THE PHOSPHOLIPID VESICLE-BASED DRUG PERMEABILITY ASSAY: 5. DEVELOPMENT TOWARDS AN AUTOMATED PROCEDURE FOR HIGH THROUGHPUT PERMEABILITY SCREENING

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Abstract

In-vitro screening for oral absorption has become an essential part of drug discovery and development. Recently, a new phospholipid vesicle-based permeation assay was developed which has shown to satisfyingly predict passive absorption of drugs in humans. The purpose of the current study was to investigate whether the assay may be further developed into a high-throughput tool by automating its most time consuming steps. The following challenges were addressed: (i) to design, build and test a heat-sealing machine for mounting of the desired type of filter support onto both single wells and 24-well titre plate inserts, and (ii) to transfer the permeability assay to a robotic work station with attached UV-reader. The workstation is able to pipette and transport both plates and filter inserts and perform on-line photometric quantification of the amount of drug permeated. In order to enable the robot to move single (Standard-Transwell®) filter inserts, an extension of the gripping arm was designed, built and tested. Furthermore, in an alternative approach 24-well filter plates (Millicell®) were used instead of single filter inserts. The latter turned out to be more suitable in terms of error-free high-throughput robotic handling. The permeability values of drugs gained by the two automated procedures were compared with those measured by manual handling of the assay. Only neglectable differences in permeability values were seen.

In conclusion, the most time-consuming steps of the assay were shown to be eligible for automation. This represents an interesting addition to the tool-box of *in-vitro* permeability screening assays running in a medium- to high-throughput format due to its easiness, its transferability to other laboratories and its good correlation with *in vivo* data on fraction absorbed of drugs in humans.

Keywords: Automation, high throughput, drug permeability, liposomes, artificial membrane.

1 Introduction

Oral bioavailability of a drug, i.e. the fraction of an orally administered dose of a drug that unchanged reaches the systemic circulation, is an important property to see if a drug is suitable for oral administration and e.g. in dose finding. The oral bioavailability of a drug is mainly determined by the solubility of the drug in the GI-fluid as well as its permeability through the intestinal barrier. In order to eliminate drug candidates with poor oral bioavailability, permeability screening is today implemented in early stages of the drug discovery and development process. Efficient strategies for screening of permeability properties of large numbers of new drug candidates are needed to facilitate the selection of the most promising candidates for further development.

The phospholipid vesicle-based permeation assay has recently been introduced as a novel method for screening of passive drug permeability through barriers mimicking biological membranes.[1-3] The permeation barrier consists of a tightly packed layer of liposomes on a filter support.[1-3] For a diverse set of compounds the apparent permeability coefficients obtained from the phospholipid vesicle-based permeation assay have been found to correlate well with literature data on human absorption *in vivo*, 18 out of 22 drug compounds were classified correctly.[2] This approach thus models the *in vivo* absorption better than the bio-mimetic PAMPA model[4] and equally well as the Caco-2[5] and the double sink PAMPA (DS-PAMPA) models[6] in the prediction of passive diffusion of drug compounds.[2]

Furthermore, the phospholipid vesicle-based barriers were demonstrated to withstand a pH range from 2.0 to 8.0 without losing their integrity, and are thus regarded suitable for permeation studies at different pH conditions mimicking the drug transit through the GI-tract.[7] The barrier has also shown to be compatible with relevant co-solvents and certain tensides. Permeability testing of drugs in presence of commonly used additives appears thus feasible.[7] Taken together, the phospholipid vesicle-based permeation assay, so far, has shown to be a promising approach for low- to medium-throughput screening of passive drug permeability.

However, in order to fully characterise the permeability properties of potential drug compounds, e.g. at different pH-values and in the presence of different additives, a large number of experiments is necessary. This is time consuming and cumbersome with the current set-up due to the lack of commercially available filter supports containing the desired type of filter membrane as well as the time needed for preparing the barriers and for performing the permeation experiments manually.

The purpose of this study was thus to investigate whether the most time consuming steps of the phospholipid vesicle-based permeability assay can be automated: (I) fusion of the desired filter material onto bare filter inserts, (II) preparation of the liposome-based permeation barriers on the filter support, and (III) performing the permeability assay to compare with the manual procedure.

Part I, the fusion of the desired filter material onto bare inserts, has so far been made by hand, insert by insert, and has been the most labour-intensive step. This step was

automated by designing, constructing and testing two prototypes of a heat-sealing machine in terms of automatically fusing the filters onto the filter inserts.

Part II, the preparation of the liposome based barriers was found difficult to automate since the preparation would require automation of centrifugation, heating (50 ºC and 65 ºC) and freezing (-80 ºC) steps, that are not easily implemented within the robotic platform used. However, the phospholipid vesicle-based barriers have earlier been shown to be stable during storage at –80 °C for up to two weeks which gives the opportunity to produce and store larger batches of the barriers beforehand.[1]

Part III of the process, the performance of the permeability assay itself, it is not very labour-intensive but is the most time consuming step as it requires manual handling for at least 7 h to move the filter inserts at certain time intervals from one well to the next. At the end of the experiment the sampling from the compartments and the UV-analysis also have to be done. If the assay and analysis steps could be automated, manpower will only be necessary to prepare the barriers and start the experiments. Automation should thus allow a significant increase in throughput. Routines were established and tested to carry out all steps on a common robotic workstation (Tecan Genesis). These comprise: pipetting of both donor and acceptor solutions to the filter inserts and wells, respectively, storage of the plates, moving of the inserts from one well to the next at given time points, sampling and quantification of drug content by UV-absorbance measurements.

2 Materials and Methods

2.1 Materials

Egg phosphatidylcholine, Lipoid E-80, was obtained from Lipoid, Germany. Caffeine, metoprolol tartrate, sulpiride, testosterone, acebutolol hydrochloride, terbutaline hemisulfate and calcein were purchased from Sigma-Aldrich Co, St. Louis, USA. Bare single filter inserts (Standard Transwell®, d = 6.5 mm) and bare 24 well filter plates (Millipore Millicell® 24-well cell culture) were custom made by Corning Inc, Corning, USA and Millipore, Billerica, USA, respectively. The mixed cellulose ester filters (0.65 µm pore size) to be mounted onto the inserts were obtained from Millipore, Billerica, USA.

