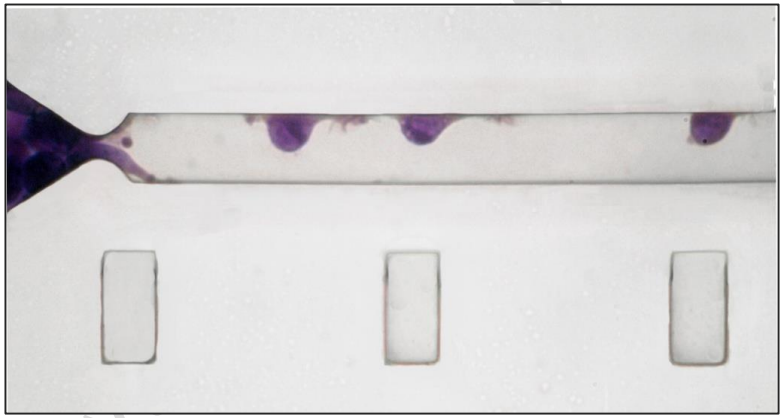
Cell Émigré

Cell Migration

Assay

Rapid quantification of mammalian cell migration



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Cell Migration assay protocol

This protocol is suitable to quantify the linear distance travelled (microns - µm) in a fixed time period or the migration rate of cultured cells (microns/hour – µm/h) of live mammalian cells during *in-vitro* culture.

PREPARATION OF THE CELL MIGRATION 'CHIP'.

Each Cell Émigré 'chip' is supplied sterile and void of all fluid (Fig.1, below). Prior to addition of cells each microchannel array will need to be primed with fluid. This should be performed **at least 1 hour before cell addition.** The Cell Émigré chip and all solutions should be brought to room temperature before use.

1. Aseptically transfer Cell Émigré migration chip from its sterile packaging within a Laminar flow cabinet.

2. Using a micropipette **add *30 µl*** of Priming Solution to the reservoir(s) labelled **[i]**. Allow 30 seconds for the fluid to fill the microchannels.

3. Now add ***60 µl*** of suitable culture medium to the reservoir(s) labelled **[ii]**. Allow 10 minutes for the culture medium to fully displace the Priming Solution from the microfluidic network.

4. Remove all solution from the reservoir(s) labelled **[i]** and dispose this fluid to waste. Immediately replace with **30 *µl*** of culture medium.

***Tip 1**: To prevent the entrapment of air bubbles in microchannels the fluid contents of individual reservoirs must be replaced immediately with the appropriate fluid volume.*

DO NOT PROCESS MULTIPLE RESERVOIRS IN BATCHES.

***Tip 2**: To ensure complete removal of fluid from access port reservoirs, tilt the chip to one side and gently withdraw media from the angle formed by the intersection of the reservoir side-wall and base.*

5. Transfer the chip(s) to an appropriate humidified environment within an incubator. **See page 7 for essential information on humidity control.**

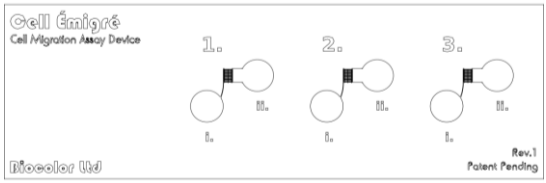


Fig.1. Overview of Cell Émigré Migration 'Chip'.

CELL LOADING OF THE FLUID-PRMED MIGRATION ARRAY(S)

Cells must be supplied to the microfluidic network as a cell suspension in culture medium. Cell clumps must be avoided as these will obstruct the microchannels and prevent single cell loading.

Enzymatic or detergent-based methods are used to release adherent cells prior to addition to Cell Émigré. Cells should encounter the minimum necessary exposure times to these release agents so that much of the membrane receptor function can be preserved. This should permit quicker cell adherence to the base of the loading bay within the microfluidic channel at the incubation stage.

