A Combinatorial Approach in Designing Hydrophilic Surfaces for Solid-Phase Peptide Synthesis

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Received 14 June 2002; revised 2 January 2003; accepted 2 January 2003

ABSTRACT: A hydrophilic surface suitable for solid-state peptide synthesis was developed on a solid support called a lantern. The split-and-mix combinatorial technique was used to prepare about 500 surfaces in a very short time. Surfaces were analyzed according to values for gel formation, percentage weight grafted, grafted copolymer composition, and number of functional groups per lantern. These values were correlated to the purity of a peptide synthesized on these surfaces. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 3371–3378, 2003

Key words: peptides; synthesis; surfaces; supports

INTRODUCTION

Merrifield[1] pioneered solid-phase synthesis of peptides, and since then polymer supports have been the subject of considerable and increasing interest as insoluble matrices in organic synthesis.[2] This conceptual revolution in organic synthesis was mainly governed by the need to rapidly generate libraries of compounds in the field of drug discovery. A traditional organic chemist mixes two or more chemicals in a vessel, provides the correct reaction conditions, and at the reaction end undertakes purification to recover the intended compound. A solid-phase chemist attaches one of the chemicals onto a solid, which is then reacted with an excess of the other reagents to drive the reaction to completion. At the end, the intended compound, still attached to the solid, is removed physically from the mixture, cleaved, and recovered. In addition to the advantages already mentioned of forcing reactions to completion and simplifying product isolation, this also has the advantage of allowing parallel processing and the possibility of automation of the reactions.

The physical and chemical properties of particular solid supports play a decisive role in which support is used for a specific synthesis. Initially, crosslinked polystyrene resins were used as solid supports. The access of reagents to the reactive sites within the polymer matrix is critical to the success of the synthesis.[3] For example, macroporous polymer beads are highly crosslinked materials with a rigid structure that remains porous even in a dry state. This enables the reagents to have access to the sites within the pores of the bead in virtually any solvent. However, only the surface of the pores of these resins have accessible reactive sites, thus limiting their loading capacity or the number of reactive sites. In contrast, the reactive sites of lightly crosslinked gels are accessible only after the beads are swollen in a suitable solvent. Merrifield resins (polystyrene crosslinked with 1% divinyl benzene) swells best in low-polarity aromatic solvents, halogenated hydrocarbons, and tetrahydrofuran. But these beads do not swell in polar solvents. The problem of reaction site accessibility in a broad range of solvents is typically addressed by incorporating a hydrophilic polymer such as polyethylene glycol onto the resins.[4]

In small-molecule synthesis medicinal and organic chemists quickly realized commercially available resins have some limitations. Greater mechanical stability and, therefore, higher crosslinking were required to cope with the more demanding synthesis conditions. There was also a greater need to maintain bead integrity and eliminate resin fragmentation. Higher crosslinking, however, limits the number of reactive sites and thus created difficulty in synthesizing sufficient quantities of the compounds.

A pellicular design, in which the actual solid support is permanently bound onto the surface of another rigid material, overcomes these problems, and the "pins" concept, developed by Geysen et al.,[5] is based on this. A mobile polymer was grafted onto rigid polyolefin pins of different shapes. Over the years Mimotopes Pty, Ltd., has developed a number of different grafted pins that have allowed increasing the quantity of chemicals to be synthesized.[6] Unlike low crosslinked swelling beads, it is the surface area of the grafted support and not the volume that determines the loading capacity. It is easy to achieve consistency

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Journal of Applied Polymer Science, Vol. 89, 3371–3378 (2003)
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Figure 1 Comparison between a lantern and the amount of resin required to obtain the same loading.

in reaction kinetics between grafted surfaces of different sizes and shapes. This is impossible with microporous beads, as the diffusion path length must change with size, and as a consequence, reaction rates must change. Using the "lantern" shape (Fig. 1) with polystyrene-grafted surfaces has been shown to produce better reaction kinetics than do the commercially available resins.[7] However, as discussed above, polystyrene is not a compatible polymer with some polar solvents, particularly for aqueous chemistry. This article reports on an approach taken to develop a hydrophilic surface of the lantern. Preparation of lanterns involves three distinguishable steps: molding a base polymer, grafting a second polymer, and attaching spacers and linkers. Figure 2 shows a schematic diagram of the surface of a lantern.

