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Can Microfluidics boost the Map of Glycome Code?

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Abstract

Proteins carry out pivotal functions in cells. Less appreciated is that the proteins are sugar coated and that glycosylation affects how the immune system recognizes the protein, as being friend or foe. Unlike proteomics, glycomics is not identified by structures and sequence of the single units is not predefined. This makes difficult and tricky the study of glycosylation and glycoproteomics. However, the role of glycome code on cellular mechanisms cannot be neglected. Glycosylation of proteins is a major event in posttranslational processing along their route, cell surface proteins are mainly glycoproteins. Hence, glycosylation changes and glycan-protein interactions feature malignant transformation and tumor progression. The distance between glycomics and proteomics is still far and it is missed the methodological approach to pinpoint the site where glycosylation takes place.

Here, glycomics and glycoproteomics are analyzed and the role that microfluidics can play in research is investigated by the description of the already reported application. The margins of improvement of microfluidics are still wide. Here, analyzing the structural hierarchical levels, we intend critically discuss the role that microfluidics might have in boosting knowledge and progress in glycoscience.

Introduction

The term "glycome" describes the complete repertoire of glycans and glycoconjugates that cells produce under specified conditions of time, space, and environment [1-4]. "Glycomics" refers to studies that profile the glycome [5,6]. Glycan refers to a polysaccharide or oligosaccharide, it can be homo or heteropolymers of monosaccharide residues and can be linear or branched. Glycans may also refer to the carbohydrates as parts of a glycoconjugate, such as a glycolipid, glycoprotein, which may contribute to several biological mechanisms [7,8]. Glycans play pivotal role in the mechanisms of cell recognition, cell interaction and communication [9-12]. They participate in almost every biological process, which ranges from organ development to tumor growth to intracellular signalling. Many of those mechanisms are still unclear and efforts must be spent to understand how the totality of glycansgoverns the related processes [13].

One of the fundamental mechanisms that still need investigation is the glycosylation of proteins. This is a recurring mechanism in cell membrane and it can be related to anomalous behavior of the cells. Glycosylation is for example a universal feature of malignant transformation and tumor progression and cancer-associated modifications [1,14-19]. The glycosylation of the proteins give up immediately a new problem that concern the sequencing of the glycans by high-throughput technologies. Furthermore the sequencing has to bring information on pinpointing of glycosylation along the peptidic sequence.

The technology for following the mentioned mechanisms were approaching the maturity and many of them such as mass spectrometry, X-Ray and NMR were the key techniques to give the answers to many of the questions [20,21]. To the other side, even if the technology for profiling the proteins and even simple post-translational modifications were approaching maturity [22,23], the most abundant post translational modification, glycosylation, still remains practically unexplored [24,25]. This is misleading and resulting from the fact that glycomics researchers profile glycan structures but ignore the proteins from which they came, and proteomics researchers profile proteins while ignoring the appended glycans [26-29].

Even though it is not yet explored in this field, high throughput microfluidics can serve to deal thousands of information and to correlate the different disciplines increasing the know-how for human health [30-33].

To the other side, microfluidics, as high throughput technology [34-36], has already been introduced as tool for glycomics investigation [37-44], even if several challenges remain unreached.

The aim of this review is to explore the glycomics code by emphasising the utility that microfluidics might have in boosting the research of glycomics and glycoproteomics. To achieve this challenge, we refer to the cells and we identify four different levels of knowledge of glycomics. We are sure that this classification might help to understand the applications of microfluidics and find information for the sequencing of the glycans.

The hierarchical levels of the glycome

The glycans can open different and valid ways to describe the biological phenomena even tough actually there is still a huge gap between the potential of glycomics and the available techniques. There is no universal "glycan structure code" akin to the genetic code [45] and the glycome code is still a challenge. In contrast to the genetic code, the glycome is not identical across the variety of live forms. This is due to the different forms that the single unit could display. In addition, the genetic base of core functions such as gene transcription and energy tends to be significantly conserved among species. To complicate glycomics and to postpone the glycome code knowledge, it is that the carbohydrates always come as a mixture of isomeric configurations (α- and β-) or as carboxylate species.

Many schemes of simplification have been proposed to decipher the glycome code.

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In principle, different levels of structures can be identified. Figure 1 displays the schematic overview of the different hierarchical levels that characterize the living organisms [46].

First level

This first hierarchical level is the essentially catalogue of structures and it is an important starting point for any comprehensive glycome analysis. How the parts in the catalogue assemble to form the intact system is also important for understanding function and it is the logical question arising from this first level.

