Biogrid—a microfluidic device for large-scale enzyme-free dissociation of stem cell aggregates

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Culturing stem cells as free-floating aggregates in suspension facilitates large-scale production of cells in closed systems, for clinical use. To comply with GMP standards, the use of substances such as proteolytic enzymes should be avoided. Instead of enzymatic dissociation, the growing cell aggregates may be mechanically cut at passage, but available methods are not compatible with large-scale cell production and hence translation into the clinic becomes a severe bottleneck. We have developed the Biogrid device, which consists of an array of micrometerscale knife edges, micro-fabricated in silicon, and a manifold in which the microgrid is placed across the central fluid channel. By connecting one side of the Biogrid to a syringe or a pump and the other side to the cell culture, the culture medium with suspended cell aggregates can be aspirated, forcing the aggregates through the microgrid, and ejected back to the cell culture container. Large aggregates are thereby dissociated into smaller fragments while small aggregates pass through the microgrid unaffected. As proof-of-concept, we demonstrate that the Biogrid device can be successfully used for repeated passage of human neural stem/progenitor cells cultured as so-called neurospheres, as well as for passage of suspension cultures of human embryonic stem cells. We also show that human neural stem/progenitor cells tolerate transient pressure changes far exceeding those that will occur in a fluidic system incorporating the Biogrid microgrids. Thus, by using the Biogrid device it is possible to mechanically passage large quantities of cells in suspension cultures in closed fluidic systems, without the use of proteolytic enzymes.

Introduction

During the last decade, various types of stem and progenitor cells have been identified and successfully cultured and expanded in vitro. Several cell types have been proven to be beneficial in a number of animal disease models. This is particularly true for neural stem and progenitor cells, which have brought hope for future treatments of a number of incurable disorders of the central nervous system (CNS), although significant hurdles to a clinical therapy remain.1,2

Neural stem and progenitor cells can be isolated from embryonic and adult CNS tissue and cultured in vitro as adherent cultures or as suspension cultures of free-floating cell aggregates—so-called neurospheres—to expand the cell population.3,4 These cultures consist of heterogeneous mixtures of stem and progenitor cells and are therefore often termed neural precursor cells (NPCs). Cultures of NPC can also be produced by neural induction and differentiation of embryonic stem cells (ESCs)5 and expanded as free-floating cell aggregates.6 Using ESC for clinical purposes requires large-scale cell cultures, which is most easily achieved with suspension cultures.7 During production of clinical grade cells, adherence to the standards of Good Manufacturing Procedures (GMP) is necessary. As a consequence, cells should if possible be maintained in closed systems. The use of exogenous substances with biological activity should also be minimized. For cell cultures, proteolytic enzymes used for dissociation during passage represent substances that should be avoided, if possible. In experimental research on neurospheres this issue has been addressed by replacing enzymatic dissociation with manual cutting of the spheres into smaller fragments using scalpels, micro-scissors or tissue choppers.8 Likewise, in applications where it is necessary to maintain a direct contact between cells, methods to cut tissue and...
cell aggregates manually are used. These procedures are however labor-intensive with potential risk for contamination and are not compatible with large scale GMP cell production. Tissue grinders such as the Potter–Elvehjem homogenizer are typically associated with extensive cell loss. Various methods to isolate stem and progenitor cells using filtration have also been developed, but for use with cell suspensions. After extensive searches in patent databases and scientific journal databases we have not found any device by which cell aggregates can be dissociated non-enzymatically in a large scale under GMP-compatible conditions.

To address this problem, we have developed Biogrid, a tissue slicer for efficient mechanical passage of free-floating cell aggregates in closed fluidic cell culture systems. The core of the device is a microgrid, the dimensions of which can be chosen to suit the cell aggregates of interest. We have applied the technique on human NPCs grown as so-called neurospheres in suspension, and we show that this method can be used to efficiently expand neurosphere cultures without the use of proteolytic enzymes during passage. We also show that with respect to neurospheres derived from the human fetal CNS, they tolerate well the pressure changes which may occur in fluidic systems into which the Biogrid can be incorporated. Moreover, we demonstrate that Biogrid can also be applied to suspension cultures of human ESCs.

**Experimental**

**Outline of the Biogrid device**

At the core of the system is a microgrid with sharp edges, mounted on the channel of an adaptor and sealed by surrounding o-rings. The channel of the adaptor is at one side connected to a culture of free-floating cell aggregates and at the other side to a syringe or to any type of device which can aspirate fluid through the channel, and then by reverse function eject the fluid through the channel back to the cell culture container.

