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Cell surface functionalization with lysine ligand-containing copolymers for fibrinolytic activity†

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Although cell transplantation has achieved great success in the treatment of many intractable diseases, due to the effect of an instant blood-mediated inflammatory reaction (IBMIR), blood clots usually form on the surface of transplanted cells, resulting in a significant reduction in the survival of transplanted cells. Inspired by the fibrinolytic mechanism in human blood, this paper constructs lysine ligand-containing copolymer-functionalized cell surfaces for fibrinolytic activity. First, a copolymer (POLF) of oligoethylene glycol methacrylate (OEGMA), 6-amino-2-(2-methacylamido)-hexanoic acid (Lys) and fluorescein *O*-methacrylate (FluMA) was synthesized by reversible addition–fragmentation chain transfer (RAFT) copolymerization. Then, the copolymer POLF was modified onto the HeLa cell surface by codeposition with dopamine. An *in vitro* study showed that compared to unmodified HeLa cells, modified cells not only have good cell viability but can also resist fibrinogen adsorption and selectively bind plasminogen from plasma. More importantly, upon activation of plasminogen adsorbed to plasmin, the modified cells were able to rapidly lyse fibrin formed on their surface. This study offers a novel proof-of-concept for constructing transplanted cells with fibrinolytic activity.

Introduction

In recent years, due to the development of cell separation and purification technology and transplantation immunology, cell transplantation, as the most promising alternative treatment for traditional organ transplantation, has achieved great success in the treatment of tumors, autoimmune diseases, nervous system diseases, and other intractable diseases. However, donor cells are usually affected by an activated instant blood-mediated inflammatory reaction (IBMIR) after transplantation into the recipient. Therefore, only a small number of transplanted cells can reach the target tissue. This seriously restricts the therapeutic effect and further development of cell transplantation.[1] In brief, the IBMIR is a nonspecific reaction mediated by the innate immune system. When the transplanted cells are in direct contact with blood, the body's coagulation and complement systems are activated, which in turn causes platelet adhesion and aggregation, and the transplanted cells are embedded in the blood clot and eventually lead to reduced survival of transplanted cells.[2]

According to the mechanism of the IBMIR described above, the activation of platelets and the coagulation system is an important factor for the initiation of the IBMIR, and the formation of blood clots on the surface of transplanted cells is the main reason for the failure of cell transplantation. Therefore, the following two ways can usually be used to inhibit the formation of blood clots on the surface of transplanted cells to improve the efficacy of cell transplantation: (1) intervention in recipients, such as using anticoagulant drugs to provide anticoagulation. However, this approach often has a greater impact on the recipient, causing the recipient to have systemic complications, bleeding tendency, and other risks.[3–6] (2) Pretreatment of transplanted cells, such as performing pretreatment on the isolated and purified cells to make the transplanted cells have good blood compatibility.[7–12] Since this approach has little impact on the recipient, it is the main direction of current development research. For instance, one of the major factors that negatively impacts transplanted pancreatic islets is blood coagulation, and pancreatic islet transplantation often causes thrombosis, which, in turn, causes inflammation and is detrimental to transplanted cells. Therefore, controlling the generation of thrombi is an important strategy to ensure the success of pancreatic islet transplantation.[1,13]

In our previous work, novel methacrylic monomer containing lysine ligands, 6-amino-2-(2-methacylamido)-hexanoic acid (Lys), were designed and synthesized, in which the 3-amino and carboxylic acid groups were free. The molecular structure of plasminogen contains five kringle domains, two of which

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(K1 and K4) provide lysine binding sites (LBSs) with high affinity for the carboxyl-terminal lysine residues of fibrin. Similarly, the t-PA molecular structure also contains two kringle fragments, among which K2 contains a lysine binding site.[14,15] Therefore, the material surfaces modified by graft copolymerization of the Lys monomer can selectively bind plasminogen and a tissue-type plasminogen activator (t-PA), which are the two key components of the human fibrinolytic system. Furthermore, the adsorbed plasminogen can be converted into plasmin *in situ* under the action of t-PA, which can effectively dissolve the blood clot formed on the surface of the biomaterial.[16–20] Therefore, we imagine that if the lysine ligands are modified onto the surface of transplanted cells, transplanted cells with fibrinolytic activity can be prepared. This will provide a good idea to effectively solve the problem of massive loss of transplanted cells caused by blood clots.

