

An antimicrobial plastic

Technical Field

The present invention relates to an antimicrobial plastic.

5 Background

Microbial infection remains one of the most serious complications. The contamination by microorganisms is of concern in numerous applications including healthcare products, medical devices, food packaging, water purification systems and manufacturing industries.

10 Polystyrene (PS), for example, is a commodity plastic that is widely used in the packaging and other fields. Much has been done to develop self-disinfection polystyrene to avoid using large amount of disinfectants and for a sustainable ecosystem. For example, PS has been co-formulated with other materials such as chitosan and inorganic materials such as titanium dioxide nanoparticles, zinc oxide
15 nanoparticles, silver compounds, carbon nanotubes and graphene oxides, just to name a few. However, many of these may exhibit leaching of toxic chemicals. Considering the immense scale of PS applications in packaging, especially the food packaging field, inherently antimicrobial plastic that has a self-disinfection surface without the drawback of leaching toxic chemicals is highly desired.

20 There is therefore a need for an improved plastic material.

Summary of the invention

The present invention seeks to address these problems, and/or provides an improved antimicrobial plastic.

According to a first aspect, there is provided an antimicrobial plastic comprising: a
25 thermoplastic polymer and a polycationic polymer, wherein the polycationic polymer forms semi-free surface nanostructures with the thermoplastic polymer, the semi-free surface nanostructures capable of electrostatically bonding with bacterial components.

According to a particular aspect, the polycationic polymer may be covalently linked within a main chain of the thermoplastic polymer via covalent bonds. According to a
30 further aspect, the plastic may have a surface roughness of ≥ 50 nm.

The polycationic polymer may be any suitable polycationic polymer. The polycationic polymer may be hydrophilic. In particular, the polycationic polymer may be a functionalised poly-imidazolium polymer. Even more in particular, the functionalised poly-imidazolium polymer may comprise an alkenyl terminal group at each of its ends.

5 The alkenyl terminal group may be any suitable group. For example, the alkenyl terminal group may comprise a vinyl group. According to a particular aspect, the functionalised poly-imidazolium polymer may comprise PIM-Vinyl.

The plastic may comprise a suitable amount of polycationic polymer. For example, the plastic may comprise 0.1-10 wt % polycationic polymer based on the total weight of the plastic.

The thermoplastic polymer may be any suitable thermoplastic polymer. For example, the thermoplastic polymer may be polystyrene, polyacrylate, copolymers or mixtures thereof.

15 According to a second aspect, there is provided a method of preparing the plastic according to the first aspect, the method comprising:

- mixing a polycationic polymer and monomer units of the thermoplastic polymer with a solvent to form a mixture;
- adding an initiator;
- heating the mixture; and
- 20 - precipitating the plastic.

The mixture may comprise a suitable amount of polycationic polymer. For example, the mixture may comprise 0.1-10 wt % polycationic polymer based on the total weight of the mixture.

25 According to a particular aspect, the heating may result in the co-polymerisation of the polycationic polymer with the monomer units of the thermoplastic polymer. In particular, the co-polymerisation may result in the polycationic polymer becoming covalently linked within a main chain of the thermoplastic polymer via covalent bonds.

According to a particular aspect, the precipitating the plastic may comprise surface phase separation of the polycationic polymer on the plastic, thereby forming semi-free surface nanostructures on the plastic.

Brief Description of the Drawings

5 In order that the invention may be fully understood and readily put into practical effect there shall now be described by way of non-limitative example only exemplary embodiments, the description being with reference to the accompanying illustrative drawings. In the drawings:

10 Figure 1 shows a schematic representation of phase separation of thermoplastic polymer surface modified with antimicrobial polycationic polymer to form nanostructures;

Figure 2 shows the synthetic scheme for the preparation of a plastic according to one embodiment, i.e. synthesis of modified polyimidazolium material (PIM-vinyl) from polyimidazolium (PIM-45) and subsequent polymerisation with styrene monomer to
15 yield functionalised polystyrene (PS*) according to one embodiment;

Figure 3 shows average contact angle distribution of different plastic film samples;

Figures 4(a) and (b) show the antimicrobial activities of PS*-1 and PS*-3 against *C. albicans* at 1/500 dilution YMB media at 1 wt. % PIM-Vinyl and 3 wt. % PIM-Vinyl, respectively. Star indicates no colony observed. The data are expressed as mean $\pm$
20 S.D. of triplicates;

Figures 5(a) and (b) show the leaching properties and antimicrobial activity, respectively of PS controls and PS*-1. The data are expressed as mean $\pm$ S.D. of triplicates; and

Figure 6 shows time-kill kinetics of PS*-1 against *E. coli* at 1/500 dilution TSB media.
25 Star indicates no colony observed. The data are expressed as mean $\pm$ S.D. of triplicates.