2.2 Methods

2.2.1 Preparation of the filter inserts

2.2.1.1 Hand-made preparation of the single filter inserts

Filter inserts were prepared as described earlier.[2] In brief, single filter inserts without filters were used. Pieces of mixed cellulose ester filters (0.65 µm pore size) were fused onto the inserts by first rubbing the fusion area of the inserts with sand paper and then fuse the filter onto the inserts by pressing the inserts with the filter, in circular movements, against a 130 ºC preheated TLC plate heater (CAMAG, Muttenze, Switzerland) for about 5 min.

2.2.1.2 Automated preparation of the filter insert

For the automated preparation of the inserts the same type of bare single filter inserts and filters were used as described for the manual method. In addition, bare 24-well filter plates were used. Automated sealing machines, the IBR Heat-Press HP80-3500 (see Figure 2, section 3.1) for the single filter inserts and the IBR Heat-Press HP60-250-ESM for the 24 well filter plates, were especially designed and built in collaboration with IBR-Ingenieurbüro*, Waldkirch, Germany. They were tested for automated fusion.

2.2.2 Preparation of the phospholipid vesicle-based barrier

The barriers were prepared according to the procedure reported earlier.[2] In brief, liposomes with two different sizes (extruded through filters with pore size 400 and 800 nm, respectively) were made from egg phospholipids and deposited on a filter support in consecutive steps, first the smaller liposomes and then the larger, by use of centrifugation. Freeze-thaw cycling was then used to promote liposome fusion to produce a tight barrier. Afterwards, the barriers could be stored up to two weeks at – 80 °C.[1]

2.2.3 Automation of the permeability experiments

The robotic platform was a Genesis RSP 150 robot from Tecan, Crailsheim, Germany. By design, the robot is equipped with four pipetting needles and is able to do all pipetting steps, as well as to move the trays and the filter inserts.[8] For moving of single inserts a custom-made extension of the gripper was employed. A UV absorbance reader (Tecan

* http://ibr-konstruktion.de

Infinite M200), which is able to measure in the wavelength range from 230 nm to 1000 nm, was connected to the robot. Figure 1 shows a picture of the robotic workstation and the different components of the system.

Figure 1

Robot software: Gemini v4.2.16.303 (Tecan Deutschland GmbH, Crailsheim, Germany). Reader software: Magellan v6.1 (Tecan Austria GmbH, Salzburg, Austria). As the reader used here does not have a fluorescence option, the samples from the experiment with calcein were measured manually on a Luminescence Spectrometer LS 50B from Perkin Elmer, Beaconsfield, Great Britain. However, a variety of readers could in principle be connected to the robot, depending on what analytical method is needed.

The resistance was measured manually as described earlier at the end of the permeability testing to control the integrity of the single barriers.[2]

2.2.4 Statistical methods

To test if the changes brought about by co-solvents and tensides were significant, student's t-tests for comparison of two means was performed. A significance level of $p=0.05$ was always used. The hypotheses determined the choice of a one or two sided t-test.

3 Results and discussions

3.1 Automation of the filter insert manufacture

Filter inserts with the desired filter type (mixed cellulose ester filters with a pore size of 0.65 µm), used as a support for the liposome based barriers are currently not commercially available. So far they have thus been prepared in-house by hand, which is labour-intensive and error-prone. A sealing machine (IBR Heat-Press HP80-3500) to make 24 (single) filter inserts in one run, including software that allows fully automated runs, was therefore designed and built in collaboration with IBR-Ingenieurbüro, Waldkirch, Germany.

The process consists of pressing the filter insert and the cellulose filter onto a hot surface and cutting off the excess filter material. Thus, the instrument included exact temperature control of the areas for sealing (heat piston), a pneumatic unit to press the inserts with the filter towards the heat piston with predefined force and duration, and a cutting device fitting exactly the insert diameter.

Figure 2

A first prototype of the new heat-sealing instrument, as shown in Figure 2, was designed to simultaneously fuse 24 filters onto inserts. To do this the filter inserts are mounted upside down on an insert holder (Figure 2A) and a filter disc is positioned on top (Figure 2B). The machine then pneumatically moves the sandwich consisting of filter holder with the inserts and filter disc until they have contact with the sealing piston, which is electrically heated to a predefined temperature (Figure 2C). While the filter holder and

piston are in contact and the sealing process is running, round knifes are pneumatically moving down cutting off the excess filter material (see Figure 2E for picture of the heat and cut piston). The sealing process is continued for a predefined time until the filter material is properly sealed to the insert base. After the sealing process is completed the insert holder and the sealing piston move back to their initial positions and the excess of filter material is removed manually (Figure 2D)

Various temperature settings of the heat piston and pneumatic pressure were tested to identify optimal sealing conditions. A piston temperature of 150 ºC and a pneumatic pressure of 2 bars applied for 30 sec were found ideal in terms of minimal numbers of leaky inserts. The fraction of leakage incidents was reduced from 12% for the inserts hand-made by an experienced person to below 2 % for the inserts made by the sealing machine.

Taken together, the process of sealing filters onto the insert bases went from being the rate limiting step of the permeability assay to the most efficient step and this consequently improves the throughput of the assay significantly. Within the same time span we earlier used for producing one insert (5 min), we can now make 48 inserts by using the sealing machine (two runs). The efficiency of the process is thus increased by a factor of almost 50 and the time used for 18 inserts, which is needed for one assay run, reduced by about 1.5 h. Furthermore, the quality of the machine-made inserts is better than those hand-made in terms of tightness of the joints between the filter and the insert base.

In parallel a second heat-sealing machine was designed in collaboration with IBR-Ingenieurbüro, Waldkirch, Germany, to handle 24-well filter plates. This second prototype (IBR Heat-Press HP60-250-ESM) works after the same principle as that described above, but here the filters are sealed one by one to the bottomless wells. All together it takes approximately 15 min to seal all the 24 wells with filters. The same sealing parameters as described above (150 ºC, 2 bars, 30 sec) were also used here. It was even more important that the sealing in the 24-well filter plate is performed properly since leakage in only one filter would lead to discarding of the whole plate.

