polymer films were placed on a CaF_2 plate. The transmittance measurements were taken in the range of wave number 400 to 4000 cm^{-1} using a 4 cm^{-1} resolution and averaged after 2000 scans.

Fourier transform infrared spectroscopy – attenuated total reflectance (ATR-FTIR)

ATR-FTIR analysis was recorded using FT-IR (Thermo scientific, Model Nicolet 6700) with GeATR arrangement (Harrick Scientific's VariGATR). Spectra were collected for the samples containing the peptide powder with an applied force of 290 N, at 4 cm^{-1} resolution with 2000 scans averaged signal and an incident angle of 65°.

X-ray photoelectron spectroscopy (XPS)

XPS measurements were performed using Kratos AXIS Supra spectrometer (Kratos Analytical Ltd., Manchester, U.K.) with Al Kα monochromatic radiation X-ray source (1486.6 eV). The XPS spectra were acquired with a takeoff angle of 90° (normal to the analyzer); the vacuum condition in the chamber was 2×10−9 Torr. The survey spectra were measured with pass energy 160 eV and 1 eV step size and high-resolution XPS spectra with a pass energy of 20 and 0.1 eV step size. The binding energies were calibrated using C 1s peak energy as 285.0 eV. High-resolution XPS spectra were collected for C 1s, O1s, F 1s, and N 1s peaks with pass energy 20 eV and 0.1 eV step size. Data were collected and analyzed by using the ESCApe processing program (Kratos Analytical Ltd.) and Casa XPS (Casa Software Ltd.).

Contact angle measurements

The water contact angle was measured using a Theta Lite Optical Tensiometer (Attension Theta, Finland). The volume of each drop was 0.5 µL. The measurements were done at 4 locations on 3 independent surfaces and averaged.

Differential scanning calorimetry (DSC)

DSC characterizations were done using DSC 8000 (Perkin Elmer, USA). Samples of polymer films (4.3-7 mg) were placed in a platinum sample pan. Then, the samples were heated at a rate of 10 °C/min under a nitrogen atmosphere and the thermograms were recorded and analyzed using Perkin Elmer Pyris™. From the resulted DSC thermograms, melting temperature (Tm) and crystallization temperature (Tc) were extracted. A degree of crystallinity (X_c) was calculated using the following equation 1.[1]

$$X_C = \frac{\Delta H_m}{f_P \Delta H_m^\circ} \quad (1)$$

Where ΔH_m (J/g) is the latent heat of fusion of the sample measured at the Tm, f_P is the LDPE weight fraction in the sample, and ΔH_m° is the theoretical latent heat of fusion for 100% crystalline LDPE, equal to 293 J/g.[1]

Optical properties

Haze, clarity, and transmission of 100 µm polymer films were measured using Haze-gard plus (BYK Gardner, USA). The average of three different measurements was taken.

Antiviral activity assay

The antiviral activity of the polymer films was done by using bacteriophage T4 as the virus for antiviral activity measurements. The preparation of the bacteriophage suspension was based on the previously described method.[2]

A starter of *E. coli* (ATCC 11303) was prepared by adding a single colony to 10 mL Lysogeny Broth (LB) nutrient broth (2% LB broth) and incubated overnight at 37 °C at 150 rpm.

The stock of the virus suspension was 10-fold serially diluted to 10^6 plaque-forming units (PFU/mL) in LB phage (0.8% LB broth, 0.5% NaCl). Then, a 16 µL drop of 10^6 PFU/mL viral suspension was placed on each polymer surface (1 cm× 1 cm). The surfaces were coated with parafilm (1 cm× 1 cm) to ensure a uniform spread of the viral suspension and prevent dehydration of the viral suspension on

the surface. Afterward, the surfaces were incubated at room temperature for 24 h. After 24h, the phages were harvested from the surface by shaking the surfaces for 15 min at 150 rpm in 2 mL of soybean casein digest broth with lecithin and polysorbate (SCDLP, 3% TSB, 0.1% lecithin, and 0.7% tween 80). The bacteriophage T4 in SCDLP was diluted ten times to a dilution of 10^{-1}. A mixture of bacteriophage T4 in SCDLP (20 µL), *E. coli* starter (25 µL), and slightly warm agarose (0.6%, 1 mL) were added into a glass test tube. The mixture was gently mixed and spread on solidified nutrient agar in 6 well plates. The plates were incubated at room temperature for 24 h to form the phage plaques. Finally, the phage plaques were counted, and the number of PFU/mL was calculated based on the number of plaques, dilution factor, and the volume of the phage solution.