Based on this idea, in the present work, the copolymer poly(oligoethylene glycol methacrylate)-*co*-(Lys)-*co*-(fluorescein *O*-methacrylate) (POLF) containing lysine ligands was first prepared by RAFT copolymerization. Due to the presence of innate amine, thiol, and hydroxyl groups on cell membranes, and the copolymer also contains amino groups of lysine units and a small amount of hydroxyl groups derived from RAFT reagents in the end groups, the copolymer POLF was modified onto the HeLa cell surface by codepositing with dopamine by forming covalent or hydrogen bonds.[21–23] The results of *in vitro* experiments show that the modified cells not only have good cell viability but also can resist nonspecific protein adsorption and selectively bind to plasminogen. Most importantly, upon activation of adsorbed plasminogen to plasmin, the modified cells also have good fibrinolytic activity to lyse nascent blood clots. In general, the cell surface modification method provided in this study has the following advantages: (1) the modification process is simple and only requires ''one-step'' codeposition; (2) the modified cells have good cell viability; and (3) the modified cells have good fibrinolytic activity. Combining the above characteristics, our study offers a novel proof-of-concept for constructing transplanted cells with fibrinolytic activity.

Experimental section

Materials

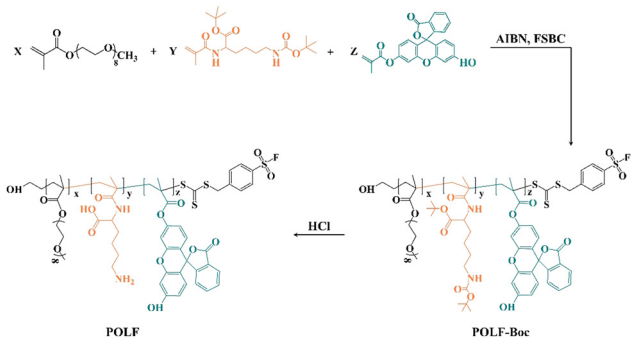
Dopamine hydrochloride (98%) and polyethylene glycol methyl ether methacrylate (OEGMA, M_n = 475) were purchased from Aladdin (Shanghai, China). Fluorescein *O*-methacrylate (FluMA, 99%) was purchased from Sigma-Aldrich (Shanghai, China). The RAFT reagents 4-(fluorosulfonyl)benzyl(2-hydroxyethyl) carbonotrithioate (FSBC)[24] and 6-*tert*-butoxycarbonyl amino-2-(2-methylacryloylamino)-hexanoic acid *tert*-butyl ester (Lys(P))[17] were synthesized as reported previously (^{1}H NMR spectra are shown in Fig. S1 and S2, respectively, ESI†). Calcium chloride ($CaCl_2$) and 2,2′-azoisobutyronitrile (AIBN) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Phosphate buffer solution (PBS, 10×, pH 7.4) was purchased from Shenggong Biological Engineering Co., Ltd (Shanghai, China). A penicillin–streptomycin mixture (100×), goat anti-human fibrinogen-fluorescein isothiocyanate (anti-Fg-FITC), anti-PLG polyclonal antibody-fluorescein isothiocyanate (anti-Plg-FITC), and goat anti-human albumin-fluorescein isothiocyanate (anti-HSA-FITC) were purchased from Solarbio (Beijing, China). The high-glucose Dulbecco's modified Eagle's medium (DMEM) and trypsin solution (0.25%) were purchased from Thermo HyClone (Logan, USA). The standard fetal bovine serum (FBS) was purchased from Thermo Gibco (New York, USA). Triton X-100, paraformaldehyde, and trypan blue were purchased from Sigma-Aldrich (Shanghai, China). The CCK-8 cell counting kit was purchased from Beyotime (Shanghai, China). Fibrinogen (Fg) and plasminogen (Plg) were purchased from Enzyme Research Laboratories (South Bend, USA). Whole pig blood was purchased from Yuanye Biological Company (Shanghai, China). Tissue plasminogen activator (t-PA) was purchased from Genentech (San Francisco, USA). The human cervical cancer cells (HeLa) used in the experiment were purchased from Shanghai Enzymatic Biotechnology Co., Ltd (Shanghai, China).

Instruments and measurements

^{1}H nuclear magnetic resonance (NMR) spectra were acquired using a Varian Mercury-400 spectrometer (Varian, USA). The optical absorption value and fluorescence spectrum were recorded using a multifunction enzyme labeling instrument (Varioskan® Flash, Thermo Fisher Scientific, USA). Fluorescence images of cells were observed using a fluorescence microscope (BX51, Olympus, Japan) and a confocal laser scanning microscope (TCS SP5 II, Leica, USA). The fluorescence intensities of cells were obtained by flow cytometry (FACVERSE, BD, USA). Scanning electron microscopy (SEM) images and energy dispersive spectrometer (EDS) spectra were obtained using a Hitachi SU8010 electron microscope (Hitachi, Japan). The molecular weight (M_n, g mol^{-1}) and polydispersity coefficient ($Đ_M$) of the copolymers were determined using gel permeation chromatography (GPC) (e2695, Waters, USA) with PBS solution containing 0.2 M $NaNO_3$ as the mobile phase.