3.2 Automation of the permeability assay by using a robot platform and single filter inserts

The requirements for the robot to be able to run the permeability experiments are that it can perform pipetting, move the plates and take off and put back the lids as well as move the inserts. To run the minimal experiment with six inserts, two 24-well plates with lids and one 96-well UV plate are needed. However, the goal of developing the automated procedure is to investigate more drugs/compounds at the same time.

The manual procedure for the permeability assay involves moving of the filter inserts to fresh acceptor wells at specific time intervals. For the automated assay we decided to stick to this procedure rather that replacing the acceptor medium in the well by pipetting. Although the single filter inserts (Standard Transwell®) used here have holes in their upper edges (see Figure 3) which principally would allow the pipettes to stick down into

the acceptor solution it has to be taken into account that each filter insert is free to rotate in the plate wells, which could potentially lead to collisions between the pipettes and the inserts. Pipetting for replacement of acceptor solution was thus regarded too risky.

Figure 3

The robot was therefore programmed to do the following: The robot first moves the 24-well plates from the storage rack to the working table to give the pipetting arm access to the wells. The lid is removed from each plate and placed on the working table next to the plate. The pipettes now fill equal amounts of acceptor medium into all wells. Single filter inserts with the beforehand prepared permeation barriers are then picked up from a 24-well plate on the working table by a especially designed finger tool and placed in row 1 (assigned t=0) on the first assay plate (see Figure 4). The donor solutions, each containing a given drug, are transferred into the donor chambers (filter inserts) by the pipettes. The inserts are then consecutively moved seven times from row 1 to row 8 usual at time points t = 1, 2, 3, 3.5, 4, 4.5 and 5h. Between each moving step the lid is put back onto the plate and the plate moved back into the hotel to clear the working table for the next operation (parallel experiments). Finally, the 96-well plate, intended for UV measurements, is moved from the hotel to the working table and aliquots are transferred from each well in the 24-well plates to the 96-well UV-plate. The UV plate is moved into the UV-reader and absorbance values are determined.

Within 30 min, which is the minimum time span for moving the inserts to the next row of wells, the robot is in principle able to handle six compounds with six replicates each in parallel. With the robot equipment used here only three drugs (six replicates each) could be handled in parallel due to limited storage capacity of the hotel (see Figure 1B). However, an increase of the hotel space would allow handling of the maximum number of compounds as well as sequential starting of more than one set of permeability assays. For a maximum of six compounds with six replicates each the automated procedure is assumed to take about 12h.

3.2.1 Design and testing of the finger-tool for moving the single filter inserts

To allow robotic moving of the small, round single filter inserts a special finger tool to extend the gripping arm (see Figure 4) was designed and built together with IBR-Ingenieurbüro, Waldkirch, Germany. To manufacture a stable and precise but light tool, the gripper was made mainly from aluminium and had a weight of 220.8 g. The new tool is small (finger length: 60 mm) to be as light as possible.

Figure 4

The finger tool is placed on the two gripping arms in a 90º angle position (see Figure 5A). The depth of the tool is 80 mm, which gives a stable contact between the finger tool and the gripper arms. When the gripper moves the arms toward each other the finger tool and the finger do the same. The width of the whole tool can thus be varied from 87-114

mm. The fingers are curved at the tip and have a soft rubber material on the inside of the fingertips to improve the gripping performance. An important aspect was that the gripper should be able to collect the finger-tool when the inserts have to be moved and park the tool when plates have to be moved. The finger-tool is therefore placed on a station when not in use, where it is freely reachable for the gripper, as shown in Figure 5B. By this design the gripping arm can serve two functions, both moving plates and lids as well as moving smaller items by fetching the finger-tool first. This tool is also very useful for handling of all kinds of round vials up to 30 mm. Due to the shape of the finger tips the gripper is optimal for vials from 10 to 20 mm diameter.

Figure 5

Gripping of the single inserts, which have only a very small and flat rim which can be used for gripping, turned out to be a critical step in the automation of the assay since the contact area between the fingers and the inserts is quite small (see Figure 3 and Figure 7). Due to the geometry of the 24-well plate the filter inserts are also placed quite close to each other as shown in Figure 6. This requires a high precision in positioning the fingers in between the inserts. To meet this demand the fingers are programmed to grip the inserts not parallel to the plate edge but at a 45° angle as shown in Figure 7. Using the finger-tool the robot was now mechanically able to move inserts from one well to the next during the permeability assay. However, the inserts are placed in wells on a 24-well plate and the single inserts can rotate 360° in the well and have some space to move out of the central axis. Thus, it turned out to be challenging to grip the inserts perfectly which in worst case led to a premature stop of the program which means loss of all results from

this run. We decided to try with another filter plate format which circumvents the need for the gripper.

Figure 6

Figure 7

3.3 Automation of the permeability assay by using 24-well filter plates

So, as an alternative for using the single filter inserts, a plate with 24 bottomless wells (see Figure 8) was tested in parallel. The advantage of using this plate is that 24 wells can be moved at once which circumvents the challenging movement of single inserts and drastically reduces the total number of moving operations within one run. As for the single filter inserts the 24-well filter plates are at present not commercially available with the desired type of filter (0.65 µm pore sized mixed cellulose ester filters) and have to be preformed by the heat-sealing instrument (section 2.2.1.2).

Figure 8

An important difference between the single filter inserts and the 24-well filter plate is the surface area of the filters. The effective filter area for a 24-well filter plate is 0.6 cm^2 per well[*], while the corresponding area for the single filter inserts is 0.33 cm^2.[×] Based on this

[*] http://www.millipore.com/faqs.nsf/docs/faq398

[×] Transwell® Permeable Supports Selection and Use Guide - http://www.corning.com/Lifesciences/technical_information/techDocs/transwell_guide.pdf

difference the preparation procedure for the barriers was adjusted so that the amount of liposome dispersion per area remained the same.

