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Abstract

Molecularly imprinted polymers (MIPs) were developed to replace natural antibodies with a cost-effective and durable synthetic material. Molecular imprinting of proteins conventionally utilizes the whole protein as the template, which is complex (as many different epitopes may be imprinted) and expensive. In this work, seven peptides (13-18 amino acids) were synthesized and used as templates for the imprinting and recognition of Regenerating Protein 1 (REG1). REG1 is involved in the proliferation and differentiation of diverse cell types, and was recently described as a urinary biomarker for pancreatic ductal adenocarcinoma (PDAC). Peptide-imprinted poly(ethylene-*co*-vinyl alcohol)s (PIPs), containing four different mole fractions of ethylene were cast on screen-printed electrodes to find the optimum composition for both the sensing and the extraction of REG1 in an *E coli* culture medium. Peptides with fewer than 16 amino acids and two or three aromatic and hydrophobic groups have a higher affinity for MIPs of poly(ethylene-*co*-vinyl alcohol) (EVAL) with 27 mole% of ethylene, while those with four aromatic and hydrophobic groups have a higher affinity for MIPs with EVALs that contain 32 mole% of ethylene. The peptide / EVAL combination that maximized both imprinting effectiveness and response to REG1B was the sequence NEDRETWVDADLY imprinted into 32 mol% EVAL. This EVAL composition and template peptide were then modified by incorporation of magnetic nanoparticles, thus extending applications for PIPs to include extraction of REG1 protein from *E coli* culture medium.

Keywords: peptide imprinting; Regenerating protein; electrochemical sensing; extraction.

1. Introduction

The sensing of proteins and their extraction from biological fluids are of great interest in the biomedical field.[1] Molecularly imprinted polymers (MIPs) are an attractive and inexpensive technology for these applications; they can be coated on the surfaces of transducers and nanoparticles and used instead of natural antibodies in the recognition of molecules in biosensing[2] and bioseparation.[3] Conventionally, the molecular imprinting of proteins (such as lysozyme and albumin)[2] is conducted using whole molecules, but the imprinting of peptide epitopes has only recently been demonstrated. For example, the imprinting of a 16-residue peptide (lysozyme C, 1.8 kDa) on a porous silica scaffold enhanced the binding of the whole protein (lysozyme, 14 kDa).[4] Li's group extensively investigated the imprinting of 9- to 15-mer albumin fragments[5] with zero, one or two mutated residues[5-6] for albumin sensing[5, 7] and adsorption.[6b] A peptide with four amino acids (YPLG) has been utilized as a template for imprinting MIPs (composed of methacrylic acid (MAA) and ethylene glycol dimethylacrylate (EGDMA)), which were then used in the recognition of the larger peptide oxytocin.[8] A 15-mer peptide (TQLRYSWKTWGKAKM) that contained 90-95 units of the Japanese encephalitis virus nonstructural protein 1 (NS1) has been used as a template for the recognition of Dengue virus protein by a quartz crystal microbalance (QCM) chip.[9] More recently, multiepitope peptides were imprinted on poly(ether sulfone) particles for the simultaneous capture of several model proteins, including human serum albumin, immunoglobulin G and transferrin.[10] Shea's group has synthesized imprinted polymer nanoparticles that have a specific binding affinity for the bee toxin melittin (Mel), a 26 amino acid peptide.[11] These nanoparticles can capture and clear a target peptide toxin in the bloodstream of living mice.[12] Molecularly imprinted polymers can be prepared for the recognition of proteins via the generation of peptide-fragment binding sites by semi-

covalent imprinting and enzymatic digestion.[13]

MIPs can be combined with electrochemical analysis to make electrochemical measurements conveniently and rapidly; this combination is thus useful in a quick screening for the optimal composition of MIPs. Piletsky and Turner,[14] Blanco-López *et al.*,[15] McCluskey *et al.*,[16] Rao and Kala[17] and Suryanarayanan *et al.*[18] have all separately reviewed MIP-based electrochemical sensors.

In this work, seven peptides (13- to 18-mers) from Regenerating Protein family (REG1A and REG1B) were used as templates for molecular imprinting. REG1 proteins were recently identified as a biomarkers in urine for early detection of pancreatic adenocarcinoma,[19] and are therefore an important and novel target for MIP development. Poly(ethylene-*co*-vinyl alcohol)s, EVALs, that contained four concentrations of ethylene from 27 mole % to 44 mole % were utilized to prepare peptide-imprinted polymers (PIPs) by phase inversion, and these were then electrochemically analyzed. EVALs were chosen as the templating polymer, owing to their advantages of low cost and ease of preparation. Additionally, they form non-covalent complexes with template molecules, which allows template removal without the use of strongly acidic or basic solvents. Finally, EVALs allow rapid screening of different co-polymer mole ratios to identify those that offer the strongest binding and/or selectivity. This approach – varying MIP composition in a study of multiple peptide templates – should help to establish guidelines for MIP design.

MIPs made using optimal ethylene mole ratios of the EVALs for the imprinting of the seven peptides were then used to sense REG1B in *E coli.* culture medium. Finally, magnetic peptide-imprinted polymer nanoparticles were prepared and characterized. These PIP-based sensors with incorporated magnetic nanoparticles were then successfully used for the extraction of REG1B from an *E coli.* culture medium.

