Reactive & Functional Polymers 69 (2009) 905–914

Contents lists available at ScienceDirect

Reactive & Functional Polymers

journal homepage: www.elsevier.com/locate/react

Functional fibrous polypropylene solid support and its application in solid phase peptide synthesis and cell specific binding

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ARTICLE INFO

Article history:
Received 12 August 2009
Received in revised form 9 September 2009
Accepted 20 September 2009
Available online 25 September 2009

Keywords:
Polypropylene fibers
Radical grafting
Solid support
Peptide synthesis

ABSTRACT

Surfaces of polypropylene (PP) fabrics were modified through thermal initiated radical graft polymerization with acrylic acid and poly (ethylene glycol) di-acrylate (PEG-DIA). Various polymerization conditions were studied to determine the optimal parameters leading to the best surface functionality. Carboxylic acid groups on the functionalized PP support were further derivatized for solid phase peptide synthesis. Peptide ligands LHPQF and sppLDI were successfully synthesized through simple coupling reactions utilizing Fmoc chemistry. Specific binding of Streptavidin–Alkaline phosphatase conjugate and Jurkat (T lymphoblastic) cell were successfully achieved on the novel functional PP substrates. The good geometrical flexibility, mechanical stability, chemical reactivity and biocompatibility of this novel planar support make it very promising substrate for various biotechnological applications.

1. Introduction

Solid supports that can satisfy a wide range of requirements for solid phase organic chemistry or biomedical materials have been increasingly needed and extensively explored for decades. These solid supports should provide suitable chemical reactivity, mechanic property, and good biocompatibility. Various forms of polymeric supports have been developed for specific applications such as combinatorial peptide synthesis, enzyme immobilization, DNA microarray [1–6]. Spherical resins made of functionalized cross-linked polystyrene have been widely used for synthesis of peptides and small molecules since the advent of solid phase peptide synthesis chemistry [7–10]. In addition, other forms of solid supports such as Merrifield MicroTube [11], poly(acrylic acid) grafted polypropylene "pins" [12], have been used in solid phase synthesis.

Planar solid supports provide another option for solid phase synthesis of peptides and small molecules. The ease of access and convenience of manipulation provided by the planar supports make them suitable substrates for either automatic or manual biological assays. Various materials have been developed, including glass substrates in spot-synthesis [13], microarray technique [14,15] and light-directed, spatially addressable parallel chemical synthesis technology [16,17], all with dimensional stability and chemical inertness. Natural polymer substrates such as cellulose paper were reported as an excellent solid-phase support for multiple peptide synthesis [18–21] and the generation of peptide libraries [22]. However, most cellulose paper could not survive vigorous mechanical shaking which is necessary to ensure a complete reaction, and has only been successfully used in a flow-through arrangement and spot-synthesis.

Synthetic polymers normally possess good mechanical strength, dimensional stability and flexibility, and organic solvent durability for a wide range of applications. Many synthetic polymers especially polyolefins are chemically inert and non-polar, and stable in many common solvent systems. However, these features often create adverse problems in surface contact of reagents, adhesion, coating, painting, colloid stabilization, etc. Especially in biomedical applications, synthetic polymeric materials often face some critical obstacles such as undesirable protein adsorption, cell adhesion, and aqueous solution compatibility.

Polypropylene and other polyolefins are widely used in biomedical applications as disposable devices, and in the form of membranes and films [23,24]. A variety of research efforts have been devoted on the modification and application of polypropylene planar substrates [1,25–30]. Surface modified polypropylene and other polyolefin textile materials (woven or non-woven fabric and fiber) have attracted more and more interest from various fields such as battery industry, air or water purification, tissue engineering, anti-bacterial materials, biomedical devices [31–34]. Fibrous textiles can be processed into three-dimensional structures, which provides very large or more accessible specific surface

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doi:10.1016/j.reactfunctpolym.2009.09.007

areas than film, sheet and membrane shapes. All these features of polymeric fabric make it a potential solid support for chemical and biomedical applications. Hydrophilic groups such as methyl methacrylate (MMA), 2-hydroxyethyl methacrylate (HEMA)/glycidyl methacrylate (GMA), acrylamide, 3-allyl-5,5-dimethylhydantoin (ADMH), and acrylic acid, have been reported to be successfully grafted onto polypropylene textile materials through plasma, radical or radiation-induced grafting [35–40].

In this study, carboxylic acid groups were introduced onto polypropylene fabric by using a radical graft polymerization process. The combination of carboxylic acid groups and PEG units could result in a mechanically stable, chemically active, hydrophilic surface on polypropylene fibers. After this treatment, two linear peptide ligands, LHPQF and sppLDI, were successfully synthesized on the surface of PP fabric. Later, enzyme (Streptavidin–Alkaline phosphatase conjugate) specific immobilization and lymphoma cell specific binding on LHPQF planar substrate and sppLDI planar substrate, respectively, were successfully carried out, which further confirmed the success of peptide synthesis and demonstrated the potential applications of this new planar polymeric support in biomedical applications.