When programming the modified assay procedure to handle 24-well filter plates again a decision had to be made whether to move the insert tray at different time points from one plate to the next or instead to replace the acceptor solutions in the wells by pipetting. However, since the shape of the wells and the design of the plates make it difficult to empty the wells completely (see Figure 9) we decided to move the 24-well filter plate to fresh acceptor wells. This alternative is also considerable faster than replacing the acceptor medium by pipetting. A four-drug script, with six replicates for each drug, was developed for 24-well filter plates after the same principles as for the single insert system, except that the whole plate with the 24 filter units was moved over to new plates at the desired time points.

Figure 9

Compared to the script for the single filter inserts, the number of movements is now drastically reduced. For example, a three-drug assay (six replicates) with single filter inserts needs 144 moving steps while for a four-drug assay (six replicates) with 24-well filter plates only a total of 28 moving steps is necessary. Another advantage with using these plates is that due to fewer moving steps and thus a faster process time it is possible to run about 20 drugs with six replicates each at the same time.

Due to the difference in filter area a systematic increase of the flux of the drugs was observed when changing from the single filter inserts to the 24-well filter plates. The flux through barriers made on the 24-well filter plates was about twice the flux through single filter inserts, which correlates with the expected value when the filter area is doubled. The increased flux and hence the increased concentration of drug in the acceptor chamber allows a decrease of the assay time by decreasing the time span between the time points for moving of the inserts or alternatively allows for detection of drugs with slow flux. Thus the 24-well permeability assay allows a considerably higher throughput.

A currently unused possibility with the robot is that the work space can be connected to an incubator. It is thus also possible to run the experiments at other temperatures than room temperature and by that get information on how the temperature is affecting the permeation across the barriers. Furthermore, connecting the robot with an automated hotel unit, it would be possible to further increase the number of drugs which could be automatically handled.

3.4 Comparison of automated and manually obtained permeability values

The automated permeability assays using single filter inserts and 24-well filter plates were run with five and four drugs, respectively, already tested in the manual permeability assay, and the P_{app}-values were compared. In addition, calcein was used as a hydrophilic marker in the assay with single filter inserts. The number of parallels for each drug is shown in Figure 10 and six inserts were used in each parallel. The results are presented in Table 1a and 1b.

Table 1a: Summary of permeability data from the automated assay using single filter inserts and 24-well filter plates and the manual run assay. The standard deviations are given in parenthesis.

Drug	P_{app} Manual runs using single filter inserts (10^{-6} cm/sec)		P_{app} Tecan Genesis RSP 150 using single filter inserts (10^{-6} cm/sec)		P_{app} Tecan Genesis RSP 150 using 24-well filter plates (10^{-6} cm/sec)	
Sulpiride	1.32[3]	(0.30)	1.59	(0.03)	1.34	(0.17)
Acebutolol	0.78[2]	(0.07)	0.76	(0.14)	0.79	(0.27)
Terbutaline	0.40[2]	(0.05)	0.33	(0.06)	0.35	(0.06)
Metoprolol	3.23[2]	(0.78)	3.77	(0.31)	-	-
Calcein	0.06[2]	(0.01)	0.05	(0.01)	-	-

Table 1b: Summary of permeability data for caffeine from the automated assay using single filter inserts and 24-well filter plates and the manual run assay. The standard deviations are given in parenthesis.

Operator	P_{app} Manual runs using single filter inserts (10^{-6} cm/sec)		P_{app} Tecan Genesis RSP 150 using single filter inserts (10^{-6} cm/sec)		P_{app} Tecan Genesis RSP 150 using 24 well filter plates (10^{-6} cm/sec)	
Operator A			7.25	(0.66)	7.57	(0.44)
Operator B (more experienced)	6.04	(0.57)	6.41	(0.81)	-	-

As can be seen in Table 1a and 1b there were only negligible differences between the mean permeability values obtained with either of the automated assays, compared to the values obtained with the manual assay. For the compounds in Table 1a no significant difference was found using the t-test for comparison of two means. This shows that it does not make a difference whether the experiment is performed manually or automatically, irrespective of the type of filter inserts used. The only significant

difference was in fact observed when two different operators with different level of experience performed the assay using caffeine. To clarify if the observed difference is due to inter-individual differences or systematic differences between manual experiments and robotic experiments, production of permeation barriers and the robotic experiments using single filter inserts were repeated for caffeine by the same operator that performed the manually run experiment (see Table 1b). The permeability value obtained from the robotic experiments by operator B ($6.41 \pm 0.81 \times 10^{-6}$ cm/sec) is closer to the result of the manual experiment of the same operator ($6.04 \pm 0.57 \times 10^{-6}$ cm/sec) than to the values of the robotic experiments by operator A ($7.25 \pm 0.66 \times 10^{-6}$ cm/sec and $7.57 \pm 0.44 \times 10^{-6}$ cm/sec, respectively). This seems to indicate that there is more an operator and/or experience-dependent difference, with respect to barrier production and/or assay handling, rather than a systematic difference between the manually and robotically obtained permeability values.* A more detailed look into the individual samples obtained using the two automated procedures and the manual assay is given in Figure 9. This was performed to see if there is a difference in reproducibility between the automated and manual procedure. No notable difference in the reproducibility was observed showing that also in this sense the manual and automatic procedures performed equally well. This experience differs from literature reports on the PAMPA assay where a comparison of different robotic systems with respect to the day-to-day variation indicated that the results

* The permeability values for caffeine reported here differ from our earlier published value from a manual run ($12.54 \pm 0.98 \times 10^{-6}$ cm/sec).[2] The reason for this difference is not clear at the time being.

obtained were more reproducible when the automated procedures were used compared to manual handling.[9]

The phospholipid vesicle-based assay seems to have a somewhat higher day-to-day variation compared to the PAMPA assays. The reason for this is most probably that the present model is based on a considerably more complicated barrier system. However, the higher day-to-day variation does not decrease the ability of the model to differentiate between drugs with different permeability values or to classify them correctly according to their in vivo absorption properties.[2]

Figure 10

4 Conclusions

This study demonstrates that the two most time consuming steps in the phospholipid vesicle-based permeation assay can be automated. Firstly, the fusion of the filters to the bare inserts has successfully been automated by using specially designed heat-sealing machines, resulting in a faster and less error prone filter insert production process. Secondly, the permeability assay was successfully transferred to a common laboratory robot. Both tested filter insert systems, the single filter inserts and the 24-well filter plate, were found suitable. It could be shown that transferring the assay to an automated system does not influence the outcome of the permeability assays. The 24-well filter plates were found to be easier and faster to handle which render them more suitable for high throughput screening. The single filter inserts allow, on the other hand, for more flexibility and are thus suitable for medium throughput screening.