Synthesis of copolymer POLF labeled with FITC

As shown in Scheme 1, POLF copolymers labeled with FITC were synthesized by RAFT copolymerization. Briefly, a calculated amount of mixtures of OEGMA, Lys(P), FluMA, and FSBC were dissolved in dry methanol, and AIBN was added as an initiator. The solutions were then degassed by bubbling with nitrogen for 30 min, and the polymerization reaction was carried out at 70 °C for 8 h. The copolymers containing Lys(P) (POLF-Boc) were finally purified by dialysis using seamless cellophane dialysis tubing (MWCO 3500) in deionized water for 3 days and then lyophilized. Afterwards, the copolymers POLF-Boc were deprotected by treatment with concentrated hydrochloric acid at 45 °C for 6 h. The obtained copolymer POLF was purified using the dialysis method mentioned above. Additionally, the synthesis process of copolymer POL without FluMA units is similar to the method mentioned above.



Scheme 1 The synthetic route of POLF copolymers.

Cell culture

HeLa cells were cultured in the DMEM containing 100 U per mL penicillin, 0.1 mg per mL streptomycin and 10% FBS at 37 °C and 5% CO_2. The medium was changed every 2 days. HeLa cells were digested and harvested by the addition of a 0.25% trypsin solution, collected by centrifugation at 1200 rpm for 5 min, and resuspended in the fresh cell culture medium prior to the experiment.

Cell surface modification by the copolymer POLF

HeLa cell suspensions (1×10^5 cells) were cultured in pore plates or Petri dishes for 12 h and then incubated in the culture medium with 1.0 mg per mL dopamine and 1.0 mg per mL POLF (pH = 8.5) at 37 °C for 1 h. After washing with PBS three times, the free polymer was removed, and the modified cells (HeLa@POLF) were obtained. After modification with POLF, the morphology of HeLa@POLF cells was characterized by scanning electron microscopy. The fluorescence image of HeLa@POLF cells was visualized by confocal laser scanning microscopy. The fluorescence intensities of HeLa@POLF cells were counted by flow cytometry. Additionally, the process of HeLa cells modified by copolymer POL without FluMA units (HeLa@POL) is similar to the method mentioned above. HeLa@Dopa cells were prepared by dopamine deposition without adding the POLF copolymer.

Cell viability assay

Cell Counting Kit-8 (CCK-8) was used to assess the viability of the HeLa@POLF cells and the cytotoxicity of the POL and POLF copolymers. Briefly, the HeLa@POLF cells were cultured in the DMEM (for cytotoxicity, HeLa cells were cultured with the two copolymers in DMEM) for 1, 2 and 3 days in 96-well plates. Then, 200 μL of the DMEM and 20 μL of the CCK-8 dye were added to each well at determined times, and the 96-well plates were kept in an incubator at 37 °C for 2 h. Two hundred microliters of the solution was transferred to new 96-well plates. The absorbance of each solution at 450 nm was recorded using a multifunction enzyme labeling instrument. The relative viability of the cells was assessed by comparing the optical density (OD) values of each experimental group (HeLa@POLF) and the control group (unmodified HeLa cells).

Cell proliferation assay

HeLa cells and HeLa@POLF cells of the same density were cultured in the DMEM in 6-well plates, and after they adhered, photographs of the cell distribution before proliferation were taken. After culturing the modified cells and normal HeLa cells for 4 days, the two cells were counted using a hemocytometer to determine whether their proliferation function was affected.

Protein adsorption test

HeLa@POL cells were first incubated with 1 mg per mL Fg protein (for Plg 0.1 mg mL^{-1}, for HSA 10 mg mL^{-1}) in the DMEM or with pooled normal human plasma as well as whole pig blood for 3 h at 37 °C and washed three times with PBS. Subsequently, 0.5 mL of anti-Fg-FITC (or anti-Plg-FITC, anti-HSA-FITC) was added to the wells at a concentration of 0.15 mg per mL anti-Fg-FITC (for anti-Plg-FITC 7.5 μg mL^{-1} and for anti-HSA-FITC 0.15 mg mL^{-1}) in the DMEM and incubated at 37 °C for 1 h. After washing three times with PBS, the fluorescence image of the HeLa@POL was observed and photographed by fluorescence microscopy. The fluorescence intensity was determined using the ImageJ software.