Second level

The second hierarchical level of analysis involves defining which glycans were associated with individual proteins or lipids. Analysis of the complete repertoire of a cell's glycoproteins, including their glycan structures and sites of attachment, lies at the intersection of glycomics and proteomics and is often referred to the term "Glyco proteomics".

Third level

A third level of complexity involves the determination of which glycans or glycoconjugates were expressed on specific cells or tissues. This level of glycomics sequencing is essential if the goal is to reveal new functions in cell–cell communication or to correlate particular glycomes with disease tissue.

Fourth level

The fourth level involves the visualization 3D of the organization relative to each other within the cell, at the cell surface, and in the extracellular matrix.

Microfluidics

The traditional approach to perform the sequencing of the glycans suffers of the low throughput [47]. Microfluidics has been already exploited as high throughput technique and successful attempts have been done to deal with tiny volume of extremely complex biological samples [48], or to integrate the whole operations sample treatment Figure 2.

Herein we do not intend to describe in details all microfluidic components and applications and we invite the readers to find some interesting lectures in Arora et al., McKenna et al., Cheong et al., Simone et al., and Rillahan et al. [49-54]. Our intention is to highlight the advantages that microfluidics might have in revealing the glycome code.

Recently the interest of biologists has been focused on cell, and microfluidics has been reorganized to handle and guest the cells. The characteristic dimensions of the microfluidic channels, the possibility

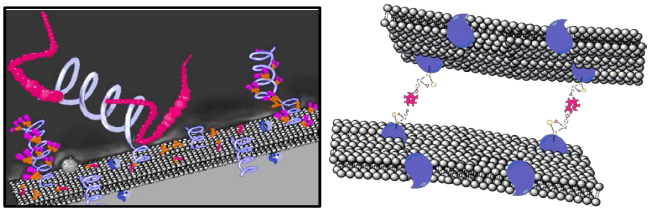


Figure 1: Schematization of the four hierarchical levels of glycomics. The spheres represent the glycans. The deciphering of the purple sphere belongs to the first hierarchical level. The deciphering of the orange and purple sphere and the pinpoint of the point of interaction belong to the second hierarchical level. Organization into the space (3D) (long pink chains) resembles the third hierarchical level. On right a detail on mechanism of cell-cell interaction (forth hierarchical level).

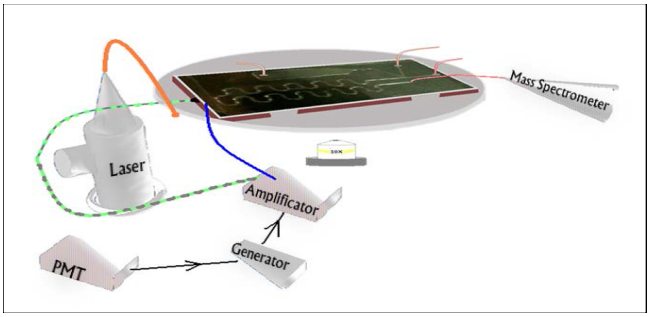


Figure 2: The microfluidic device integrating all operations to perform the sequencing of the glycans upon different hierarchical levels.

to modify the surface and mimic 3D environment makes 'lab on chip' deeply intriguing to handle the cells and study their behavior. To date, to follow the interest of the biologists was just natural consequence of the microfluidic science. Recently, the possibility to compartmentalise the single cell and to study them has made much more interesting microfluidics, due to the possibility to analyze each single cells of huge population in few minutes to collect thousands of information. The know-how gained in cell handling, culture and analyze them pave the way to the high throughput sequencing of the glycans [55-58].

The structure of glycans-level first: high throughput microfluidics

The standard method to describe the first level of the carbohydrate structure consists in the identification of the different isomers of the glycans and identification of the single components of the long chains. The sequence of the carbohydrates is like LEGO blocks, the first level of sequencing enables to define the single block and in particular the terminal of the sequence. Recognition of the single monosaccharides occurs by the formation of 'specific interaction' between the carbohydrate and the lectins. The lectins are the proteins (extracted from vegetal or animal) that bind the single monosaccharide of the glycomics sequence forming a unique complex that is given from the specific interaction between the carbohydrate and the lectins.

Protein-carbohydrate interactions as well as carbohydrate-carbohydrate interactions exhibit low intrinsic affinity and high specificity (K_D values of 1 μM^{-1}) and they get a biological effect only through multivalent interactions [59]. Owing to low avidity, to exploit the affinity and the specificity of the interactions, additional valence or multivalency is required. Investigators have observed that signal intensity for different glycans reflects their relative affinity for the moiety. By varying the concentration of the moiety, high-affinity and low-affinity ligands can be distinguished. The differences between these ligands are minimized as a result of the saturation of the signal during scanning. As the concentration of the moiety is decreased, only high-affinity ligands are detected.