EXPERIMENTAL

Materials

Different grades of polyethylene (PE) and polypropylene (PP) were obtained from Montell (Melbourne, Australia). 4-Methyl-1-pentene (TPX) was obtained from Mitsui Company (Sydney, Australia). Ethylene vinyl acetate (EVA) was obtained from Qenos (Melbourne, Australia). The monomers—acrylic acid, methacrylic acid, hydroxyethyl acrylate, hydroxy ethyl methacrylate, dimethyl acrylamide, polyethylene glycol methacrylate, *n*-vinyl pyrrolidinone, and vinyl acetate—were obtained from Aldrich and were used without purification. The linkers were obtained from the companies indicated: Fmoc Rink and hydroxymethyl phenoxy acetic acid handle (SC-HMP) from Senn Chemicals, hydroxymethyl phenoxy pentanoic acid handle (LC-HMP) and 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid (BAL linker) from Advanced ChemTech, 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid (hyperlabile linker) from Novabiochem, and 4-α,α-diphenylhydroxymethyl)benzoic acid (trityl linker) from PepChem. The spacers were obtained as indicated: Butoxycarbonyl (Boc)-HMD (hexamethylenediamine) from Fluka, 9-fluorenylmethoxycarbonyl (Fmoc) amino acids from Senn Chemicals, poly(ethylene glycol) from Merck and jeffamine from Fluka. Fmoc chloroformate, used as a blocking agent, was obtained from Aldrich. The chemical initiators, benzoyl peroxide (BPO) and tertiary butyl peroxy hexanoate (TBPEH), were obtained from Interox (Sydney, Australia) and were used as obtained. Potassium bromide and sodium metabisulfite were obtained from Aldrich to be used as redox initiators. *N,N*-Dimethyl-para toluidine, used as an activator for peroxide initiation, was obtained from Aldrich.

Injection molding

The polyolefins were injection-molded to the lantern shape using a Kawaguchi 45-ton B-type injection-molding machine and a sliding core tool. Samples were prepared using different barrel temperatures, cooling times, injection speeds, and injection pressures.

Grafting

Solutions of the monomers in the required solvent and concentration were prepared and added to a vessel containing the lanterns. Each lantern had 0.42 mL of solution. This solution was degassed by passing nitrogen through it, and the lid was tightly closed. Initiators were added for thermal initiation during the preparation of the monomer solutions. The polymerization/grafting was effected either by heating the sample or by exposure to gamma radiation, which was carried out in a gamma cell at a dose rate of 1.5 Kgy/h.

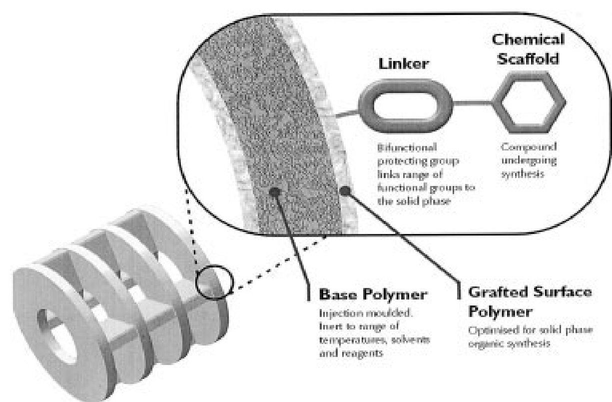


Figure 2 Surface of the lantern.

A variable dose rate was achieved by using attenuators.

After polymerization/grafting the solution was decanted, and the lanterns were washed with a "good" solvent of the polymer to remove the homopolymer. After the final wash the grafted lanterns were dried in a vacuum oven overnight at 25°C. The graft percentage was calculated using the initial and final weights.

Infrared spectra

ATR spectra of the lantern surface were obtained by using a split-pea accessory[8] attached to a Perkin–Elmer 2000 series instrument. A total of 16 scans with a spectral resolution of 16 cm^{-1} were accumulated. The lanterns were used without further sample preparation; hence, this was a nondestructive method.

Coupling spacers/linkers

The first spacer was attached to the functional group on the functional grafted monomer. For example, for methacrylic acid the functional group was carboxylic acid, and for hydroxy ethyl methacrylate it was the hydroxy group. The spacers were attached using chemistry described elsewhere.[6,9] The final spacer was always a Fmoc-protected amino acid. The Fmoc group was then deprotected, and the linker was attached using HOBT and DIC as activating agents. After every coupling reaction the lanterns underwent two dimethylformamide (DMF) washes, three methanol washes, and a final DCM wash to remove any unattached chemicals.