2. Experimental Section

2.1 Reagents and chemicals

In Table 1, peptides NEDRETWVDADLY (Peptide 7), KESGTDDFNVWIG (Peptide 5), KSWGIGAPSSVNPGYCVS (Peptide 3), SSTGFQKWKDVPCEDK (Peptide 1) of REG1A; SCSGFKKWKDESCEKK (Peptide 2), KSWDTGSPSSANAGYCAS (Peptide 4), KESSTDDSNVWIG (Peptide 6) of REG1B were ordered from Yao-Hong Biotechnology Inc. (HPLC grade, New Taipei City, Taiwan). Sodium dodecyl sulphate (SDS) and poly(ethylene-*co*-vinyl alcohol) (abbreviated as EVAL) with ethylene 27, 32, 38 and 44 mole % were from Sigma-Aldrich Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO) was purchased from Panreac (Barcelona, Spain). Potassium ferrocyanide and potassium ferricyanide were both from J.T. Baker Chemical Co. (Center Valley, PA). Potassium chloride was from Showa Chemical Industry Co., Ltd. (Tokyo, Japan). De-ionized water, produced by a PURELAB Ultra (ELGA, Albania), used in the preparation of buffers and for rinse solutions was 18.2 MΩ-cm in resistivity. Human REG1B cloned in Escherichia coli (*E. coli*) (#g1004044D06) was purchased from Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan. The bacterial culture medium was prepared by adding 25g LB broth powder (BD Difco^{TM}) in 1L deionized water, which was autoclaved for 2 h at 121 ^{0}C and 1.25 atm, and cooled to room temperature. Ampicillin (Sigma-Aldrich) 0.1 g was added to the culture medium before use.

2.2 Preparation and characterization of peptide-imprinted polymers coated sensing chips

The preparation protocol for forming peptide-imprinted (PIPs) and non-imprinted (NIPs) polymeric thin films on the working electrodes were adapted from previous reports,[20]

and include three steps; (1) Two microliters of the EVAL solution with or without 1.0 mg/mL of template peptides were pipetted on the working gold substrate of a screen-printed electrode (4 mm diameter, DropSens, Spain); (2) Electrodes were placed in an oven at 50 ^{o}C for 6 h to enhance the evaporation of DMSO; and then (3) the template peptide was removed by washing with 10 mL of 0.1 wt % aqueous SDS and DI water three times.

The electrochemical analysis was performed by sample injection into a flow-cell (DRP-FLWCL, DropSens, Spain) for screen-printed electrodes (4 mm diameter, DropSens, Spain). The working, counter and Ag/AgCl reference electrodes were covered by injecting ca.10 ⌈L of an aqueous solution of 500 mM KCl, 20 mM $K_4Fe(CN)_6$ and 20 mM $K_3Fe(CN)_6$. The electrochemical reactions were controlled and monitored with a potentiostat (608-1A, CH Instruments, Inc., Austin, TX). The current response of the imprinted polymeric sensing electrodes was assessed using cyclic voltammetry. The potential was scanned from -0.6 V to +0.6 V at 0.1 V/s and the effects of imprinted peptides, interferent molecules and real samples on the peak currents for the ferri-/ ferrocyanide system were recorded. All measurements in this work were carried out with at least two replicates; data are expressed as means and standard deviations.

Peptide- and non-imprinted EVAL films were freeze-dried before examination by a scanning electron microscope (Hitachi S4800, Hitachi High-Technologies Co., Tokyo, Japan) and electron spectroscopy for chemical analysis (ESCA). ESCA (Axis Ultra DLD, Kratos Analytical Inc., Manchester, UK) was employed to measure the elemental composition of the PIP and NIP films.

2.3 Collection of E coli. Culture Medium Containing REG1B

One milliliter *E. coli* (10^6 cell/mL) was added to 200 mL fresh LB medium in a 500 mL flash bottle and incubated overnight. The *E. coli* culture was further incubated at 30°C for 10 days in a shaking incubator, by which time the medium became noticeably turbid owing to the growth of the *E. coli* culture. The *E. coli* was then separated from the medium by centrifugation (Hsiangtai, CN-1000, Taiwan) at 2000 rpm for 15 min.

2.4 Synthesis and Characterization of Magnetic Peptide-imprinted Polymer Composite Nanoparticles (MPIPs).

Magnetic nanoparticles (MNPs) were prepared by co-precipitation using a mixture of iron (III) chloride 6-hydrate and iron (II) sulfate 7-hydrate by adding sodium hydroxide (Massart method).[21] MNPs were freeze-dried, modified with hexamethyldisilazane (HMDS) (ca. 5 ⌈L/mg) and mixed with 0.1 wt% EVAL/DMSO solution at 20 mg/mL. The mixture with and without 1 mg/mL of peptide was dispersed in 10 mL non-solvent solution (deionized water/isopropanol 2/3 in weight) for EVAL at 5 ^{0}C. Peptides were removed from magnetic nanoparticles using a magnetic plate and washing with 0.1 wt % SDS solution 10 min and then deionized water 10 min three times.

Magnetic and peptide-imprinted polymer composite nanoparticles were monitored by a dynamic light scattering (DLS) particle sizer (90Plus, Brookhaven Instrument Co., New York).[21] The magnetic nanoparticles, peptide-imprinted magnetic EVAL composite nanoparticles before and after removal of template were freeze dried and their magnetization monitored with a magnetic property measurement system (MPMS XL-7, Quantum Design, San Diego, CA) at 298 K in ±15000 Gauss. Magnetic peptide-imprinted composite particles were stained with uranyl acetate and then examined and photographed in transmission electron microscopy (TEM, Hitachi-700, Hitachi Co., Japan) operated at 100 kV accelerating

voltage.