**Microfabrication**

A tissue slicing microgrid was fabricated in (110)-silicon, n-doped, 1–3 Ω cm, and double side polished. Standard anisotropic wet etching in potassium hydroxide was employed (80 °C, 20 g KOH/100 ml H2O).

The microknife edges were arranged in an array configuration to avoid the risk of clogging and also to ensure that excessive pressure build-up is avoided. The initial microgrids (1st generation) were designed as a 3 x 3 mm microgrid with a central knife edge area of 2.5 mm² (Fig. 1A). These microgrids were subsequently used for all experiments on NPCs reported here. In order to simplify handling and avoid fracture of the thin 1st generation grids when mounting them on the adaptor, a second generation was designed. In these microgrids the thickness of the grid and the width of the peripheral rim were increased (Fig. 1B). The increased slicing area was implemented to reduce clogging as the cell clusters populate the grid area in the slicing process. The critical geometries of the two chip generations are listed in Table 1. Fig. 1C shows a 3” wafer with more than 300 microgrids. Fig. 1D shows a scanning electron microscope close-up image of the grid structure, with silicon knife edges of 30 μm spaced 200 μm apart.

**Adaptors**

An adaptor was machined to enable clamping of the microgrid in a Delrin manifold. The manifold was designed to allow either a direct mounting of a standard Luer needle or coupling to 1/16” Teflon tubing via a chromatography ferrule. To ensure a tight sealing of the microgrid, o-rings were mounted on the Delrin fixtures (Fig. 1E and F).

The adaptor was designed to allow the use of the Biogrid device in different types of fluidic systems, including automated fluidic systems driven by syringe pumps. All experiments on NPCs in this study were performed using this version of the Biogrid device. For easier use of the Biogrid when the syringe was handled manually, a smaller version of the adaptor with Luer fittings was designed and manufactured for the second generation of microgrids (Fig. 2C and D), which was used for the ESC aggregates. However, the basic principle is identical for the two adaptors, and only the dimensions of the microgrids used differ.

**Human neurosphere cultures**

The feasibility of the Biogrid device was studied using human NPCs, cultured as neurospheres. Human neurospheres were
The procedure was approved by the Regional Ethical Committee of Stockholm. Briefly, the embryonic-fetal tissue was retrieved from clinical first trimester routine abortions with informed consent from the patient. The subcortical forebrain and the spinal cord were dissected from the identified CNS tissue, homogenized with a glass-Teflon homogenizer, and cultured at 100 000–200 000 cells per ml in a humidified 5% CO2 atmosphere using a cell medium containing DMEM/F-12 supplemented with 1% N2, 5 mM Hepes, 0.6% glucose, 25 U/ml of penicillin and streptomycin (Cambrex), supplemented with 20 ng/ml human epithelial growth factor (EGF, R&D Systems), 20 ng/ml bFGF (R&D Systems), and 10 ng/ml human ciliary neurotrophic factor (CNTF, R&D Systems). Free-floating neurospheres appeared during the first week of culture, and they were split into

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Fig. 2  (A) Schematic illustration of the first generation adaptor with a mounted microgrid, connected on the left side to a syringe via tubing with chromatography fitting and on the right side to tubing for aspiration of cell aggregates from a cell culture dish. (B) Cell aggregates are cleaved into smaller fragments as they pass through the microgrid by the flow of the aspirated cell culture medium. (C) Design of the second generation adaptor shown before assembly and (D) after assembly mounted on a syringe, with a needle attached.
two flasks to establish parallel cultures which were passaged every 7–14 days, either by enzymatical dissociation with TrypLE Express (Invitrogen), 4–5 minutes at 37 °C as previously described, or by mechanical cutting of the neurospheres with the Biogrid device.

**Passage of neurospheres using Biogrid**

For mechanical cutting at passage of cultures using Biogrid, the free end of the Teflon tubing at one side of the device was connected to a 10 ml plastic syringe and the tubing at the other side of the device was used to aspirate the neurospheres from the cell culture flask. The cell culture medium with neurospheres ranging in size from 50 to 500 μm was aspirated at a flow of 0.5–1 ml s⁻¹ through the Biogrid device. The suspension was then immediately ejected through the device into a new cell culture flask, completing a cycle back and forth through the microgrid, which was often sufficient for cutting the neurospheres. If large fragments could still be seen in the culture medium, these fragments were once again aspirated with the Biogrid and then ejected back into the flask. The device was thereafter flushed with 5–6 ml of fresh cell culture medium, which was also ejected into the cell culture flask.