Loading determination

The loading at any stage of the coupling was defined as the amount of Fmoc-protected group (amino acid or linker) attached to the lantern. It is expressed in micromoles per lantern. After the final wash the lanterns were dried in a fume hood for 1 h. Each lantern was treated with 10 mL of 20% piperidine–DMF for 45 min, and then 1 mL of this solution was diluted with 10 mL of the original piperidine–DMF solution, and the UV absorbance was read in a 1-cm cuvette at 301 nm. Loading was calculated from the following equation.

$$\text{Loading } (\mu\text{mol/lantern}) = [(A_{301}/\varepsilon) \times 11 \times 10^4]$$

where $\varepsilon = 7800^{-1}$ cm^{-1}.

This method was explained in more detail in a previous article.[10]

Peptide synthesis

Peptides were synthesized on a Symphony Multiplex peptide synthesizer (Rainin Instrument Co., Inc., Protein Technologies., Inc.) using Fmoc-protected amino acids. A maximum of 12 lanterns was included in one vessel during synthesis; hence, 12 surfaces could be evaluated simultaneously. Fmoc deprotection was done twice for 2.5 min with 20% piperidine–DMF. Amino acids were coupled twice for 20 min using N-methyl morpholine (NMM)/N-hydroxy benzotriazole (HOBT)/2-(1-H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) for activation in DMF. The coupling mixture was composed of 75 mmol AA, 200 mmol NMM, 75 mmol HOBT, and 72 mmol HBTU (ratio 1:2.6:1:0.97). Cleavage of the peptides was performed manually using a cleavage solution of trifluoroacetyl (TFA), H_2O, thioanisole, anisole, ethane dithiol, and triisopropylsilane (81.5:5:5:2.5:2.5:1.0 v/v/v/v/v/v).

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) were run on a Waters 2690 system equipped with a Waters Millennium 2020 client/server control, a Waters 996 PDA photodiode array UV-vis detector, and a Monitor 5-μm C18M (150 × 4.6 mm) column. The operating temperature was 10°C in the sample carousel and 35°C in the column heater. The following buffer systems were used: A—100% water + 0.1% TFA, B—90% acetonitrile + 10% water + 0.1% TFA. The protocol for eluting the samples was: 0–1 min isotactic at 10% B, 1–16 min linear gradient to 66.6% B, 16–18 min isotactic at 66.6% B, 18–19 min linear gradient to 100% B, 19–25 min wash, and reequilibration to starting conditions. Detection was done at 214 and 254 nm.

Mass spectroscopy

Mass spectra were obtained using a PE Sciex API-III(+) mass spectrometer equipped with a Perkin–Elmer Sampler 200 autosampler and an Applied BioSystems 140B solvent delivery system. Operating conditions were: flow rate of 40 μL/min, scan range of 200–2200 AMU, scan step of 0.2 AMU, and scan duration of 2.5 min (14 scans). The buffer solution used was 25% water, 75% acetonitrile, and 0.1% acetic acid.

RESULTS AND DISCUSSION

As mentioned above, there are three distinct steps in the preparation of a lantern surface: (1) injection-molding a polyolefin into the shape, (2) grafting a second polymer, and (3) coupling spacers and linkers. It is possible to use many variables during these three

steps, and it was the intention in this work to prepare as many surfaces as possible for evaluation. The effects of the following variables were examined:

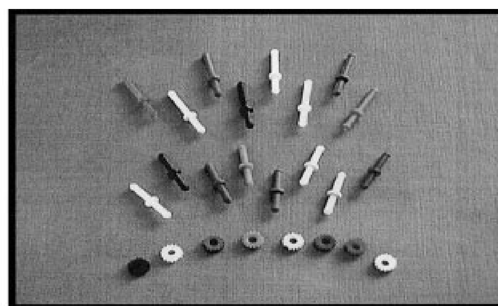
1. Injection molding- type of polymer, barrel temperature, injection time, injection pressure, cooling time, cooling temperature;
2. Grafting—monomer type, comonomer composition, method of grafting, temperature of grafting, time of grafting, initiator type and concentration in thermal grafting, radiation dose in radiation grafting, solvent, solution concentration, mixed solvents;
3. Coupling—spacer and linker type, concentration, reaction time, reaction temperature, activator type and concentration.

