BE-Gradient Barrier-Free Applications

Confinement of collagen hydrogels at different concentrations and their diffusion profile.

Introduction

Our new design BE-Gradient Barrier-Free is a device designed for 3D culture where a central chamber is linked with two fluidic lateral channels (Figure 1). The innovation we present is the absence of any physical barrier between the central chamber and the lateral channels.

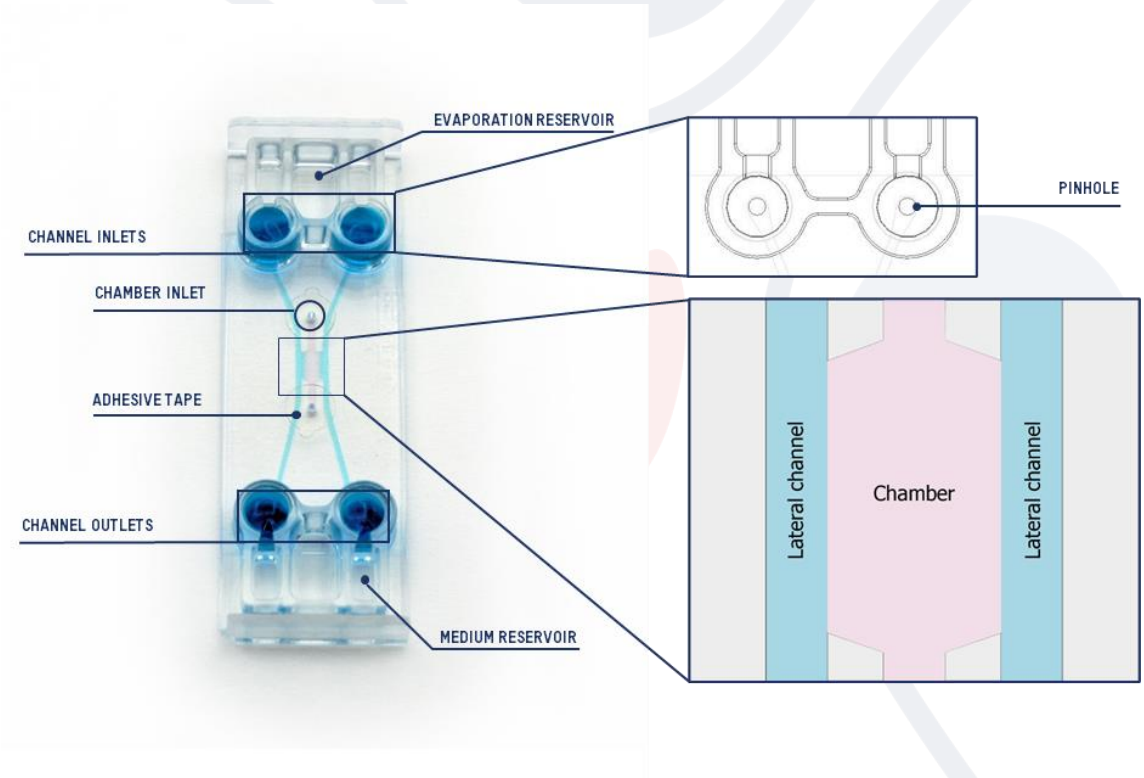

Figure 1 BE-Gradient Barrier-Free device with the description of each section.

BE-Gradient Barrier-Free is designed for the application of electrochemical gradients as well as barrier and vasculature models with 3D cell cultures. It is compatible with any type of optical microscopy (inverted phase contrast, confocal, fluorescence, etc). For barrier and vasculature models, the lateral channels are meant to simulate blood vessels, where endothelial cells can be cultured. The creation of a chemical gradient is also possible by changing the concentration of one element between the channels on both sides of the central chamber.