In case the volume of the cell culture before passage exceeded 10 ml, or if the cell culture medium showed early signs of color shift, the content of the flask was transferred to a 50 ml Falcon tube, centrifuged at 1000 × g for 2 min, all but 1–2 ml of the supernatant discarded, and the pellet carefully resuspended by adding 6–7 ml of the fresh medium. The Biogrid device was then used to aspirate the neurospheres from the Falcon tube and eject them into a new cell culture flask. The device was flushed as described above.

After use, the Biogrid device was opened and the microgrid inspected. In case tissue debris remained in the microgrid, it was placed over-night in liquid tissue dissolve (BTS-450, Beckman Coulter). The next day the microgrid was rinsed in water and then transferred to a commercial isopropyl alcohol sterilizing solution.

**Calculating expansion rates**

To follow the expansion of the neurosphere cell cultures, the total number of NPCs in enzymatically dissociated neurospheres were assessed at each passage using Trypan blue vital staining and cell counting using a Bürker chamber. To count cells in neurospheres passaged using Biogrid, the successful expansion allowed us to use one of the duplicate flasks for cell counting after enzymatic dissociation. The cells in these cultures were counted and multiplied by the total number of duplicate culture flasks to get an estimate of the total number of cells of the particular NPC culture. Biogrid cell cultures used for cell counting were then discarded. At the end of the experiment, the neurospheres were dissociated using TrypLE Express, cells counted and the total number of cells calculated.

To measure the sizes of neurospheres and fragments present in the medium after passage, an inverted microscope equipped with an eye-piece ruler was used. For spheres the diameter was measured, and for ellipsoids, prisms and irregularly shaped fragments the major axis and the axis perpendicular to the major axis were measured.

**Embryonic stem cell suspension cultures**

Human ESCs (cell line HS360) were derived from the inner cell mass of supernumerary blastocyst-stage embryos from the in vitro fertilization unit, Karolinska University Hospital Huddinge, Sweden. Approval for all procedures was obtained from the Regional Ethical Committee of Stockholm. Human ESCs were first cultured on mitotically inactivated human foreskin fibroblasts (CRL-2429, ATCC) in knockout Dulbecco’s modified Eagle’s medium, 20% knockout-SR, 2 mM GlutaMax, 0.1 mM β-mercaptoethanol (all from Invitrogen), 0.1% minimal essential medium non-essential amino acids, 50 U ml⁻¹ of penicillin/streptomycin (both from Cambrex Bio Science), and 8 ng ml⁻¹ human basic fibroblast growth factor (bFGF, R&D Systems) as previously described. Human ESC colonies were passaged by mechanical splitting every 5 to 7 days and re-plated on fresh fibroblasts.

To establish suspension cultures as described by Steiner and collaborators, large cell aggregates were taken from colonies of undifferentiated ES cells with a normal karyotype grown on a feeder layer using a scalpel. The aggregates were placed in a 6 well ultra low attachment plate in 2 ml of the suspension medium containing the Neurobasal medium with 14% knockout-SR, 2 mM L-glutamine, 50 U ml⁻¹ of penicillin/streptomycin, 1% minimal essential medium non-essential amino acids and 4 ng ml⁻¹ human bFGF. The medium was replaced every second day by tilting the plate and removing 50–80% of the medium and adding a fresh medium. Cell aggregates were passaged every 7 or 8 days using a 1000 μl pipette to break up the aggregates into smaller clusters, until dissociation instead was performed using the Biogrid device. After replacing most of the cell culture medium, each well with cell aggregates was aspirated through a device equipped with a microgrid with 10 μm knives and 120 μm spacing at an approximate flow rate of 1 ml s⁻¹ and immediately ejected back into the well. The ESCs were monitored for two passages with the Biogrid to ensure continued cell growth and sphere formation.