Binding force and avidity are related each other by the equation (1).

$$F_{binding} = -log(k_D/sec^{-1}) \quad (1)$$

Low avidity of the binding is translated in weak force of binding whilst high avidity implicates strong binding force.

In microfluidics assay where the binding between the ligand and the receptor play the fundamental role, microfluidics enables the control of shear stress and the shear force [60,61] and consequently is a strong tool to measure the binding force that characterizes the complex. Hence, microfluidics gives chance to control the shear stress

and the shear force and measure the constant of association and dissociation (K_D) of the binding interrogating at the same time the samples on different ligands.

Inside the microfluidic environment, to account the adhesion force, the cell receptor–surface ligand interaction has been represented by a linear spring exerting adhesive force on the target cell Figure 3A. The experimental investigation with the W6/32, an antibody that binds specifically to MHC class I molecule and which has an important role in the recognition of the tumor cells from the immune system, has been simulated by the numerical model with a constant spring $K_s = 7.5 \pm 10^{-8}$ N/s. More details of the model were provided [60], the deformation is provided by the equation (2)

$$X = U_{\infty} + M(F_{nd} + F_{BX})$$

Where both vectors, the cell velocity X and the unperturbed flow field U_{∞}, have six components including three translational and three rotational degrees of freedom. Accordingly, the shear force F_{hd} and the external force vector F_{Bx} also have six components, which are three force and three torque vector components acting on a cell, while M is a 6 × 6 mobility matrix. We have defined F_{Bx} as the binding force of the cells to the surface, and F_{Sx} as the shear force exerted on the cell. At a flow rate $F_{Bx}>F_{Sx}$ the cells are prevalently adherent to the substrate, whilst at a flow rate $F_{Bx}<F_{Sx}$ the cells start to detach and move inside the channel with the fluid flow.

Microfluidic design and chemistry are the two elements that need to be optimized in order to study the behavior of the carbohydrate based complex.

We designed Hele-chamber to study the possibility first to specifically identify the α-galactose immobilized on microbeads covalently glued to the bottom of the microfluidic assay Figure 3B [62], then we isolated successfully CTCs from carcinoma affected patients [63].

Beyond the microfluidics to investigate the role of the cells, microfluidics has been exploited to resolve the isomeric mixtures of carbohydrate. To get this challenge, microcolumns were filled by the stationary phase and conjugated by lectins in order to reproduce on micro-scale the selective chromatography [64]. The sensitivity of separation based on the different enantiomeric configuration is high and confirms the results reached from different methods of separations operate in microfluidic environment [65].

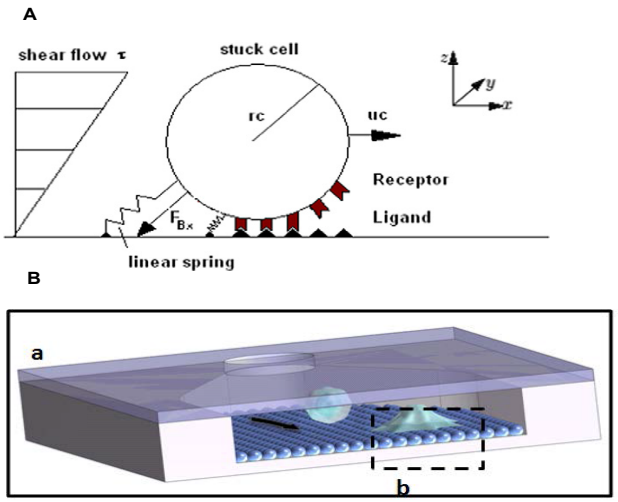


Figure 3: Design of the experiment, principle and suggested mechanism of cell adhesion. The samples were manually injected into the microfluidic assay and incubated. Following, the PBS was perfused up to 30 min to wash the assay. During the washing the contaminants were removed and only the targeted cells were captured; B) Mechanisms of interaction between the cells and the β-galactose.

This is the borderline between the first and the second hierarchical level. The first hierarchical level of sequencing has still several challenges; the most important is to get high-throughput analysis. In standard protocols, multiplexed plates and arrays of spotted lectins have replaced by the single spotted assay and microcolumn [66,67]. The assays display the possibility to run at the same time the samples and interrogate them with different ligands. Multiplex, high throughput microfluidics is excellently represented by microdroplets. They can stably isolate single molecules or single cells, whereas the multiplex can be due to the possibility to include inside each droplet a library of epitopes and interrogating the single compartmentalized sample for the same library [68].