EVA, though more hydrophilic, was found to be unsuitable for this application as it was soluble in many common solvents. PE gave a slightly heavier lantern than PP molded under the same conditions. The molding conditions used had no effect on the grafting efficiency for individual polyolefins. PP lanterns produced a greater weight increase compared to PE after grafting under the same conditions.

Thermal and radiation grafting were the two methods investigated. Temperatures between 50°C and 75°C were used with peroxide initiators, whreas lower temperatures such as 30°C–40°C were used with redox-initiating systems and peroxide initiation activated by an amine. *In situ* radiation grafting was carried out only at room temperature, and dose rates between 0.75 and 1.5 kGy were evaluated. The preirradiation method was applied with an initial irradiation of the molded polyolefin for doses between 20 and 100 kGy and subsequent polymerization at temperatures between 50°C and 75°C.

It is known[11] that solvents affect the grafting efficiency of monomers onto polyolefins. This may have a significant effect on copolymer systems. The effect of solvents such as DCM, water, methanol, THF, DMF, cyclohexane, and their mixtures with different ratio were investigated.

Taking into account all these variables, it was possible to prepare thousands of different surfaces for evaluation. Preparing and analyzing the surfaces one by one was not practical. To evaluate multiple surfaces concurrently, we adapted techniques developed in the drug discovery industry. Over the past decade the simultaneous synthesis of large numbers of compounds has become an important tool in the drug discovery process. Using the high-throughput techniques developed for this new branch of chemistry (termed combinatorial chemistry), we have synthesized and evaluated many hundreds of grafted surfaces. Importantly, in order to track the many different surfaces concurrently, a method of physical tagging is required. Unlike resin solid supports, the lantern shape allows physical tagging. There are several tagging methods available,[12] but color coding is the simplest. It is possible to attach a color tag to the lanterns as shown in Figure 3. Once the color tag is attached, it is possible to trace a particular Lantern during and after any treatment. An advantage of color coding is that it allows for a synthesis method called split and mix.[13]



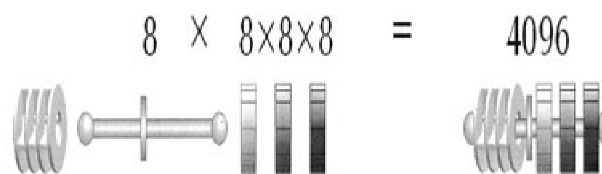


Figure 3 Color coding.

The split-and-mix method is shown in Figure 4. In the example shown, the first step could be the grafting reaction carried out in five vessels in parallel, each with a different condition. After washing and drying, the color tags are attached and mixed. The second step may be the coupling of the same spacer to the lanterns prepared in all five conditions. Once this reaction is completed, the lanterns are divided into three, this time one lantern from each of the five grafting conditions into one vessel. This way, three different linkers can be attached in parallel. At the end of the three reaction steps, 15 different surfaces have been prepared. The number of vessels used in each step, the order of split/mix steps, and the number of steps can be varied as the experiment designer wants. Taking this approach, it is possible to prepare many different surfaces in a short time.

It is also important to use high-throughput analysis methods to screen the surfaces that have been developed. Obviously, results of all the surfaces cannot be reported in an article like this. Hence, results from only selected examples are given to demonstrate the point. The initial screening of the grafting condition was done depending on the gel formation. As given in the Table I, some grafting conditions resulted in a gel. As shown in Table I, the MA–DMA graft carried out in