Plasma clot lysis assay

The modified and unmodified HeLa cells were incubated with pooled normal human plasma at 37 °C for 3 h in 96-well plates to adsorb plasminogen (Plg) in the plasma and then washed with PBS three times. Subsequently, 200 μL of t-PA was added to the wells at a concentration of 0.1 mg mL^{-1} and incubated for 30 min to activate the cell surface-bound Plg into plasmin. After washing three times with PBS to remove any unbound proteins, 100 μL of plasma was added to the wells containing the HeLa cells. Following 5 min of equilibration at 37 °C, 100 μL of 0.025 M $CaCl_2$ aqueous solution was added to produce fibrin clots, simulating the coagulation and fibrinolysis processes in blood under natural conditions. The absorbance of the plasma at 405 nm was measured at 30 s intervals over 1 h using a multifunctional microplate reader.

Results and discussion

Synthesis of copolymer POLF

As shown in Scheme 1, the fluorescence labeled copolymer POLF was synthesized in two steps. The three monomers OEGMA, Lys(P), and FluMA were first copolymerized by RAFT to obtain copolymer POLF-Boc. Afterwards, the *tert*-butyl ester protecting group (*t*-Boc) of the side chain of the Lys (P) monomer unit in the POLF-Boc copolymer is then removed by hydrochloric acid to obtain the copolymer POLF containing lysine ligands. Fig. 1 shows a typical ^{1}HNMR spectrum of the synthesized copolymer POLF-Boc and POLF. For POLF-Boc, the characteristic peak with a chemical shift at 1.37 ppm belongs to the proton on the *t*-Boc of the Lys(P) monomer unit side chain, the characteristic peak with a chemical shift at 3.5–3.6 ppm belongs to the methylene proton peak of the side chain of the OEGMA monomer unit, and the characteristic peak with a

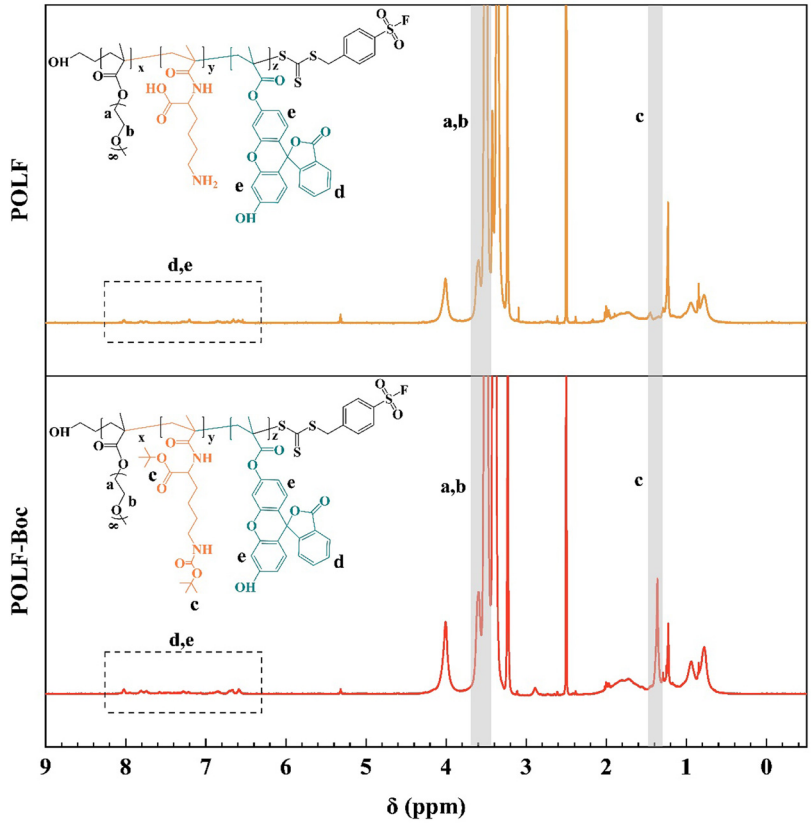


Fig. 1 ^{1}H NMR spectra of the copolymer POLF-Boc and POLF in DMSO-d6.