Several different hydrogels are used in *in vitro* biological models, such as Matrigel, collagen and fibrin. Collagen is the most abundant protein in the human body and plays an essential role in maintaining the integrity and elasticity of tissues. It serves as support and anchor for cells to migrate and survive[1]. The concentration of collagen found in different tissues and models varies depending on the organ and the pathophysiology the model aims to mimic. Therefore, it is crucial to validate that the BE-Gradient barrier-free device allows for the use of different hydrogel concentrations, such as collagen in this case.

In this study, we evaluated the spatial distribution of different collagen hydrogels concentrations in the central chamber and confirmed the proper confinement of the hydrogel in it. Additionally, we examined the diffusion profile of rhodamine through the three collagen hydrogels.

Methodology

Filling and handling

Before seeding:

- Prewarm the device in the incubator overnight to avoid the appearance of air bubbles.
- The central culture chamber must be completely dry to confine the hydrogel in it.

1. Prepare the collagen type I solutions following the protocol described in González-Lana et al.[2]. For this assay, we used **0.5**; **1**; **2** and **4** mg/mL. Keep the solution at 4°C until the time of seeding.
2. Add FluoSpheres™ (Invitrogen™, Fisher Scientific, 10513463) to the collagen solution in a ratio of 100:1.
3. Pipette 10 µL of the solution and slowly inject it through the inlet of the central chamber until filling it midway (Figure 2 A. 1-3). Then, pipette the rest of the solution from the outlet of the chamber until the chamber is fully filled (Figure 2 A. 4-6). When changing the tip from the inlet to the outlet to finish injecting the solution, keep pressing the pipette plunger so air does not enter the pipette tip. The central chamber can also be completely filled by one side only if performed slowly and carefully (Figure 2 B).

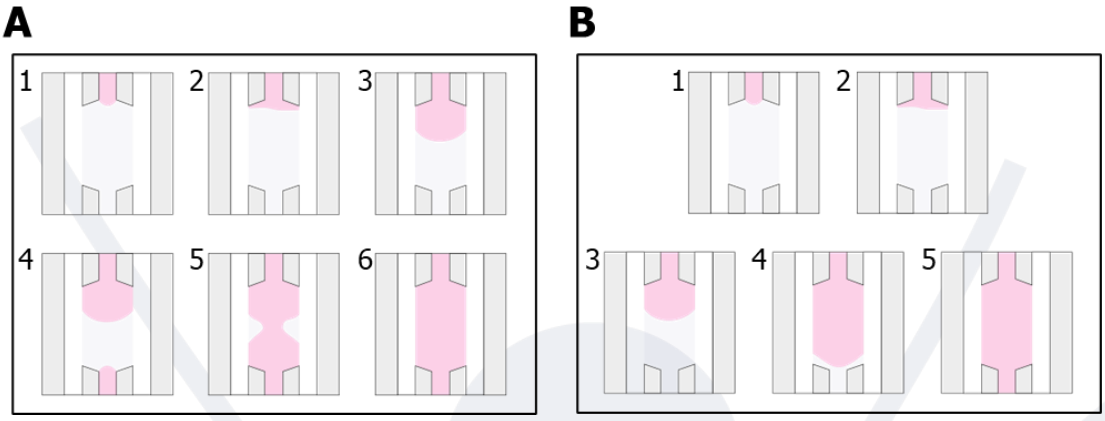

Figure 2 Different options to seed the central chamber of the BE-Gradient Barrier-Free. (A) Filling the chamber from both inlet and outlet. (B) Filling the chamber from the inlet only.

4. Flip the chip upwards and downwards every 5 minutes several times for homogeneous 3D distribution during the polymerization of the hydrogel while keep the device at 37 °C, for a total of 15 minutes.
   Disclaimer: this step is dependent on the hydrogel. Check manufacture's recommendations for polymerization and apply to this protocol.
5. Seal the central chamber inlet and outlet with the adhesive provided. Make sure that the surface is clean and dry to assure a proper adhesion. Do not seal the central chamber before the hydrogel is fully polymerized, otherwise the hydrogel can leak into the lateral channels when the tape is placed.
6. Within the lateral channels, place an aqueous solution with rhodamine (1 µg/mL; MW 479.01; Sigma-Aldrich, R6626). To introduce the solution in the channels, first pipette around 250 mL in the inlet well of the channel and gently aspirate from the outlet, ensuring that the tip of the pipette is vertical and in the pinhole. After the channel is filled with the medium, there's no need to keep aspirating; it will flow naturally from the inlet to the outlet.