Glycoproteomics- level second: microfluidics support to handle underivatised sample

The second hierarchical level merges proteomics and glycomics with special care to pinpoint the site where the glycans chains bind the peptide.Mass Spectrometry is still considered the gold technique to perform analysis. Glycoproteomics (glycomics + proteomics) has the important role to advance the understanding of system biology by identifying new biomarkers or new molecules of interest and to potentially improve medical technologies. The profile of glycosylation changes significantly during cell life and modification. Progress of the disease can be also detected by sequencing of the glycans [69]. For example, the increased activity of N-acetylglucosaminyltransferase V, which is an enzyme responsible for the formation of branching N-linked glycans, was linked to invasion of tumors and metastasis in several cancers [70,71].

However, to put glycomics and proteomics together makes the analysis exponentially greater, a reflection of the fact that glycoproteomics encompasses two completely different classes of molecules—molecules with very different chemistries, compositions, and structures.

There are two main types of protein glycosylation: (i) N-linked glycosylation whereby the glycan is attached to the amide nitrogen of asparagine in a consensus Asparagine-X-Serine/Threonine (Asp-XSer/ Thr) sequence, where X can be any amino acid except proline and (ii) O-linked glycosylation in which the glycan is attached to the hydroxyl oxygen of serine or threonine in the protein Figure 4A.

A typical glycoproteomics pipeline consists of glycoprotein enrichment techniques Figure 4B, followed by multidimensional chromatographic separation, mass spectrometry and data analysis. The approach can follow a top-down or bottom up sequence [72]. The technique used will depend on the specific research question asked, but Mass Spectrometric analysis communes top and bottom down investigation [73,74]. Most of the available approaches interrogate derivatised serum glycans [75], desialylated samples [76]. However, for biomarker-related applications, minimal sample manipulation and processing are preferred, so as to preserve the original glycan profile and maximise method reproducibility. Thus, the challenges of glycoproteomics are 1. Increase the efficiency of the separation; 2. Reduce the steps of derivatisation.

