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Advancing the burgeoning field of glycomics

by Dr Daniel M. Ratner

Utilising existing automated high-throughput screening techniques, carbohydrate microarrays are joining DNA and protein microarrays as an enabling technology for biomedical research. Recent synthetic advances and new methods in microarray fabrication have positioned the glycochip to make significant contributions to the growing field of glycomics.

While conventional DNA and protein microarray technologies have been a boon to industrial and academic research, investigators interested in studying the biological roles played by glycans have been frustrated by a lack of similar tools. Carbohydrates and glycoconjugates are known to be involved in inflammation, cell-cell interactions, pathogen-host adhesion, signal-transduction, development, and a myriad of other processes [1]. With half or more of all proteins containing some carbohydrate modification, the challenges associated with their study have been a considerable hindrance to the field of glycobiology and to our understanding of biology as a whole.

New methods in synthetic carbohydrate chemistry and microarray technology are beginning to address this need for novel biophysical methods in glycomics [Figure 1]. In the past five years, several research groups have independently developed promising prototype glycochips. All the while, progress continues towards standardising automated solid-phase oligosaccharide synthesis to address difficulties associated with tackling structures as diverse and complex as carbohydrates and glycoconjugates [2]. It remains to be seen if a single platform will emerge as the standard for glycan microarray technology, but commercial and academic interests - including Glycominds Ltd. and the Consortium for Functional Glycomics - have already made their glycochips available to an eager research community.

The chemistry and applications of a few representative systems are described herein to illustrate progress in the development of carbohydrate microarrays (additional detailed reviews of this subject are available in the literature [3]). In the drive to pioneer the first generation carbohydrate microarrays, many contributors deserve mention. The following article will highlight past and present efforts by Wang (Columbia University), Feizi (Imperial College), Mrksich (University of Chicago), Shin and Park (Yonsei University), Seeberger (MIT and ETH Zürich) and the Consortium for Functional Glycomics (Scripps Research Institute). For the burgeoning field of glycomics, these groups - among others - have made the vision of carbohydrate microarrays a reality.

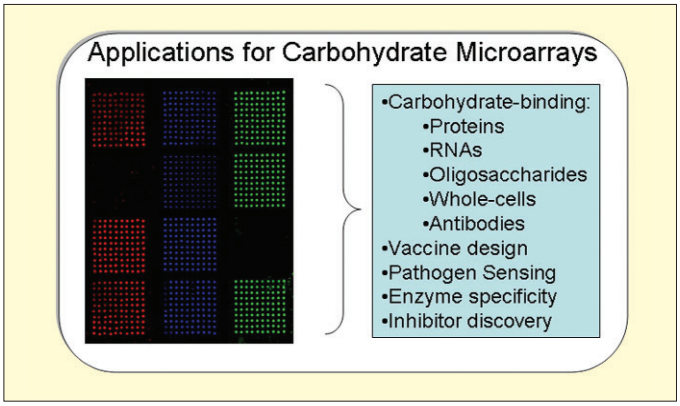

Figure 1. Carbohydrate microarrays have introduced high-throughput and parallel screening techniques to accelerate research in glycomics.

Carbohydrate microarrays: considerations

Carbohydrate recognition often consists of low affinity interactions between glycans and their associated binding partners. Therefore, a successful microarray platform must be able to probe protein-carbohydrate, carbohydrate-carbohydrate, nucleic acid-carbohydrate, and