2.5 Extraction of REG1B by Magnetic Peptide-imprinted EVAL Nanoparticles.

Extraction of REG1B from E coli culture medium was performed using a KingFisher™ mL purification system (Thermo) with steps as follows: (1) MPIP particle collection: peptide 7 MPIPs (1mg) were placed in the first tube with 1 mL deionized water. The magnetic bars together with the tip comb(s) move slowly up and down into the tubes for 10 min. MPIPs were then collected on the walls of the tips. The magnetic rods together with the tip comb(s), having collected the magnetic particles, can be lifted out of the tubes and transferred into the next tubes. (2) MPIPs particles release and extraction: The magnetic bars are lifted off and the tip combs are lowered into the *E. coli* culture medium. MPIPs are released by moving the tip comb(s) up and down for 10 min to complete the mixing and adsorption of REG1B from the *E. coli* culture medium. (3) Release of REG1B: MPIPs adsorbed with REG1B were then transferred to 1 mL deionized water from the previous tube and vigorously shaken for 10 min. An enzyme-linked immuno-sorbent assay (ELISA) kit SEK11638 (Sino Biological Inc.; http://tw.sinobiological.com/) was employed to examine the REG1B concentration;[22] the total protein was measured using a Protein Quantification Assay (740967.50, MACHEREY-NAGEL GmbH & Co. KG; http://www.mn-net.com/tabid/10972/default.aspx). All measurements in this work were carried out with at least two replicates; data are expressed as means and standard deviations.

3. Results and Discussion

Peptides of Regenerating Protein 1 (REG1) that have been studied are shown in Table 1. The candidate peptides were taken from various internal sequences within the full protein and

were 13-18 amino acids long. A small difference in non-homologous amino-acids of REG1A and REG1B is in position 54 where a positively-charged Arginine (R) in REG1A is proline in REG1B. REG1A is a polar protein, with negatively-charged amino acids in positions 52/30 (secreted protein/crystal structure positions), 53/31, 55/33 and 59/37 (EDED) that have been proposed to form a contiguous parallel stretch on the protein surface. The position 54/32 (R) is also proposed to contribute to the charge on the protein surface.

Peptides 2, 4, and 6 (from Reg1A) have been used as templates in a previous study.[22] The current work studied peptides 1, 3, 5, and 7 (from Reg1B), with a focus on peptide 7. The solubility of peptide 7 should be very good owing to its 6 charged (EDREDD) and 3 polar (NTY) amino acids. Also, there are 2 bulky aromatic amino acids (WY) within the sequence. The difference between REG1A/REG1B compared to REG3G/REG3A is substantial, thus cross-reaction with REG3 is unlikely. Structurally, the first part of the peptide 7 (7 amino acids, p51-57) forms an extended loop, whereas the second part of the peptide 7 (positions 58-63) has an alpha-helix structure.

Cyclic voltammetry was used to screen for the optimal composition (ethylene mole %) of the EVALs. Figure 1(a) shows that the peak current occurred at a potential of 300 mV (for *E. coli* culture medium). Figure 1(b) shows the variation in current density with EVAL composition, for PIPs recognizing peptide 7, when electrochemically measuring a peptide concentration of 1.0 ng/mL. The EVAL that contained 32 ethylene mole % yielded the largest current density differences: 68.26 ± 8.45 and 26.38 ± 5.62 $\mu A/cm^2$, for the PIP and the NIP respectively. Composition screening was also performed on peptides 1,3, and 5; the results for all peptides are summarized in Figure S1. Dark bars indicate current densities with non-imprinted polymers; total bar height shows the current density with the imprinted films. Current density using non-imprinted films was generally 20-24 $\mu A/cm^2$; imprinting

effectiveness (the ratio of the current density difference on MIPs to that on NIPs) varied from < 2 to better than 3 for optimal ethylene contents. Uncertainties (from repeated measurements) were ±1-4 μA/cm^2. Table 1 lists the optimal compositions of EVALs of peptides for imprinting. Though a clear trend is difficult to discern, it appears that among the shorter peptides (< 18 amino acids), those with lower aromatic / hydrophobic content are better imprinted with the lower ethylene content EVAL (27 mole %). This is perhaps unsurprising: lower hydrophobicity implies greater hydrophilicity, and the vinyl alcohol group adds hydrophilicity (and hydrogen-bonding capabilities) to the polymer. Peptides with more than 16 amino acids may undergo intramolecular interactions, which would influence their interaction with imprinting polymers.

The effect of the concentration of peptides and REG1B on MIP-coated electrodes was then examined by cyclic voltammetry. Figure 1(c) plots the cyclic voltammograms (CVs) of various concentrations of peptide 7 on PIP electrodes, showing that the current density significantly increases with the concentration of peptide 7. Figure 1(d) depicts the comparison of the electrochemical signals of REG1B and peptide 7 titrated to the peptide 7 MIP sensor. As reported previously,[22] at lower concentration (less than 1.0 pg/mL), the response of REG1B to peptide MIP sensors is very close to that of peptides, suggesting that the entire protein can participate in electron transfer processes. At higher concentrations, the response falls off slightly for the protein compared to the imprinted peptide. In both cases, the current response varies roughly logarithmically with the peptide or protein concentration.

The surface morphologies and compositions of PIPs were examined by SEM, ESCA and AFM, and shown in Figs. 2(a)-(b), (c) and (d)-(f), respectively. The surface morphologies of peptide 7-imprinted polymers before and after template removal, Figs. 2(a) and (b), were obtained by SEM, which revealed that the fabricated thin film was almost flat. Fig. 2(c)

shows an ESCA analysis, which demonstrated that peptide 7 could be almost entirely removed after the solidification of EVALs by the SDS washing solution. A few peptides may become trapped in the EVAL, but this is not likely to affect the sensing of REG1B; these sites would be inaccessible to the protein. Figs. 2(d)-(e) show that peptide removal increased the mean surface roughness from 1.05 to 1.14 nm; rebinding with 1.0 ng/mL of peptides for 30 min dramatically increased roughness to 2.77 nm.