**Immunocytochemistry**

For immunocytochemical analyses of the NPCs at the end of the culture period, some neurospheres were dissociated with TrypLE Express and plated on poly-d-lysine and fibronectin-coated cover slips at a density of 20 000 cells per cm². After differentiation for 14 days in DMEM/F12, 1% N2, 5 mM Hapes, 2 mM Glutamax, 0.6% glucose and 5% fetal calf serum, neurons and astrocytes were visualized using antisera to β-tubulin type III (1:800, Sigma) and glial fibrillary acidic protein (1:500, Dako) as described previously. From the same cell cultures, intact neurospheres were fixed, cryo-sectioned, treated for antigen retrieval (10 mM citrate buffer, pH 6.0, 120 °C for 20 minutes) and immunostained for the proliferation marker Ki-67 (1:100, Dako). Cell nuclei were counterstained with Hoechst 33342. To perform quantitative analyses, Ki-67 immunoreactive cell nuclei, or β-tubulin type III, and GFAP immunoreactive cytoplasm surrounding Hoechst...
3342-labeled nuclei by more than 75% were counted in a Zeiss Axiophot fluorescence microscope.

Exposure to transient pressure changes

To measure pressure changes occurring across the Biogrid during passage, neurospheres were passaged using a Biogrid device directly attached to a 10 ml syringe at the one end and a three-way connection with Luer fittings at the other. A short piece of fluid-filled Teflon tubing was connected to the three-way connection, a pressure transducer and a 0.9 × 90 mm spinal needle. The needle had the original sharp bevel cut off perpendicularly and grinded smooth, for aspiration of neurospheres from cell culture flasks. With this set-up, pressure changes in the fluid during aspiration and ejection at normal passage were recorded.

To study the possible harmful effects of pressure changes in the fluid on NPCs, 1 ml of the medium containing neurospheres was aspirated into the syringe. The three-way connection was switched to connect the syringe only to the pressure transducer, and high and low pressures were created, respectively, by compressing or aspirating the medium in the syringe against the closed connection and the transducer. The pressure change was established during 1 second and maintained for 20 seconds. The pressure was then abruptly normalized, the medium and neurospheres were ejected into 24 well plates, and 200 µl of the medium were collected for measurement of LDH (Cytotoxicity Detection Kit, Roche Diagnostics GmbH) as a marker of cell injury before and after exposure to a mechanical treatment.

Results and discussion

Slicing principle

Fig. 2A and B show a schematic illustration of the Biogrid system set-up. The basic principle of Biogrid was to force cell aggregates such as the neurospheres against thin microfabricated knife edges of a microgrid by means of a fluid driven pressure, thereby cutting larger aggregates into smaller fragments (slices), while aggregates smaller than the openings of the microgrid pass through without being affected. By passing the aggregates back and forth through the microgrid, similarly sized fragments are obtained for further culturing. The final size of the fragments will depend on the chosen dimensions of the microgrid, the size of the original aggregate and the number of times the fragments are passed through the microgrid. Large neurospheres will result in larger “first-passage” slices, which can be further reduced by repeatedly passing them through the microgrid.

Cell culturing and proliferation studies

The Biogrid device was evaluated by applying it to a mechanical passage of human neurospheres, comparing them to neurospheres passaged using proteolytic enzymes. All cell culture results reported herein were generated using the 1st generation microgrid. Follow-up studies were also performed using the 2nd generation microgrids and adaptor, which did not show any deviation in terms of cell survival or proliferation (data not shown). Handling of the device was however substantially simplified.

For application on human NPCs cultured as neurospheres, microgrids with a grid spacing of 200 µm and a knife width of 20 µm was chosen, since the neurospheres are typically passaged when the largest spheres have reached a diameter of approximately 500 µm. The initial experiments showed that the Biogrid device could indeed be used to cut human neurospheres into smaller pieces and further cultured and expanded (Fig. 3A and B). Passing the neurospheres back and forth once was often sufficient to achieve a homogeneous suspension of fragments and small neurospheres, but when larger neurospheres were abundant, repeating the procedure once or twice was necessary.

We also observed that after Biogrid-assisted passage of some neurosphere lines, a substantial number of single cells appeared in the cell culture. Although the number of single cells was lower if a microgrid with more narrow knives (10 µm) was used, we found that similar release of single cells from some neurosphere lines occurred if they were dissociated with razor blades or scalpels.

To evaluate the applicability of Biogrid, a number of duplicate human neurosphere cell cultures were established according to previously published methods. At the time of first passage and split of cultures, one of the duplicate neurosphere cultures was passaged using enzymatic dissociation, while mechanical passage using Biogrid was applied to the other. The neurosphere cultures were studied for 10–13 weeks. During this time, the number of cells in mechanically dissociated cultures increased from 2–3 million to 100–300 million, i.e. a 100-fold increase. The growth of enzymatically passaged cultures was more variable. At the end of the study period, some of these cultures contained...
80–200 million, while others reached over 10 billion, representing a 10 000-fold increase (Fig. 4A).