The antiviral activity assay was repeated three times in three independent experiments (a total of nine repeats for each surface).

Antibacterial activity assay

The antibacterial activity of the polymer films was assessed using *E-coli* (ATCC 25922). A starter of *E.coli* was prepared by adding a single colony to 20 mL of TSB solution and incubated overnight at 37 °C at 150 rpm. Then, the bacterial suspension was centrifuged at 4,000 rpm for 3 min and washed three times using PBS buffer (10 mM, pH 7.0, 154 mM NaCl). The bacterial suspension was diluted to 106 colony forming units per mL (CFU/mL) by measuring the optical density (OD) at 600 nm. A 15 µL drop of *E. coli* suspension, at a concentration of 10^6 CFU/mL, was placed on each film (1 cm× 1 cm). Then, the surfaces were coated with parafilm (1 cm× 1 cm) to ensure the uniform spread of the bacterial suspension and prevent dehydration of the suspension on the surface. The surfaces were incubated at 37 °C for 24 h. Afterward, they were sonicated in 3 mL PBS buffer for 1 min, vortexed, and decimally diluted to 10^{-3}. The bacterial suspension at different dilutions was seeded on solidified nutrient agar and incubated at 37 °C for 24h. Finally, the colonies were counted, and the number of CFU per mL was calculated based on the number of colonies, dilution factor, and the volume of the bacteria solution.

The antibacterial activity assay was repeated three times in three independent experiments (a total of nine repeats for each surface).

Antifungal activity assay

The antifungal activity of the polymer films was done by using three fungi: *Pythium* sp., *Fusarium oxysporum*, and *Sclerotium rolfsii*. The polymer films with a diameter of 6 cm were perforated (10

pinholes/cm^2) using an 8 cm metal plate in which 25 blades were installed. The perforated plastic film was laid on Czapek- Dox agar (55% sucrose, 2.0% K_2HPO_4, 3.7% $NaNO_3$, 0.90% KCl, 0.92% $MgSO_4 \cdot 7H_2O$, 0.020% $FeSO_4$, 0.46% Chloramphenicol, 37% nutrient agar) in a Petri dish.[3] The fungi were grown for five days on Czapek medium, thereafter discs were cut around the edge of the colony to ensure equal fungal age when transferred to the tested polymer film. Then, the mycelium disc (5 mm) of either *Pythium* sp. *Fusarium oxysporum* or *Sclerotium rolfsii* was placed in the center of the polymer film and incubated at 25 °C. In this way, the fungus was on direct contact with the polymer films and the nutrient agar. After 5 days, the diameter of each colony was measured, and the colony area was calculated. The inhibition ratio was calculated based on the surface area of the fungi growth on neat LDPE with no additional additives.

The antifungal activity assay was repeated five times in two independent experiments (a total of ten repeats for each surface).

Live/dead assay of bacteria on the polymer film

The bacteria cell viability of *E-coli* (ATCC 25922) was assessed using a live/dead assay. *E-coli* suspension, at a concentration of 10^6 CFU/mL was placed on the polymer films at 37 °C for 24h, as described above. Then, the polymer films were immersed in PBS buffer (1 mL) three times to wash the nonadherent bacteria. The stain mixture of propidium Iodide (0.02 mM) and SYTO 9 (0.02 mM) was prepared in PBS. A 100 µL drop of the stain mixture was placed on the polymer films and was left for 15 min in the dark at room temperature. Then, the films were washed with PBS to remove the excess stain on the surface. Afterward, the polymer films were placed on a glass-bottom dish and the images of stained bacteria cells were observed by CLSM with excitation at 488 nm and 561 nm.