Detailed Description

As explained above, there is a need for an improved plastic material.

In general terms, the present invention provides a plastic material with inherent antimicrobial properties by incorporating antimicrobial components into polymer chains during polymerization processes. In particular, active functional groups may be added at both ends of the active antimicrobial chain, where the functional group can subsequently co-polymerize with plastic monomers, and be integrated within the polymer chain via strong covalent bonds. In this way, leaching of the antimicrobial component is prevented, while at the same time, the antimicrobial component is able to exhibit the antimicrobial properties effectively, despite being integrated within the polymer chain.

According to a first aspect, there is provided an antimicrobial plastic comprising: a thermoplastic polymer and a polycationic polymer, wherein the polycationic polymer forms semi-free surface nanostructures with the thermoplastic polymer, the semi-free surface nanostructures capable of electrostatically bonding with bacterial components.

The thermoplastic polymer may be any suitable thermoplastic polymer. In particular, the thermoplastic polymer may be a hydrophobic thermoplastic polymer. For example, the thermoplastic polymer may be polystyrene, polyacrylates, copolymers, or mixtures and combinations thereof. According to a particular aspect, the thermoplastic polymer may be polystyrene.

The polycationic polymer may be any suitable polycationic polymer. For the purposes of the present invention, a polycationic polymer may comprise a main-chain polycationic polymer.

According to a particular aspect, the polycationic polymer may be hydrophilic. For example, the polycationic polymer may be a polyquaternary ammonium polymer. In particular, the polycationic polymer may be a functionalised poly-imidazolium polymer. Even more in particular, the functionalised poly-imidazolium polymer may comprise an alkenyl terminal group at each of its ends. The alkenyl terminal group may be any suitable group. For example, the alkenyl terminal group may comprise a vinyl group. According to a particular aspect, the functionalised poly-imidazolium polymer may comprise PIM-vinyl.

According to a particular aspect, the polycationic polymer may be covalently linked within a main chain of the thermoplastic polymer via covalent bonds. In particular, the

functionalised polycationic polymer with the functional groups at its ends enable the polycationic polymer to co-polymerize with the thermoplastic monomer, thereby being integrated within the polymer chain of the antimicrobial plastic via strong covalent bonds instead of simply being embedded within the plastic. In this way, the polycationic polymer may not leach out from the plastic.

The antimicrobial plastic may comprise semi-free surface nanostructures formed from phase separation of the polycationic polymer on the thermoplastic polymer surface. Accordingly, the antimicrobial plastic may have a surface roughness. The plastic may have a surface roughness of ≥ 50 nm. In particular, the surface roughness may be 55-1000 nm, 60-900 nm, 65-800 nm, 70-700 nm, 90-600 nm, 100-550 nm, 200-500 nm, 300-400 nm. Even more in particular, the surface roughness may be 200-600 nm.

In particular, the surface nanostructures allow the polycationic polymer which are on the tip of the surface nanostructures to have flexibility and freedom to interact with surrounding microbes and bacterial components. The surface nanostructures on the antimicrobial plastic, which may be cationically charged, may attract bacteria, which may be anionically charged. In this way, the bacteria may be captured via electrostatic bonding with the polycationic polymer on the surface nanostructures. A schematic representation of the antimicrobial plastic capturing a bacteria component is shown in Figure 1.

The surface nanostructures may have a suitable structure. For example, the surface nanostructures may have a structure comprising hairs, a honeycomb-like structure, or a combination thereof. According to a particular aspect, the surface nanostructures have a structure comprising hairs. The height of the hairs may be any suitable height. For example, the height of the hair may be about 500-600 nm. According to another particular aspect, the surface nanostructure may have a honeycomb-like structure. The honeycomb-like surface structure may comprise a drop from peak to valley of about 700-900 nm, particularly about 800 nm.