Table 3 shows data that were obtained using *E. coli* culture medium that contained REG1B. Peptides 1, 3, 5 and 7 from sequences of REG1B (in Table 1) were synthesized and imprinted onto EVAL-coated electrodes, and calibration curves were made using the respective peptides. When measuring REG1B in culture medium, the current density differences obtained using peptides 1, 3, and 5 as templates were less than 50 $\mu A/cm^2$; these corresponded to assayed protein concentrations of < 10.52 ± 2.12 ng/mL, respectively. (The actual concentration of REG1B was estimated at 78-213 ng/mL using an ELISA assay.) Using peptide 7 as template, however, gave a current density difference of 61.56 ± 0.17 $\lceil A/cm^2$, (three samples), corresponding to a REG1B concentration of 112.76 ± 3.99 ng/mL. Although all templates were about equally able to recognize themselves, peptide 7 yielded an EVAL film with the best response to REG1B. Thus, the epitope recognition of REG1B using peptide 7-imprinted polymer-coated electrodes may have potential in making measurements on real urine samples.

The high affinity for peptide 7 of EVAL that contained 32 mole % of ethylene was exploited in the preparation of peptide 7-imprinted polymer-coated magnetic nanoparticles (MPIPs). Figure 3 displays the characteristics and reusability of MPIPs, obtained using DLS, TEM, and SQUID. Figure 3(a) compares the size distributions of MPIPs. The mean sizes of magnetic nanoparticles and magnetic non-imprinted polymeric particles were 50 and 90 nm,

respectively. Template removal reduced the mean sizes of MPIP nanoparticles from around 200 nm to almost that of NIPs. Interestingly, rebinding peptides on MPIPs may have induced partial aggregation of MPIPs. (It is possible that the aggregation may be inhibited by either limiting the number of imprinted surface cavities or the concentration of peptide.) This phenomenon was also evident from the TEM image (Fig. 3(b)). The magnetic nanoparticles inside the MPIPs were superparamagnetic and coating with PIPs reduced their saturated magnetization from approximately 61.2 emu/g to 40.0 emu/g. The binding of peptides on MMIP reduced the saturated magnetization from 45.4 to 44.3 emu/g.

Finally, the *E coli.* culture medium was repeatedly extracted with the MPIP nanoparticles and REG1B was then released to the ferri-/ ferrocyanide solution for electrochemical measurement. Fig. 3(d) presents the reusability of the MPIPs, showing that for at least the first five cycles, the recovery of REG1B in the *E. coli* culture medium exceeded 80%. The recovery of REG1B was calculated (with calibration curve shown in Fig1(d)) from the REG1B concentration measured by the PIPs-coated electrodes in the released solution, compared to that obtained in the first extraction by MPIPs nanoparticles. Moreover, the purity of the extracted REG1B, which was defined as the percentage of REG1B to total protein amount in the extracted solutions (as determined by an ELISA assay and a total protein kit, see Materials & Methods), was as high as 83.03 ± 9.30 % in the first cycle; further purification is also possible by the same protocols.

4. Conclusions

Owing to the potential importance of proteins that have been identified as markers for cancers, molecularly imprinted polymers (MIPs) have been generated for the sensing and extraction of biomarker proteins in biological fluids. Using peptide epitopes instead of

complex proteins reduces the cost and may also enhance the exposure of the MIP binding sites. However, issues such as the peptide length and hydrophobicity must be examined; longer peptides may form inappropriate secondary structures and thus affect the selectivity of the MIP. Using electrochemical screening, we found that fewer aromatic and hydrophobic amino acids in peptides from REG1B formed better MIPs when a lower mole % of ethylene EVAL was used. Although it remains to be seen whether this holds true for other proteins as well.

Finally, it is important to note that peptide-imprinted films show dramatic differences in their ability to recognize the parent protein, even when those films all recognize their target peptides well. Thus, screening a peptide film for its response to the original target protein is crucial. After identifying the most effective peptide and EVAL combination for the recognition / binding of REG1B, we constructed imprinted polymer particles incorporating magnetic nanoparticles. We have also shown that MIPs with incorporated magnetic nanoparticles can be employed in the extraction and separation of REG1B protein, which proved to be an effective and robust protein isolation method.

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Legends

Figure 1. (a) Cyclic voltammograms (CVs) of REG1B measured using peptide 7-imprinted polymer coated electrodes in a solution of 20 mM potassium ferricyanide ($K_3[Fe(CN)_6]$), 20 mM potassium ferrocyanide ($K_4[Fe(CN)_6]$), and 0.5 M KCl with/without *E. coli* culture medium. (b) Current density difference for the peptide 7-imprinted and non-imprinted polymers coated electrode for 1.0 ng/mL and buffer solution of target molecules when voltages of 300 mV were applied. The imprinting effectiveness was defined as the ratio of the current density difference of peptides on the PIPs to that on the NIPs of the same composition. (c) Cyclic voltammetry of peptide 7 solutions measured using peptide 7-imprinted coated electrodes using a potentiostat. (d) The calibration curve of peptide 7 and REG1B to peptide 7-imprinted polymer based sensors, using an applied voltage of 300 mV.

Figure 2. The surface morphology of peptide 7-imprinted polymers (PIPs) prepared using 32 mole% of ethylene EVAL (a) before and (b) after template removal of peptide 7. (c) Nitrogen atomic analysis of above surface by electron spectroscopy for chemical analysis (ESCA). The atomic force images of peptide 7-imprinted polymers prepared using 32 mole% of ethylene EVAL (d) before, (e) after template removal and (f) rebinding with 1.0 ng/mL of peptide 7.

Figure 3. (a) Size distribution of magnetic nanoparticles (MNPs), magnetic non-imprinted nanoparticles (MNIPs) and magnetic peptide-imprinted nanoparticles (MPIPs) before and after template removal, and rebound with peptide. (b) TEM image of the magnetic peptide-imprinted nanoparticles (MPIPs), the scale bar is 10 nm. (c) The magnetization of magnetic nanoparticles (MNPs), and magnetic peptide-imprinted nanoparticles (MPIPs) before and after template removal, and rebound with 1.0 ng/mL peptide. (d) The relative recovery of REG1B with extraction cycles of REG1B from E coli. culture medium and desorption in

ferric/ferrocyanide solution, which was measured electrochemically. (The recovery above 100% in cycle 2 is within the experimental uncertainty, as shown by the error bars.)