This phospholipid vesicle-based permeation assay thus represents an interesting alternative to the established intestinal permeability screening tools due to its easiness, transferability to other laboratories and its good correlation to *in vivo* data on fraction absorbed in humans.[2]

As automation increases the throughput considerably the assay is also useful for studies of the influence of a range of factors on drug permeability. For example, the effects of varying the pH in the donor compartment can be efficiently studied, also varying the lipid composition in the liposome barrier can be performed to model various epithelial barriers.

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Figure texts

Figure 1: The robotic system, Tecan Genesis RSP 150

A – Robot arm containing four pipettes (needles)
B – Hotel for multi titer plates (MTP)
C - Gripper for moving single filter units (plus support to park this tool)
D - Robot arm (RoMa) for moving plates and filter inserts
E – Working table, nine positions for MTP plates
F - The UV absorbance reader, Tecan Infinite M200

Figure 2: The IBR Heat-Press HP80-3500 to automatically make 24 single filter inserts at once
A – Standard Transwell® Permeable supports without filters on the support plate
B – Standard Transwell® Permeable supports with the filter on top
C – Fusion of the filter to the insert bases
D – Filter fused inserts
E – The heat and cut pistons (round knifes with heat piston inside) for fusing the filter to Standard Transwell® Permeable supports

Figure 3: Photograph of the Standard Transwell® inserts. Holes which could be used for emptying the respective MTP-wells can be seen in the upper parts of the inserts.

Figure 4: Photographs of the specially designed finger-tool enabling gripping of filter inserts. A: from the front; B: from the side; C: from beneath; D: from above

Figure 5: Photographs of the specially designed finger-tool in the robotic system. A: the finger-tool mounted on the gripping arm; B: the finger-tool parked at the station from where it can be picked up by the gripper.

Figure 6: Photograph of a 24-well plate with single filter inserts, shown from the top

Figure 7: Photograph of the finger tool gripping one single filter insert from a 24-well plate

Figure 8: Millicell® cell culture plates: insert tray (on top) and receiver tray (in the middle) (reprinted with the permission form Millipore)

Figure 9: The insert tray at the Millicell® cell culture plates (reprinted with permission from Millipore)

Figure 10: Results from the single parallels with the automated assay using single filter inserts and 24-well filter plates together with permeability data from the single parallels obtained with the manually run assays. Standard deviations of the results from 6 inserts are reported for each parallel.