2. Experimental

2.1. Materials

Polypropylene fabric #976 (PP) was purchased from Testfabrics Inc. (West Pittiston, PA). *N*-Hydroxybenzotriazol (HOBt) and *N*,*N*′-diisopropylcarbodiimide (DIC) were purchased from Advanced ChemTech (Louisville, KY). Fmoc-protected amino acids were obtained from SynPep Corporation (Dublin, CA), Chem-Impex International, Inc. (Wood Dale, IL, USA), and NeoMPS, Inc. (San Diego, CA). *N*,*N*-Dimethylformamide (DMF) was purchased from VWR (Brisbane, CA). Dichloromethane (DCM), methanol (MeOH), diethyl ether, acetonitrile (CH_3CN), acrylic acid (AA), benzoyl peroxide (BPO), poly (ethylene glycol) di-acrylate (PEG-DIA), and 1,3-diaminopropane were purchased from Acros (Pittsburg, PA). 5-Bromo-4-chloro-indolyl-phosphatase (BCIP) was purchased from Bio SynthAG (Zurich, Switzerland), and alkaline phosphatase conjugated streptavidin was purchased from Rockland (Gilbertsville, PA). Jurkat (T lymphoblastic) cell was obtained from American Type Culture Collection (Rockville, MD). The cells were maintained in RPMI 1640, 10% fetal bovine serum, 50 units/mL penicillin, and 50 mg/mL streptomycin (Life Technologies Inc., Rockville, MD). All other chemicals were purchased from Sigma–Aldrich.

Benzoyl peroxide was recrystallized twice from a chloroform and methanol mixture at room temperature and dried in a desiccator.

2.2. Thermal grafting

A piece of PP fabric (about 1 g) was washed by acetone for overnight by extraction in a Soxhlet apparatus followed by washing in boiling water with soap for 30 min. Then, distilled water was used to wash off any residual soap reagent on fabric before it was dried in vacuum for 3 days. PP fabric sample was then soaked in 50 mL pure acetone containing a known amount of poly (ethylene glycol) di-acrylate (PEG-DIA). A known amount of initiator (BPO) was dissolved in 50 mL of acetone and added to the above PP fabric/acetone solution. A defined amount of monomers (acrylic acid) were added into the PP fabric/initiator/acetone mixture. The whole mixture solution was stirred at room temperature for 30 min. The PP fabric was taken out, drained and placed into a preheated curing oven under nitrogen protection at preset temperature for a certain time to pursue the monomer grafting and curing reactions.

Various grafting solution composition and polymerization processing parameters were utilized for comparison to find the optimal modification conditions. The concentration of acrylic acid in grafting solution varied from 0.1 M to 2 M. The weight percentage of BPO in grafting solution varied from 0.01% to 0.2%. PEG-DIA weight percentages in grafting solution were 0%, 0.2%, 0.5%, 1%, 2% and 4%. Heating temperatures were tested at 90 °C, 100 °C, 110 °C, 120 °C, and 130 °C in varied duration of 10 min, 20 min and 40 min, respectively.

The treated fabric was extracted with acetone in a Soxhlet apparatus for overnight to remove any remaining monomers, initiators and ungrafted polymers. The fabric was rinsed by large amount of hot distilled water and dried at 60 °C for overnight. The dried fabric sample was placed in a desiccator for 72 h until it reached a constant weight.

2.3. Amination of carboxylic acid groups

The modified PP fabric (1 g) was cut into pieces of 1 cm × 1 cm and mixed with equivalent molar HOBt and DIC in 50 mL DMF solution for 15 min at room temperature. The fabric was washed by shaking in DMF (3 × 30 mL, 5 min each time) at room temperature. The activated PP fabric was soaked and shaken with extra amount of 1,3-diaminopropane in 50 mL DMF for overnight. After the amination reaction, PP fabric was washed thoroughly with DMF (3 × 30 mL), Methanol (3 × 30 mL), and DMF (3 × 30 mL). The amination result was examined by a Kaiser test. Since each active –COOH on PP surfaces could react with 1 equiv. diamine to produce 1 equiv. primary amine, the amount of primary amine groups resulted from this reaction could represent the amount of active carboxylic acid groups on the materials.

2.4. Peptide synthesis on aminated PP fabric

Peptides were synthesized on the aminated polypropylene fabric using Fmoc chemistry and DIC, HOBt activation agents. Every amino acid unit contained protected amino ends and side chains. Five equivalents of amino acid, HOBT and DIC were mixed with DMF for 10 min before adding aminated PP fabric. Coupling reaction of PP fabric with amino acids was carried out for overnight. After the coupling reaction was over, the fabric was washed with DMF (3 × 30 mL), MeOH (3 × 30 mL), DMF (3 × 30 mL), respectively. Kaiser test [41] was carried out to monitor the amino acid coupling reaction. Fmoc protecting group on amino end was removed by shaking PP fabric in 25% methyl piperidine/DMF solution for 20 min twice. The PP fabric was then washed three times with DMF, MeOH, and DMF sequentially before proceeding to the next amino acid coupling. Again, Kaiser test was used to monitor the success of Fmoc cleavage from amino terminal of amino acid. The above coupling and Fmoc deprotection procedures were repeated until the whole sequence was completed as illustrated in Scheme 1. After the completion of the last amino acid coupling and Fmoc deprotection, PP fabric was thoroughly washed twice with DMF (3 × 30 mL), MeOH (3 × 30 mL), and DCM (3 × 30 mL). The washed PP fabric was allowed to air dry at room temperature for 30 min to remove DCM and placed in a desiccator for overnight. The side chain protection groups of the amino acids in the peptide sequence were removed by shaking in a TFA side chain cleavage cocktail composed of 82.5% TFA, 5% water, 5% phenol, 5% thioanisole and 2.5% EDT for 2 h. PP fabric was then washed by TFA (2 × 30 mL), water (2 × 30 mL), DMF (3 × 30 mL) and DCM (3 × 30 mL). The fabric sample was air dried for 30 min and stored in desiccator. The sequence of synthesized peptide on PP fabric was verified by Applied Biosystems Procise 494 Protein Sequencer.