Tables:

Table 1. The peptides (colored) of Regenerating Proteins used to imprint onto poly(ethylene-*co*-vinyl alcohol)s. The last column indicates the results from screening of EVALs for the highest imprinting effectiveness.

Protein	**Amino acid sequence**	**Peptide**	Amino acids		Optimal EVAL (ethylene %)
			Total	Aromatic & Hydrophobic	
Reg1A	MAQTSSYFMLISCLMFLSQSQ GQEAQTELPQARISCPEGTNA YRSYCYYFNEDRETWVDADL YCQNMNSGNLVSVLTQAEGA FVASLIKESGTDDFNVWIGLH DPKKNRRWHWSSGSLVSYK SWGIGAPSSVNPGYCVSLTS STGFQKWKDVPCEDKFSFVC KFKN	Peptide 7	13	4	32
		Peptide 5	13	4	32
		Peptide 3	18	6	27
		Peptide 1	16	6	32
Reg1B	MAQTNSFFMLISSLMFLSLSQ GQESQTELPNPRISCPEGTNA YRSYCYYFNEDPETWVDADL YCQNMNSGNLVSVLTQAEGA FVASLIKESSTDDSNVWIGLH DPKKNRRWHWSSGSLVSYK SWDTGSPSSANAGYCASLTS CSGFKKWKDESCEKKFSFVC KFKN	Peptide 6	13	3	27
		Peptide 4	18	3	32
		Peptide 2	16	2	27

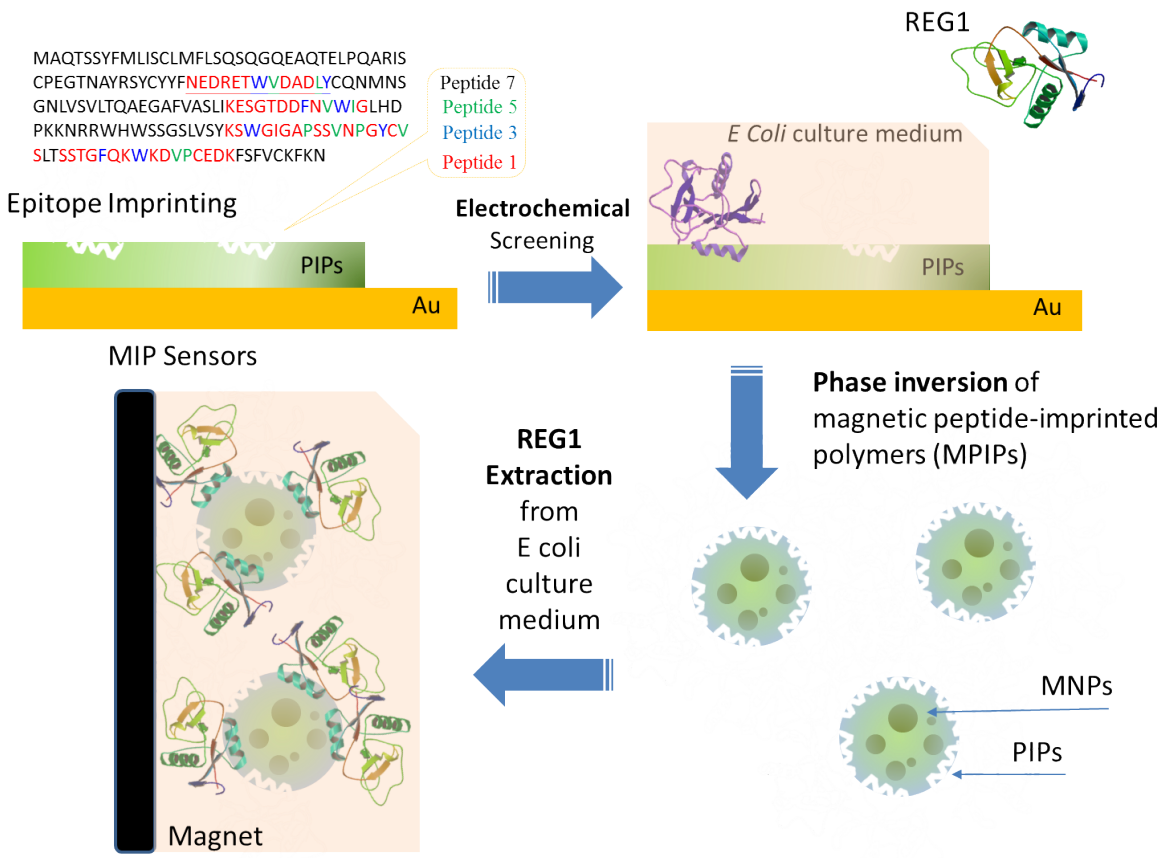
Table 2. The comparison of Peptide 7 in Regenerating Islet-Derived 1 Alpha (REG1A) protein with non-homologous peptides from REG1B and REG3G. REG3 proteins may also relate with hepatocellular carcinoma.[23]

29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44		(Secreted protein/crystal structure sequence)
51	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**66**		
N	***E***	***D***	***R***	***E***	T	W	V	***D***	A	D	L	Y					REG1A
N	E	D	**P**	E	T	W	V	D	A	D	L	Y					REG1B
F	L	S	P	K	S	W	M	D	A	D	L	A					REG3G

Table 3. The current measurement of *E. coli* culture medium by peptide-imprinted EVAL coated electrodes. The same *E. coli* culture medium containing REG1B was pre-diluted thousand times before electrochemical analysis.

