Published in final edited form as:

Curr Protoc Protein Sci. 2011 February ; CHAPTER: Unit12.9. doi:10.1002/0471140864.ps1209s63.

High-Throughput Lectin Microarray-Based Analysis of Live Cell Surface Glycosylation

Yu Li¹, Sheng-ce Tao^{2,3,4}, Heng Zhu^{2,3}, and Jonathan P. Schneck¹

¹Department of Pathology, Oncology & Medicine and Institute for Cell Engineering, Johns Hopkins University, Baltimore, Maryland ²Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland ³The High-Throughput Biology Center, Johns Hopkins University School of Medicine, Baltimore, Maryland ⁴Shanghai Center for Systems Biomedicine, Shanghai Jiaotong University, Shanghai, China

Abstract

Lectins, plant-derived glycan-binding proteins, have long been used to detect glycans on cell surfaces. However, the techniques used to characterize serum or cells have largely been limited to mass spectrometry, blots, flow cytometry, and immunohistochemistry. While these lectin-based approaches are well established and they can discriminate a limited number of sugar isomers by concurrently using a limited number of lectins, they are not amenable for adaptation to a high-throughput platform. Fortunately, given the commercial availability of lectins with a variety of glycan specificities, lectins can be printed on a glass substrate in a microarray format to profile accessible cell-surface glycans. This method is an inviting alternative for analysis of a broad range of glycans in a high-throughput fashion and has been demonstrated to be a feasible method of identifying binding-accessible cell surface glycosylation on living cells. The current unit presents a lectin-based microarray approach for analyzing cell surface glycosylation in a high-throughput fashion.

Keywords

microarrays; lectins; glycosylation; cancer stem cells; immunology; infectious diseases

BASIC PROTOCOL

Lectins, plant-derived glycan-binding proteins, have long been used to detect glycans on cell surfaces. However, the techniques used to characterize serum or cells have largely been limited to mass spectrometry, blots, flow cytometry (Batisse et al., 2004; Venable et al., 2005), histochemistry (Carter and Brooks, 2006), and immunohistochemistry (Wearne et al., 2006). While these lectin-based approaches are well established and can discriminate a limited number of sugar isomers by concurrently using a limited number of lectins (Hirabayashi, 2004), they are not amenable for adaptation to a high-throughput platform. Fortunately, given the commercial availability of lectins with a variety of glycan specificities, lectins can be printed on a glass substrate in a microarray format to profile accessible cell surface glycans. This method is an inviting alternative for analysis of a broad range of glycans in a high-throughput fashion and has been demonstrated to be a feasible method of identifying binding-accessible cell surface glycosylation on living cells (Tao et

al., 2008). The current unit presents a lectin-based microarray approach for analyzing cell surface glycosylation in a high-throughput fashion.

The lectin microarray is simple in concept similar to other array technologies. Whereas other arrays analyze binding interactions on molecules of similar scales, lectin microarrays can be used to ascertain lectin-glycan interactions for live cells. Nevertheless, the overarching concept of the lectin microarray is direct. The fundamental interaction that captures the cells is between the printed lectin and the cell surface glycans that are accessible to those lectins. Although lectin-glycan interactions are of low affinity (10^{-6} to 10^{-7} range for most glycoproteins), the multivalency of lectins can achieve sufficiently high avidity to retain cells on the lectin spot. However, having a low affinity is also advantageous since nonspecific interactions can be easily overcome by the application of shear forces.

Materials

Lyophilized lectins (available from EY Laboratories and Vector Laboratories)

Storage buffer (PBS plus 0.02% Tween and 25% glycerol)

2× printing buffer (see recipe)

Slide washing solution (see recipe)

Slide blocking buffer (see recipe)

$\geq 1 \times 10^6$ cells of interest

Phosphate-buffered saline (PBS; Invitrogen)

CFSE cell tracking dye (Invitrogen)

Cell binding buffer (see recipe)

384-well plates (e.g., Nunc Millipore)

Schott Nexterion^R H slides (Schott North America)

Microarray printer (e.g., Bio-Rad ChipWriter Pro)

Laboratory marker

Nalgene wash bottles *or* 25-ml pipets

37°C water bath

Adhesive chambered gasket (e.g., Grace Biolabs FlexWell gaskets)

Paper towels

Microarray slide scanner (e.g., GenePix 4000B)

Print the lectin microarray

1. Dissolve lyophilized lectins to 2 mg/ml in storage buffer as recommended by the manufacturer of the lectins.
2. Dilute the lectin stock solution (from step 1) 1:1 with 2× printing buffer to achieve a final concentration of 1 mg/ml in 1× printing buffer.
3. Transfer 7- μ l lectin solution into each well of a 384-well plate for printing. When necessary, include positive controls (e.g., Cy3 and Cy5 dyes).