Microfluidics is important in the step of sample preparation for

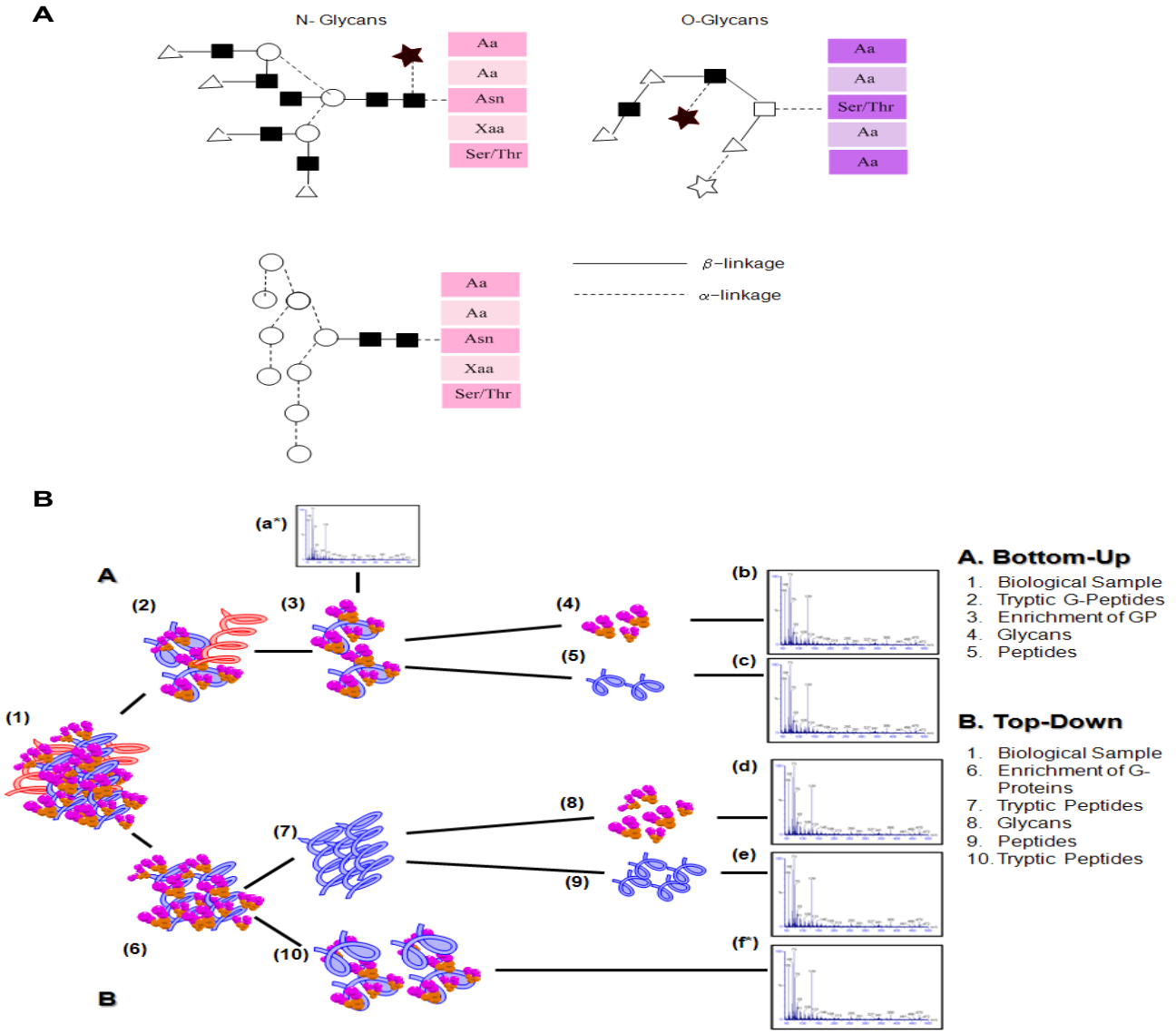


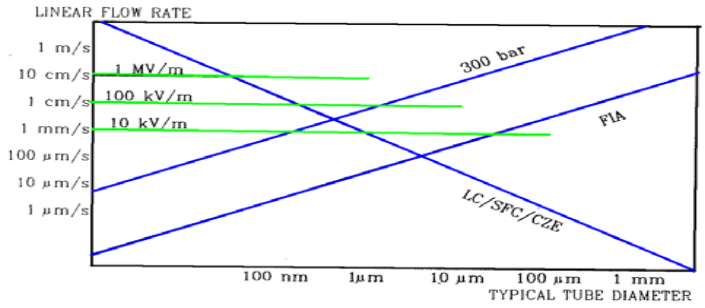
Figure 4: N-Glycans and O-glycans spectra. B). Pipeline of glycoproteomics analysis A shows the Bottom Up pipeline and B the Top Down. The analysis from (a) to (f) is the Mass Spectrometric Spectra of Peptides or Glycans. (a*) and (f*) keep information of glycoproteomics.

quantitative subsequent analysis. However, microfluidics can expose the sample to on line analysis, avoiding the step of collection and off line input and output to the subsequent analysis. This second approach has not been yet exploited to deal glycomics and glycoproteomics but it is in the focus of scientists of miniaturization. Here we discuss in particular the first approach as this has been directly applied to glycomics and glycoproteomics.

Microfluidics might have a deep role in improving the methods of glycoproteomics. By exploiting microchromatography, scaling down the characteristic time of process is available, it reduces the number of plates of the column; still it reduces the efficiency in handling the tiny volume of the sample. Manz et al. accurately describes the influence of the diffusive regime on the performance of the flow and separation [77]. Figure 5A shows the laminar flow rates required for time constant (flow injection analysis) and diffusion controlled tubing systems (chromatography and electrophoresis). A pressure gradient yields flow rates proportional to those needed in a time constant system, regardless of time space scale. Figure 5B and 5C show the results of separation efficiency as depending on the number of theoretic plates and the limits of detection respectively. It can be observed that the range of detection of the microchromatography has been located below 1 picoliter, displaying the high expected results from this technique. The advances in such microfluidic technologies allow flow switching between cross-interconnected channels by adjusting electrical potentials at various channel terminals.