When we analyzed the sizes of fragments immediately after passage of neurospheres with diameters between 50 and 500 μm, it clearly showed that the large neurospheres were cleaved into smaller fragments, while neurospheres up to 300 μm diameter remained intact (Fig. 4B). The largest remaining neurospheres illustrate the elastic nature of the neurospheres. Although the largest fragments according to the graph are much larger than the openings of the microgrid, these fragments are slices which are much thinner than the dimensions of the axes shown in the graph. All three axes could however not be measured and displayed.

Thus, the growth rate of neurospheres passed with Biogrid, as well as most of those enzymatically passed, would suffice for producing NPCs for a clinical application, but it is not clear to us why the most extreme growth only occurred when enzymatic dissociation was used. Importantly, with the use of Biogrid the rate of expansion of mechanically passed NPC cultures was more reproducible than that of the enzymatically passed cultures.

We subsequently investigated the cellular constituents of neurospheres passed mechanically or enzymatically. Considering the varying growth rate, we quantified the number of cells immunoreactive for the proliferation marker Ki67 in cryo-sectioned neurospheres. In both types of spheres, about 40% of the cells were Ki67-IR, with no indications of a difference between the groups (Table 2).

Neurospheres contain a heterogeneous cell population, and a small proportion of cells in the neurospheres are relatively mature progenitor cells, expressing neuronal or astroglial markers in cells which extend cell processes. We hypothesized that these progenitor cells would more easily survive mechanical passage, since there is no complete dissociation into single cells, leading to a relative increase of such cells in mechanically dissociated neurospheres. We therefore calculated the number of differentiated cells immunolabeled with markers for neurons (β-tubulin III) and astrocytes (GFAP). As we and others have reported previously, the vast majority of cells were immunoreactive for the early neural marker nestin (data not shown), while 3–4% were GFAP-IR with a morphology typical for astrocytes, and 6% of the cells were β-tubulin III-IR with a neuronal morphology. We found no differences in the relative frequency of these different cell types in neurospheres passed using the two methods (Table 2). Thus, our hypothesis was rejected, and the data suggest that mechanically and enzymatically passed neurospheres contain similar cell populations. It should be emphasized that to ascertain that neurospheres passed enzymatically and mechanically are identical, expression of a large number of other markers such as transcription factors for positional identity would be necessary. However, such an extensive analysis is beyond the aim of this study.

If the Biogrid device is to be used in a fluidic system for large-scale production of cells, the cells used have to withstand the pressure changes, which inevitably will occur in such a system. Initially we determined the typical pressure changes in the fluid when the Biogrid device was used for passage of neurospheres. At the flow rate used which was high enough to avoid clogging, the absolute change from atmospheric pressure was typically 50–60 mm Hg, i.e. 700–710 mm Hg during aspiration and 810–820 mm Hg during evacuation of the syringe. The pressure changes never exceeded 100 mm Hg.

**Table 2** Relative proportion of cells immunostained for the proliferation marker Ki-67, the astrocyte marker GFAP and the neuronal marker β-tubulin type III (β-Tub) in neurospheres passaged for 12 weeks using enzymatic dissociation or mechanical dissociation using the Biogrid device. Results are expressed as mean ± SD, n = 5