Temperature fluctuations and movement of loaded slides must be kept to a minimum during at least the first 2-hours of the assay. This is to prevent dislodging of cells from the loading bays during the attachment phase.

6. **Cell Sample:** Dilute the cell suspension to between ***7x10^5*** *and* ***9x10^5*** *cells / ml* using suitable growth medium. Cells sourced from solid masses such as tissues or organoids may need to be passed through a cell strainer to obtain single cell suspensions.
   All cell suspensions should be gently re-suspended using a micropipette immediately prior to loading.

7. **Cell Loading:** Take the pre-filled chip from the incubator and remove all culture medium from reservoir **[ii]**. Replace with ***50 µl*** of the same growth media as used in step 3. Now remove all culture medium from reservoir **[i]**. Replace with ***65 µl*** of cell suspension in growth medium.
   *If using Silicone Oil to prevent evaporation then at this point gently layer **10 µl** over the fluid at top of each reservoir (first add oil to reservoir **[ii]**, then reservoir **[i]** to avoid placing excessive hydrodynamic stress on the cells)*

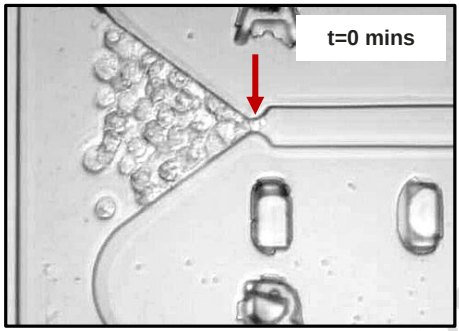
8. Incubate the chip at 37ºC, taking care to ensure sufficient humidity (see page 7). Record the time of placement of the chip in the incubator. This may be used as the assay start time for the calculation of the migration rate: *cell migration distance / migration time* (you may wish to subtract cell attachment time to obtain a more accurate rate value). **See page 5-6 for quantification information.**

9. **Optional Step**: Post-migration cell morphology may be preserved by first removing all fluid from reservoir **[i]**, and replacing with ***60 µl*** of Fix & Stain solution. Remove ***25 µl*** of fluid from reservoir **[ii]** to initiate *in-situ* fixing & staining of migrating cells. Incubate until desired intensity is reached (~30 mins at room temperature). Following this, remove all fluid from reservoir **[i]** and replace with ***60 µl*** of diH_2O to flush excess stain from microchannels. Prior to storage, remove all fluid from reservoirs and dry chips at 60 - 85ºC for 1 hour.

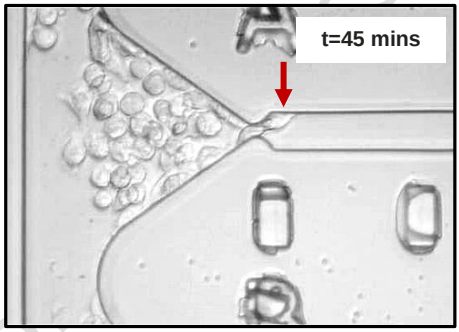
Fig.9. Three stages in the timeline of cell loading to cell migration.
Cell line: HT1080 Human Fibrosarcoma cells (Magnification: x100)

Arrow indicates position of lead cell

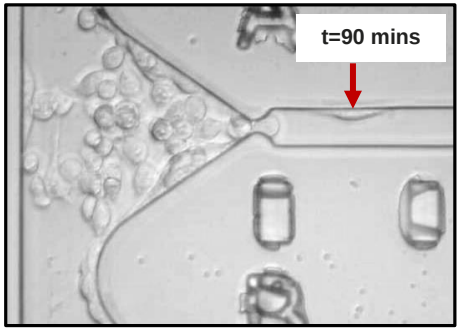
a.



b.



c.



Cell Émigré

Cell Migration Assay Kit

TECHNICAL INFORMATION

The assay has been designed for research work only and should be used only within the 'Clean Room' of a Cell Culture Unit.

Handle the Cell Émigré Assay using Good Laboratory Practice.