For long-term storage, keep the lectins divided into aliquots up to 3 months at -20°C .

4. Print lectins in triplicate per block with replicate blocks on Schott Nexterion^R H slides.

Printing parameters, such as spot/pin size, spot distance, etc., should be optimized based on the arraying technology used. For example, Tao et al. (2008) used a BioRad contact printer with 120- μm diameter quill-style pins and 230- μm spot spacing to print lectins in triplicate per block. Consult the operation guide of the array printer being used for the printing procedure.

5. Keep the printed lectin microarrays at 4°C and protected from light overnight prior to use.

For storage, the printed lectin microarrays can be maintained for up to 6 months at 0°C , without losing activity.

Prepare the lectin microarray for use

6. Prior to washing, mark the boundaries (using a laboratory marker) of the printed areas on the reverse side of the microarray slide. Gently flow slide washing buffer over the printed slide surface to remove excess lectins using Nalgene wash bottles or a 25-ml pipet.

This is important for the application of silicone gaskets later, since washing the lectin microarray will make printed spots invisible to the naked eye.

7. Submerge the slide surface completely in blocking buffer for 1 hr at room temperature. Remove the slide from the blocking buffer from one side slowly.

The slide surface will not retain any liquid except at the edge, which can be easily removed by gently tapping the slide on a paper towel.

8. While the microarrays are in the blocking buffer, prepare the cells of interest. Label cells with CFSE tracking dye by resuspending the cells in $1\times\text{PBS}$ with 10 μM CFSE at 1×10^6 cells/ml. Place the cells in a 37°C water bath for 1.5 min.

From this point forward, keep samples away from light as much as possible.

9. Wash the cells two times, each time with 15 ml $1\times\text{PBS}$ to remove excess CFSE dye. Wash once more with 15 ml cell binding buffer.

10. Resuspend the washed cells in cell binding buffer at 5×10^6 cells/ml.

Probe the lectin microarray

11. Apply adhesive silicone gasket (e.g., Grace BioLabs 16-well silicone gasket) to the printed slide surface.

12. Allow the cells to bind at room temperature for 2 hr total.

- a. *For static cell incubations:* Seed each block with 5×10^5 labeled cells of interest up to a block area of 100 mm^2 .
- b. *For agitated incubations:* Agitate the cells during incubation at 30-min intervals by shaking at 250 rpm for 10 sec each time. Cell numbers per block may be reduced to as low as 1×10^5 per block if cells are agitated during incubation. However, if sufficient cells are available then using 5×10^5 cells per block is recommended.

The number of cells per block can be adjusted for both availability, as well as printed area size. The number of cells needed can be estimated by accounting for

the area of the printed block with the approximate size of the cells used to probe the lectin microarray.

13. Submerge the slide in a large volume of $1 \times$ PBS. Carefully remove the incubation gasket while keeping the slide completely submerged. Invert the slide and shake gently while still submerged to dislodge and wash away unbound cells.

Some gasket adhesives may be too adherent, making delicate removal difficult. The authors used FlexWell silicone gaskets from Grace Biolabs with adhesive specifically designed to work with the Schott Nexterion slide surface. While using FlexWell gaskets is not mandatory, the authors recommend performing a mock run with the gasket and slide.

14. Remove the slide from the slide washing buffer edgewise to reduce the amount of slide washing buffer on the slide.

15. Blot away excess slide washing buffer using a paper towel, avoiding the areas where lectins have been printed. Remove as much slide washing buffer as possible.

After the lectin microarray is removed from the slide washing buffer, lectin spots with bound cells should be visible to the naked eye.

16. Allow the slide to air dry; it will take <5 min.

17. Acquire the slide image on a microarray scanner at $5\text{-}\mu\text{m}$ resolution in the FITC fluorescence channel. Apply appropriate laser power and gain settings while avoiding signal saturation.

Typical cell binding images are shown in Figure 12.9.1.

18. Quantitate signal intensities for each lectin spot (e.g., Axon GenePix 5.0).

Analyze the data

A binary mode of analysis can be performed according to the methods outlined by Tao et al. (2008).