Release of the peptide assemblies from the polymer films

The leakage of blended peptide assemblies from the polymer films (1 cm× 1 cm) was tested under two conditions: immersing the polymer films in 95 % ethanol solution (0.5 mL) and immersing scratched polymer films in TDW water (0.5 mL). In the later condition, the films were scratched four times using a diamond knife. Each film was then immersed in the solvent for different immersion times (immediate exposure, 1h, and 24h). Then, the solvent was collected, and the UV-absorbance of the solvent was measured (Shimadzu, UV-1650PC, Kyoto, Japan). For each condition and time exposure, three repeats were done and the absorbance was measured. In addition, polymer films after the release experiment were collected and analyzed by SEM as previously described.

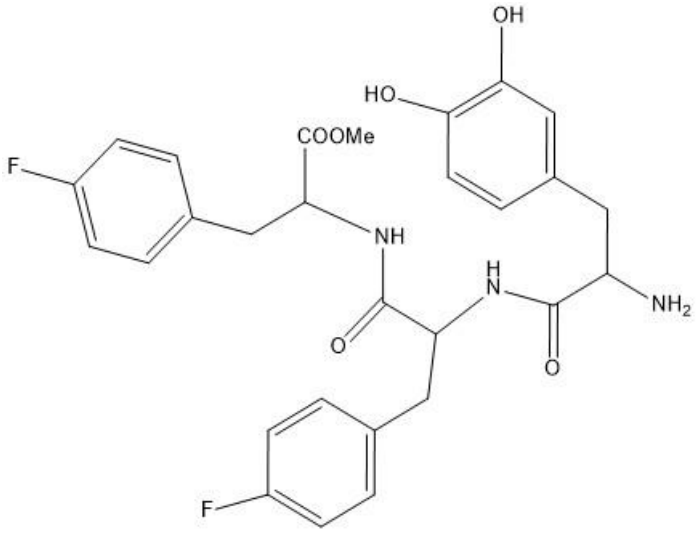

Fig. S1 The chemical structure of the peptide Dopa-Phe(4F)-Phe(4F)-OMe with a molar mass of Mw=542 gr/mol.

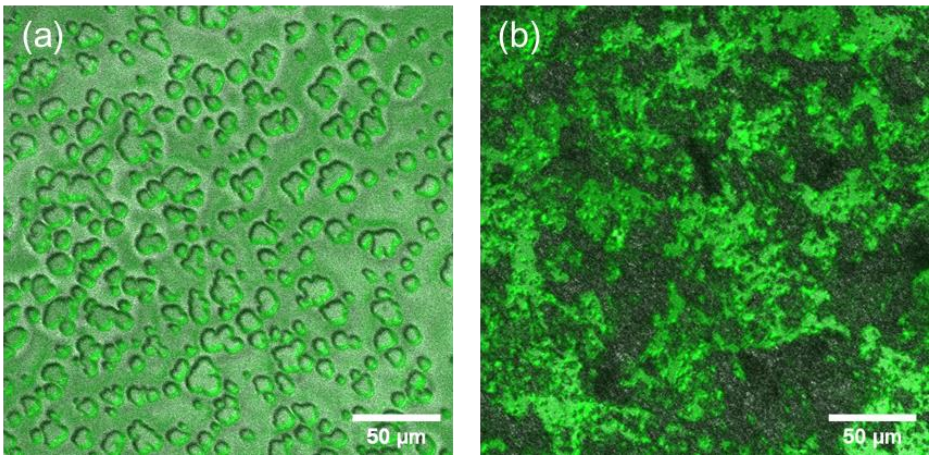

Fig. S2 CLSM images of the peptide assemblies. (a) before and (b) after lyophilization.