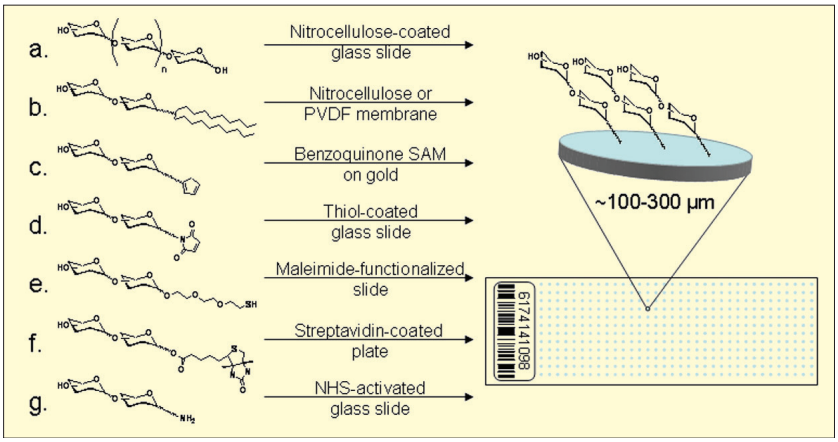

Figure 2. Immobilisation chemistry for the fabrication of carbohydrate microarrays: a. microbial polysaccharides printed directly onto nitrocellulose-coated glass slides; b. semi-synthetic neoglycolipids printed onto nitrocellulose or PVDF blotting membranes; c. cyclopentadiene-linked sugars are coupled via a Diels-Alder reaction to a benzoquinone SAM; d. maleimide-linked glycans are bound to thiol-coated glass slides; e. thiol-derivatised carbohydrates printed onto maleimide chips; f. biotin-linked glycans bound to streptavidin microtitre plates; g. amine-modified glycans bound to commercial NHS-activated glass slides.

intact cell-carbohydrate interactions with sufficient specificity to detect weak binding. To achieve this, the glycan moiety should be displayed in a fashion that is readily accessed by the analyte, and the system must strive to achieve low background signal due to non-specific adsorption of analyte to non-binding glycans and underivatised portions of the array. Carbohydrate-modified surfaces are ideal for interrogating these weak interactions, as monolayers of sugars on the surface of a chip are analogous to the multivalent display of glycan frequently found on the cell surface. This multivalency serves to amplify the binding event, increasing the level of the desired signal.

Several factors must be taken into consideration when designing a microarray. First, a suitable surface must be selected for displaying the glycan library. This can include microtitre plates, functionalised glass slides, nitrocellulose, and gold surfaces. Second, a linking chemistry, whether covalent or non-covalent, must be adopted. This requires careful consideration of the source of the glycans (synthetic vs. isolated), and whether a spacer will be incorporated to increase the accessibility of the sugar in solution above the surface. Numerous linking chemistries are possible, such as lipid modified glycans for non-covalent adsorption to nitrocellulose and hydrophobic surfaces, and sulphydryl, amino, and maleimido derivatised sugars for covalent attachment to functionalised surfaces [Figure 2]. Third, a protocol must also be developed for blocking potential sites for non-specific binding of analyte to the surface. And finally, a method must be established to detect weak binding events on the surface of the array. Most often, this will entail a fluorescent probe, although some groups have had success with surface plasmon resonance (SPR) and matrix-assisted laser desorption/ionisation and time-of-flight mass spectrometry (MALDI-TOF MS).

Immobilisation chemistries

Two early microarray platforms developed by Wong *et al* and Feizi *et al* utilised nitrocellulose surfaces for the immobilisation of carbohydrates and neoglycolipids. One method used nitrocellulose coated glass slides to array a series of microbial polysaccharides [4]. Similarly, neoglycolipids prepared from isolated oligosaccharides were arrayed onto nitrocellulose membranes [5]. In both instances, the surfaces were 'blocked' with bovine serum albumin (BSA) to reduce non-specific binding of interrogating proteins to the nitrocellulose.

Mrksich and colleagues developed one of the early methods to covalently attach synthetically defined sugars to an array. This was accomplished using linker modified saccharides bound to derivatised self-assembled monolayers (SAM) of alkanethiolate on gold surfaces [6a]. These microarrays were based on redox tunable hydroquinone ↔ benzoquinone SAMs; cyclopentadiene-coupled saccharides were covalently bound via a Diels-Alder reaction. In addition to providing a platform for detecting carbohydrate-binding proteins, Mrksich's arrays were used for on-chip enzymatic glycosylations, SPR to profile carbohydrate-protein binding, and direct analysis of protein binding partners by MALDI-TOF MS [6b].

