Using the Dunn Chemotaxis Chamber (Hawksley DCC100)

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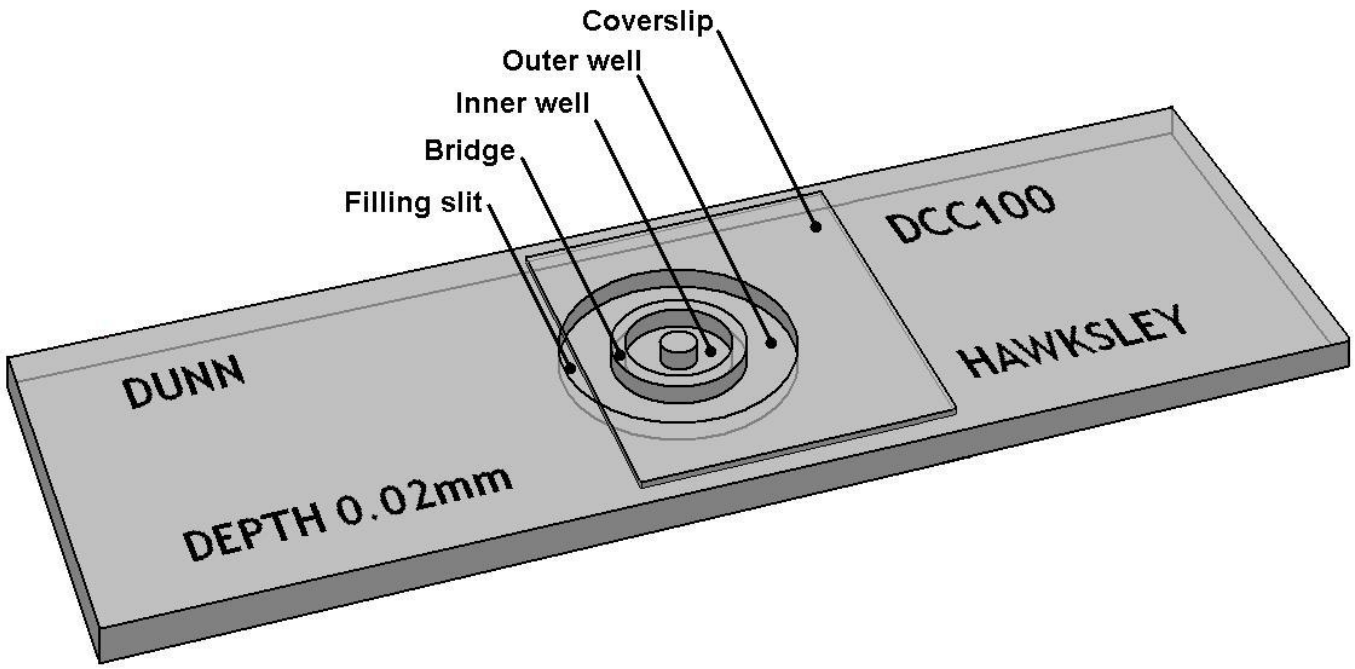

Design

The Dunn Chemotaxis Chamber (DCC100) allows the behaviour of cells subjected to a linear concentration gradient of chemoattractant to be observed directly in the light microscope. The chamber was designed to have good optical properties and long-term stability of the gradient, thus permitting time-lapse recording of cell behaviour over many hours.

The chamber consists of a glass microscope slide with two concentric annular wells ground into the centre of one face to a depth of about half the thickness of the slide (see figure). The annular platform that separates the wells (the bridge) is about 1mm wide. This bridge and the central pip are optically polished to lie precisely 20 µm below the slide's face. Thus when the wells are covered with a coverslip carrying the cells to be studied, there is a gap between coverslip and bridge of 20 µm. If the inner well of the chamber is filled with control medium and the outer well filled with a medium containing chemoattractant, a radially directed linear diffusion gradient becomes quickly established in this gap and is subsequently maintained for several hours. Being made of glass, with care, the chamber can be cleaned, sterilised and reused indefinitely.