Cell Émigré Assay

Cell Émigré takes its name from the French for "migrated out" which aptly describes the journey taken by each migrating cell. The assay is designed to enable user-friendly quantification of migration in attachment-dependent mammalian cells. The assay kit comprises a set of single-use microfluidic migration chips together with support reagents and protocol.

What is Cell Migration?

Cell migration is the name given to the active process of cell movement, occurring in response to intra and extracellular signals. Coordinated and controlled migration of specialised cell types is necessary for the growth, maintenance and repair of an organism.

Among mammals, cell migration is involved from the initial stages of embryonic development. Under physiologically normal conditions this process is tightly regulated. However in pathological states aberrant cell migration can lead to serious dysfunction of the organism.

Pathology

Associated pathologies include Vascular disease, Psoriasis, Lupus and Cancer. In the latter case a tumour can remain localised; however a subset of tumour cells may develop an aggressive pro-migratory phenotype. Such cells are then able to migrate from the primary tumour mass and colonise other secondary sites within the whole organism, a process known as metastasis.

Assay Development

Migration assays may be used to study the process of migration or influence of a treatment on the response of cells. One *in-vitro* approach has been to allow adherent cells to attach and migrate across a flat surface - such as glass or treated plastic. This is effectively a 'two-dimensional' culture area and on such a surface some adherent cells will exhibit 'random walk' migration behaviour.

This migration is characterised by seemingly random and continuous direction changes and is readily observed yet difficult to quantify. Approaches to quantification typically use time-lapse photographic or video tracking of individual cells, followed by computer image analysis to determine each cells migration path. The associated hardware requirements for cell tracking can complicate simultaneous assays.

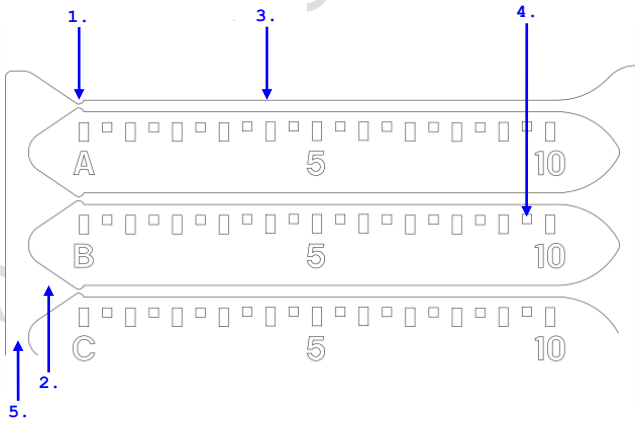
Other approaches – perhaps best typified by the Boyden Chamber technique - rely on the ability of migrating cells to move from the upper side of a porous membrane through the pores and onto the membrane underside. These assays can be time-consuming and technically demanding to perform with a reliable level of consistency.

Cell Émigré: Technical Innovations (refers to Figs.1 - 3)

1. **A 'self-loading' assay chip.**
   Each Cell Émigré assay chip comprises three identical migration arrays (labelled 1 – 3, see **Fig.1**). Each array contains ten microchannels (labelled A-J, see **Fig.2**). A sample of cells in fluid suspension is applied via the pipette access port where it is automatically distributed via the supply channel **(5)** across all cell cluster holding zones **(2)** in each array. No external pumps are required. The result is ten, functionally identical 'cell clusters', all aligned to the same horizontal gate restriction **(1).** This acts as a defined **'START'** position for cell migration.

2. **Linearly constrained cell migration.**
   Following cell attachment (37°C), migration can then occur. The microchannel wall **(3)** continuously guides each cell along a **linear migration path,** by means of the phenomenon of contact guidance. Quantification requires only the measurement of a cells **linear** cell displacement from the **'START'** position.

3. **Direct Quantification: Using an integrated measurement scale.**
   Each array on the chip incorporates a measurement scale **(4)** integrated alongside each microchannel. The scale is calibrated in microns and is clearly visible under microscopic examination. This allows direct measurement of a cells total migration distance by comparison of it alignment with graduations on the scale (see Fig.3).