19. Briefly, normalize lectin intensities for each spot to background fluorescence and plot.

The spots with signal intensities ≤ 6 standard deviations above 0 are considered nonbinding events; spots with signal intensities >6 standard deviations above 0 are considered positive binding events. Based on the number of replicates per lectin-cell pair, a voting system can be used to determine if the total binding event is positive or negative. A hierarchically clustered binary code of lectin-cell interactions can be constructed for each cell type analyzed.

REAGENTS AND SOLUTIONS

Use Milli-Q-purified water or equivalent in all recipes and protocol steps. For common stock solutions, see APPENDIX 2E; for suppliers, see SUPPLIERS APPENDIX.

Cell binding buffer

1 $\times$ Phosphate-buffered saline (PBS) containing:

0.5 mM CaCl_2

0.5 mM MnCl_2

10 mg/ml BSA

Prepare fresh

Supplying divalent cations is necessary for certain lectins to bind their respective glycan ligands. BSA is added to reduce the nonspecific binding.

Printing buffer, 2×

2× Phosphate-buffered saline (PBS) containing:

0.04% (v/v) Tween-20

50% (v/v) glycerol

0.1 mg/ml BSA

Prepare fresh

Printing buffer is used to dissolve lectin stock solutions for printing. Tween-20 and BSA are added to improve spot morphology. Glycerol is added to reduce evaporation and enhance stability in storage. Percentages of reagents in printing buffer are optimized for lectin microarrays but may be adjusted.

Slide blocking buffer

50 mM ethanolamine in 50 mM sodium borate, adjust to pH 8.0 using 1 M HCl. Store up to 1 year at room temperature.

If using stored buffer, verify the pH prior to usage.

Ethanolamine is used to block any remaining active amine-chemistry sites on the Schott Nexterion^R H slide, which is important to eliminate non-specific cell capture.

Slide washing solution

0.5% (v/v) Tween-20 in 1× PBS

Store indefinitely at room temperature

COMMENTARY

Background Information

The surfaces of all vertebrate cells are decorated with a broad selection of glycans with considerable structural diversity called the “glycocalyx.” Due to the abundance of glycans on cell surfaces, it comes as no surprise that many cellular interactions with the surrounding environment are affected by glycosylation. Interactions with neighboring cells, as well as interactions with the extracellular matrix, are two facets of cellular communication that are heavily dependent on glycan binding. Changes in glycosylation have been shown to correlate to cellular differentiation, malignant transformations (Lau and Dennis, 2008), tumor progression (Mayoral et al., 2008; Zhao et al., 2008), and metastasis (Yuan et al., 2008), suggesting the importance of characterizing cell surface glycosylation states for research, diagnostic, and potential therapeutic purposes. Unfortunately, the structural diversity of the cell surface glycome, which confers the biological functional diversity, is in fact responsible for impeding its analysis.

Traditional means of investigating glycosylation using flow cytometry, blotting, histochemistry, and mass spectrometry either address singular glycosylation specificities or fail to scale to high-throughput applications. Lectin microarrays help overcome both limitations by using the wide range of available lectins with a correspondingly wide range of glycan specificities to profile cell surfaces (Tao et al., 2008). Lectin microarrays showed

promise in early studies analyzing glycosylation states of cell lysates, bacteria, as well as proof of principle experiments using Lec mutant Chinese Hamster Ovary (CHO) cells (Hsu et al., 2006; Tateno et al., 2007). Although lectin microarrays have been used to profile glycoproteins and cell lysates (Kuno et al., 2005; Ebe et al., 2006; Lee et al., 2006; Pilobello et al., 2007), their use for profiling live cells have been limited to proof-of-principle studies (Zheng et al., 2005; Hsu et al., 2006; Lee et al., 2006; Tateno et al., 2007). For example, lectin microarrays accurately and predictably distinguish between glycosylation-defective CHO mutants (Tateno et al., 2007), and Hsu and colleagues used a 21-lectin microarray to distinguish O-antigens on lipopolysaccharides (LPS) of bacterial cell surfaces (Hsu et al., 2006). However, extensive profiling of mammalian cell lines, as well as primary cell populations, was still largely unexplored territory until recently. Tao and colleagues showed that in addition to the profiling potential of lectin microarrays, the lectin binding profile can also be used for multiple biologically relevant applications including development into potentially clinically relevant diagnostic tools with predictive power (Tao et al., 2008).