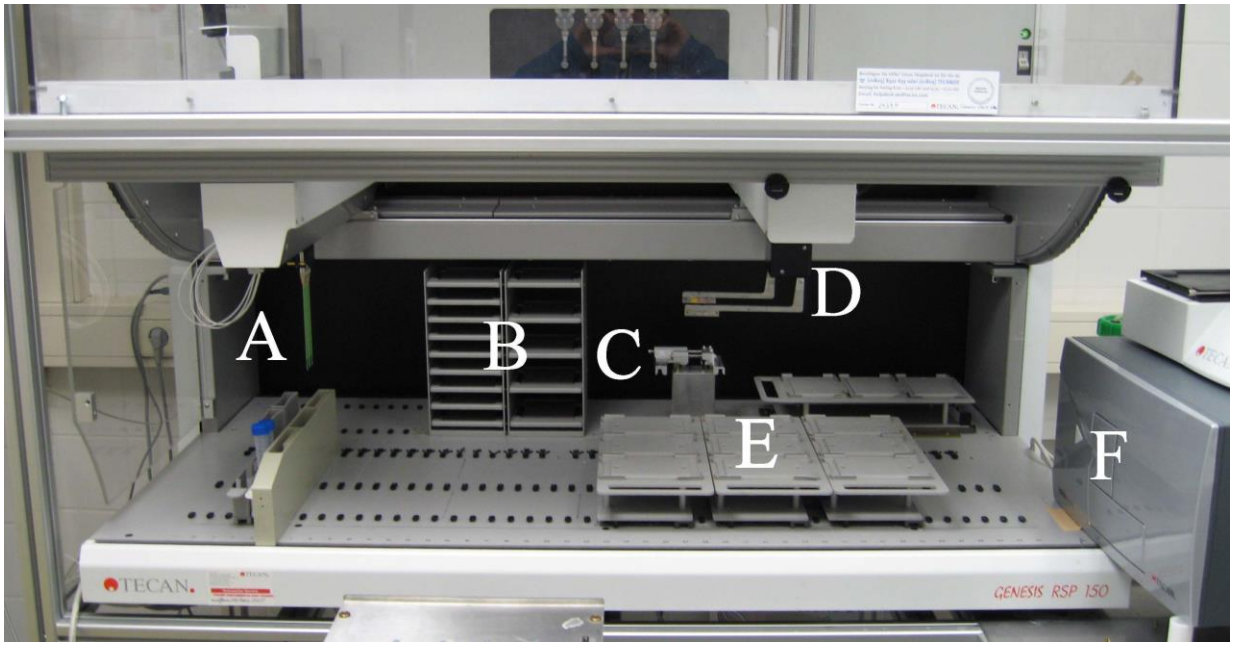

Figure 1

Figure 2

Figure 3

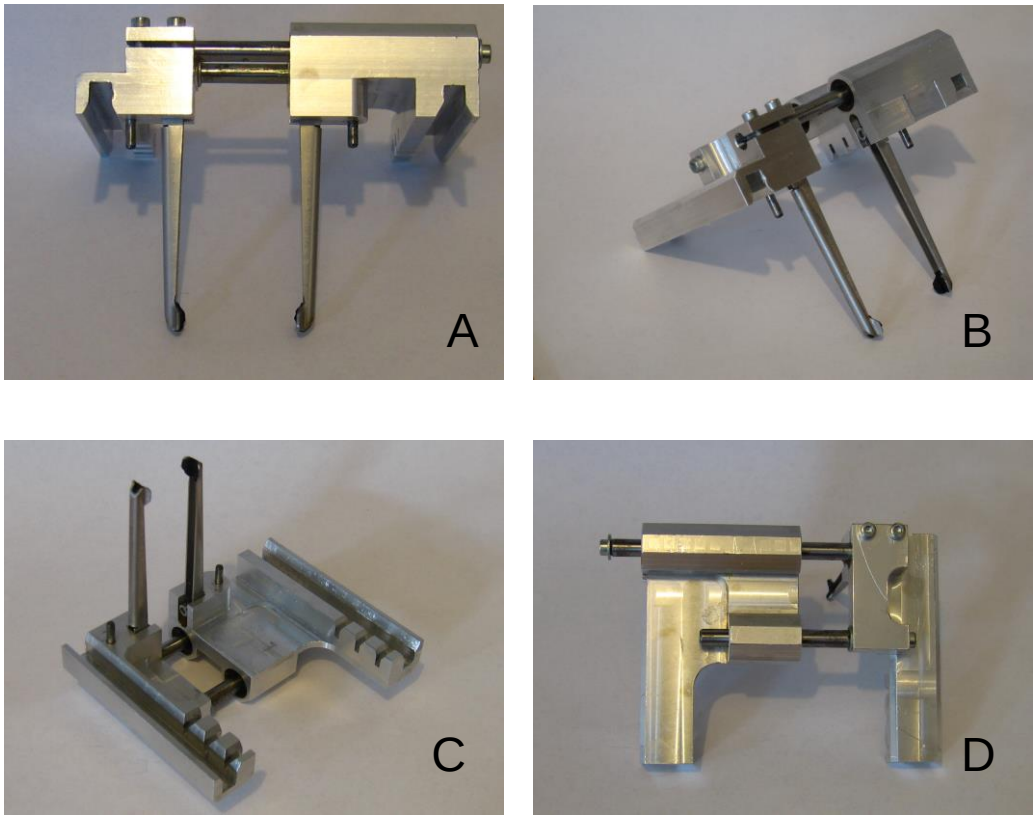

Figure 4

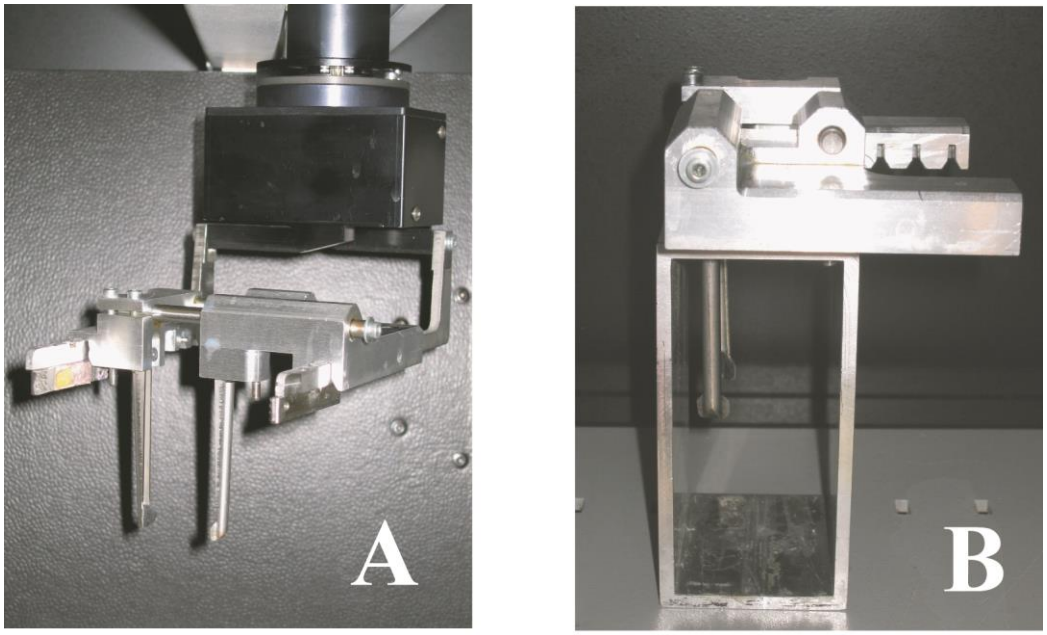

Figure 5

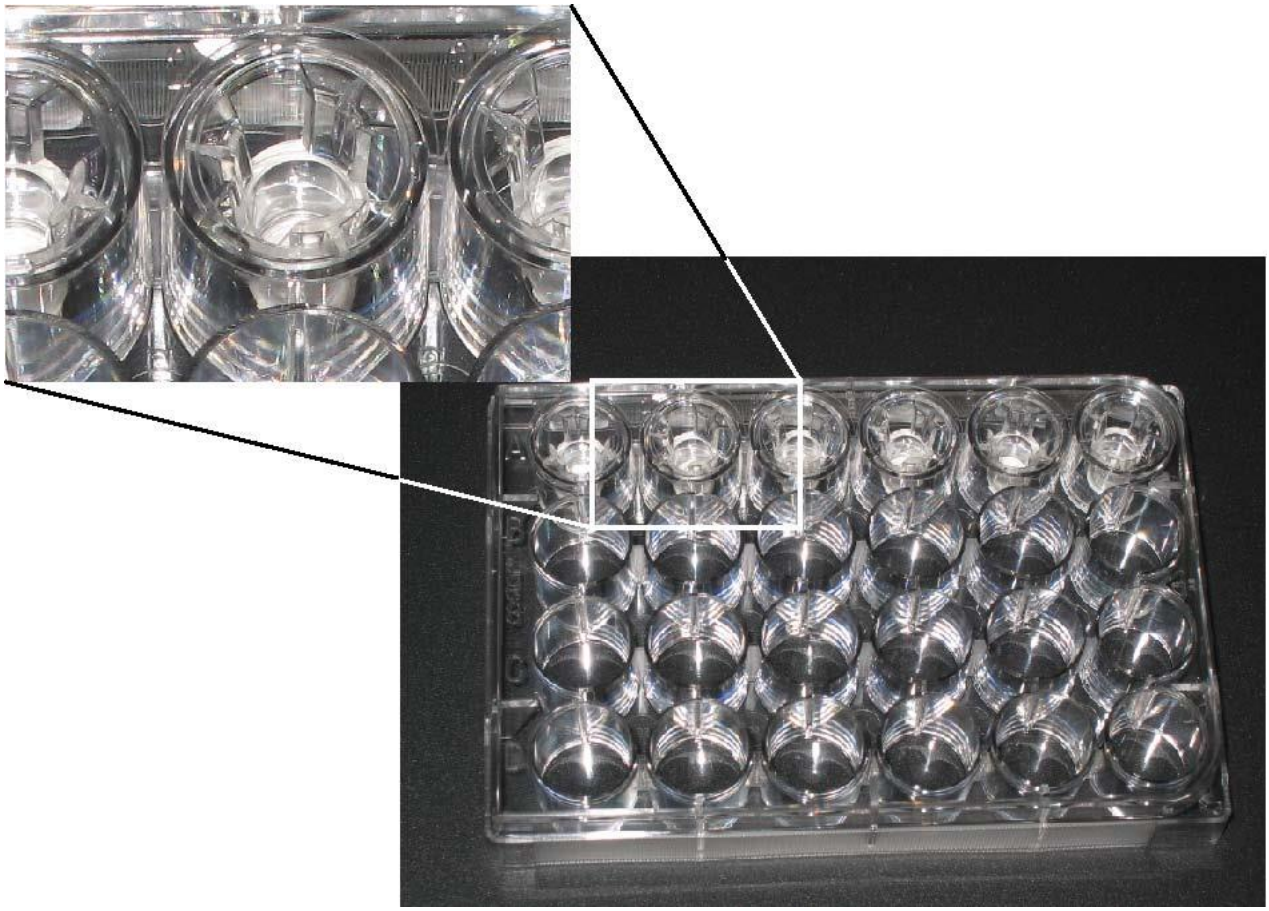

Figure 6

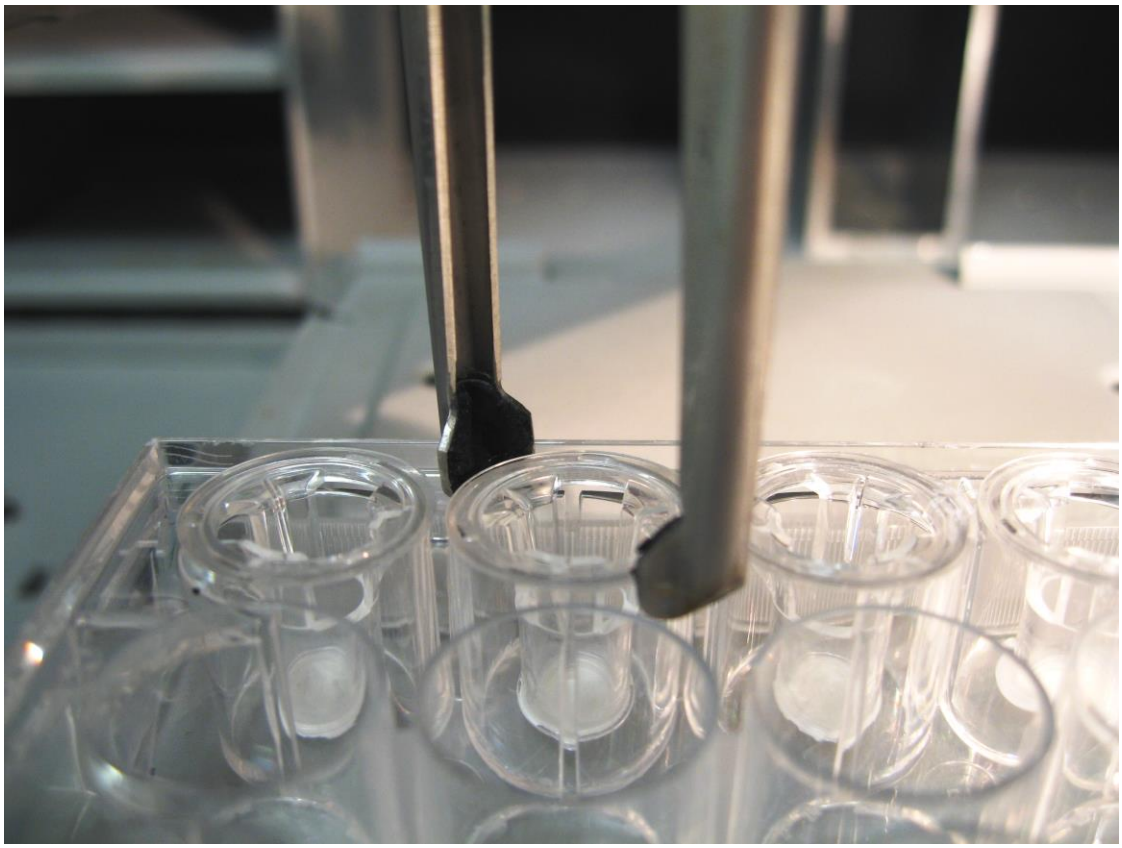

Figure 7

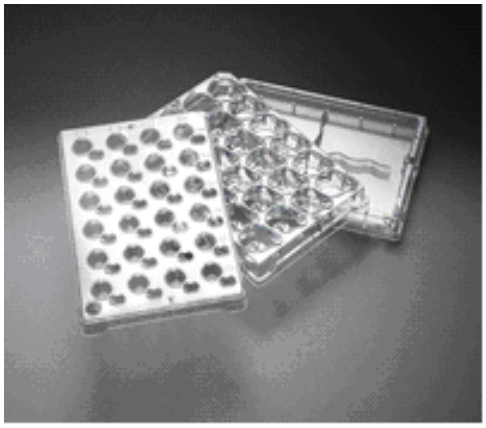

Figure 8

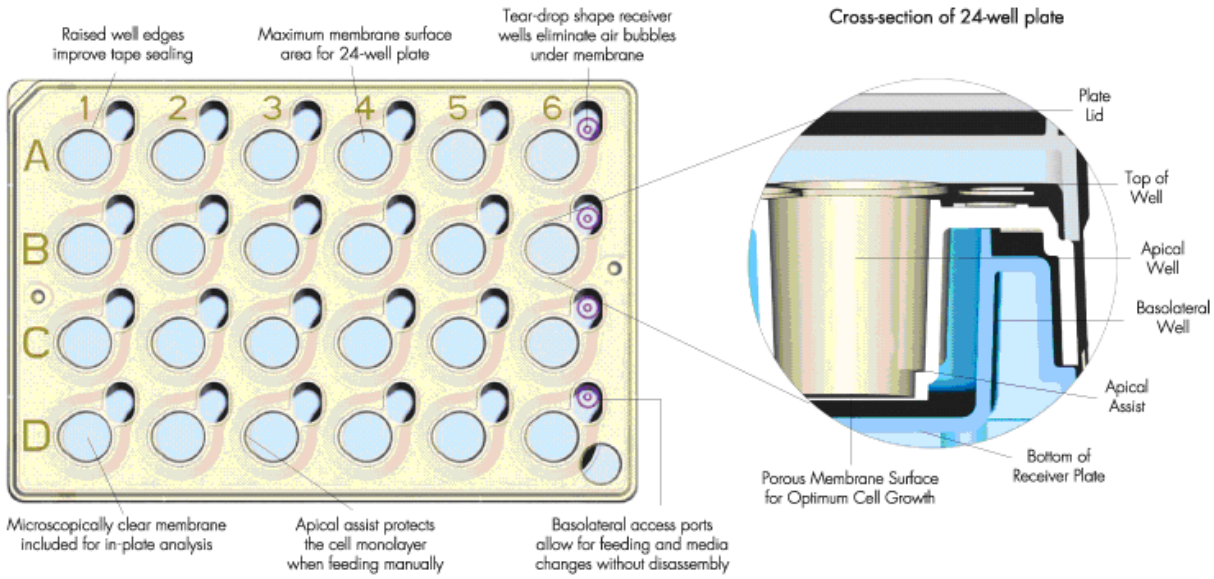
Raised well edges improve tape sealing
Maximum membrane surface area for 24-well plate
Tear-drop shape receiver wells eliminate air bubbles under membrane