1. Gate restriction.
2. Cell Cluster holding zone.
3. Linear Measurement Microchannels.
4. Integrated Measurement Scale.
5. Supply Channel.

Fig.2. Diagram of a single Cell Émigré microchannel array.

Intended Applications:

1. To directly determine the migration of individual cells using the calibrated scale attached to each microchannel. Different cell lines can be assayed and compared within the one migration chip.

2. The effect of gene knockout, antibody or chemical treatment can be simultaneously compared against control (untreated) cells.

3. Examining the effect of soluble compounds on cell migration. These can be supplied within the culture medium surrounding the cells. The assay can be used to rapidly screen previously uncharacterised or poorly characterised compounds for anti- and pro-migratory effects.

4. Evaluate the interaction of migrating cells with extracellular compounds such as Collagen, Laminin or xenobiotic molecules. Compounds can be coated onto or attached to the surface of the microchannels before the live cells are added.

Assay Kit Components:

1. **Cell Émigré migration chips.**
   Produced as a transparent & biocompatible silicone top attached to a glass microscope slide. Supplied sterile in packaging. Keep sealed until ready to use.

2. **Priming Solution (0.75 or 1ml).**
   Contains 70% isopropanol. Supplied in a sterile vial. Formulated to prime the microchannel network with fluid without the trapping of air bubbles.

3. **Cell Fix & Stain Solution (1.5 or 2.75ml).**
   Contains Crystal Violet dye in neutral buffered solution. Contains 10% formalin to allow cells to be fixed and stained at the same time for preservation of a permanent migration record.

Other Components required – not supplied:

1. Trypsin or alternative cell detachment agent. Cells MUST be brought into an aggregate-free single cell suspension prior to loading the migration chip.

2. A chamber suitable for maintaining migration chips within a localised humid environment within the incubator, (see Page 7 for further information).

Cell Émigré Kit Pack Sizes & Storage Conditions:

1. **M1000 -** Contains 5 chips, providing microchannel arrays for 15 assays.
2. **M2000** - Contains 12 chips, providing microchannel arrays for 36 assays.

All components are sterile for up to one year unopened when stored at 25°C.

Using Cell Émigré to quantify cell migration

a. Selection of cells to measure

The Cell Émigré assay can be performed either at multiple time points (kinetic assay) or at a single, pre-determined assay endpoint (endpoint assay).

The position of initial cell entry into the microchannels represents the zero-displacement (START) position and is indicated by the capital letters - **A to J** on the measurement channel scale. This is the position from which migration is measured. The '***5***' and '***10'*** labels on the scale indicate the 500 and 1000 micron positions respectively. The periodic graduations on the axes (ticks) of the measurement scale are each spaced 100 microns apart.

During an assay additional cells from the cell cluster holding zone will follow the lead cell (first cell) into the microchannel. We suggest recording the displacement of the **lead cell only**, since the microchannel entry time can only be accurately determined for this cell. In the case of multiple cells, each subsequent cell entering the microchannel represents an unknown entry time and hence its migration rate cannot be reliably determined.

b. Selection of optimal assay time

Each Cell Émigré microchannel has a linear cell migration measurement portion, accommodating a migration distance of up to 1000 microns, followed by an open-ended portion (to allow for fluid flow during cell loading. If the analyst does not keep migration within the linear portion of the microchannel, then cells may migrate out of one microchannel and enter the adjacent microchannel from the opposite direction.

Should this happen it is no longer possible to reliably assay the lead cells, the result being a skewing of the average migration distance for the array in question.

To prevent this the total assay time must be managed. We suggest running a control experiment to determine the migration rate for the cell line being studied. The optimal assay time can then be calculated to keep migration within the 1000 micron limit. We suggest that cells should be allowed to migrate within the range: **150 – 650 microns.**

By keeping migration assays involving highly motile cells to within 4-8 hours the chance of direction reversal through cell collision is decreased.