Optimized microfluidic column, the stationary phase affects the performance of the separation, the HILIC is the more successfully applied. Zaia and coworkers have developed the novel N-linked glycan derivatization method where stable isotopes are incorporated into the reductive amination reagents in order to perform relative quantification of glycans from different samples. This method is the first to use tetraplex stable isotopes and quantified in the same mass spectrum. Fractionation of samples is crucial to this experiment so that the isotopic envelopes of different glycans do not overlap causing error

A



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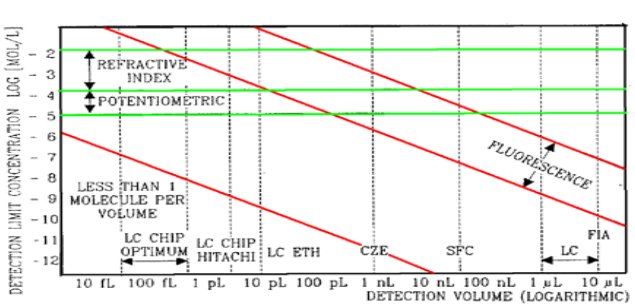

B

Parameters		Electroosmotic Chromatography			Liquid Chromatography		Supercritical fluid Chromatography	
Theoretical Plates	***N***	100k	1M	10M	100k	1M	100k	1M
Analysis time	***t, min***	1	1	1	1	1	1	1
Heating power	***P/L, W/m***	1.1	1.1	1.1				
Capillary id	***d, ☒ m***	2.4	7.6	2.4	2.8	0.9	6.9	2.2
Capillary length	***L, cm***	6.5	21	6.5	8.1	26	20	64
Pressure Drop	***P, atm***				26	2600	1.4	140
Detection Volume	***V, pl***	47	4.7	0.47	0.8	0.08	1.2	12
N Response time	***t, ms***	21	6.5	2.1	16	5	16	5
Stop time	***t, s***	3.3	3.3	3.3	5.1	5.1	5.1	5.1

Figure 5: Linear flow rate as a function of the inner diameter. B) Calculated parameters calculated for separation performances. C) Limits of detection for given techniques (adapted from Sensors and Actuators: B).

in quantification. Thus HILIC online separation has been performed. By combining HILIC and ESI it has been done the sequencing of samples from healthy and tumor samples and the differences have been identified [78]. The study has been followed by the integration of high throughput cleavage and MS analysis of N-linked glycans. Zaia's group has also tried to increase the hydrophobicity of the glycans, which will increase the detection of glycans in nanospray MS and in future capable of incorporating stable isotopes for relative quantification of glycans [79,80].

However, the separation of biomolecules even though requires the use of the mentioned protocols to prepare the samples, it is possible to start from cell culturing to glycan sequencing avoiding external contamination, loosing of the samples and at the same time picovolume of sample can be handle without un-useful dilution and sample.

The possibility to deal with the microsurface of contact and the high control of the microflow increases the efficiency of separation and the throughput.

The challenge, at this point, becomes to analyze the cell fingerprint without modification of the living organisms. Many routinely used techniques tend to partially or completely destroy the sample or even miss potentially important modifications such as sulfation and O-acetylation. The handling of underivatized glycans pushes to move the attention to microfluidics. Attempts to perform online analysis in microfluidic full integrate devices are reported in literature. Cells can be cultured in biomimetic environment with online change of the culture medium. To the other side, enriched medium, containing the released molecules can be analyzed and profiled as well as the cells. To the other sides, when the analysis is aimed to the readout of the fingerprint of the cells, microdroplets have been envisioned to perform high throughput analysis of single cells. Water in oil droplets can be used to compartmentalise single cell and the targeted molecules constituting the assay. After encapsulation, droplets of different elements can be pooled into a "droplet library," ready for subsequent use in a single screening assay that includes all library elements. Specific examples to identify the glycome profile of the cells once they are encapsulated inside the microdroplets are not reported yet, whilst examples of sequencing of the glycocode directly from the cells are documented in microfluidic environment as well.

Cell membrane profile-level third: microfluidics towards on-chip investigation

The third hierarchical level offers a global view of the distribution of certain glycan epitopes on cells and tissues. The sequence of cell glycans is still staticbut takes advantages of the multivalency of the interaction. Considering the interest for the cell as whole, the second and the third hierarchical level shares the same interest in handling the native samples. This is to keep the original structure, and in particular for the third hierarchical level, the knowledge of the 3D structure is required.

The analysis of a higher level of organization is required to perform the glycomics sequencing at the cell membrane.

The advantages of cellular investigation are

1. Single cell

2. Reduction of the time of cell handling (in environment diverse from the extracellular microenvironment). The analysis on the single cell keeps the 3D organization of the glycans and the cross linked structure, as consequence the analysis by MS gives only limitative results losing the information on the space structure of the glycans, the conformation that could promote the crosstalk with other organisms. The standard techniques to perform 3D analysis of the cells can be done by NMR and X-ray techniques that also can exploit the advantage of miniaturization, even if the available examples are at the moment negligible [81,82].