A chemoselective coupling reaction between thiols and the maleimide group has been exploited by several investigators for microarray fabrication. Park and Shin *et al* described coupling maleimide-linked sugars to thiol-derivatised glass surfaces [7]. The Seeberger laboratory developed the inverse method by incorporating a thiol-terminated tri(ethylene glycol) spacer on the reducing end of a series of synthetic oligosaccharides [8]. These synthetically defined structures could be printed onto maleimide functionalised glass slides, and fluorescent encoded latex microspheres. Mrksich *et al* had similar success with thiol-modified sugars printed on SAMs of maleimide on gold surfaces [6c].

The Consortium for Functional Glycomics currently possess a library of over 200 synthetic and natural glycans for microarray fabrication [9]. Two primary linking chemistries have been employed for the Consortium's arrays. An ELISA-based array was prepared using biotinylated glycans bound to steptavidin-coated plates [9a]. More recently, the consortium has adopted amino-functionalised linkers to covalently couple their impressive glycan library to N-hydroxysuccinimide (NIH)-activated glass slides [9b].

Biological applications

While ongoing research continues to refine the existing microarray platforms, biomedical studies have already found numerous applications for the current generation of carbohydrate microarrays. Arrays of carbohydrates have been used to identify the glycan-specificity of proteins, nucleic acids, and intact cells (pathogen adhesion and cell-cell recognition). Glyochips have also been employed to examine human serum for carbohydrate reactivity, and to profile glycan-dependant enzymes and enzyme inhibitors.

Carbohydrate microarrays have found particular application in the study of HIV glycobiology [8]. The predominant surface envelope protein of HIV, glycoprotein gp120, plays a vital role in viral entry. The gp120 molecule possesses numerous sites for N-linked glycosylation, and the glycans are known to partic-

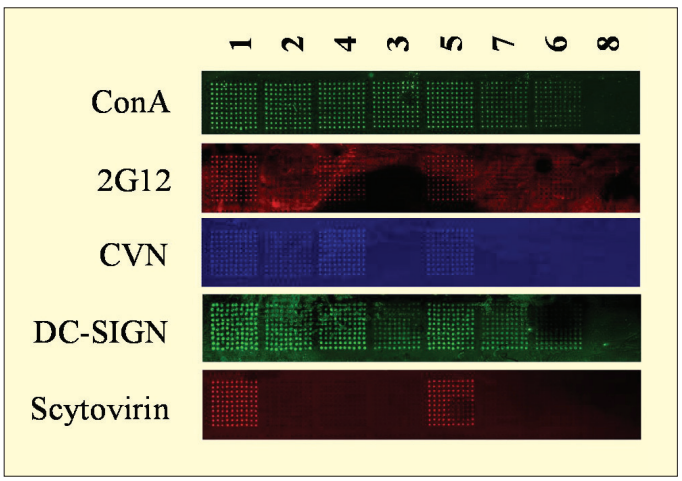

Figure 3. Carbohydrate microarrays containing a panel of synthetic mannans (Man1 through Man9) 1 through 7 and galactose 8, printed at 2 mM. False colour image of incubations with fluorescently labelled ConA, 2G12, CVN, DC-SIGN and Scytovirin (Reprinted from reference 8, with permission of Wiley-VCH).