See more at: https://youtu.be/hLhDuOolVwk

Assessment of the fluorescence

To evaluate the hydrogel spatial distribution, we recommend using a fluorescence or confocal microscope capable of 3D reconstruction.

1. Place the device with the fluorescent hydrogel in the central chamber when the hydrogel is polymerized but the channels are still empty (step 5 from the previous section).
2. Acquire images on the microscope at various z-planes throughout the chamber's height to ensure the hydrogel is fully filling the whole chamber.
3. After, fill the channels with the fluorescent solution and repeat the image acquisition, preferably in the same zone or similar.

Results

One of the most important features of BE-Gradient Barrier-Free is the ability to contain a hydrogel in the central chamber while having direct contact to two lateral channels. This feature aims to create a closer environment for cell-cell interaction in barrier models or gradient formation.

It is important to ensure the suitability of this device with hydrogels of different consistencies. Collagen is extensively used in *in vitro* models both as coating and as 3D matrices. The consistency of this material is different if the concentration is as low as 0.5 mg/mL or as high as 4 mg/mL. The former is more fluid and the latter more viscous and denser.

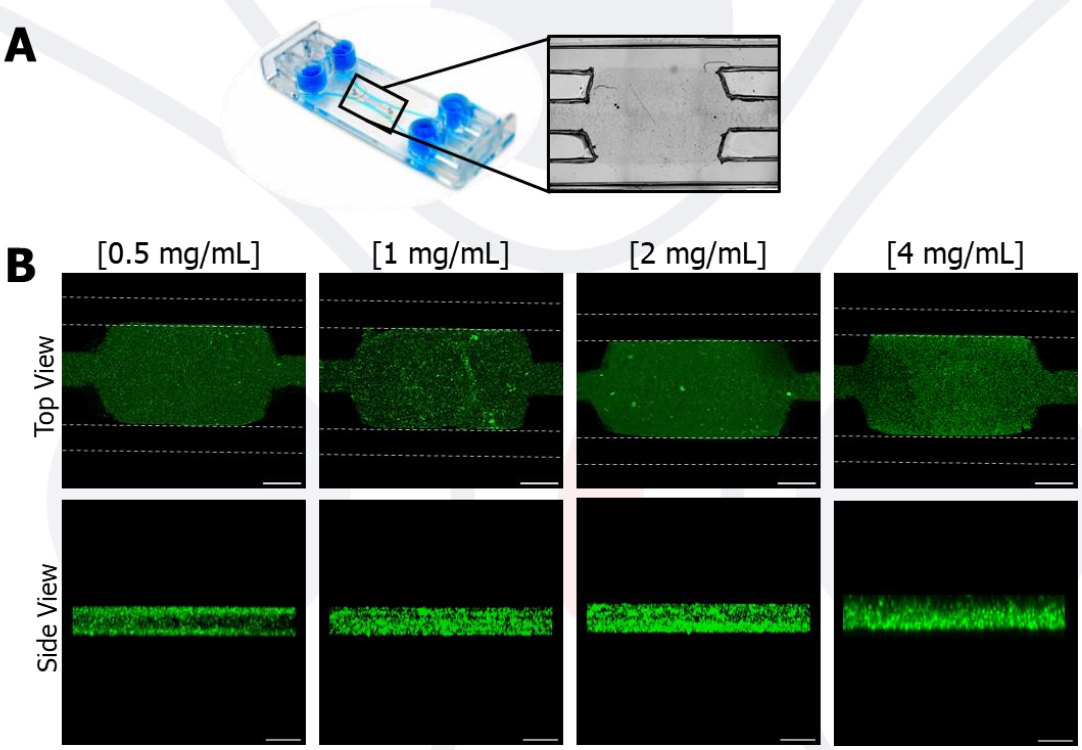

Figure 3 Confinement of different collagen concentrations in BE-Gradient Barrier-Free central chamber. (A) Brightfield image of the area of the device with the hydrogel confined in the central chamber and the lateral channels. (B) Confocal images of different collagen concentrations (0.5, 1, 2 and 4 mg/mL) loaded to the chamber of BE-Gradient Barrier-Free device. The first line shows the top view, and the second line shows the side view of the devices. The dotted lines in green represent the lateral channel positions. Scale bar: 1 mm (first row); 200 µm (second row).

Four different concentrations of collagen were tested, 0.5; 1; 2 and 4 mg/mL by staining the hydrogel with fluorescent particles and loading it within the central chamber of the device. The confocal microscopy images demonstrated that all concentrations were able to be loaded, filling the chamber but not the lateral channels (Figure 3 Top view). The assessment of the lateral side of the device showed that the hydrogel reached the whole chamber, ensuring a correct confinement. These findings provide evidence of the device's adaptability to hydrogels with varying concentrations and therefore diverse mechanical properties including stiffness and pore size.

We also assessed the diffusion profile across hydrogels of different concentrations (0.5; 1 and 2 mg/mL) by perfusing a rhodamine solution into one of the lateral channels (Figure 4). It was observed that with the lowest concentration (0.5 mg/mL) the fluorescence diffusion through the hydrogel was faster than 1 and 2 mg/mL, traversing the central chamber completely after 25 minutes and generating a fluorescence gradient as depicted in Figure 4C. Hydrogels at concentrations of 1 and 2 mg/mL exhibited nearly identical fluorescence diffusion, reaching approximately halfway across the chamber. After 15 min all concentrations had red fluorescence in the central of the channel which indicated that the rhodamine solution had reached that region (Figure 4). It can be extrapolated that, if both channels were filled, 15 min would be needed for the complete diffusion of this solution across the chamber.