The plastic may comprise a suitable amount of polycationic polymer. For example, the plastic may comprise 0.1-10 wt % polycationic polymer based on the total weight of the plastic. In particular, the plastic may comprise 0.2-8.0 wt %, 0.3-5.0 wt %, 0.5-4.0 wt %, 0.7-3.0 wt %, 0.8-2.0 wt %, 1.0-1.5 wt % polycationic polymer based on the total weight

of the plastic. Even more in particular, the plastic may comprise about 0.5-1.0 wt % polycationic polymer based on the total weight of the plastic.

According to a second aspect, there is provided a method of preparing the plastic according to the first aspect, the method comprising:

- 5 - mixing a polycationic polymer and monomer units of the thermoplastic polymer with a solvent to form a mixture;
- adding an initiator;
- heating the mixture; and
- precipitating the plastic.

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The polycationic polymer and the thermoplastic polymer may be any suitable polycationic polymer and thermoplastic polymer. In particular, the polycationic polymer and the thermoplastic polymer may be as described above in relation to the first aspect.

- 15 The mixing may comprise mixing a suitable amount of polycationic polymer with the monomer units to form the mixture. For example, the mixture may comprise 0.1-10 wt % polycationic polymer based on the total weight of the mixture. In particular, the mixture may comprise 0.2-8.0 wt %, 0.3-5.0 wt %, 0.5-4.0 wt %, 0.7-3.0 wt %, 0.8-2.0 wt %, 1.0-1.5 wt % polycationic polymer based on the total weight of the mixture. Even
- 20 more in particular, the plastic may comprise about 0.5-2.0 wt % polycationic polymer based on the total weight of the mixture.

The solvent may be any suitable solvent. According to a particular aspect, the solvent may be a polar solvent. For example, the solvent may be, but not limited to, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), or a mixture thereof.

- 25 The adding may comprise adding any suitable initiator. For example, the initiator may be any suitable initiator to initiate the polymerisation of the polycationic polymer and the monomer units of the thermoplastic polymer. In particular, the initiator may comprise, but is not limited to, dibenzoyl peroxide, azobisisobutyronitrile (AIBN), or a mixture thereof.

According to a particular aspect, the heating may result in the co-polymerisation of the polycationic polymer with the monomer units of the thermoplastic polymer. In particular, the co-polymerisation may result in the polycationic polymer becoming covalently linked within a main chain of the thermoplastic polymer via covalent bonds.

- 5 The heating may be carried out at any suitable temperature. For example, the heating may be at a temperature of 50-150°C. In particular, the temperature may be 60-140°C, 65-130°C, 70-120°C, 75-100°C, 80-90°C. Even more in particular, the temperature may be about 60-120°C.

- 10 The heating may be carried out for a suitable period of time. For example, the heating may be carried out for 10-20 hours. In particular, the heating may be for 12-18 hours, 13-17 hours, 14-16 hours. Even more in particular, the heating may be for 16-18 hours.

The heating may be carried out under suitable conditions. According to a particular aspect, the heating may be carried out under nitrogen or argon atmosphere.

- 15 According to a particular aspect, the precipitating the plastic may comprise surface phase separation of the polycationic polymer on the plastic, thereby forming semi-free surface nanostructures on the plastic.

- 20 The surface nanostructures formed may have a suitable structure. For example, the surface nanostructures formed may have a structure comprising hairs, a honeycomb-like structure, or a combination thereof. According to a particular aspect, the surface nanostructures formed have a structure comprising hairs. The height of the hairs may be any suitable height. For example, the height of the hair may be about 500-600 nm. According to another particular aspect, the surface nanostructure formed may have a honeycomb-like structure. The honeycomb-like surface structure may comprise a drop from peak to valley of about 700-900 nm, particularly about 800 nm.

- 25 The antimicrobial plastic formed comprising the semi-free surface nanostructures may have a surface roughness. According to a particular aspect, the plastic formed may have a surface roughness of ≥ 50 nm. In particular, the surface roughness may be 55-1000 nm, 60-900 nm, 65-800 nm, 70-700 nm, 90-600 nm, 100-550 nm, 200-500 nm, 300-400 nm. Even more in particular, the surface roughness may be 200-600 nm.

