SmarTTransfuser
A Biochip System for the Final ABO Compatibility Test

Karine Charrière[1], Jean Sebastien Guerrini-Chappuis[1], Bruno Wacogne[1,2], Alain Rouleau[2], Celine Elie-Caille[2], Christian Pieralli[2], Lionel Pazart[1], Pascal Morel[3] and Wilfrid Boireau[2]

[1] *INSERM-CIT 808, Besançon University Hospital, Besançon, France*

[2] *Institute FEMTO-ST, University of Franche-Comté, Besançon, France*

[3] *French Blood Transfusion Center, Besançon, France*

Keywords: Blood transfusion, Lab-on-chip, Microarray, Nanobiotechnology, Surface functionalization, Biosensor, Bedside test, ABO compatibility, Crossmatch.

Abstract: Before each transfusion of red blood cell concentrate, a final ABO compatibility test is carried out at the patient's bedside on a piece of card and interpreted visually. Despite this ultimate test, transfusion accidents still occur due to group incompatibility, which can be lethal. In order to improve this test, we have developed a specific device based on microarrays for the validation of a smart and safe transfuser in the context of critical transfusional situations. This miniaturized device incorporates a biochip to analyze ABO compatibility in order that the hemagglutination reaction of red blood cells with IgMs in solution be replaced by specific capture and concentration of IgMs on microarrays. Results indicate that a specific immunocapture is obtained with globular concentrates and with different total blood. Smarttransfuser is a smart device developed in collaboration with the French Blood Transfusion Center for the optimization at the patient's bedside of an ultimate test prior to transfusion.

1 CONTEXT

A variety of blood components are available including red cell concentrate (RCC), platelet concentrate (PC) and plasma.

About 24 million components were transfused and about 15 million whole blood or RCC were transfused in the USA in 2008 (National Blood Collection and Utilization Survey 2009). In France, almost 3 million labile blood products, of which 79% are RCC, are distributed annually for just over 500.000 patients who are transfused.

In 2009 there were 3 patient deaths following a reaction to ABO-incompatible blood in France, and this reaction may have contributed to the deaths. Last year, there were two major ABO-incompatible reactions, one of which led to death (Afssaps, 2010). In the UK, a total of 14 ABO-incompatible red cell transfusions were given, 10 resulting from bedside administration errors, 2 from wrong blood in tube phlebotomy errors and 2 due to laboratory errors in which the wrong sample was used for crossmatch (SHOT, 2009). According to the Fatalities Reported to the FDA following blood collection, in combined fiscal years 2005 through 2010, ABO incompatibilities account for 9% of the transfusion-related fatalities reported. There was a decrease in ABO haemolytic reactions from 4 in FY2009 to 2 in FY2010 (FDA, 2010).

Attentive analysis of these attribution errors has shown that these mistakes are often multiple, occurring throughout the transfusional process.

2 ULTIMATE CONTROL

Before each transfusion of RCC in France, a final ABO compatibility test is carried out at the patient's bedside on a piece of card and interpreted visually. The final test, carried out by nurses or doctors, aims to verify the identity of the recipient (Figure 1). It also aims to verify compatibility between ABO grouping and the red blood cell units to be transfused. This final compatibility test at the patient's bedside, the last security measure, is frequently called into question and other countries

Charrière K., Sebastien Guerrini-Chappuis J., Wacogne B., Rouleau A., Elie-Caille C., Pieralli C., Pazart L., Morel P. and Boireau W..
SmarTTransfuser - A Biochip System for the Final ABO Compatibility Test.
DOI: 10.5220/0003852402570262
In *Proceedings of the International Conference on Biomedical Electronics and Devices* (BIODEVICES-2012), pages 257-262
ISBN: 978-989-8425-91-1

choose other identification methods before administering red blood cells.