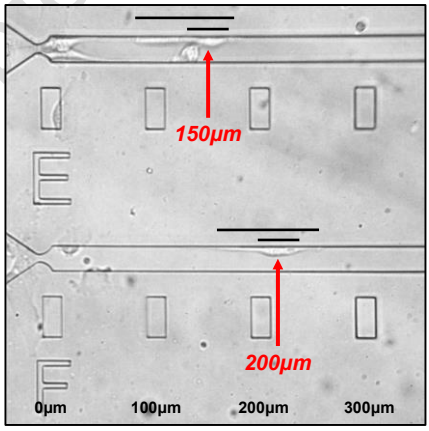
c. Selection of a Migration Alignment Position

Quantification of migration requires that alignment of the lead cell in each microchannel be visually compared to the parallel measurement scale. This should be performed under x100 - x400 magnification.

A linearly migrating cell displays considerable morphological change during its migration, particularly due to the dynamic extension of the cells filopodia (we have observed migrating HT1080 cells ranging from between 10 – 150 microns in overall length!). The transient nature of leading edge protrusions excludes them as a suitable 'alignment point' for comparison with the measurement scale.

For this reason we recommend standardising on the use of the '**centroid**' position of the cell body for determining measurement scale alignment. This corresponds to the **centre of the rounded portion (only) of the cell body** (see **Fig.3** below for examples). The centroid remains constant (in the short term), regardless of whether a cell is in a rounded or elongated phase of a migration cycle. The alignment of the centroid region of the cell can be rapidly and consistently compared against this scale 'by eye', as indicated below.

The measurement scale has been produced with 100 µm axis ticks for visual clarity. We recommend that the migration value be recorded to **the nearest 50 µm** for each **lead cell** in channels **A – J** of the array (see top cell at the 150 µm position in Fig.3). This value represents the displacement from the START position (gate restriction) and is a direct, linear measurement of the cells migration along the microchannel.



- **Longer bar (black):** Indicates total cell length.
- **Short bar (black):** Indicates length of rounded portion of cell body.
- **Arrow (red):** Indicates alignment of centroid position on cell with measurement scale.

Fig.3. Quantification by alignment of cell 'centroid' to measurement scale.

Maintaining stability of the migration chip environment

a. Control of evaporative losses

The large surface area to volume ratio of an unsealed microfluidic assay chip means that water can be rapidly lost due to evaporation from the reservoirs – even in a pre-humidified incubator used for cell culture. This places undesired osmotic stress on the cells.

Evaporation can be controlled in two ways:

1. **Use of a 'Humid Chamber' <u>WITHIN</u> the incubator.**
   The use of a humidity chamber such as that supplied by Sigma (*#H6644*) is recommended - even in an already humidified incubator. This holds 6x Cell Émigré migration chips with a reservoir in the base for water. Alternatively migration chips can be placed within a lidded Petri-dish to which a piece of damp filter paper has been added to raise the local humidity.

2. **Use of Anti-Evaporation Fluid.**
   A small (10µl) volume of low density Silicone Oil such as Sigma Silicone Oil AR 200 (#85419) can be layered over the top of the culture medium in each reservoir. This biologically inert fluid prevents evaporative fluid loss while allowing gas exchange for cellular respiration. Using this fluid the assay can be extended for up to 48 hours in the incubator. Silicone oil is not recommended if cells are to be further processed or treated after migration.

b. Prevention of pH shifts within migration chips

A number of different pH buffering systems are commonly used to keep cells within a physiological pH range. A common buffering agent is sodium bicarbonate and typically media utilising this buffer require a 5% CO_2 atmosphere within the incubator to control pH levels.

The low fluid volume within a microfluidic chip means that there is little spare gas-buffering capacity within the chip, relative to the cells. Culture medium within a chip that is moved often between the incubator and the laboratory may be subject to pH shifts. These can be large enough to cause cell stress. If an experimental protocol requires frequent recording of results over an extended time period then the use of culture medium that does not require an external supply of CO_2 could be considered. This would include medium containing Hanks' Balanced Salt Solution or a HEPES buffering system.