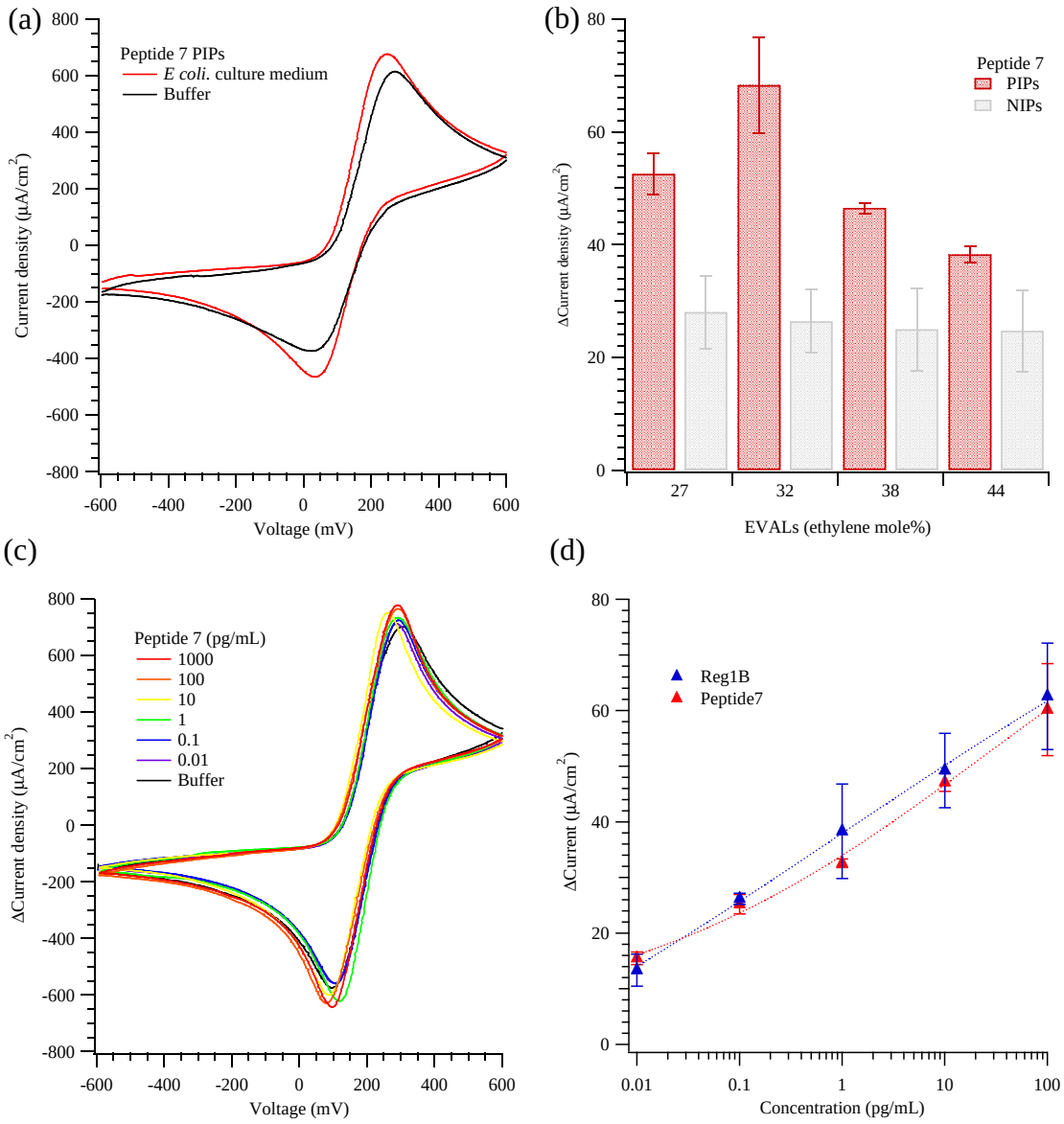
Real Sample	Peptide imprinted	ΔCurrent (μA/cm^2)	Convert concentration (pg/mL)	Mean concentration (pg/mL)
E.coli culture medium	7	61.36	109.05	112.76±3.99
		61.53	112.24	
		61.78	116.98	
	5	47.85	10.25	9.09±1.30
		47.29	9.35	
		46.09	7.68	
	3	47.40	10.25	9.51±0.73
		46.92	9.51	
		46.41	8.78	
	1	48.13	12.54	10.52±2.12
		46.96	10.70	
		45.14	8.32	