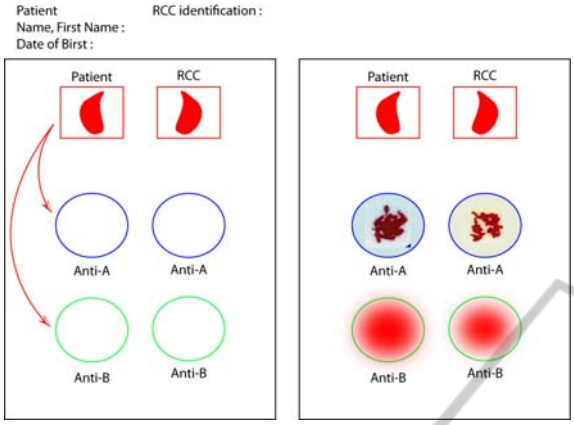

Figure 1: Representation of a bedside card. To use these cards, it is necessary to rehydrate each reaction field with one drop of isotonic saline solution (blue and red circles). One drop of patient blood should be deposited on the "patient field" and one drop of RCC on the "RCC field". Each drop should be deposited in the correspondents' reaction fields, with a new spatula for each to avoid cross reaction. The card should be shaken to see whether a hemagglutination reaction occurs. Nurses should decide whether RCC can be transfused according to this rule: for circles of the same color (same antibody), a positive reaction with blood cells and a negative reaction with the patient's blood forbid transfusions. In all other cases, transfusion is allowed. In this example, transfusion is allowed.

To eliminate these difficulties, the most frequently studied solutions aim to ensure compatibility between the information on the blood product to be transfused and the recipient. The technology developed consists of reproduced code systems: bar codes which are read and radio frequencies, to verify that the code attributed to the patient (on a bracelet or in his/her file), corresponds to the code attributed to the RCC (on a label) in terms of ABO bulking (Aandahl et al., 2007). These techniques constitute a higher level of transfusional safety but nonetheless do not overcome human error, for example when the bracelet is allocated or during recipient grouping, etc. (Dzik, 2005). Dzick outlines in a review some other obstacles: resistance to change, confusion as to the best technology and uncertainty concerning investment returns (Dzik, 2007).

In the UK, for example, the final administration check must be performed at the patient's side immediately before administering the blood component by matching the patient details attached to the blood component with the details on the patient's identification band (or equivalent) (British Committee for Standards in Haematology 2009).

None of these is entirely satisfactory at the present time (Levy, 2008), and the frequency of accidents is noticeably identical, no matter which test device is used.

The studies described in this position paper aim to demonstrate the benefit of applying immunocapture techniques on microarrays to the development of an "intelligent" blood transfusion device.

3 IMMUNOSENSOR ENGINEERING

A great challenge in biosensors and diagnostic devices is "how to obtain relevant biological mechanisms on the surface of microarrays and which analytical tools are convenient for providing accurate and rapid information on the structures of captured biological entities of interest?"

To fulfill these aims, many skills must be combined for a general approach. Successful immobilization of biomolecules on a solid base requires several critical factors to be controlled. The biomolecule must be linked to the surface with appropriate orientation, false positive signals must be avoided by minimizing non-specific interactions and ligands must remain active after binding (no denaturation, folding, etc.). To control the chemical functionalization of chips and the self-assembly processes of chemical monolayers for a highly controlled surface, biophysical investigations are needed.

3.1 Design and Prodution of Homemade Chips

Design and production of homemade chips compatible with Surface resonance plasmons (SPR) (from BiacoreTM) have been performed as previously described with the help of the MIMENTO technological platform, Besançon, France (Boireau et al., 2009).

A 2-nm thick chromium (Cr) layer was deposited on a SiO_2 wafer (width: 13 mm; thickness: 0.17 mm from AGAR) with plasma sputtering technology to optimize the adherence of gold to the substrate. The 40 nm thick Au layer was deposited onto the top of the Cr layer using plasma-sputtering technology. The deposition time and the argon flow pressure were optimized to obtain a suitable gold surface. The deposition time for the Cr and Au layers were

respectively 5 and 21 s. For all depositions, the argon flow pressure and current were 7 µbar and 0.3 A respectively.

With these deposition parameters, highly efficient SPR biochips were produced (in terms of complex optical thickness, surface roughness and pseudo-periodic nano-structuration) as demonstrated by Mangeat et al (Mangeat, 2009).