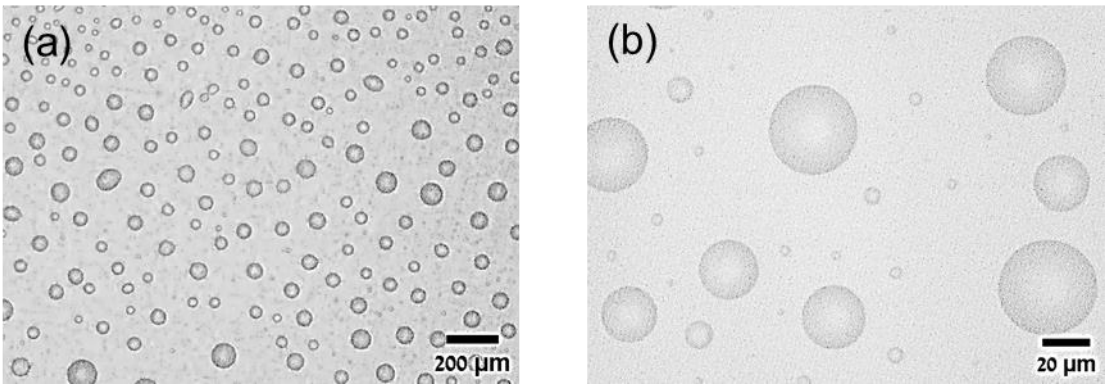

Fig. S3 Optical microscope images of peptide assemblies at different magnification.

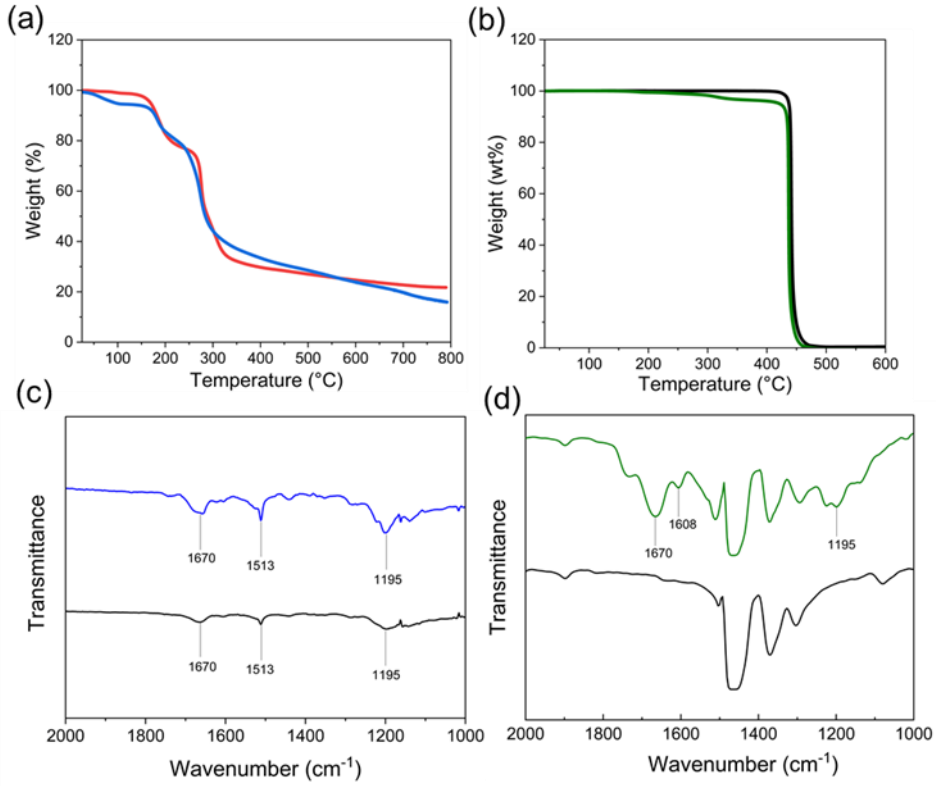

Fig. S4 Physical characterization of the polymer films. (a) Thermogravimetric analysis of the peptide (blue) and peptide assemblies (red). (b) neat LDPE (black) and LDPE/peptide (green). (c) ATR-FTIR spectra of peptide assemblies before (blue) and after (black) freeze-drying and heating at 160 °C for 11 min. (d) FT-IR spectra of neat LDPE (black) and LDPE/peptide (green).