Scheme

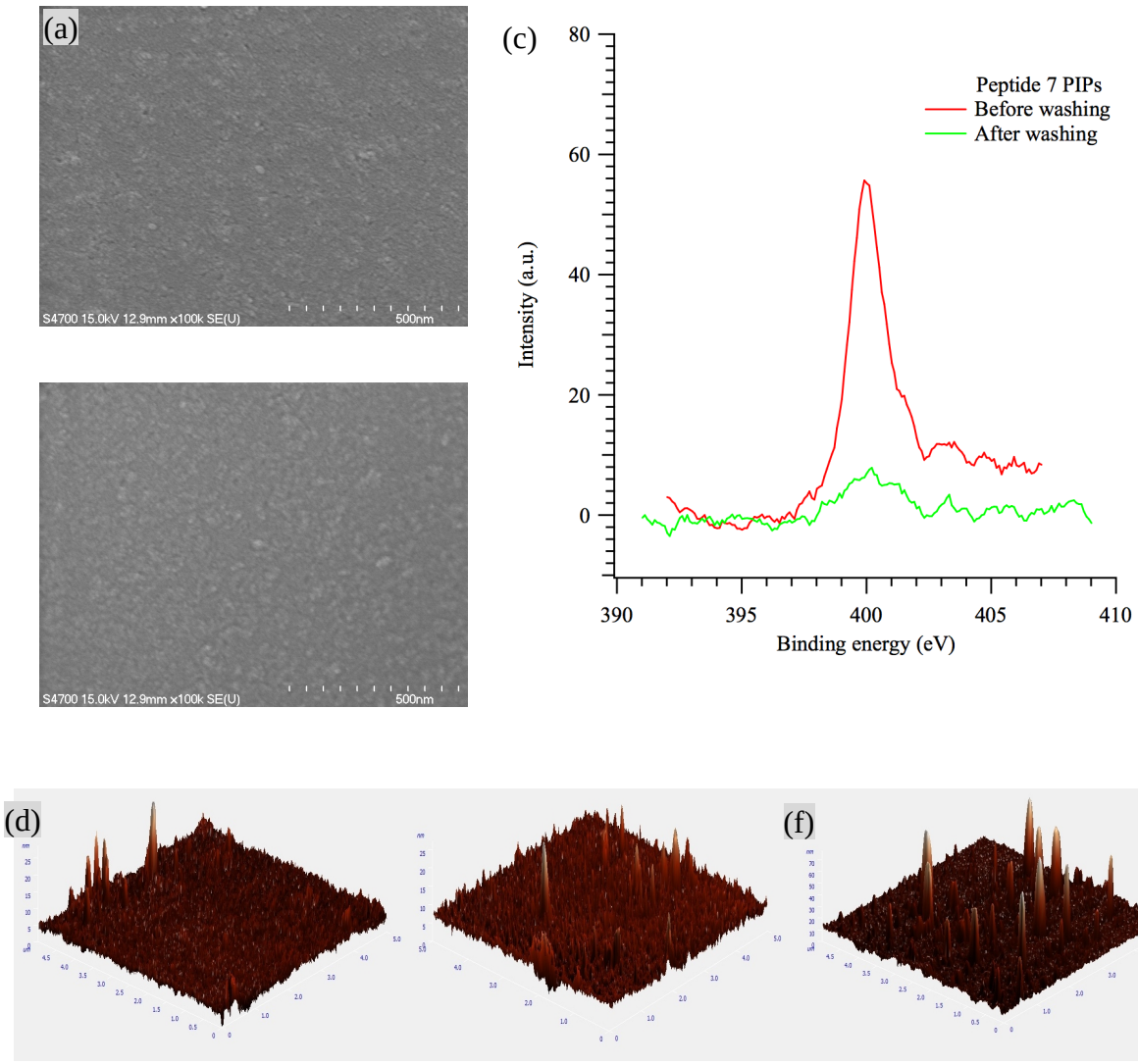


Scheme 1. The preparation of peptide-imprinted polymers (PIPs) coated electrode and magnetic nanoparticles for the epitope recognition of REG1 in *E coli* culture medium.