Low cost system for Time-Lapse images

The Cell Émigré assay is fully compatible with existing time-lapse microscopy systems. These can be used to observe the process of migration in one or more of the microchannels of the array, depending on the magnification used.

A time interval between images of 0.5 and 2 minutes should be optimal for migrating mammalian cells.

Typically time-lapse recording requires a costly heated microscope stage. As an accessible alternative Biocolor has successfully used a digital microscope for time-lapse recording of migrating cells. Although the optics systems are typically basic these microscopes have the considerable advantage of being able to be placed directly INSIDE a 37°C incubator. Such a microscope should have an inbuilt megapixel camera and internal **battery-powered** LED illumination. Digital images (using either the 5x or 10x objective lens) may be transferred via a USB cable to a computer (located outside the incubator). By using a thin, flexible cable the incubator door can remain closed throughout the incubation.

Fig.4. Time-lapse microscope in incubator

Visit http://www.biocolor.co.uk/index.php/cell-emigre-cell-migration-assay/to view examples of time-lapse migration and for further instructions on how to assemble such images into video file.
Suitable microscopes would include the CELESTRON Micro 360+ (#44126) or indeed any **BATTERY** illuminated microscope of physical dimensions to fit within the incubator. It should offer at least x100 magnification and output digital images via a usb cable. **Electrical supply cables MUST NOT be placed within an incubator.**

Cell Émigré: Trial Experiments

Trial experiment: Stimulation of cell migration.

Agent: Culture medium supplemented with Foetal Bovine Serum (FBS).

Test concentrations: 0% - 15%.

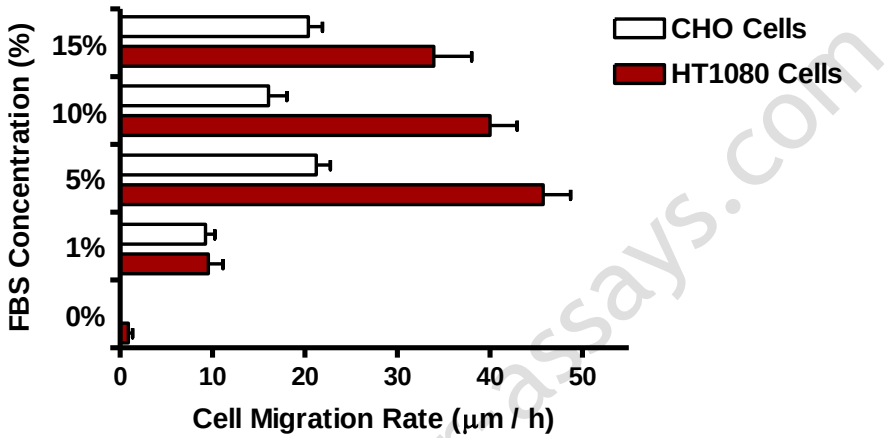


Fig.5: Effect of FBS concentration on the migration rate of HT1080 and CHO cells.

Results are the mean of 3 x microchannel arrays per column +SEM

Methodology:

Cells from HT1080 [ATCC® CCL-121] or CHO [ECACC 85050302] were grown to approximately 70% confluency and then starved (overnight) by replacing the 10% FBS-containing growth medium with growth media containing 0% FBS.

Cells were released from the monolayer using Trypsin and suspended in PBS containing 0.1% sterile Bovine Serum Albumin (BSA). A cell count was performed and the suspension centrifuged to form a pellet. The supernatant was removed and growth medium (without serum) containing 0.1% BSA was added to adjust the cell concentration to $1.2x10^6$ cells/ml. Aliquots (500 µl) of this cell suspension were removed and FBS/growth medium was added directly to each to achieve a range of 0%, 1%, 5%, 10%, 15%. An additional cell-free set of tubes was prepared. These were used to flush the chip after priming (steps 2-4 of loading protocol).