Critical Parameters and Troubleshooting

Microarray quality control—The fabrication process of the lectin microarray is similar to that of existing protein microarrays (Zhu et al., 2001; Chen et al., 2008; Hu et al., 2009). Printing buffers described in this protocol have been optimized for the lectins used in Tao et al. (2008). The composition of the printing solution can be adjusted if spot morphology is unacceptable. If there is no cell binding and a printing error is suspected, lectin spots can be validated by probing the microarray with an amine-reactive dye, such as 549 nm NHS-Ester (Invitrogen). Moreover, individual lectins can also be tested via flow cytometry, using fluorescently conjugated lectins (e.g., using FITC), and cells in solution analyzed by FACS.

Cell probing—The authors have found that one of the most important considerations in probing the microarray is the number of cells used. Each lectin block should be seeded with cells at the recommended density when possible to ensure proper coverage of the printed spots with cells. As mentioned in the protocol, the addition of periodic agitation can reduce the minimum number of cells necessary, but a static incubation is preferred when possible. Fluorescent labeling of cells can be verified by analyzing labeled cell samples on a flow cytometer.

Anticipated Results

Cell binding will vary based on numerous factors including lectin stability, glycan availability, lectin binding avidity, and cell density. See Tao et al. (2008) for examples of data obtained from lectin microarrays for both spot binding, as well as binary data analysis.

Time Considerations

Duration of the assay can be separated into three segments: fabrication, experimental, and data acquisition. The time necessary for the fabrication process will depend on the equipment used, the size of the printing batch, and the number of lectins used per slide. Minimally, the overnight incubation requires that the preparation of the lectin chip will take one day. The experimental stages of the lectin microarray will vary in time based on the number of samples used. For two to six samples, expect the slide blocking, cell labeling, and microarray probing to consume ~2.5 hr. Lastly, the length of time for data acquisition and analysis will vary based on the equipment used and the number of slides to be analyzed. Scanning at 5- μ m resolution, each slide should take ~30 min. Time duration for data analysis is entirely dependent on the user.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from the NIH AI 44129, CA 108835, & P01 AI072677 to J.P. Schneck, U54RR020839-01 to H. Zhu, and the State Key Development Program for Basic Research of China (grant 2010 B 529205) & SRF for ROCS, SEM to S.C. Tao. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. Yu Li and Sheng-ce Tao contributed equally to this article.

Literature Cited

- Batisse C, Marquet J, Greffard A, Fleury-Feith J, Jaurand MC, Pilatte Y. Lectin-based three-color flow cytometric approach for studying cell surface glycosylation changes that occur during apoptosis. *Cytometry A*. 2004; 62:81–88. [PubMed: 15536639]
- Carter TM, Brooks SA. Detection of aberrant glycosylation in breast cancer using lectin histochemistry. *Methods Mol. Med*. 2006; 120:201–216. [PubMed: 16491603]
- Chen CS, Korobkova E, Chen H, Zhu J, Jian X, Tao SC, He C, Zhu H. A proteome chip approach reveals new DNA damage recognition activities in *Escherichia coli*. *Nat. Methods*. 2008; 5:69–74. [PubMed: 18084297]
- Ebe Y, Kuno A, Uchiyama N, Koseki-Kuno S, Yamada M, Sato T, Narimatsu H, Hirabayashi J. Application of lectin microarray to crude samples: Differential glycan profiling of lec mutants. *J. Biochem*. 2006; 139:323–327. [PubMed: 16567396]
- Hirabayashi J. Lectin-based structural glycomics: Glycoproteomics and glycan profiling. *Glycoconj. J*. 2004; 21:35–40. [PubMed: 15467396]
- Hsu KL, Pilobello KT, Mahal LK. Analyzing the dynamic bacterial glycome with a lectin microarray approach. *Nat. Chem. Biol*. 2006; 2:153–157. [PubMed: 16462751]
- Hu S, Xie Z, Onishi A, Yu X, Jiang L, Lin J, Rho HS, Woodard C, Wang H, Jeong JS, Long S, He X, Wade H, Blackshaw S, Qian J, Zhu H. Profiling the human protein-DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling. *Cell*. 2009; 139:610–622. [PubMed: 19879846]
- Kuno A, Uchiyama N, Koseki-Kuno S, Ebe Y, Takashima S, Yamada M, Hirabayashi J. Evanescent-field fluorescence-assisted lectin microarray: A new strategy for glycan profiling. *Nat. Methods*. 2005; 2:851–856. [PubMed: 16278656]
- Lau KS, Dennis JW. N-Glycans in cancer progression. *Glycobiology*. 2008; 18:750–760. [PubMed: 18701722]
- Lee MR, Park S, Shin I. Protein microarrays to study carbohydrate-recognition events. *Bioorg. Med. Chem. Lett*. 2006; 16:5132–5135. [PubMed: 16875819]
- Mayoral MA, Mayoral C, Meneses A, Villalvazo L, Guzman A, Espinosa B, Ochoa JL, Zenteno E, Guevara J. Identification of galectin-3 and mucin-type O-glycans in breast cancer and its metastasis to brain. *Cancer Invest*. 2008; 26:615–623. [PubMed: 18584353]
- Pilobello KT, Slawek DE, Mahal LK. A ratiometric lectin microarray approach to analysis of the dynamic mammalian glycome. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:11534–11539. [PubMed: 17606908]
- Tao SC, Li Y, Zhou J, Qian J, Schnaar RL, Zhang Y, Goldstein IJ, Zhu H, Schneck JP. Lectin microarrays identify cell-specific and functionally significant cell surface glycan markers. *Glycobiology*. 2008; 18:761–769. [PubMed: 18625848]
- Tateno H, Uchiyama N, Kuno A, Togayachi A, Sato T, Narimatsu H, Hirabayashi J. A novel strategy for mammalian cell surface glycome profiling using lectin microarray. *Glycobiology*. 2007; 17:1138–1146. [PubMed: 17693441]
- Venable A, Mitalipova M, Lyons I, Jones K, Shin S, Pierce M, Stice S. Lectin binding profiles of SSEA-4 enriched, pluripotent human embryonic stem cell surfaces. *BMC Dev. Biol*. 2005; 5:15. [PubMed: 16033656]