3.2 Chemical Functionalization and SPR Experiments

Protein immobilization is a crucial point which conditions the properties of specificity, stability and usability of biosensors. Most of the macromolecular coupling strategies on biosensor surface layers are based on deposition, functionalization and activation of polymer cushions with an expected high density of probes. We chose to control the immobilization and the homogeneity of the antibody layer on a bidimensional surface. This was done by SPR biophysical experiments in order to obtain quantitative information (level of immobilization).

The homemade chips were chemically functionalized as follows. The chemical functionalization was obtained using a mixture of 11-mercapto-1-undecanol (11-MUOH) and 16-mercapto-1-hexadecanoic acid (16-MHA) (purchased from Sigma–Aldrich). The mixture of 11-MUOH/16-MHA (97/3 by mole) at 1mM in absolute ethanol was sonicated for 10 min using an Elma sonicator (power 90W, frequency 50/60 Hz). Surfaces were rinsed by ethanol and ultra-pure water. Then, the carboxyl groups were activated using 240 µL of N-hydroxysuccinimide (NHS) at 10 mM and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) at 48 mM (Amine Coupling Kit from Biacore AB, Uppsala, Sweden) and incubated for 30 min at RT. Surfaces were rinsed by ultra-pure water. This procedure prepares the chips for the immobilization step.

Biacore experiments were performed with the Biacore™ 2000 apparatus at 25°C at a rate of 2 µL/min. The antibodies used were IgM anti-A or IgM anti-B (DIAGAST, provided by the French Blood Transfusion Center, Besançon). The running buffer was saline phosphate buffer (PBS, 100 mM at pH7.4 with NaCl 50 mM). The degree of protein immobilization and the level of interactions in the Biacore technology apparatus were plotted on a sensorgram (response unit (RU) versus time (s)). One thousand RU correspond to a shift in the resonance angle of 0.1°. Calibration of the apparatus gives a correlation between the shift in angle and the surface mass density deposited on the biochip surface, ranging from 0.1 to 1 ng/mm^2 (Stenberg, 1991). After exposure to the analytical solution, the chips were removed from the Biacore unit *via* an undock procedure with empty flow cell command.

We showed that after this chemical treatment of the gold surface, it was possible to immobilize anti-A and anti-B IgMs. First, different immobilization pH were tested and the optimal pH conditions for promoting functionalized antibody/surface interactions were established. For each antibody, the best immobilization was obtained with pH 4.65 (Figure 2). In this way, we were able to implement efficient conditions for grafting IgM on biochips. For each antibody, the surface was nearly saturated after the first injection, showing that our grafting conditions are optimized (Figure 3). The grafting rate reaches 1500 IgM/µm^2 on average, which could potentially involve 100 000 antibodies for each captured red blood cell.

On these functionalized surfaces, we demonstrated erythrocyte capture with Atomic Force Microscopy. With erythrocytes from group A globular concentrate, we achieved a specific interaction with surface grafted with anti-A IgMs.

These initial results make way for an *ex-vivo* development.

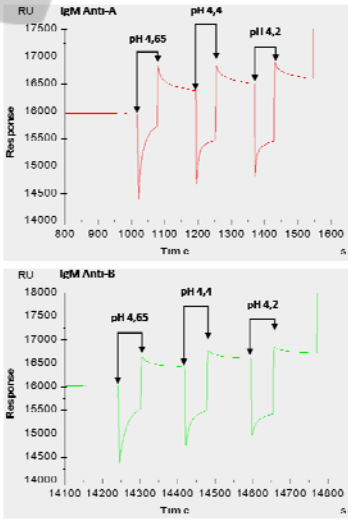

Figure 2: Immobilization of IgM anti-A and IgM anti-B measured by SPR. Three pHs (4.65; 4.4; 4.2) were tested and the resonance surface plasmon, expressed in units of resonance (RU), was measured in real time. For each antibody, optimum immobilization pH is 4.65.