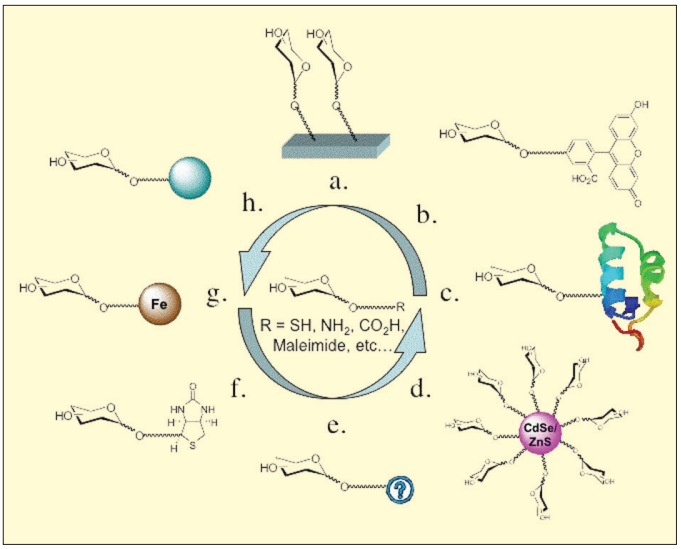

Figure 4. Tools for glycobiology: a. modified surfaces for microarrays and SPR; b. monovalent fluorescent conjugates; c. neoglycoproteins and carbohydrate vaccines; d. multivalent quantum dot conjugates; e. future neoglycoconjugates; f. affinity tag (biotin, etc.) conjugates; g. magnetic particle conjugates; h. latex microsphere and sepharose affinity resin conjugates (Reprinted from reference 8, with permission of Wiley-VCH).

ipate in host-cell adhesion and immunoevasion. Utilising a microarray comprised of a panel of synthetic high-mannose oligosaccharides, Seeberger *et al.* profiled the mannose-binding specificities of two anti-HIV proteins (Cyanovirin-N and scytovirin), the HIV-inactivating human monoclonal antibody 2G12, and the dendritic C-type lectin DC-SIGN [Figure 3]. The Consortium for Functional Glycomics has also used their microarray to study the carbohydrate ligand-binding properties of DC-SIGN and a related endothelial cell receptor DC-SIGNR [9].

Continuing efforts to develop carbohydrate-based vaccines for pathogens (including malaria and HIV) and cancer have highlighted additional uses for the glycochip. Microarray analysis of serum anti-carbohydrate activity will facilitate vaccine design and dose trials. Microarrays have already been used to show anti-carbohydrate activity in healthy individuals [9b]. These results detected the expected anti-ABO blood group response (varying by the individual's blood type), as well as antibodies towards glycans representative of Gram-negative bacterial polysaccharide (suggesting past exposure to these antigens).
The repertoire of novel applications for glycochips continues to grow. Whole-cell assays have been reported for both mammalian and bacterial adhesion. Schnaar *et al* (Baltimore) have published on intact cell adhesion of hepatocytes to Glycominds' commercial glycochip. Seeberger *et al* have used their own microarrays to detect bacterial-adhesion to cell surface glycans. In addition, Seeberger and Disney (Buffalo) have also used an aminoglycoside glycochip to study antibiotic resistance, antibiotic toxicity, and to detect RNA binding.

Future developments

Synthetic spacer-modified glycans displaying amine or thiol functionalities are emerging as useful tools for microarray fabrication and a host of spin-off technologies. Once a covalent linking strategy has been established, the possibilities are vast [Figure 4]. In addition to the microarray, these glycans can be used to prepare a veritable tool chest for glycomics: fluorescent conjugates, neoglycoproteins, multivalent quantum dot conjugates, affinity tagged saccharides, derivatised magnetic particles, latex microspheres, and sepharose affinity resins [8]. Many of these methods are beginning to receive attention in the literature as more groups grow aware of the successful applications of carbohydrate microarrays to glycobiology.

Conclusions

The field remains crowded with competing strategies for carbohydrate microarrays. With nearly a dozen reported methods for preparing the glycochip, the lack of standardisation may intimidate some from investing in this new technique. Only time will tell whether a single linking chemistry will be agreed upon by the community, but the versatility of the available microarrays suggest that one need not wait any longer for the technology to mature. Carbohydrate microarrays, in their many forms, are emerging as a major force to drive research in the new era of glycomics.

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