Cell Émigré assay chips were primed and loaded with media containing variable FBS concentrations according to the protocol (Inside covers). Cells from the corresponding aliquot of culture media were then added (see step 7 inside back cover of manual).

Cell Émigré: Trial Experiments

Trial experiment: Inhibition of cell migration.

Agent: Doxorubicin Hydrochloride (Sigma 44583)

Test concentrations: 0 – 20 nM

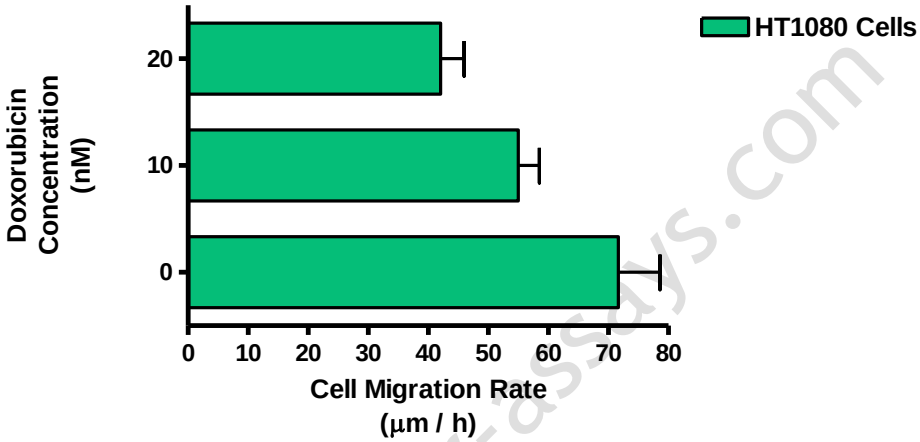


Fig.6: Effect of Doxorubicin on the migration rate of HT1080 cells.

Results are the mean of 3 x microchannel arrays per column +SEM. CHO cells did not show any response to Doxorubicin treatment (results not shown).

Methodology:

Cells from HT1080 [ATCC® CCL-121] or CHO [ECACC 85050302] were grown to approximately 70% confluency. The cells were released from the monolayer using Trypsin, before being suspended, counted and adjusted to a concentration of $8x10^5$ cells/ml. A Doxorubicin stock solution was prepared in growth medium. Aliquots of cell suspension (500 µl) were removed to sterile tubes and sufficient Doxorubicin stock was added (up to 10% volume) to achieve either a 10 or 20 nM final concentration. If required, aliquots were supplemented with medium to maintain identical cell concentrations in each tube.

An additional cell-free set of tubes was prepared. These were used to flush the chip after priming (steps 2-4 of loading protocol).

Cell Émigré assay chips were primed and loaded with media containing variable Doxorubicin concentrations according to the protocol (Inside covers). Cells from the corresponding aliquot were then added (see step 7 inside back cover of manual).

Cell Émigré: Trial Experiments

Trial experiment: Inhibition of cell migration.

Agent: Cytochalasin D (Sigma C8273)