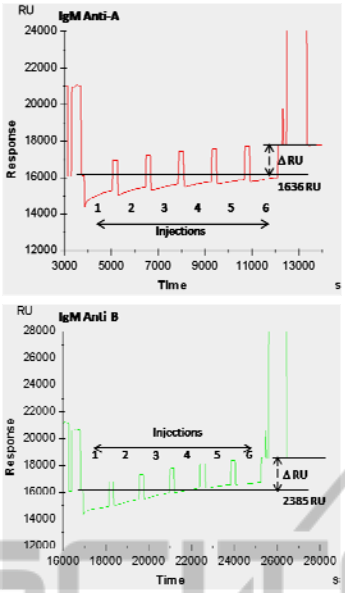

Figure 3: Immobilization of IgM anti-A and IgM anti-B measured by SPR. Six successive injections were realized and the resonance surface plasmon, expressed in units of resonance (RU), was measured in real time. We observed an echo variation of 1636 RU for the IgM anti-A and of 2385 RU for the IgM anti-B at the end of injections.

3.3 Feasibility Tests

After controlling functionalization of gold surfaces and immobilization of erythrocytes, we checked the ability of the biochips to work with other blood groups. To do this, we conducted surface plasmon resonance imaging (SPRi) experiments.

The chemical functionalization was performed as described above. Four spots of IgM antibodies were grafted onto the surface. Antibodies anti A or anti B (purchased from Diagast) were diluted (1/10) in acetate buffer (0.1 mg/mL, pH 4.5) and 2 spots of each specy (2 µL/spot) were deposited on each surface and incubated for 1 hour at room temperature in a humid chamber. Then a blocking agent (Rat Serum Albumine 40 µg/mL, pH 5.2) was used to passivate the surface by incubation at room temperature for 30 min. Incubation in ethanolamine (0.2 M) was then used to target the free NHS entities in order to desactivate the surface. Finally, the biochips were rinsed with ultra pure water and used for SPRi experiments. They were performed using a SPRi-Plex imager (Horiba Scientific, France) equipped with a 660 nm wavelength LED and a CCD camera.

Experiments were carried out at room temperature, in physiological serum (NaCl 0.9 %). The flow rate in the chamber was 50 µl/min. Ligands (red blood cell concentrate group A or whole blood groups O, A, AB and B) were injected (volume 200 µl) and the biochip surface was rinsed to remove unbound ligands. Whole blood and red blood cell concentrate were provided by the French Blood Transfusion Center, Besançon).

The biochip was tested with different blood groups in order to assess its specificity. Between two measurements, the surface of the biosensor was treated with PBS-n-Octyl-beta-D-glucopyranoside for 1 min to dissociate the probe/target adducts previously formed (regeneration of the surface before a second injection). The system has proven to be selective. Indeed, each target was bound only to its corresponding antibody. No significant signal was observed on the non-corresponding antibody and reference spots. This shows the absence of undesired non-specific binding (i.e., cross reactivity) and/or adsorption on the surface. As seen in figure 4, a very intense signal is observed on anti-A IgM spots with whole blood group A (Figure 4A) whereas no significant signal is observed on the two other spots. In figure 4B, erythrocytes are immunocaptured only by anti-B IgMs.

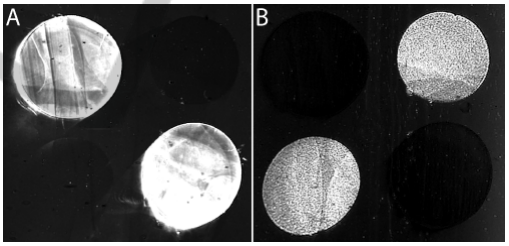

Figure 4: Different photographs of one biochip after injection of whole blood group A (A) or whole blood group B (B).

This high selectivity demonstrates that it is possible to determine whole blood / red blood cell concentrate groups by using a biochip in order to replace the hemagglutination reaction of red blood cells by IgM in solution.

4 TOWARD A MEDICAL DEVICE

The sensor should now be incorporated in the main device, called “SmarTTransfuser” and developed for clinical trials.

We have previously demonstrated that our homemade biochips can specifically immunocapture red blood cells of different blood groups. The next step of the development of the SmarTTransfuser is to design a medical device which could automatically perform the compatibility test at the

patient's bedside. Indeed, a mobile device is required which contains the following elements: a fluidic system, four biochips and an optical reading module.

4.1 General Concept

Our SmarTTransfuser system consists of a secure patient blood sampling system combined with a biochip and a mobile reader used to perform an ultimate blood compatibility test at the patient's bedside (Pazart, 2010).