chemical shift at 6.0–8.1 ppm belongs to the proton peak of the benzene ring of the FluMA monomer unit. The content of the monomer unit in the POLF-Boc copolymer was calculated according to the integrated area of these three groups of characteristic proton peaks. After the POLF-Boc copolymer was reacted with hydrochloric acid, the disappearance of the characteristic proton peak with a chemical shift of 1.37 ppm indicated that *t*-Boc was removed, and the copolymer POLF containing lysine ligands were obtained. As a representative example, the GPC results showed that the outflow curve of the copolymer POLF-2 is a relatively symmetrical unimodal distribution (Fig. S3, ESI†), and the M_n of the obtained POLF increased with the increasing feed ratio of OEGMA and Lys(P) monomers, while the dispersity $Đ_M$ was maintained well at approximately 1.8 (Table 1). These data indicate that the copolymerization is controllable well by using FSBC as the RAFT reagent and that the content of lysine ligands in POLF can be adjusted by the feed ratio of monomers. In addition, POLF-2 aqueous solutions have strong fluorescence properties and can be used as a fluorescent labeling means to directly observe the interaction between the polymer and cells by fluorescence microscopy and flow cytometry (Fig. S4, ESI†).

Table 1 Composition of the copolymer POLF and POL

Polymer	Feed ratio OEGMA : Lys(P) : FluMA	Composition OEGMA : Lys : FluMA[a]	$M_{n,GPC}$[b] (g mol^{-1})	$Đ_M$[b]
POLF-1	25 : 4 : 1	24 : 2 : 1	5700	1.77
POLF-2	25 : 2 : 1	20 : 2 : 1	9100	1.81
POLF-3	45 : 2 : 1	40 : 1 : 1	10 200	1.69
POL	25 : 2 : 0	14 : 1 : 0	8400	1.83

[a] Determined by ^{1}H NMR. [b] Determined by GPC.

POLF modification of the cell surface

As a representative mammalian cell, HeLa cells were used to verify the feasibility of the copolymer POLF for cell surface modification. After HeLa cells were modified by only dopamine deposition (HeLa@Dopa), in contrast to the relatively smooth cell surface of unmodified HeLa cells by SEM imaging (Fig. 2a), a certain rough cell surface of HeLa@Dopa was found (Fig. 2b). However, unlike HeLa@Dopa cells, the cell surface modified by codepositing dopamine and POLF-2 (HeLa@POLF) was very rough and distributed with different particle sizes (Fig. 2c), which was consistent with previous studies.[21]

In addition, the HeLa@POLF cells were also observed by a phase contrast image and a confocal fluorescence image.

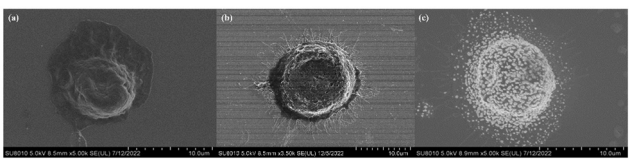


Fig. 2 SEM images of (a) HeLa cells, (b) HeLa@Dopa and (c) HeLa@POLF.

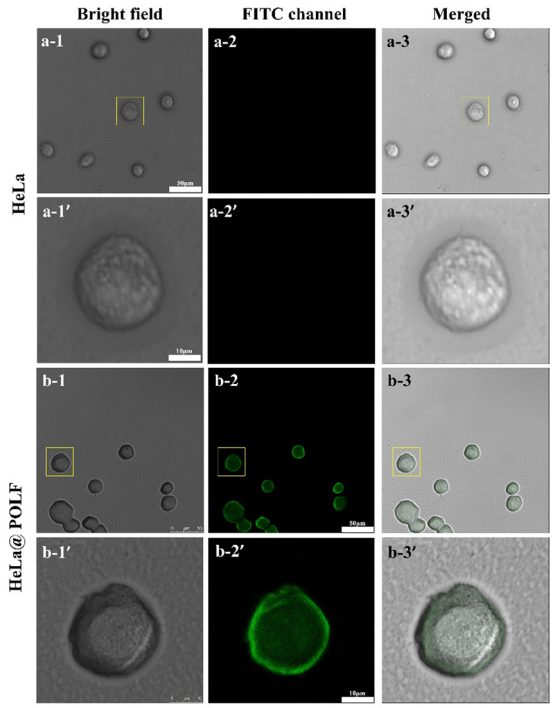


Fig. 3 Confocal fluorescence images of (a) HeLa cells and (b) HeLa@POLF cells.