Cross-section of 24-well plate
1
2
3
4
5
6
A
B
C
D
Plate Lid
Top of Well
Apical Well
Basolateral Well
Apical Assist
Bottom of Receiver Plate
Porous Membrane Surface for Optimum Cell Growth
Microscopically clear membrane included for in-plate analysis
Apical assist protects the cell monolayer when feeding manually
Basolateral access ports allow for feeding and media changes without disassembly

Figure 9

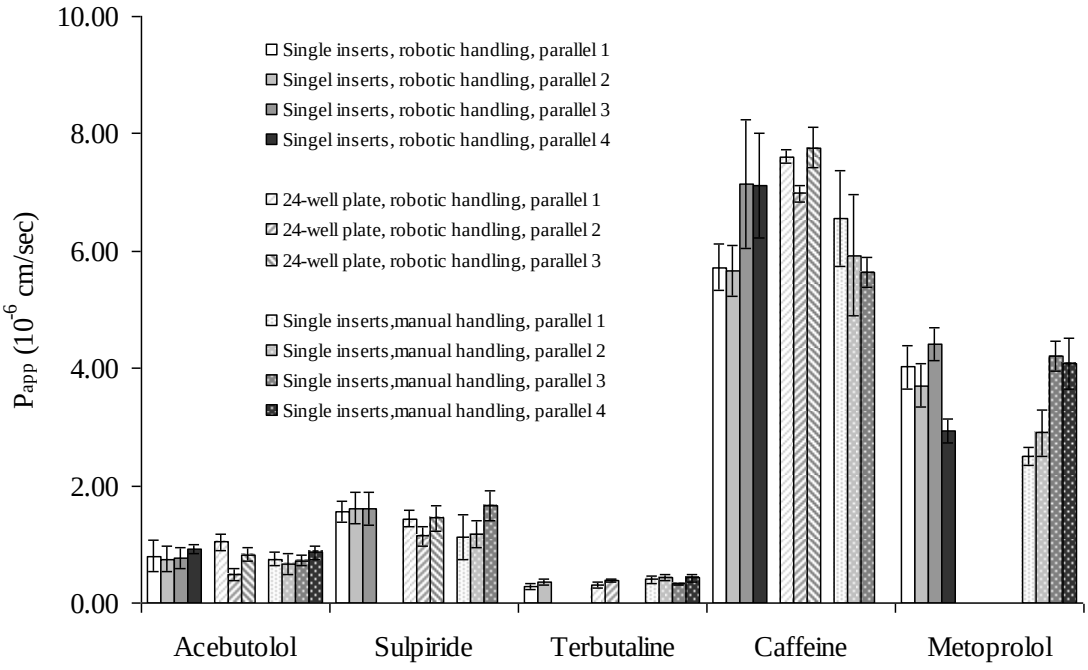
10.00
8.00
6.00
4.00
2.00
0.00
Papp (10-6 cm/sec)
Single inserts, robotic handling, parallel 1
Singel inserts, robotic handling, parallel 2
Singel inserts, robotic handling, parallel 3
Singel inserts, robotic handling, parallel 4
24-well plate, robotic handling, parallel 1
24-well plate, robotic handling, parallel 2
24-well plate, robotic handling, parallel 3
Single inserts,manual handling, parallel 1
Single inserts,manual handling, parallel 2
Single inserts,manual handling, parallel 3
Single inserts,manual handling, parallel 4
Acebutolol
Sulpiride
Terbutaline
Caffeine
Metoprolol

Figure 10