Immunocapture of red blood cells (patient and RCC) is performed on 4 biochips (2 for the patient and 2 for the RCC). These biochips are inserted into a cartridge that includes fluidics arrangements used to drive the patient's blood and RCC towards the chip surfaces. The cartridge is inserted into a mobile reader/actuator that controls the flow of fluids, the optical reading of the immunocapture reaction and allows (or forbids) blood transfusion (Pazart et al., 2011) (Figure 5).

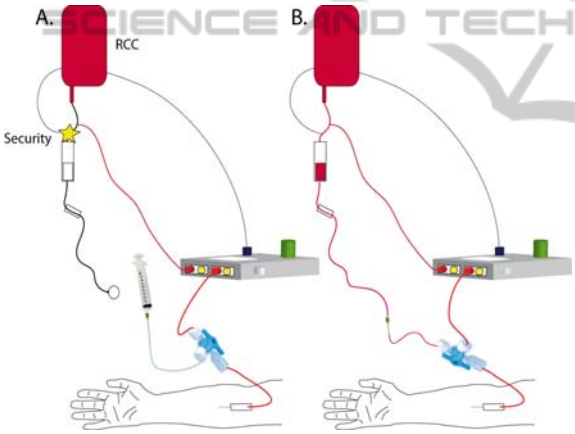

Figure 5: General concept of the SmarTTransfuser. The SmarTTransfuser is on the transfusional line, and the compatibility test is automatically realized (A). If the test is correct, a green light comes on, the safety measure is switched off and medical staff continue with the transfusion as usual (B).

4.2 Immunosensor

The microsystem presented here and developed under the coordination of the FEMTO-ST Institute is an embedded detection device, which uses a microsystem, including the surfaces, for detection of blood group (biochips).

In order to design the medical device, the first step was to determine which configuration will allow for a specific immunocapture. Several prototypes were designed. The best results were obtained with the prototype presented in figure 6. This configuration allows for a good blood flow, with very little retention of red blood cells.

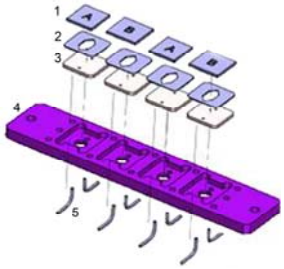

Figure 6: Representation of the reading zone of the SmarTTransfuseur system. There are four biochips for the ABO compatibility test: two for patient blood and two for red blood cell concentrate (1). To ensure the system was sealed with no retention of red blood cells, there is a two part joint: a silicone sheet with a hexagonal window (2) and a base in polycarbonate (3). All these parts are inserted in a plastic base (4) and fluids can flow via part 5.

We tested immunocapture with this configuration. The experimental design was as follows: NaCl 0.9 % was used as course buffer (50 µL/min) and 250 µL of red blood cell group A (dilution 1/4) was used (20 µL/min). We showed that in these conditions, immunocapture is strong and specific (Figure 7). Optical detection of the red blood cells has been validated once and the result is yet to be confirmed. We will therefore not describe this issue in further detail in this position paper.

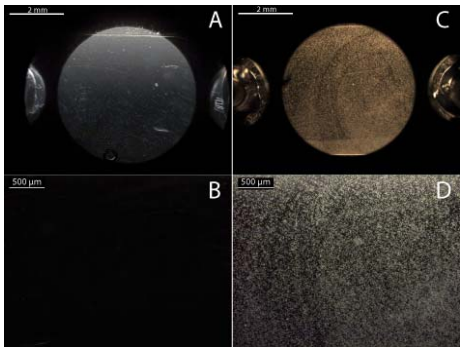

Figure 7: Photography of two biochips after immunocapture reactions with red blood cell concentrate. Very few red cells are observed on biochip graft with anti-B antibodies (A and enlargement B). Almost all of the surface of the biochip grafted with anti-A antibodies is covered with red blood cells (C and enlargement D).

Detection of the red blood cell capture relies on optical absorption. Blue LEDs and optical detectors are positioned on each side of each reading zone. Optical elements are parts of the mobile reader/actuator. Optical detection of the red blood cell trapping has been validated once and the result

has yet to be confirmed. It is therefore too early to describe this issue in further detail in this position paper.