Compared to unmodified HeLa cells without fluorescence (Fig. 3a), the green fluorescence derived from FITC was clearly observed at the periphery of the HeLa@POLF cells (Fig. 3b). Furthermore, the results of flow cytometry analysis also showed that the HeLa@POLF cells moved to a higher fluorescence intensity (Fig. S5, ESI†). All these results indicated that by this codepositing method, the copolymer POLF containing lysine ligands could be successfully immobilized on HeLa cells. This may be due to the presence of innate amine, thiol, and hydroxyl groups on cell membranes, and the copolymer also contains the amino groups of lysine units and a small number of hydroxyl groups derived from RAFT reagents in the end groups. The copolymer POLF could be modified onto the HeLa cell surface by codepositing with dopamine by forming covalent or hydrogen bonds.[23,25–28]

Effect of POLF modification on cell viability

In cell surface engineering, modification of the cell surface cannot affect the normal physiological activities of cells, such as cell proliferation and differentiation.[7,29] Therefore, the cell viability of HeLa@POLF cells and the cytotoxicity of the POL and POLF copolymers were assessed by the CCK-8 method. The results showed that both polymers exhibited negligible cytotoxicity and cell viability of more than 90% (Fig. S6, ESI†). As shown in Fig. 4, after 24 h of incubation, the HeLa@POLF cells were maintained more than 80% viability. Upon further extending the incubation time to 72 h, the HeLa@POLF cell viability was increased to 91%, and no significant difference was found between unmodified HeLa cells and HeLa@POLF cells. The

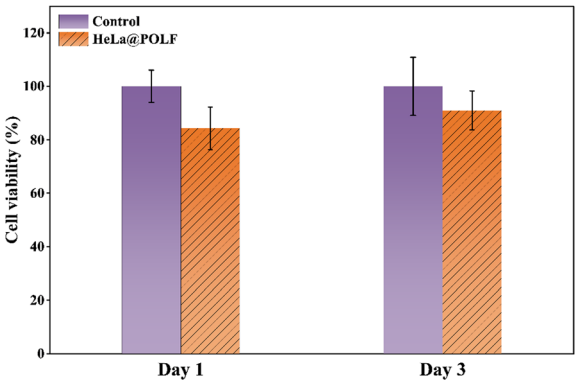


Fig. 4 Cell viability of HeLa cells and HeLa@POLF by the CCK-8 assay.

results of the cell proliferation experiment also showed that the original proliferation function of the HeLa@POLF cells was not affected (Fig. S7, ESI†). The results indicated that the functionalization of the cell surface with POLF by this codeposition method had good biocompatibility.

Protein adsorption properties

Fibrinogen (Fg), a key protein in the human coagulation cascade, is known to play a leading role in mediating platelet adhesion to biomaterials.[30] Fg adsorption on the modified cell surface from the DMEM, plasma and whole blood was measured to provide indications of protein resistance and blood compatibility. To exclude the influence of the green fluorescence of the copolymer POLF on the subsequent fluorescence of anti-Fg-FITC bound to the cell surface, HeLa cells were modified by a copolymer POL (HeLa@POL) with a molecular weight similar to that of POLF-2 and without FluMA monomer units. As shown in Fig. 5 and 6, after HeLa@POL cells were incubated with Fg in the DMEM or with pooled normal human plasma, as well as whole blood and subsequently bound to the anti-Fg-FITC antibody, compared with the large amount of green fluorescence on the surface of unmodified HeLa cells and HeLa@Dopa cells, the green fluorescence on the surface of HeLa@POL cells was relatively low for all media. Especially, for whole blood, the surface fluorescence intensity of HeLa@POL cells was ~13% as that of unmodified HeLa cells. Additional protein adsorption data for other high-abundance plasma proteins, such as human serum albumin (HSA), also further verified that the HeLa@POL cells can effectively resist nonspecific protein adsorption (Fig. S8, ESI†). The results indicated that the HeLa@POL cells could effectively resist fibrinogen adsorption whether in whole blood, plasma or buffer. This is presumably due to the well-known protein-resistant properties of OEGMA units in the copolymer POL.[31,32]

Plasminogen (Plg), the key zymogen of the fibrinolytic pathway, is a single-chain glycoprotein that circulates in the blood at a concentration of 180–200 μg mL^{-1}.[18] The biomaterial surface-localized plasminogen has the potential for clot dissolution if it can be converted to plasmin (human thrombolytic enzyme). The Plg adsorption on the modified cell surface from the DMEM and plasma was measured to provide indications of