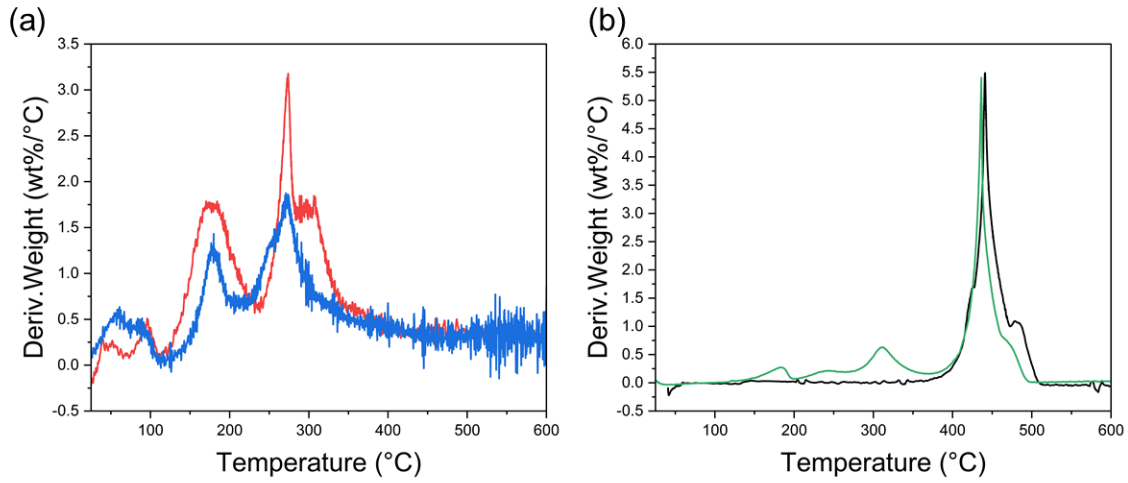

Fig. S5 Differential thermogravimetric analysis of the (a) peptide assemblies (red) and non-assembled peptide (blue). (b) neat LDPE (black) and LDPE/peptide (green).

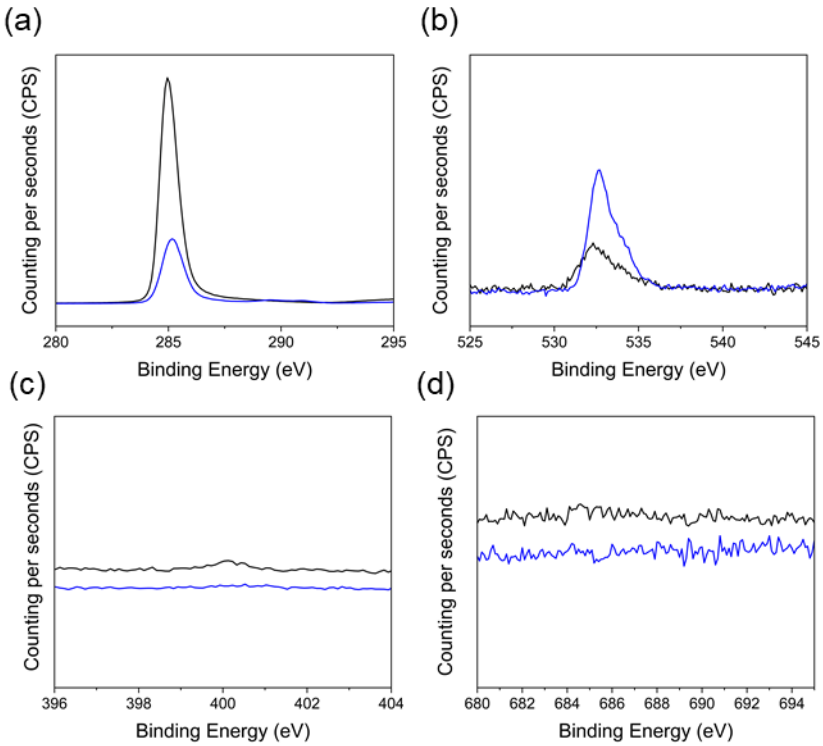

Fig. S6 XPS analysis of (a) carbon, (b) oxygen, (c) fluorine, and (d) nitrogen of neat LDPE (black) and LDPE/peptide (blue).

Table S1 Surface elemental composition and contact angle of LDPE and LDPE/peptide.