5 CONCLUSIONS

The ABO compatibility test is compulsory in France. Studies led by the national hemovigilance network show persistent attribution errors of blood groups leading to transfusional accidents, with the potential for death in each case (Afssaps, 2004). The same observations have been made abroad (Stainsby, 2005).The current procedure for performing an ultimate pre-transfusional check is not uniform. Although machines are sent to the patient's bedside for an automatic analysis of ABO grouping, and technology has been developed to attribute a code to the pouch of blood and to the patient (Aandahl, 2007), these developments cannot prevent human errors and transfusional accidents, which can be lethal.

We have been working on a new system based on biochips. We have shown through SPRi techniques that these biochips are very selective and can replace current hemagglutination test.

With our first tests with the SmarTTransfuseur, we have shown that immunocapture is specific in our conditions and that optical detection can be carried out by absorption measurement. This medical device could become a new uniform procedure to carry out the ABO compatibility test. Because tests will be carried out automatically, human errors would be avoided and the additional safety will involve only minor changes to current practices.

We now need to repeat these tests in order to cover all transfusional situations before clinical trials in real-life situations.

The aim of this position paper is not only to present the system we are developing, but also to discuss the possibilities and techniques of ultimate ABO compatibility tests outside France.

ACKNOWLEDGEMENTS

The authors would like to thank the French Blood Transfusion Center, INSERM, the DGOS, the CNRS, OSEO and the "innovative project maturation" programme for their financial support.

REFERENCES

Aandahl, G. S., Knutsen, T. R., Nafstad, K., 2007. Implementation of ISBT 128, a quality system, a standardized bar code labeling of blood products worldwide, electronic transfusion pathway: four years of experience in Norway. *Transfusion*, 47(9), p.1674-1678.

Afssaps, 2010. Rapport annuel hémovigilance. Available at: http://www.afssaps.fr/.

Afssaps, 2004. Rapport annuel hémovigilance. Available at: http://www.afssaps.fr/

Boireau, W. et al., 2009. Revisited BIA-MS combination: entire «on-a-chip» processing leading to the proteins identification at low femtomole to sub-femtomole levels. *Biosensors & Bioelectronics*, 24(5), p.1121-1127.

British Committee for Standards in Haematology, 2009. Guideline on the Administration of Blood Components. Available at: http://www.bcshguidelines.com/documents/Admin_blood_components_bcsh_05012010.pdf.

Dzik, W. H., 2007. New technology for transfusion safety. *British Journal of Haematology*, 136(2), p.181-190.

Dzik, W. H., 2005. Technology for enhanced transfusion safety. Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program, p.476-482.

FDA, 2010. Fatalities Reported to FDA Following Blood Collection and Transfusion. Available at: http://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/TransfusionDonationFatalities/UCM254860.pdf.

Levy, G., 2008. [The pretransfusion bedside agglutination test is not a «Gold Standard»]. *Transfusion Clinique Et Biologique: Journal De La Société Française De Transfusion Sanguine*, 15(5), p.318-321.

National Blood Collection and Utilization Survey, 2009. The 2009 national blood collection and utilization survey report. Available at: http://www.aabb.org/programs/biovigilance/nbcus/Documents/09-nbcus-report.

Pazart L., Wacogne B., Pieralli C., Boireau W., Morel P., "Secure perfusion system", Patent WO 2011055031.

Pazart L., Wacogne B., Pieralli C., Boireau W., Morel P., "Device for taking a sample of a body fluid and method for implementing same", Patent WO 2011055029.

SHOT, 2009. Annual Report. Available at: http://www.shotuk.org/

Stainsby, D., 2005. ABO incompatible transfusions--experience from the UK Serious Hazards of Transfusion (SHOT) scheme Transfusions ABO incompatible. *Transfusion Clinique Et Biologique: Journal De La Société Française De Transfusion Sanguine*, 12(5), p.385-388.

Stenberg, E., 1991. Quantitative determination of surface concentration of protein with surface plasmon resonance using radiolabeled proteins. *Journal of Colloid and Interface Science*, 143(2), p.513-526.