The method is a simple method which may be easily scaled up. Further, the method is effective in ensuring that the polycationic polymer is covalently incorporated into the polymer chain of the thermoplastic polymer while still being able to retain its antimicrobial activities.

- 5 Having now generally described the invention, the same will be more readily understood through reference to the following example which is provided by way of illustration, and is not intended to be limiting.

Example

Synthesis and characterisation of polystyrene (PS) with modified polyimidazoliums

- 10 A polyimidazolium (PIM) compound (PIM-45, Mn ~2500 kDa) was selected as an active component to be incorporated into a polystyrene chain. PIM-45 is a main-chain antimicrobial polymer, with high selectivity and broad spectrum of activity. It has been described in WO 2014/025314. It was modified with styrenyl functional groups at both ends for further copolymerization. The synthetic approach is illustrated in Figure 2,
15 where the resultant polymer PIM-Vinyl was subsequently polymerized with styrene, and integrated within the polymer chain.

- In particular, to a solution of PIM-45 (250 mg, 0.122 mmol) in 1.25 mL ethanol, 4-vinylbenzyl chloride (3.0 eq) was added. The mixture was stirred and heated at 65°C for 16 hours in a sealed vial. After the reaction, the compound was purified by repeated
20 precipitation with tetrahydrofuran (THF) and dried under reduced pressure at 90°C. An off-white powder of PIM-45 with vinylbenzyl terminal groups (PIM-vinyl) was obtained.

- A series of functional polystyrene film samples (PS*-X, X = wt% of PIM-Vinyl in polymer) were synthesized by copolymerization of styrene and PIM-Vinyl. PS-PIM 45 was also synthesized by simply mixing styrene with PIM-45 (with no styrenyl end
25 group) as a control. The free PIM-45 can be completely washed away during the sample preparation process. The average molecular weight of PS*-1 was about 49.9 kDa which is slightly lower than PS control of 52.2 kDa.

- In particular, co-polymerization of PIM-Vinyl with styrene monomer was carried out in dimethyl sulfoxide using dibenzoyl peroxide as an initiator. Pre-determined quantities of
30 styrene (10 mL, eq 42), PIM-Vinyl (X=1 wt. %), DMSO (monomer:solvent = 1:1), and

dibenzoyl peroxide (eq 1) were mixed in a round-bottomed flask equipped with mechanical stirrer. The reaction mixture was heated at 120°C under N₂ atmosphere with constant stirring for 16 hours. The mixtures were then poured into a large excess of MeOH to precipitate the polymer. Solid polymers were purified by repeated precipitation with MeOH and subsequently re-dissolved in toluene. The polymer solution was poured onto glass petri dish and finally dried in oven at 90°C. A transparent functionalized polystyrene plastic film (PS*-1) was formed. Nuclear magnetic resonance (NMR) data showed the presence of PIM-Vinyl within the PS structure as small peaks observed indicating 1 wt. % of PIM-Vinyl incorporated.

The molecular weight of functionalized polystyrene (PS*-1, 1 wt% of PIM-Vinyl) and control samples was characterized by gel permeation chromatography (GPC) equipped with a static light scattering detector as shown in Table 2. PS-control and PS-PIM 45 have higher number average molecular weight of 57kDa respectively as compared to functionalized PS*-1 with Mn of 49 kDa. The lower average molecular weight of PS*-1 was due to the integration of active antimicrobial component within the polymer crosslinks, resulting in more short chain polystyrene with PIM-Vinyl formed. The introduction of PIM-Vinyl with vinylbenzyl terminal groups on both ends induced polymerisation between polymer chains and prevented the polymer chain growth.

PS-control and PS-PIM 45 showed similar average molecular weight as the latter was prepared by simply mixing styrene and PIM-45 with no reactive end groups. Thus, active component PIM-45 was simply embedded within the polymer and washed out of solution during the precipitation step. The resultant polystyrene formed was the same as PS-control, reflected by their similar number average molecular weight (Mn) and weight average molecular weight (Mw) values. The polydispersity index of all three samples were within a range of 2.23 – 2.29, indicating a broad molecular weight distribution which corresponds to chain reactions that typically yield values of Mw/Mn of around 2.0.