Surface	mass concentration [%]				contact angle [degree]
	C_{1s}	O_{1s}	N_{1s}	F_{1s}	
LDPE	98.9±0.3	0.9±0.3	0.2±0.1	0	100±3
LDPE/peptide	94.7±0.3	5.2±0.4	0.13±0.08	0	101±2

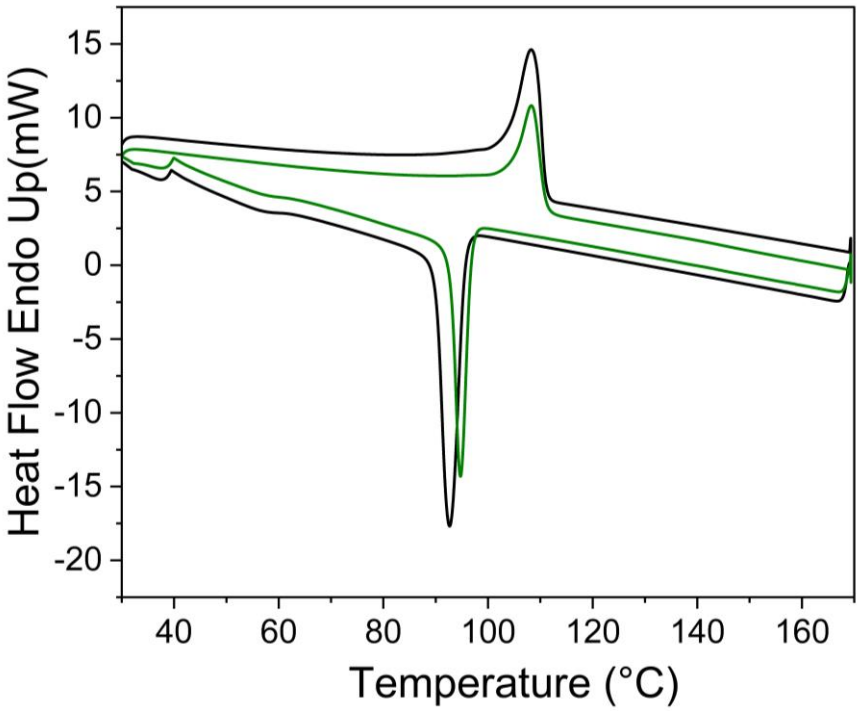

Fig. S7 DSC thermograms of the neat LDPE (black) and LDPE/peptide (green).

Table S2 DSC data for the LDPE and LDPE/peptide films.

Surface	Tm (°C)	Tc (°C)	ΔHm (J/g.)	Xc (%)
LDPE	108.3	92.6	90.6	31
LDPE/peptide	108.3	94.7	94.3	33

Table S3 Optical properties of LDPE and LDPE/peptide films.

Surface	Transmission (%)	Clarity (%)	Haze (%)
LDPE	91±0	95±0	15±3
LDPE/peptide	91±0	90±1	37±4

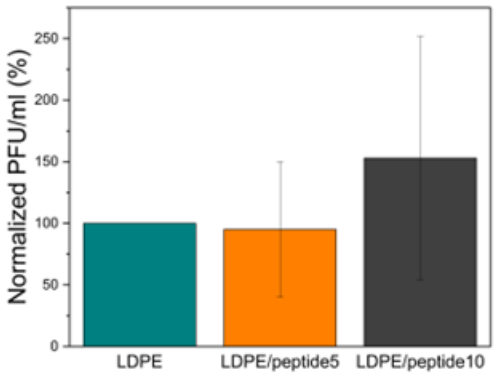

Fig. S8 Antiviral assay of the polymer films: neat LDPE, LDPE films compounded with the non-assembled peptide (5 wt%, LDPE/peptide5), and LDPE films compounded with the non-assembled peptide (10 wt%, LDPE/peptide10). Standard deviation (SD) is based on three independent experiments with triplicates (n = 9).