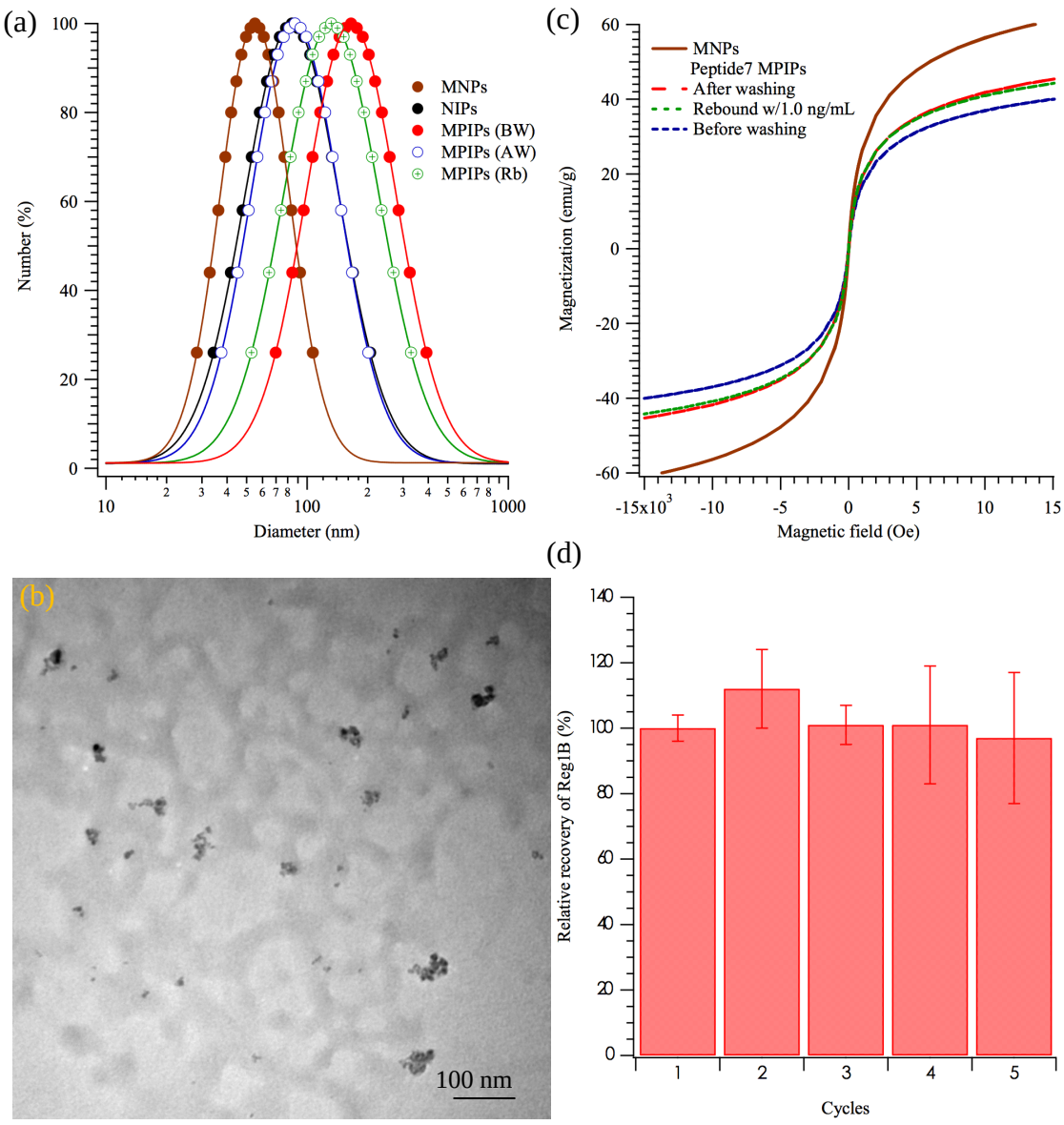
Figures



Lin et al.- Figure 1

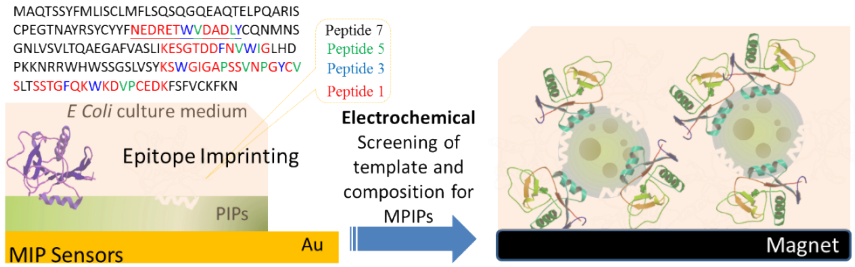


Lin et al.- Figure 2



Lin et al.- Figure 3

TOC



Seven peptides (13-18 amino acids) were synthesized and used as templates for imprinting and recognition of Regenerating Protein 1 (REG1). Peptide-imprinted poly(ethylene-*co*-vinyl alcohol)s, PIPs, containing four different mole fractions of ethylene were cast on screen-printed electrodes to find the optimum composition for both the sensing and the extraction of REG1 in an *E coli* culture medium.

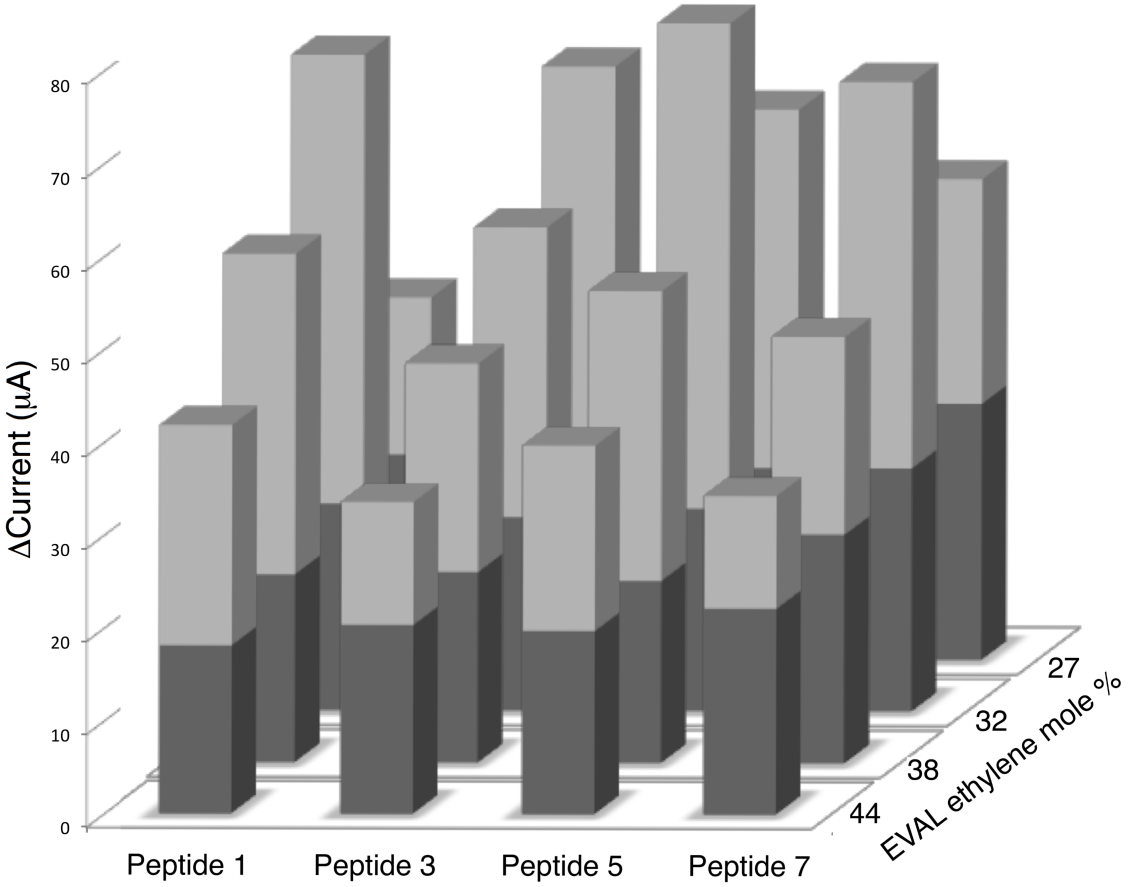


Figure S1. Summary of EVAL ethylene content screening of MIPs for peptides 1,3,5, and 7. Dark bars indicate current densities with non-imprinted polymers; total bar height shows the current density with the imprinted films. Uncertainties (from repeated measurements) were ±1-4 μA/cm^2.