Sample	Mn	Mw	Polydispersity (Mw/Mn)
PS-control	57224	127802	2.23
PS-PIM 45	57236	131452	2.29
PS*-1	49884	113705	2.28

Table 1: Average molecular weight of synthesized polystyrene

Hydrophobicity and hydrophilicity properties

The hydrophobicity and hydrophilicity properties of PS synthesized was characterized by water contact angle measurement. The presence of active antimicrobial compound within the functionalized polystyrene (PS*-1) can be reflected on the hydrophilicity of PS. All measurements were taken at room temperature. Each sample was repeated two times on three random spots on the film surface with the same thickness, and the measurement was tabulated in Table 2.

Sample	Contact Angle (°)						
	Sample 1			Sample 2			Ave
PS-comm	104.9	98.3	100.1	96.5	97.0	107.5	100.7
PS-control	91.2	101.8	93.5	107.5	100.6	106.2	100.1
PS-PIM 45	98.7	95.2	96.0	95.8	102.3	91.2	96.5
PS*-1	77.9	92.2	76.4	81.6	80.6	78.9	81.3

Table 2: Average contact angle of PS films (Key: PS-comm: commercial PS control; PS-control: lab synthesised PS control; PS-PIM45: PS with PIM-45; PS*-1: PS with PIM-Vinyl)

The contact angle, θ that a water droplet makes with the PS films were observed to change significantly with the presence of active antimicrobial compound PIM-Vinyl in PS*-1. All PS controls (PS-comm, PS-control, and PS-PIM 45) showed high hydrophobic properties with contact angle, θ ranging between 91.2°-107.5°. In contrast, PS*-1 had an average contact angle, $\theta = 81.3^\circ$.

Poly-imidazolium antimicrobial compound PIM-Vinyl has high polarity with positive charged N^+ ions within its structure. As PIM-Vinyl was evenly distributed and crosslinked within the polystyrene structure, small amount of imidazolium with N^+ ions was exposed on the surface of functionalized PS*-1 polystyrene film. The exposed N^+ ions on surface were able to form hydrogen bonds when in contact with water, thus resulting a slightly hydrophilic surface of PS*-1 film.

Antimicrobial property evaluation

The minimum inhibitory concentrations (MICs) of PIM-Vinyl and PIM-45 against the four microbes were evaluated before they were co-polymerized with styrene monomer. *Escherichia coli* (ATCC 8739, Gram-negative), *Staphylococcus aureus* (ATCC 6538,

Gram-positive), *Pseudomonas aeruginosa* (ATCC 9027, Gram-negative), and *Candida albicans* (ATCC 10231, fungus) were used as representative microorganisms to test the antimicrobial functions of the PIM-Vinyl.

All bacteria and fungus were frozen at -80°C, and were grown overnight at 37°C in Mueller Hinton Broth (MHB) prior to experiments. Fungus was grown overnight at 22°C in Yeast Mold Broth (YMB). Subsamples of these cultures were grown and diluted to give an optical density (O.D.) value of 0.07 at 600 nm, corresponding to 1.5×10^8 CFU/mL for bacteria and 3×10^6 CFU/mL for fungus (McFarland's Standard 0.5; confirmed by plate counts).

Test compounds were dissolved in MHB at a concentration of 4 mg/mL and the minimal inhibitory concentrations (MICs) were determined by microdilution assay. Bacterial solutions (100 μ L, 1×10^6 CFU/mL) were mixed with 100 μ L of antimicrobial agent solutions (normally ranging from 4 mg/mL to 2 μ g/mL in serial two-fold dilutions) in each well of the 96-well plate. The plates were incubated at 37°C for 24 h with constant shaking speed at 300 rpm. The MIC measurement against *Candida albicans* was similar to bacteria except that the fungus solution was $\sim 10^4$ CFU/mL in YMB and the plates were incubated at room temperature.

The MICs were taken as the concentration of the materials at which no obvious microbial growth was observed. Medium solution containing microbial cells alone were used as control (100% microbial growth). The assay was performed in four replicates. The MICs results of PIM-45 and PIM-Vinyl is shown in Table 3.