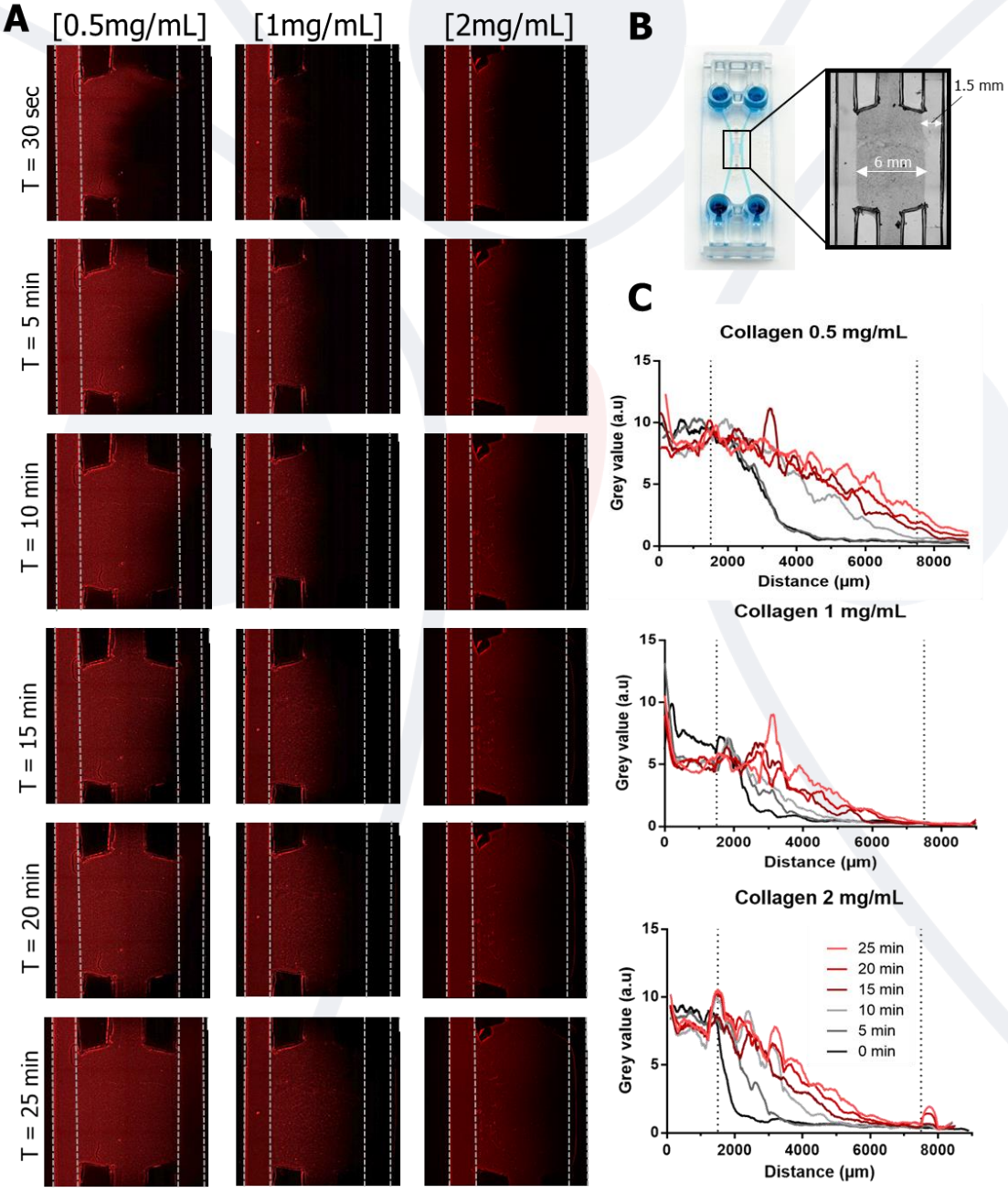

Figure 4 Diffusion test. (A) Fluorescence images of rhodamine diffusion from the left channel into the central chamber using three different collagen concentrations (0.5; 1 and 2 mg/mL) in the central chamber over 25 minutes. Scale bar: 1 mm. (B) Area of the device that was measured. (C) Plot profiles of the images acquired.

The knowledge of the diffusion rate in the hydrogel is useful to understand how much time the media would need to reach seeded cells as well as a staining solution for protocols such as immunostaining. The time of diffusion varies according to the type of hydrogel and its concentration since its porosity directly influences the speed of diffusion of the liquid from the channels to the chamber. The number of cells seeded is also a factor that can influence the diffusion velocity though the central chamber, with a denser culture having a smaller diffusion speed than a culture with less cells.

Conclusions

BE-Gradient Barrier-Free is a new device launched by Beonchip. It is composed of a central chamber and two lateral channels in direct contact, designed for 3D culture and the creation of electrochemical gradients and/or barrier models. Collagen is one of the most used extracellular matrix proteins in 3D culture. In this technical note, different concentrations of collagen hydrogels were loaded in the devices and proven to be confined in the chamber, independently of the concentration. The diffusion of a rhodamine solution was also introduced in the system, validating the diffusion without damaging the confinement and revealing the diffusion profile across the hydrogel.

Bibliography

1. Sung, K. E. *et al.* Control of 3-dimensional collagen matrix polymerization for reproducible human mammary fibroblast cell culture in microfluidic devices. *Biomaterials* **30**, 4833–4841 (2009).

2. González-Lana, S. *et al.* Surface modifications of COP-based microfluidic devices for improved immobilisation of hydrogel proteins: long-term 3D culture with contractile cell types and ischaemia model. *Lab Chip* **23**, 2434–2446 (2023).