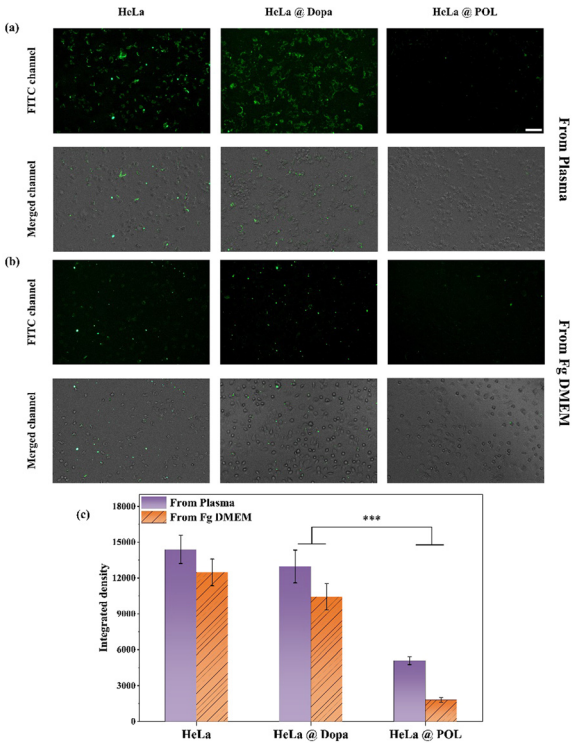


Fig. 5 Fluorescence and bright field merged images of Fg adsorbed on the surface of HeLa cells, HeLa@Dopa and HeLa@POL: (a) adsorption from plasma and (b) adsorption from 1 mg per mL Fg DMEM solution (the scale bar is 50 μm). (c) The average fluorescence intensity obtained by ImageJ statistics ($n > 4$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

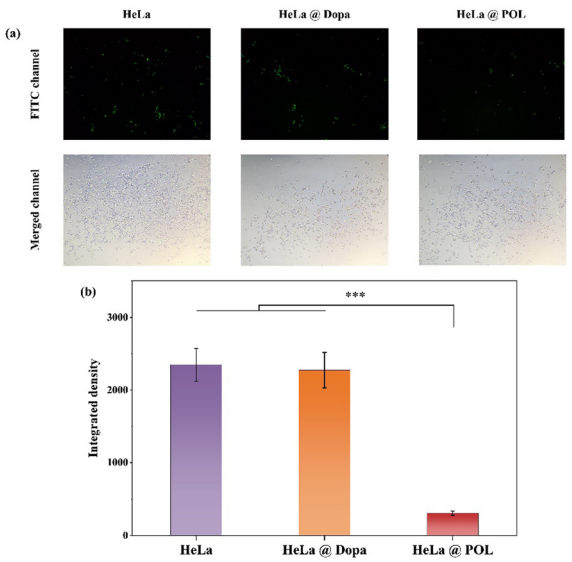


Fig. 6 Fluorescence and bright field merged images of Fg adsorbed on the surface of HeLa cells, HeLa@Dopa and HeLa@POL: (a) adsorption from whole blood (the scale bar is 200 μm). (b) The average fluorescence intensity obtained by ImageJ statistics ($n > 4$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

specific protein binding and the ability to dissolve clots, respectively. As shown in Fig. 7, after HeLa@POL cells were

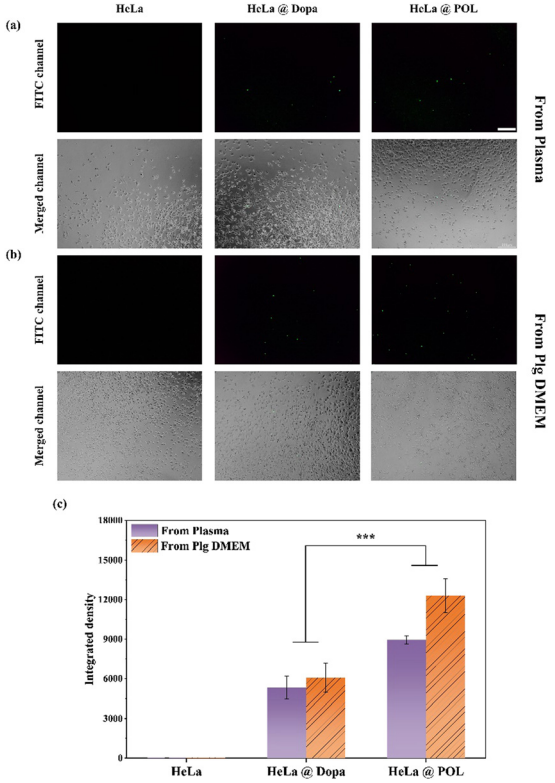


Fig. 7 Fluorescence and bright field merged images of Plg adsorbed on the surface of HeLa cells, HeLa@Dopa and HeLa@POL: (a) adsorption from plasma and (b) adsorption from 0.1 mg per mL Plg DMEM solution (the scale bar is 200 μm). (c) The average fluorescence intensity obtained by ImageJ statistics ($n > 4$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