Setting up the chamber

Cells are seeded onto a suitably washed sterile coverslip and allowed to settle prior to assembling the chemotaxis chamber. Special thick coverslips are provided that ensure dimensional stability, though other types can be used with care. Initially, both annular wells are filled with control medium and the coverslip seeded with cells is inverted onto the chamber in an offset position in order to leave a narrow filling slit at one edge for access to the outer well (see figure). After firmly seating the coverslip on the face of the chamber and blotting up surplus medium, it is sealed in place using hot wax mixture (Vaseline : paraffin : beeswax - 1:1:1) applied with a paintbrush around all the edges except for the filling slit. Take care not to apply any pressure directly over the bridge or you will crush the cells! In order to set up a chemotactic gradient, the medium is then drained from the outer well using a syringe with a fine-bore needle and replaced with medium containing the chemoattractant. The slit is finally sealed with the hot wax mixture. If the chemoattractant is a globular protein of around 20 kDa, the gradient becomes linear within approximately 20 min and has a half-life of about 24 h (Zicha *et al.*, 1991). Large changes in molecular weight will have only a small effect on these times.

Recording cell behaviour

The chamber is placed on a temperature-controlled microscope stage with suitable provision for time-lapse recording of cell migration in the gradient. It is usually positioned by locating the outer edge of the bridge to coincide with the upper margin of the recording field, so that the direction of increasing chemoattractant concentration is vertically upwards in the image. The useful recording field is limited by the width of the bridge and can have a maximum size at the specimen of about 1 mm in the vertical direction. However, a motorised X-Y stage under software control will allow cell behaviour in several different fields over the bridge to be recorded during each time-lapse interval, thus maximising the data collected in a single experiment. Cells over the central pip region are not subjected to a gradient and simultaneous recording of their behaviour may be a useful control in some experiments. The design of the chemotaxis chamber, especially the blind inner well, helps to ensure stabilty of the gradient but it is advisable not to handle the chamber roughly during the course of an experiment.

Cell tracking and analysis of chemotaxis

The method of tracking cells in the recordings in order to obtain trajectories of cell locomotion will depend on the software available and specific details cannot be given here. Chemotaxis can be evaluated by assessing directional clustering of cell migration using standard methods for the statistical analysis of directional data (Zicha *et al.,*1997; Wells and Ridley, 2005).

Comparison of design considerations in direct observation chemotaxis chambers

The Dunn chamber is a member of the class of bridge-type, direct observation chemotactic chambers which are derivatives of the original Zigmond chamber (Zigmond 1977). The main aim of these chambers is direct observation of chemotactic cell behaviour under controlled gradient conditions. An alternative approach is to use one of several microfluidics systems that have recently been developed. The considerable extra expense of these systems may be justified when precise control and dynamic modification of the chemotactic gradient is required.

However, they can be so complex and tailored to specific requirements that a detailed discussion of comparative advantages is impractical.

The Zigmond chamber was the dominant method for directly observing chemotaxis for many years. Although it suffered from a lack of dimensional stability - which could lead to unpredictable variations in the gradient - this was not so important for its original use in studying fast moving leucocytes. However, a need for greater stability arose for studying the much slower migrations of normal and malignant tissue cells and cell lines.

In mechanical terms, the Dunn chamber achieves this additional stability by being a sealed, rigid, glass enclosure entirely filled with the incompressible medium. Once the narrow filling slit is sealed with a stiff wax mixture, there can be no volume change within the chamber and this stabilises the critical gap between bridge and coverslip. The initial blotting of excess medium requires practice since incorrect seating of the coverslip can lead to variations in the gap. The actual size of the gap, however, is not critical in most applications (within, say, +/- 5 µm) and can be checked using a dry objective on a microscope with calibrated fine focus control (remember to multiply the reading by the refractive index of the aqueous medium). The enclosed design is intended to minimise pressure difference between the wells and to suppress dynamic changes in the gap size – either of which can quickly destroy the gradient.