Compound	MIC (μ g/mL)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
PIM-45	8	8	32	32
PIM-Vinyl	4	2	16	32

Table 3: Average minimum inhibition concentrations (MICs) of poly-imidazolium compound PIM-45 and functionalised poly-imidazolium compound PIM-Vinyl

The test results of PIM-Vinyl was compared with the results of PIM-45 to investigate the effect of the addition of reactive end groups to PIM-45 on the antimicrobial activity

of the compound. Clearly, both PIM-45 and PIM-Vinyl polymers exhibited antimicrobial activity against the tested microbes. Both compounds showed effective antibacterial activity where PIM-Vinyl showed superior antibacterial activity with MICs against *E. coli* and *S. aureus* at 4 µg/mL and 2 µg/mL, respectively. This indicates that PIM-Vinyl possesses an alkenyl moiety that retained the excellent antimicrobial property as it contains a fraction of PIM-45 with high antimicrobial property in its structure.

The antibacterial properties of synthesized PS film surfaces were evaluated according to Japanese Industrial Standard JIS Z 2801/ISO 22196 test method. Tryptic soy broth (TSB) and Yeast Mold Broth (YMB) were used to prepare the broths for bacteria and fungus growth according to the manufacturer's instructions. Gram-negative bacteria *E. coli* (ATCC 8739), Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), and fungus *Candida albicans* (ATCC 10231) were re-cultured. Prior to the experiment, test microbes were suspended in 5 mL of respective broths and adjusted to OD₆₀₀ = 0.07. The solution was further diluted 102 times for antibacterial testing. In order to cover the surface, 100 µL of cell suspensions was placed on each 2.5 cm X 2.5 cm sample surface and covered with thin plastic sheets. After incubation at 37°C with the surfaces, the respective cell suspensions were washed and diluted, and each dilution spread on two nutrient agar plates. Resulting colonies were then counted using standard plate counts techniques, and the number of colony forming units per mL was calculated. The number of colony forming units was assumed to be equivalent to the number of viable cells in suspension.

The antimicrobial activities of PS*-1 and other control samples are summarized in Table 4.

Test microbes	Log10 (CFU/mL) of microbe at 0h	Log10 (CFU/mL) of microbe after 24h incubation				Log reduction (CFU/mL)
		PS-comm	PS-control	PS-PIM45	PS*-1	
<i>E. coli</i>	6.43	7.35	7.16	6.90	0.00	> 7 log reduction
<i>S. aureus</i>	6.05	7.63	7.37	7.62	0.00	> 7 log reduction
<i>C. albicans</i>	5.50	5.75	5.83	5.72	5.64	NA

Table 4: Antimicrobial activities of PS*-1 film surfaces at 1/500 dilution media

The antimicrobial activities of PS films against *E. coli*, *S. aureus*, and *C. albicans* with initial inoculum concentration 10⁶ CFU/mL were evaluated at 1/500 dilution media according to JIS test standard. PS*-1 exhibited excellent bactericidal property against

E. coli with log reduction of more than 7 after 24 h incubation. The bactericidal activity against *S. aureus* was comparable to the activity against *E. coli* where no colony growth was observed after 24 h incubation with log reduction more than 7. The good bactericidal activity indicates PS*-1 can effectively kill bacteria even when the antimicrobial compound was within its polymer matrix. However, PS*-1 with 1 wt. % loading of PIM-Vinyl within the material did not demonstrate fungicidal properties against *C. albicans* at 1/500 dilution YMB media.

Considering the low loading of PIM-Vinyl in PS*-1, new PS samples with 3 wt. % PIM-Vinyl (PS*-3) was synthesized and the fungicidal activity against *C. albicans* at 1/500 dilution media was compared as demonstrated in Figure 4. PS*-1 with 1 wt. % loading of PIM-Vinyl did not exhibit antifungal activity at 1/500 dilution media. However, no colony was observed on PS*-3 with log reduction > 4 when the loading of PIM-Vinyl was increased to 3 wt.%. A higher loading of antimicrobial compound PIM-Vinyl was needed in order to exhibit fungicidal activity.