incubated with Plg in the DMEM or with pooled normal human plasma and subsequently bound to the anti-Plg-FITC antibody, compared with no green fluorescence on the surface of unmodified HeLa cells and weaker green fluorescence on the surface of HeLa@Dopa cells, the green fluorescence on the surface of HeLa@POL cells was relatively strong for both media, indicating the specific binding of plasminogen. The results indicated that the HeLa@POL cells not only could effectively resist nonspecific protein adsorption but also possessed specific plasminogen binding. This can be attributed to the specific plasminogen binding of lysine ligands (with free ε-amino and carboxyl groups) in the copolymer POL.[14,33,34]

Plasma clot lysis

When a primary thrombus (fibrin clot) occurs in the blood, the ε-lysine residues exposed on the surface of the protein clot can specifically adsorb Plg and t-PA, forming a ternary complex. This complex can accelerate the activation of Plg by t-PA, thereby producing plasmin, which degrades the fibrin clot formed in the blood, that is, the primary thrombus.[17,35] McClung *et al.* demonstrated that surfaces incorporating lysine ligands with free ε-amino and carboxylic acid groups exposed to plasma and then to t-PA are able to lyse plasma clots that begin to form on them (incipient or nascent clots). A similar plasma recalcification clotting assay was performed to assess the clot

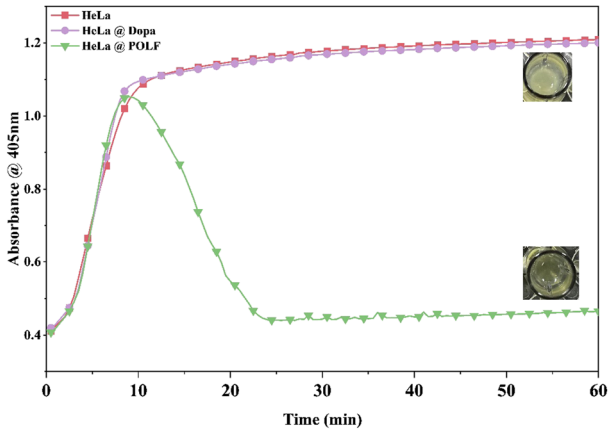


Fig. 8 Clot formation in human plasma expressed as absorbance at 405 nm *versus* time for HeLa cells, HeLa@Dopa and HeLa@POL (Insets are the photographs of plasma after the plasma recalcification test).

lysing the potential of the HeLa@POLF cells. As shown in Fig. 8, unmodified HeLa cells and HeLa@Dopa cells showed a typical thrombus formation curve; that is, with the prolongation of time, the OD405 absorbance gradually increased, peaked at approximately 20 min and formed a plateau, indicating that the cell surface produced stable fibrin clots. This is mainly because these two types of cell surfaces can only nonspecifically adsorb very little Plg from plasma.[22,28] However, the OD405 absorbance of HeLa@POLF cells was increased to a certain level in approximately 10 min and then rapidly returned to the baseline, indicating that the primary fibrin clot was partially but then lysed by the action of plasmin on the surface of HeLa@POLF cells. The attached photographs of the plasma also prove this point. After the plasma recalcification experiment, the plasma of cells containing unmodified HeLa cells and HeLa@Dopa cells was very turbid, while the plasma of cells containing HeLa@POLF cells remained clear. These data indicated that HeLa@POLF cells have fibrinolytic activity.

Conclusions

In summary, we successfully prepared lysine ligand-containing copolymer-functionalized HeLa cells. The modified cells showed good resistance to fibrinogen adsorption and a high capacity for plasminogen adsorption. Moreover, when exposed to plasma and t-PA, the modified cells showed the rapid lysis of incipient clots formed on them. In addition, the modification method presented here can also achieve good cell viability. We anticipate that our study will provide a novel proof-of-concept to address the massive loss of cells in cell transplantation caused by the IBMIR effect by constructing transplanted cells with fibrinolytic activity.

Author contributions

Shengjie Liu: experimental data collection and writing – original draft. Xingyu Heng: cell experiment guiding and problem solving. Wenjin Wang: assisted in the experiments. He Yang: confocal microscopy experiment guiding and problem solving. Wei sun: problem solving. Zhaoqiang Wu: funding acquisition, supervision, project administration, resources, and writing – review and editing. Hong Chen: supporting supervision.

Conflicts of interest

There are no conflicts to declare.

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