Test concentrations: 0 – 100 nM

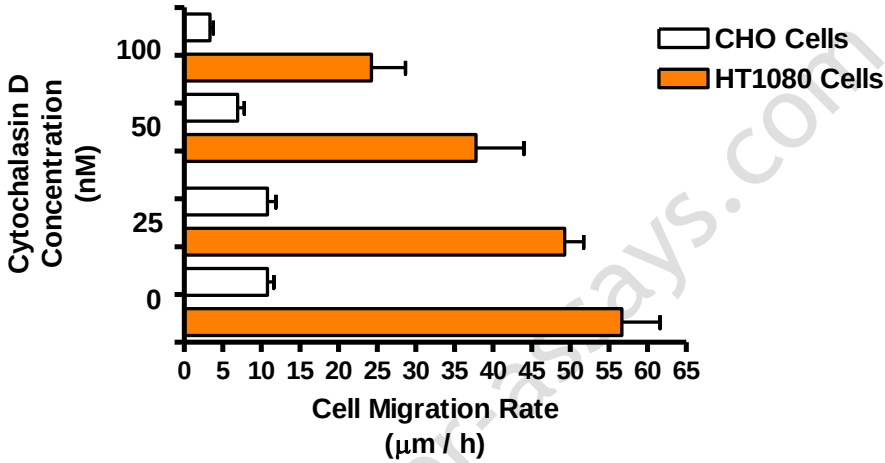


Fig.7: Effect of Cytochalasin D on the migration rate of HT1080 and CHO cells.

Results are the mean of 3 x microchannel arrays per column +SEM

Methodology:

Cells from HT1080 [ATCC® CCL-121] or CHO [ECACC 85050302] were grown to approximately 70% confluency. The cells were released from the monolayer using Trypsin, before being suspended, counted and adjusted to a concentration of $8x10^5$ cells/ml.

A Cytochalasin D stock solution was prepared in DMSO and further diluted in culture medium. Aliquots of cell suspension (500 µl) were removed to sterile tubes and sufficient Cytochalasin D stock was added (up to 10% volume) to achieve a final concentration in the range (**0-100 nM**). If required, aliquots were supplemented with medium to maintain identical cell concentrations in each tube.

An additional cell-free set of tubes was prepared. These were used to flush the chip after priming (steps 2-4 of loading protocol).

Cell Émigré assay chips were primed and loaded with media containing variable Cytochalasin D concentrations according to the protocol (Inside covers). Cells from the corresponding aliquot were then added (see step 7 inside back cover of manual).

Cell Émigré: Trial Experiments

Trial experiment: Coating of **Cell Émigré migration surface.**

Agent: Laminin (Sigma L2020).

Test concentrations: 0 – 100 µg / ml

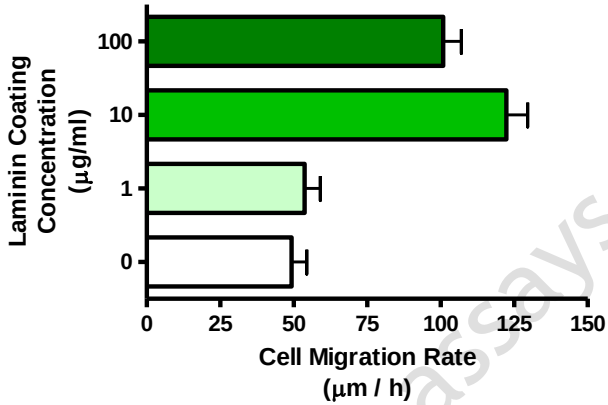


Fig.8: Rate of HT1080 migration on variable Laminin coating concentrations.

Results are the mean of 3 x microchannel arrays per column +SEM

Methodology:

Cell Émigré assay chips were primed following Steps 1-4 (inside front cover) with minor modification: In steps 3 & 4, instead of culture medium, a solution containing the desired coating compound was used, in this case Laminin (0 – 100 µg / ml in PBS). Chips primed with Laminin coating solution were then incubated at room temperature in a humidified dish for at least 12 hours to allow coating of the glass substrate to occur.

Unattached coating was removed through a rinsing step - all fluid was removed from reservoir **[ii]** and replaced with ***50 µl*** of culture medium. Similarly, all fluid was removed from reservoir **[i]** and replaced with ***30 µl*** of culture medium. Allow 10 minutes for rinsing to occur. The cell loading protocol was then resumed from Step 6 (inside back cover).

Note 1. Coating solutions should not take the form of a high viscosity fluid or gel as these can prevent cell loading by blocking the measurement microchannels.

Note 2. Some surface coatings may exhibit excessive adhesion. The result is inconsistent chip loading performance as cells (in fluid suspension) cannot freely flow from the supply channel to the microchannel arrays. In these cases a reduced coating concentration should be trialled.

A coating protocol may be downloaded from the Biocolor Website.