In order to determine the microorganism killing mechanism of the synthesized antimicrobial copolymer PS*-1, leaching assay was used to assess the leaching properties of PS*-1 following GB 15979-2002 method with minor modifications. A total amount of 0.2 g PS*-1 plastic film was placed in 2 mL microcentrifuge tubes containing 1 mL of phosphate-buffered saline (PBS) solution and incubated at shaking incubator at 300 rpm for 1 h. After 1 h, the solution was expected to contain leached antimicrobial compounds if PIM-Vinyl was not chemically attached within the polystyrene structure via strong C-C bond. The solution was then transferred to a new 2 mL microcentrifuge tube and was inoculated with *E. coli* to a final concentration of 10^4 CFU/mL. The inoculum was then incubated at shaking incubator for another 1 h at 300 rpm. A 10-fold dilution was performed on the inoculum before plating 100 μ L on LB agar plate and incubated at 37°C for 24 h. The antibacterial efficiency of the leached compounds and bacterial survivability were determined by counting the viable colonies on plate after 24 h. A parallel test using the same PS samples was conducted following the GB 15979-2002 test method where the 0.2 g test material was added together with bacterial solution and incubated for 1 h. A side by side comparison of leaching properties and bactericidal properties of PS*-1 against *E. coli* is shown in Figure 5.

The results from leaching assay of PS control, PS-PIM45 and PS*-1 against *E. coli* are as shown in Figure 5(a). No bactericidal activity against *E. coli* was observed after 1 h of incubation in leached solution. All samples showed high percentages of > 90 % bacterial survivability after 1 h, which concluded the absence of antimicrobial compounds in test solution. Antimicrobial compound PIM-Vinyl was well incorporated in PS*-1 rather than simply embedded within the PS film, thus it did not leach out from the material to cause antibacterial activity.

PS-PIM 45 did not give any bactericidal activity, which indicates the washing process of material synthesis procedure is sufficient. PIM-45 with no reactive end groups was washed off during the repeated precipitation steps, and the resultant sample did not contain any remaining antimicrobial materials that may leach out to the test solution causing bactericidal activity. It further confirms that PIM-Vinyl is chemically incorporated in PS*-1.

In contrast, PS*-1 exhibited antibacterial activity against *E. coli* with initial inoculum concentration 1.92×10^4 CFU/mL where more than 80% bacterial reduction was observed as shown in Figure 5(b). PS*-1 demonstrated strong antibacterial properties when it was added together in the bacterial solution for 1 hour incubation. This demonstrated the antimicrobial activity of PS*-1 was not caused by release of the active compound PIM-Vinyl from PS. PS*-1 with chemically bonded PIM-Vinyl inhibited the bacterial growth while in contact with bacteria in the solution.

A time-kill method was performed to assess the relative efficacy of PS*-1. Time-kill kinetics of PS*-1 was determined according to JIS Z 2801/ISO 22196 test method by reducing the incubation time of bacterial culture on sample surface from 24 h to 6 h, 8 h, and 16 h respectively. The antimicrobial activity against *E. coli* with initial inoculum concentration 10^6 CFU/mL was evaluated at 1/500 dilution media. The bactericidal activity against *E. coli* with different incubation period was compared as shown in Figure 6. PS*-1 exhibited strong bactericidal property against *E. coli* with log reduction of more than 7 after 24 h incubation, and log reduction of more than 6 after 16 h and 8 h incubation at 37°C respectively. However, when the exposure time of bacteria to PS*-1 surface reduced to 6 h, no bactericidal activity was observed on samples where log reduction of less than 1 was observed.

Based on the results, inhibition of bacteria growth was observed between 6-8 h of incubation. Hence, the inhibitory phase of PS*-1 was within 6-8 hours, but after incubation of more than 8 hours, complete killing of bacteria was observed due to extended contact with PS*-1 surface. Incubation time of ≥ 8 hours provided sufficient time for test bacteria to contact with PS*-1 in order for PS*-1 to demonstrate bactericidal properties against *E. coli*.

Surface nanostructures

The surface structure formed on PS-control and PS* samples was observed using atomic force microscopy (AFM) images. The PS-control samples all generally had a smooth surface with average roughness (Ra) in the range of $1 \text{ nm} < \text{Ra} < 20 \text{ nm}$.