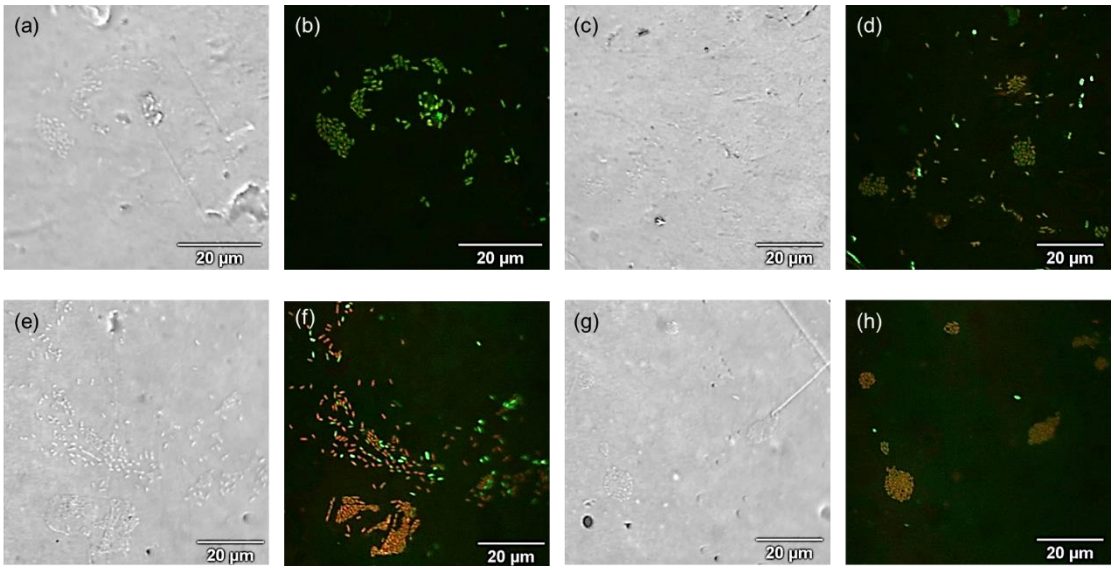

Fig. S9 CLSM images of live/dead bacteria viability assay of *E. coli.* (b and d) LDPE and (f and h) LDPE/peptide film at two different locations. Live bacteria are in green and dead bacteria in red. Bright-field images of (a and c) LDPE and (e and g) LDPE/peptide film at two different locations.