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<th>Ki-67</th>
<th>GFAP</th>
<th>β-Tub</th>
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<tr>
<td>Biogrid</td>
<td>37.1 ± 7.8%</td>
<td>3.5 ± 1.6%</td>
<td>6.2 ± 4.3%</td>
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<tr>
<td>Enzymatic</td>
<td>40.9 ± 9.7%</td>
<td>4.2 ± 1.6%</td>
<td>5.9 ± 4.9%</td>
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Fig. 4  (A) Expansion curves of individual neurosphere cultures, maintained for 11–13 weeks and passaged with the Biogrid device (solid lines) or enzymatically (dashed lines). (B) Plot of the distribution of sizes of spheres and fragments immediately after passage of a culture of neurospheres, with sphere diameters between 50 and 500 μm. Empty circles represent individual neurospheres which passed through the microgrid without being cleaved, while filled diamonds represent fragments (ellipsoids, rectangular prisms or irregularly shaped) of cleaved neurospheres. Note that to improve visualization the major axis of fragments was randomly denoted as “Axis-1” or “Axis-2” and the data point thus randomly distributed on either side of the y = x line. The microgrid used for these experiments had 200 μm wide openings and 20 μm wide beams.
Thereafter we investigated the tolerability of neurospheres to transient over- and underpressure. Neurospheres were exposed to transient pressure changes, exceeding the pressure changes occurring during cell passage by several fold, and potential cell injury was monitored by acute LDH release as well as by delayed morphological changes. Although increased LDH release was found in occasional cultures, there was no consistent effect on cell viability, even at pressure changes as large as \( \pm 700 \text{ mm Hg} \). Nor did we observe morphological signs of cell injury 4 days after the pressure changes evaluated (Fig. 5). Thus, the NPCs tolerated large and rapid changes in pressure surprisingly well. There are very few reports on how cells tolerate short-time pressure changes. In a previous study on co-cultures of neurons and Schwann cells, significant LDH release indicative of cell injury was seen in cell cultures exposed to an increased pressure of only 62 mm Hg.\(^{15}\) However, the exposure time was 24 h while we exposed the NPCs to 20 seconds of increased pressure with no signs of increased acute LDH release or delayed loss of structural integrity. In a cell culture system employing a fluidic system with the Biogrid device for passage, the time of exposure to increased (and decreased) pressure will be even less than 20 seconds. Hence, it is highly unlikely that any damage of cells will occur under conditions that may be present.

With regard to exposure to negative pressure, we have not been able to find any reports of studies in neural stem or progenitor cells. In a recent study on the mechanisms of vacuum-assisted closure of soft tissue wounds, human bone marrow mesenchymal stem cells were exposed to a 50 kPa lowering of pressure (corresponding to \(-375 \text{ mm Hg}\) for 30 min, twice a day for two weeks.\(^{16}\) A significant increase in apoptosis and reduced proliferation was observed, but again, the reduced pressure was maintained for a much longer time than the 20 seconds we apply, and also combined with lowering of the oxygen tension to 2%. It is not possible to determine which factor(s) was important for inducing apoptosis. Our data clearly demonstrate that a transient lowering of pressure by as much as 700 mm Hg does not

![Fig. 5 Exposure of neurospheres to transient pressure changes. (A) Relative concentrations of LDH in cell culture media from three different human forebrain- or spinal cord-derived neurosphere cultures, 24 h after a transient increase or decrease in the pressure. Each point represents one well containing 12–15 neurospheres. (B) Representative phase contrast images of human spinal cord-derived neurospheres, 48 h after exposure to 20 s of decreased or increased pressure. There were no signs of cell degeneration in any of the cultures. The relative changes in pressure to which each set of neurospheres was exposed are indicated.](image-url)
compromise the survival of human NPCs cultured as neurospheres. Together, these data show that human NPCs tolerate pressure changes which are several fold larger than what are expected to occur in a fluidic system using the Biogrid device for large-scale mechanical passage, even when flow rates are high through the microgrid to ensure that clogging of the microgrid is avoided.

During the last years, the possibilities for large-scale production of embryonic stem cells have been greatly improved through the development of methods for culturing mouse ESCs and human ESCs as free-floating aggregates in suspension. Similar to NPCs derived from the fetal tissue, closed cell culture systems incorporating devices for mechanical passage would greatly facilitate compliance with GMP standards in the ESC culture. When we applied a second generation Biogrid device to suspension cultures of human ESCs, the aggregates were cut into evenly sized 150–200 µm fragments by two cycles of aspiration–ejection using a microgrid with 110 µm openings and knife widths of 10 µm (Fig. 3C and D). Since ESC spheres are denser than neurospheres, using the sharper 10 µm knives was necessary. With regard to this rather rare cell type, we want to mention that with the present design of Biogrid, a minimum volume of a few ml is necessary to operate it, and 1–2 ml of the cell culture medium are also necessary for rinsing the pump/syringe, device and connections afterwards. Thus, for cultures of a few spheres, the method needs to be modified for smaller volumes.

Hence, Biogrid may also serve as an efficient tool for mechanical cutting of human ESCs in the suspension culture, and allow production of clinical grade ESCs in closed cell culture systems without the need for addition of proteolytic enzymes. With the use of Biogrid, a computer controlled pump and a bioreactor suitable for the suspension ESC culture, a fully automated GMP-compatible large-scale cell culture system for ESCs can be designed.

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