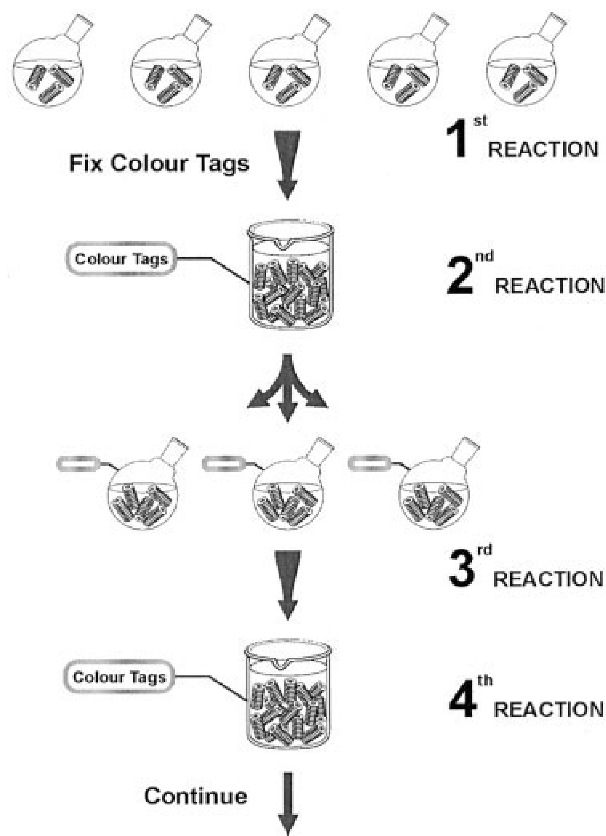


Figure 4 Split-and-mix technique.

30% methanol using high-energy radiation produced no gel, and thus separation of the lantern from the solution was easy. However, when the radiation grafting was carried out in 20% water, the solution gelled, and washing and recovery of the lantern was not possible. Any grafting condition that resulted in gelled solution was rejected, and no further purification or work carried out.

Lanterns from conditions that resulted in no gelation were washed to remove any homopolymer, dried, and weighed. The percentage of weight increase was calculated by using the graft and ungrafted weights of the lantern. Any grafting condition that resulted in a percentage weight increase of less than 10% or more

TABLE I
Examples to Show the Reaction Conditions on Gel Formation During Grafting

Monomer system	Solvent system	Concentration	Result
MA–DMA	Methanol	30%	No gel formation
MA–DMA	Water	20%	Gel formed
HEA	Methanol	15%	Gel formed
HEMA–DMA	Methanol	30%	Gel formed
HEMA–DMA	Methanol–Cyclohexane	20%	No gel formation

TABLE II
Examples to Show the Effect of Reaction Conditions on Graft Percentage

Monomer system	Solvent system	Concentration	Time of grafting	Graft (%)
MA–DMA (10–20)	Methanol	30%	7	35
MA–DMA (10–20)	Methanol –DCM	30%	4	68
HEMA–DMA (10–20)	Methanol	30%	7	6
HEMA–DMA (20–10)	Methanol	30%	7	28
MA–styrene (20–10)	Methanol	30%	7	4
MAC–styrene (20–10)	Methanol	30%	7	17

than 30% was rejected. A percentage weight increase of less than 10% was considered insufficient to give acceptable loadings, which we have determined through our experience in working with lanterns. A weight increase of more than 30% distorted the shape of the lantern. A fraction of the results are given in Table II. Of the samples shown, only HEMA–DMA and MAC–styrene at 30% concentration and 20:10 ratio irradiated for 7 h resulted in an acceptable condition. It is worth mentioning that either by increasing or reducing the time of grafting or solution concentration, it was possible to obtain acceptable graft weights with most solvents and monomer ratios. For example, by increasing the solution concentration to 50%, HEMA–DMA grafts in a 10:20 ratio achieved a weight increase of between 10% and 30% weight increase.

It was also possible to change the percentage of grafting by changing the solvent type. For example, a 10% MA–DMA solution (ratio of 7:20) in methanol produced no significant grafting. However, partly replacing methanol with cyclohexane gave 19%, 47%, and 64% weight increases with 5:1, 2:1, and 1:1 ratios of methanol:cyclohexane, respectively. No such effect was observed when ethanol was used instead of methanol. Changing the monomer ratio also changed the weight grafted under the same conditions. For example, changing the MA–DMA ratio to 4:21, 7:18, and 8:17 (in a 10% solution of methanol:cyclohexane at a 1:1 ratio) yielded weight increases of 43%, 53%, and 54%, respectively.

It was found that the grafting of vinyl acetate in methanol was very minimal. But the addition of small quantities of water improved the weight increase. The addition of 160 μL to a 1-mL solution of 75% VA–methanol improved the weight grafted by 100%. It is therefore possible to manipulate the experimental parameter to obtain a weight increase within the required range. However, even with the same monomer system the parameters needed to be changed when using different ratios.