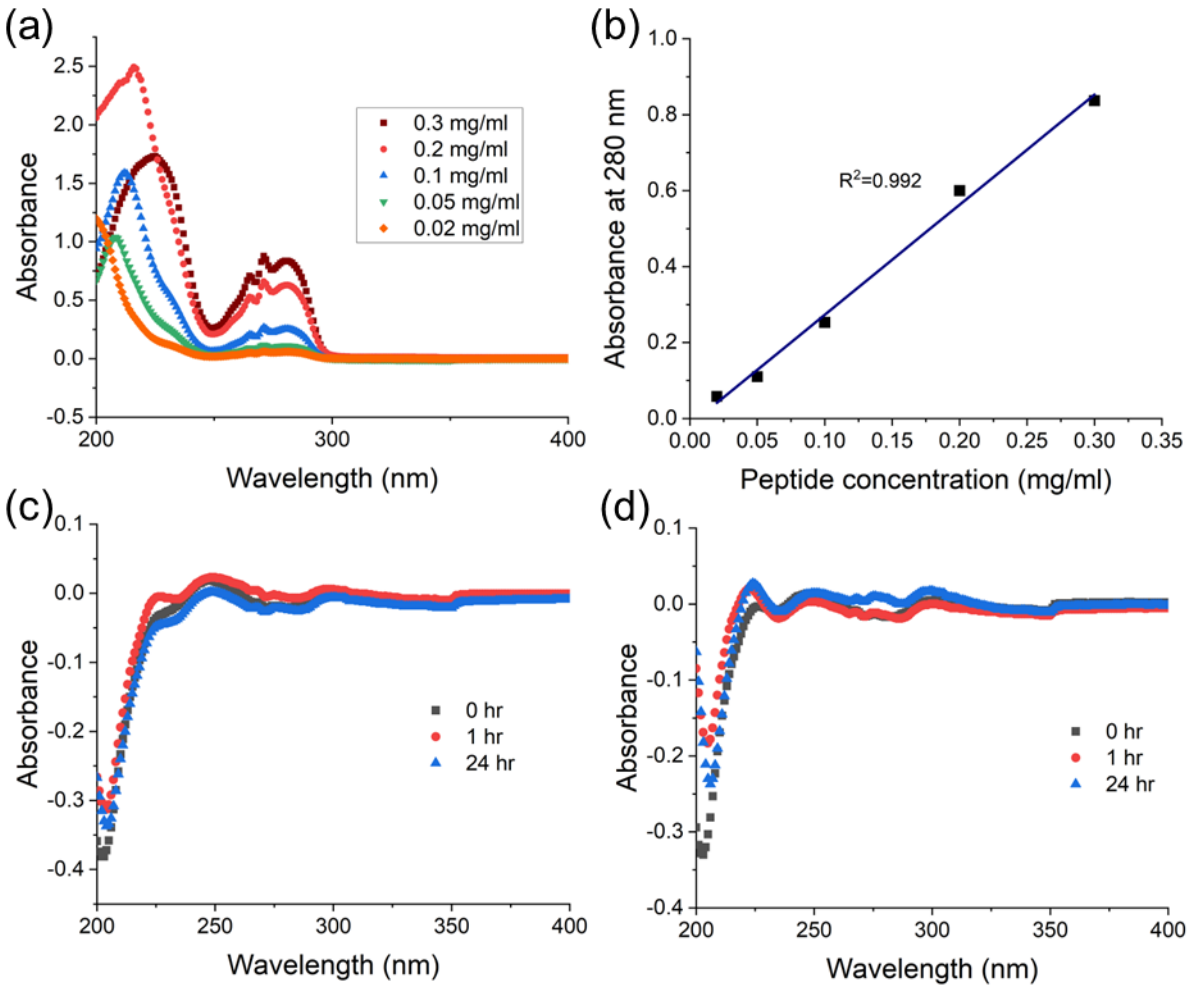

Fig. S10 (a) Absorption spectra of peptide solutions with different concentrations in water measured by UV Spectrophotometer, (b) Calibration curve of peptide in the water (absorption values at λ_{max}=280 nm), and absorption spectra of (c) ethanol, and (d) TDW from the leakage experiment of the peptide assemblies from the polymer films.

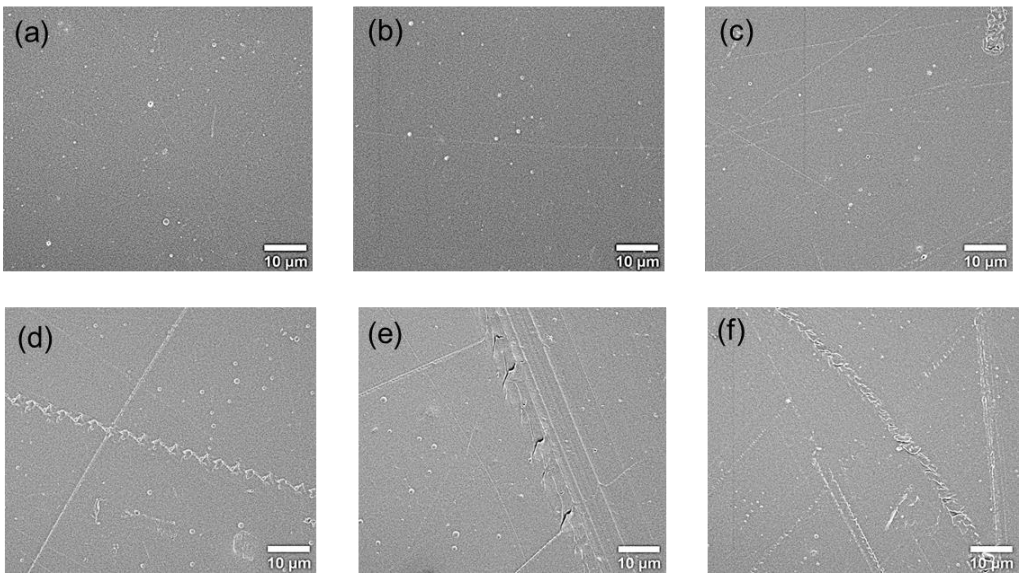

Fig. S11 HR-SEM images of the LDPE/peptide films after exposure to different conditions. (a) immediate exposure to ethanol (b) 1h exposure to ethanol, (c) 24h exposure to ethanol, (d) immediate exposure to TDW after scratching, (e) 1h exposure to TDW after scratching, and (f) 1h exposure to TDW after scratching.

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