- Wearne KA, Winter HC, O'Shea K, Goldstein IJ. Use of lectins for probing differentiated human embryonic stem cells for carbohydrates. *Glycobiology*. 2006; 16:981–990. [PubMed: 16809438]
- Yuan K, Kucik D, Singh RK, Listinsky CM, Listinsky JJ, Siegal GP. Alterations in human breast cancer adhesion-motility in response to changes in cell surface glycoproteins displaying alpha-L-fucose moieties. *Int. J. Oncol.* 2008; 32:797–807. [PubMed: 18360707]
- Zhao YY, Takahashi M, Gu JG, Miyoshi E, Matsumoto A, Kitazume S, Taniguchi N. Functional roles of N-glycans in cell signaling and cell adhesion in cancer. *Cancer Sci.* 2008; 99:1304–1310. [PubMed: 18492092]
- Zheng T, Peelen D, Smith LM. Lectin arrays for profiling cell surface carbohydrate expression. *J. Am. Chem. Soc.* 2005; 127:9982–9983. [PubMed: 16011345]
- Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, Lan N, Jansen R, Bidlingmaier S, Houfek T, Mitchell T, Miller P, Dean RA, Gerstein M, Snyder M. Global analysis of protein activities using proteome chips. *Science*. 2001; 293:2101–215. [PubMed: 11474067]

Figure 12.9.1.

Two repeated probings of HEK 293 cells on lectin microarrays. Lectins were printed in triplicate on the substrate slides. The lower panel shows the lectin layout on the microarray and the binary lectin binding profile for HEK293 cells. For clear visualization, the lectins with positive binding, binary code equal to 1, were marked in red and the lectins that did not bind the HEK293 cells, binary code equal to 0, were marked in white on the layout table. The data analysis was performed according to the methods outlined by Tao et al. (2008). Briefly, to determine cell binding to lectin spots on the microarray, the background intensity was subtracted from each lectin spot. A histogram of the net signal intensities for each microarray was plotted, centered on zero, and fitted to a normal distribution. Positive intensity (above background) appeared as a long tail in the histogram, representing a range of positive binding intensities. If the intensity of a spot was 6 standard deviations (SD) above 0, it was scored positive. For each lectin, the scores of the six replicate spots (duplicate microarrays and triplicate spots on each microarray) were used in a voting scheme to determine whether cells bound to that lectin. If more than four spots of a lectin were scored positive, it was defined as positive. Most of the lectins (80%) had either zero or six spots scored positive. The number 1 was assigned to all the positive lectins while 0 was assigned to all the negative lectins, thus a binary lectin binding profile was generated for a given type of cell that has been tested against all the lectins on the microarray. For the color version of this figure go to <http://www.currentprotocols.com/protocol/ps1209>.