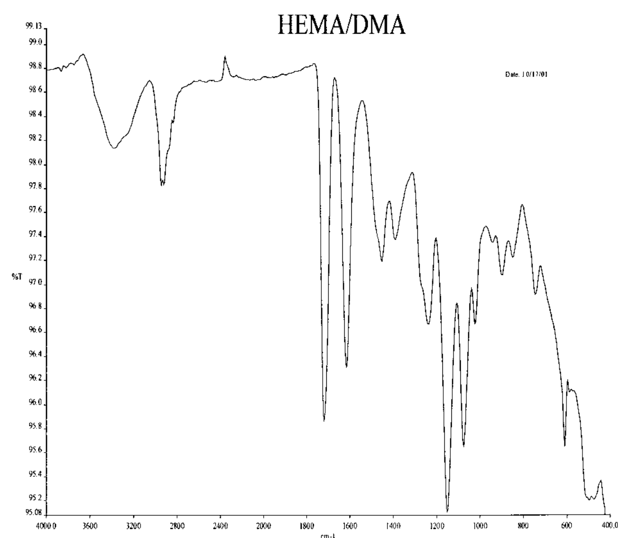


Figure 5 ATR spectrum of surface-grafted copolymer of HEMA and DMA.

ATR spectra of the lanterns (from the conditions screened positive to percentage graft weight) were obtained using a nondestructive method called the split-pea technique. A typical spectrum obtained for a HEMA–DMA graft is shown in Figure 5. The carbonyl peaks of the methacrylate and amide are readily assignable as shown in Figure 5. This allowed the estimation of the monomer ratio in the grafted polymer. This estimation was not accurate but was sufficient for the purpose described below.

All the lanterns that passed the initial two screening tests (gel and weight gain) were then used for coupling different spacers and linkers. If the functional monomer was an acid (acrylic or methacrylic acid), then the first spacer was a mono-protected diamine. Different reaction conditions for the coupling reaction were tested. The protected amine was then deprotected, and the second spacer, preferably an Fmoc-protected amino acid [alanine (A) or glycine (G)] was coupled. Different reaction conditions for the amino acid coupling were tested. Depending on the reaction condition, the amount of amino acid coupled varied. The balance of the amine groups of the diamine was blocked by acetylation. The Fmoc group was then cleaved and the loading determined by measuring the amount of Fmoc chromophore released by UV. The Fmoc-deprotected amine group of the amino acid was then used to couple the Fmoc-Rink linker.

Similarly, if the functional monomer was hydroxy (HEMA or hydroxy-PEGMA), then Fmoc alanine or glycine was coupled to the hydroxy group, and after acetylation of the uncoupled hydroxy groups, the Fmoc group was cleaved and the loading determined. The deprotected alanine or glycine was then coupled with Fmoc-Rink linker, acetylated, the Fmoc group cleaved, and loading determined as before.

Using different reaction conditions, the amount of Fmoc-Rink linker coupled could be varied and the balance of the amine groups acetylated as before. Finally, the Fmoc groups in the Rink were cleaved and the amount determined to estimate the Rink loading. Because of the acetylation, the Rink could be coupled only to the amino acid; hence, the Rink loading was always similar to or lower than the amino acid loading. Any lanterns resulting in a Rink loading of less than 5 μmol were rejected. A fraction of the results can be found in Table III to show the effect of type of monomer and amount grafted on the Gly and Rink loading. As there was a drop of at least 1 μmol of loading from the first coupling (glycine or alanine) to the second coupling (Rink amide), any lanterns with first coupling loading of less than 6 μmol were also rejected.

Out of 500 surfaces created, only about 200 surfaces passed the screening tests up to this point. The lanterns that passed the screening of the loading test were then used in synthesis of two peptides, namely, VQAAIDYING (ACP 65–74) and CYFQNCPKG (Vasopressin, Lys 8). Each letter in the peptide sequence represents an amino acid. These two peptides were selected because of their ease of preparation. It is known that the peptide ACP 65-74 is a difficult peptide to synthesize on solid supports.[14] After synthesis

TABLE III
Examples to Show the Effect of Type of Monomer and Amount Grafted on Loading

Monomer system	Graft (%)	Gly loading (μmol/lantern)	Rink loading (μmol/lantern)
MA–DMA	26	9.3	8.4
MA–PEGMA	12	6.5	<1
MA–VP	11	7.2	<1
MA–VA	25	6.8	<1

TABLE IV
Dependence of the Purity of the Peptide VQAAIDYING Synthesized on MA–DMA Surfaces with Different Loadings