The use of a thicker, more rigid coverslip is preferable for ensuring a more accurate setting of the gap size and greater dynamic stability. For low power observation this presents no problem but spherical aberration can degrade the image when using a thick coverslip with a high power, dry objective (40x or more). The best solution is to use an objective with compensation for coverslip thickness. Failing this, the use of a thinner coverslip should not present a problem since problems mainly arise when using oil immersion objectives. In fact standard #1.5 coverslips are routinely used in our laboratories. In the case of oil immersion, a thicker coverslip does not introduce spherical aberration but it may be too thick for focussing on the cells if the lens has a short working distance. However, even a thick coverslip cannot easily withstand the forces exerted by the viscous oil during focussing. The coverslip will tend to follow the movements of the objective in both directions and vigorous movements will quickly destroy the gradient and may even crush the cells onto the bridge. The solution is to focus very gently and to allow the gradient to reform after focussing. Changing to water immersion may help and the compensating ring of a multi-immersion objective will be able to compensate for a thicker coverslip when using water immersion (try different settings between oil and water).

The Insall chamber (Muinonen-Martin et al., 2010) is a more recent development that was specifically designed to ensure a better stability of the gap between bridge and coverslip when using thin coverslips with high aperture, immersion objectives. Like the Zigmond chamber, it is made from polymethyl methacrylate and relatively flexible. This construction limits the possibility of reuse – it is intended to be cheap and disposable. The bridge structure is a square arrangement with two linear bridges of differing widths on opposite sides and the other two sides are large coverslip supports – additional small supports being incorporated into the bridges. The outer well has two channels that extend beyond the coverslip for convenience of changing the medium. These are intended to be sealed with tape.

The stability of the Insall chamber therefore does not rely on it being a rigidly enclosed system. It achieves stability because the supports prevent the coverslip from approaching closer to the bridges than the specified 20 µm. This is an advantage when using oil immersion objectives and it eliminates the possibility of crushing cells onto the bridge when focussing too vigorously. Nevertheless, care must still be taken when focussing since the supports will not prevent the

coverslip being pulled away from the bridges by the viscous oil when focussing away from the chamber.

The additional supports of the Insall chamber may thus prove to confer a decisive advantage for high resolution microscopy and the linear bridges and arrangement for refilling the outer well are more convenient than in the Dunn chamber. Nevertheless, it cannot compete with the Dunn chamber for the unequivocal confirmation of chemotaxis and analysis of chemotactic behaviour. This is because the additional supports of the Insall chamber will, during assembly, inevitably crush a large number of cells in the immediate vicinity of the cells being studied. The intracellular factors thus released will remain in the inner well and will form a gradient over the bridge after the medium in the outer well is replaced with an experimental medium. It has long been speculated that dead or injured cells of many types can release potent chemotactic factors (a phenomenon known as necrotaxis) and the released factors may have chemokinetic or chemotactic activities (positive or negative) or synergistic effects in combination with other factors. Control experiments may be performed in the Insall chamber to determine whether any chemotactic factors are released by each type of cell but the possibility that the ever present cell debris has unknown synergistic effects can never be entirely discounted.

In the Dunn chamber, in contrast, only those cells that come to lie outside the outer well are crushed during assembly and it is possible to avoid crushing cells entirely by ensuring that all cells seeded onto the coverslip come to lie within the outer limit of the outer well. Even if no such precautions are taken, the released factors are unlikely to reach the inner well - and subsequently form a gradient over the bridge - since they will be washed out immediately after assembly when replacing the medium in the outer well.

In conclusion, if you need to study the mechanism of chemotaxis under highly controlled gradient conditions then a microfluidics system may be your answer. Alternatively, if you need to study the molecular components involved in chemotaxis, and their dynamics, using high resolution fluorescence microscopy then the Insall chamber may prove to have decisive advantages. However, if you need an unequivocal confirmation and analysis of chemotaxis by direct visual observation then the Dunn chamber is still the simplest and most effective solution.

References

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