However, PS*-1 sample gave very interesting submicron scale surface topographic structures. AFM topography images showed that the overall average surface roughness of PS*-1 prepared with toluene was $\text{Ra} = 102.9 \text{ nm}$. AFM height images showed that PS*-1 sample prepared in toluene had aligned hair structure with the height of hairs about 500 – 600 nm.

PS*-1 samples prepared with chloroform were also formed as a basis for comparison. The method of making these samples were the same as described above, except chloroform was used as the re-dissolving solvent instead of toluene. AFM topography images showed that the overall average surface roughness of PS*-1 prepared with chloroform was $\text{Ra} = 55.1 \text{ nm}$. Further, PS*-1 sample prepared in chloroform exhibited a honeycomb-like surface structure with the drop from peak to valley being about 800 nm.

The surface nanostructures of PS*-1 in toluene or chloroform are PS-PIM co-polymer induced phase separation. The PIM-Vinyl loading in PS*-1 sample was 1 wt%, where the number average molecular weight (Mn) of PIM-45 and PS*-1 was about 2.5 kDa and 50 kDa, respectively. About 20% of polymer chains were decorated with PIM-45 block and the highly hydrophilic PIM-45 block successfully induced phase separation on the surface to create submicron scale structures. To further verify the relationship between surface nanostructures and PIM component in functionalized polystyrene, PS* with different PIM-Vinyl loadings were investigated. Surface roughness and the height of the hair structure of PS* increased as the PIM-Vinyl loading increased from 0.2 to 1

wt%. When the PIM-Vinyl loading was further increased, the PS* surface roughness reached a plateau. The density of nanostructures (amount of hair structures) on the surface was well correlated with the PIM-Vinyl loading. In particular, the PIM components in the copolymer could spontaneously assemble on the surface, thereby
5 induce phase separation and build up surface nanostructures.

Whilst the foregoing description has described exemplary embodiments, it will be understood by those skilled in the technology concerned that many variations may be made without departing from the present invention.

Claims

1. An antimicrobial plastic comprising: a thermoplastic polymer and a polycationic polymer, wherein the polycationic polymer forms semi-free surface nanostructures with the thermoplastic polymer, the semi-free surface nanostructures capable of electrostatically bonding with bacterial components.
2. The plastic according to claim 1, wherein the polycationic polymer is hydrophilic.
3. The plastic according to claim 1 or 2, wherein the polycationic polymer is a functionalised poly-imidazolium polymer.
4. The plastic according to claim 3, wherein the functionalised poly-imidazolium polymer comprises an alkenyl terminal group at each of its ends.
5. The plastic according to claim 4, wherein the alkenyl terminal group comprises a vinyl group.
6. The plastic according to any of claims 3 to 5, wherein the functionalised poly-imidazolium polymer comprises PIM-Vinyl.
7. The plastic according to any preceding claim, wherein the polycationic polymer is covalently linked within a main chain of the thermoplastic polymer via covalent bonds.
8. The plastic according to any preceding claim, wherein the plastic comprises 0.1-10 wt % polycationic polymer based on the total weight of the plastic.
9. The plastic according to any preceding claim, wherein the thermoplastic polymer is: polystyrene, polyacrylate, copolymers or mixtures thereof.
10. The plastic according to any preceding claim, wherein the plastic has a surface roughness of ≥ 50 nm.

11. A method of preparing the plastic according to any preceding claim, the method comprising:

- mixing a polycationic polymer and monomer units of the thermoplastic polymer with a solvent to form a mixture;
- adding an initiator;
- heating the mixture; and
- precipitating the plastic.

12. The method according to claim 12, wherein the mixture comprises 0.1-10 wt % polycationic polymer based on the total weight of the mixture.

13. The method according to claim 11 or 12, wherein the heating results in the co-polymerisation of the polycationic polymer with the monomer units of the thermoplastic polymer.

14. The method according to claim 13, wherein the co-polymerisation results in the polycationic polymer becoming covalently linked within a main chain of the thermoplastic polymer via covalent bonds.

15. The method according to any of claims 11 to 14, wherein the precipitating the plastic comprises surface phase separation of the polycationic polymer on the plastic, thereby forming semi-free surface nanostructures on the plastic.