Loading (μmol/lantern)	Purity (%)
6.5	51.3
7.7	52.2
8.5	47.0
12.8	26.5

TABLE V
Dependence of the Purity of The peptide VQAAIDYING on the Monomer Ratio

Monomer system	Loading	Purity (%)
MA	8.3	12.1
MA–DMA ratio 7–20	8.5	42.0
MA–DMA ratio 10–20	8.5	47.0

TABLE VI
Effect of the Monomer System on the Purity of the Peptide VQAAIDYING

Monomer system	Loading	Purity
HEMA–DMA	10.7	13.0
MA–DMA	12.8	26.5
MA–DMA	7.7	52.2
MA–VP	5.4	31.1
HEMA	7.0	21.0
HEMA–PEGMA	7.2	28.0
PEGMA	7.0	61.0
PEGMA–MMA	7.7	48.2

the peptides were cleaved and analyzed for purity by HPLC and mass spectrometry and the yield calculated by weight. The aim was to improve the purity and yield of peptide 1 without compromising too much on peptide 2 purity and yield.

Some of the results are summarized in Tables IV–VIII. Table IV indicates the effect of increased loading on purity. On the MA–DMA surface, 8.5 μmol/lantern was a critical loading, above which the purity dropped significantly. However, we noticed that this critical loading depended on the surface. The effect of the MA–DMA ratio on the purity is evident in Table V. Increased DMA in the feed composition increased the DMA in the grafted surface, as observed by Fourier transform infrared spectroscopy. This results in improved purity. However, a further increase in DMA content in the composition limited the final loading. In addition, there was a maximum amount that could be grafted because of distortion of the lantern shape.

Table VI shows the effect of some of the different monomer sets on the final purity of the synthesized peptide. HEMA–DMA was a very poor surface. The purity on the surface at 10.7 μmol of loading was lower than that on the MA–DMA surface at a higher loading. HEMA, HEMA–PEGMA, and MA–VP surfaces also were found to be not as good as the MA–DMA surface. In the HEMA–PEGMA graft, HEMA is the functional monomer, whereas PEGMA is dormant, with a methoxy group at the end of the PEG chain. However, PEGMA and PEGMA–MMA surfaces showed comparable or better results than those of the MA–DMA surface. The PEGMA in these cases was a functional monomer with an hydroxy group at the end of the PEG chain.

TABLE VII
Effect of Spacer Type on the Purity

Spacer Type	Loading (μmol)	Purity (%)
HMD–G	7.7	52.0
HMD–GAG	8.0	56.0
HMD–GGG	7.9	53.0
DEGA–A	8.1	48.0
DEGA–G	8.0	59.0

TABLE VIII
Development over Time

Stage	Loading (μmol/lantern)	Purity of peptide VQAAIDYING	Purity of peptide CYFQNCPKG
1	6.0	22.3	57.6
2	8.0	35.8	49.9
3	8.0	49.6	49.9
4	7.0	74.2	67.1
	15.1	57.1	62.3

Table VII shows some results of the effect of spacers on the purity of the peptide. This was an MA–DMA graft where the acid group was first coupled with either hexamethylene diamine (HMD) or diethylene glycol amine (DEGA). They were then coupled with either a single amino acid (G or A) or a three-member sequence of G and A. Final coupling was the linker Rink. The results indicated a very marginal effect on the length of the amino acid sequence. But the DEGA spacer showed a significant increase in purity over the HMD spacer if the glycine were coupled.

The purity and yield values were correlated with the graft weight increase, monomer ratio, spacer type, and loading, as discussed above, and were optimized. The results obtained at different stages of the project are given in Table VIII. Stage 1 is the polyHEMA-grafted crowns. Prior to the design of the lanterns, the grafted supports marketed by this company were called crowns. Stage 2 is the result after optimizing the monomer ratio; stage 3 is the result after optimizing the percentage graft, and the final stage is the result after optimizing the spacers and linkers.

CONCLUSIONS

It is possible to use high-throughput methods to develop many surfaces in a short time. If proper methods are developed to characterize these surfaces, then the time of development of a targeted surface can be drastically reduced.

The purity of the peptides synthesized depended on the surface properties and the loading on the surface. A surface suitable for solid-phase peptide synthesis was developed. The purity of the peptides synthesized was comparable or better than commercially available resins.

Using proper high-throughput experimental methods, a significant improvement in the hydrophilic surface for peptide synthesis was achieved in a relatively short time.

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