Organisation between cells-level fourth: microfluidics to mimic in vivo mechanisms

The last hierarchical level we are going to investigate involves the dynamic interaction between the cells. The cell membrane is decorated by the glycans. Glycan-protein complexes are responsible for myriads of interactions and communications between cells and their environments. Cells interact with the surrounding during the lymphocyte rolling and host-pathogen recognition. The binding interactions are of the low-affinity, high-avidity variety that arises for the interaction of many carbohydrate-protein molecule pairs on cell surfaces.

Some year ago Hakomori schematized his theory on cell-cell interaction, highlighting the adhesion based on interaction of several combinations of glycosphingolipids (GSLs) at the surface of interfacing cells ("trans interaction") [83-85].

Clustering of GSLs or glycoproteins organized with signal transducers at the cell surface resulted in the formation of microdomains. Those, which were involved in adhesion and coupled with signal transduction to alter cellular phenotype, were called "glycosynapse".

Microfluidics has been already exploited to study cell-to-cell and cell-to-ECM interactions [86,87], but how the adhesion of the tumor cells to the endothelium, and the subsequent transmission of information, involves the selectins and the sialic acid SLex in mechanisms of glycosynapses remain unrevealed. Microchannels and micro chambers have been functionalized by selectins or endothelial cells and tumor cells were perfused inside the microenvironment to study the mechanisms the rolling and extravasation [88,89].

A target cell on a biofunctionalized surface under shear flow experiences rolling adhesion when $0 < u_{c0} < u_0$, where u_{c0} is the initial velocity of the cells and u_0 is the initial velocity of the fluid. In the rolling adhesion regime, the hydrodynamic flow is not large enough to drag the cells; consequently, target cells adhesively roll forming interactions with new bonds being continuously formed downstream that compensate the dissociation of old bonds. To study the adhesion–detachment characteristics of the cell with the substrate, we undertook a preliminary investigation to determine optimum flow rates at which to obtain the condition $0<u_{c0}<u_0$, and to define the hydrodynamic conditions that balance the binding force.

Deterministic cell rolling was investigated over a surface bearing P-selectin to mimic the process of the leukocytes in inflammatory process [90].

Elegant experiments were performed to study the cell movement involving the glycosfingolipids (GSL), the microfluidic design resembled the blood vessel and adhesion and rolling of the cells has been measured for the native and infected red blood cells. Microfluidic channels in fact have been coated with selectins and different lectins to simulate the mechanism that the cells follow once in contact with the surface. Figure 6A and 6B shows a cartoon of the blood vessel and the translation of the mechanisms in microfluidic environment. However, during the flow, the cells interact with the wall of the vessel and are put in contact with the receptor/ligands of the endothelium. Rolling and extravasation have been studied and still are in the focus of biologists, however, right now microfluidics has been exploited to study the origin of the domain that are formed or the information that the cells transmit between them or toward their own nucleus [91].

Microdomain formation is represented by the syalisation of erythrocyte membranes. The erythrocyte membrane presents five major membrane glycoproteins, three of which are heavily sialylated and two that are not, but are the major carrier of the ABH(O) blood group. At the highest hierarchical level, the question is how the terminal glycan sequence and the variations in glycosylation among members of the same species could differentiate the individual.

The classic example is the ABH(O) blood group system, which is a glycan-defined polymorphism and found in all human populations. Despite its great clinical importance for blood transfusion, this polymorphism appears to cause no major differences to the intrinsic biology of individuals of the species. Similar to other blood groups, the ABO polymorphism is accompanied by the production of antibodies against the other variants. These antibodies cause complement-mediated lysis of enveloped viruses generated within other individuals.

They can express the target structure for the antibody. It has therefore been suggested that an enveloped virus generated in a B blood group individual might bear this structure and be susceptible to lysis when contacted with antibodies of an A or O blood group individual, who would express anti-B antibodies.

Another possibility for the diversity between species is the number of pathogens that recognize glycans as targets for attachment and entry into cells. Most likely is this mechanism operative in generating the diversity of sialic acid types and linkages. The analyses have tried to combine the two mechanisms: the possible frequency dependent protection from glycan-exploiting extracellular pathogens, such as Noroviruses and Plasmodium falciparum malaria and the antibody-mediated protection from intracellular viruses [92]. Multiple agglutination assays in parallel without cross-contamination and using only microliter volumes of blood were performed by using a plug based microfluidic approach. Here the device was designed such that aqueous streams of antibody, buffer, and red blood cells (RBCs) were combined to form droplets 30-40 nL in volume surrounded by a fluorinated carrier fluid. The proof-of-concept was performed by the ABO and D (Rh) blood typing and group A subtyping by screening against multiple antigens without cross-contamination. Additionally, on-chip subtyping distinguished common A1 and A2 RBCs by using a lectin based dilution assay. The assay enables also to differentiate rare and weakly agglutinating RBCs of A subtypes just by analyzing agglutination avidity as a function of shear rate. This device is suitable for point of care applications where sample volumes were small, such as by, blood typing of newborns, and general blood assays in small model organisms.

Concluding remark

It is already widely accepted that the word of the glycans (from the simplest unit to the more complex) might aid in understanding several unraveled phenomena. Many investigations showed that glycosylation of the protein are significant of cellular changes.

To the other side, glycans manage the crosstalk between cells and the transfer of communication. Alzheimer, diabetes, tumor and metastasis have been correlated to the anomalous glycosylation, the mechanisms that drive the proteins from Golgi to the extracellular membrane.

Something is still missed to accurately describe the glyco-code and this is due to the complexity of the code and the high number of combination. Exemplification has been done distinguishing between hierarchical level of chain complexity but over position of the information from the proteomics and glycomics need different tools.

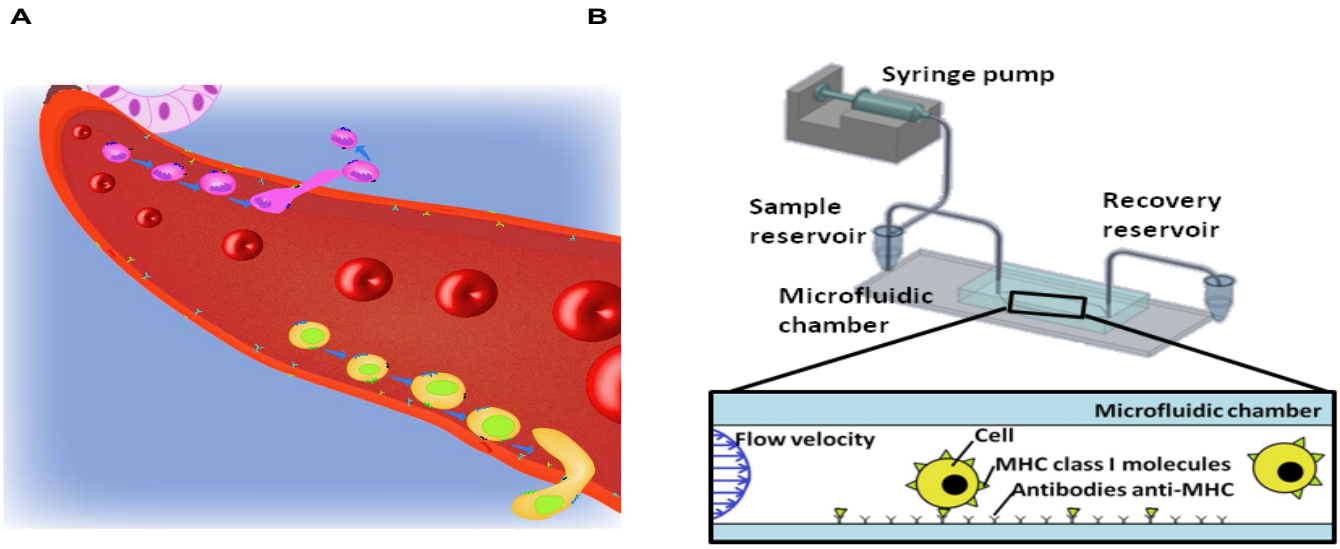

Figure 6: A) Cartoon of the blood vessel showing several ligands for the flowing cells. B) Example of Microfluidic Device where the cell rolling has been investigated and transmission of the information inside the cytoskeleton of the cells is transmitted.

Hence, in order to keep the information of the native chains, the investigation is even more moved to the cellular target. High throughput and high sensitivity of the assay are required, this comport that the steps of sample preparation need to be optimized to deal picovolume of sample, to increase the yield of separation and finally to increase the throughput. Microfluidics has already shown the high sensibility of separation, the high throughput and we believe that it can be a powerful tool to deal sequencing of the glycans over the four hierarchical levels.

Some approaches have been already touched from the scientists some others are still missed and just tested for better know and simpler molecules (i.e. proteins).

The authors hope this present review has highlighted the potential advantages and new applications that microfluidic can provide, and how glycans provide a target to describe unraveled biological mechanism.

Conflict of interest

No conflict of interest has to be declared.

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