VIA Extractor™ tissue disaggregator

GENTLE, EFFICIENT TISSUE DISAGGREGATION FOR OPTIMIZED CELL VIABILITY AND YIELD

The VIA Extractor™ tissue disaggregator* (Fig 1A) is a first-in-kind device for the disaggregation of human and animal solid tissue and tumor samples into viable, single cells. The VIA Extractor™ tissue disaggregator uses a mild processing approach for consistently high cell viability, yield, and preservation of cell integrity relative to the parent sample. The standardized, closed system provides a semi-automated process for use in high-throughput omics research (genomics, proteomics, metabolomics etc.) giving reliable results in single-cell sequencing and flow cytometry applications.

- Gentle: Optimized cell viability and yield from low impact disaggregation for optimal results in omics and single-cell sequencing applications.
- Standardized: Consistent process, output and yield reduce sample-to-sample variation.
- Semi-automated: Simpler process with fewer components and steps for tissue dissociation than traditional methods.
- Fast: Solid tissue to single cell suspension in as little as ten minutes.

Following disaggregation, cells can be preserved by controlled-rate freezing using VIA Freeze™ Uno controlled-rate freezer to maintain viability for further downstream applications.

The fresh tissue or tumor sample is processed within the Omics pouch, a barcoded, multi-compartment single-use bag. The sample is inserted into the chosen compartment using the Omics applicator. The sample bag is placed into the Omics clamp for extra stability and then the bag is heat sealed for further protection against contamination. A digestive enzyme solution is easily added to the Omics pouch via the ports using a syringe. The Omics pouch and clamp are carefully placed into the VIA Extractor™ tissue disaggregator (Fig 1B). The VIA Extractor™ tissue disaggregator is placed into the top of the VIA Freeze™ Uno controlled-rate freezer (Fig 1C) and the tissue disaggregation protocol is selected. The VIA Freeze™ Uno controlled-rate freezer offers flexibility by allowing the optimal speed, temperature and time settings to be selected depending on the sample type and size to maximize cell viability. The heating system of the VIA Freeze™ Uno controlled-rate freezer is used to control temperature. The gentle movement of the paddles in the VIA Extractor™ tissue disaggregator completes the mechanical disagreggation process resulting in a viable cell suspension, representative of the original sample.

(A)

(B)

(C)

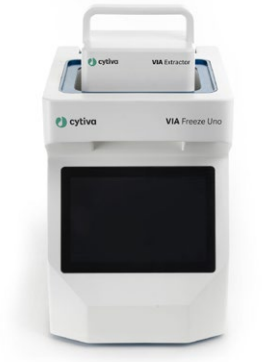

Fig 1. (A) The VIA Extractor™ tissue disaggregator, (B) the Omics pouch placed into the VIA Extractor™ tissue disaggregator and held in place with the Omics clamp, and (C) the VIA Extractor™ tissue disaggregator placed into the top of the VIA Freeze™ Uno controlled-rate freezer.

The following data was generated to demonstrate the effectiveness of the VIA Extractor™ tissue disaggregator for disaggregation of biological tissues into a viable cell suspension that is suitable for use for a wide range of applications, including tumoroid growth, flow cytometry, and single cell sequencing and analysis. The VIA Extractor™ tissue disaggregator was used alongside the gentleMACS™ Dissociator (Miltenyi Biotec) and a popular manual method to disaggregate a selection of commonly studied tissues according to standard protocols to determine relative performance in generating viable cell suspensions.

*For research use only (RUO). Not for diagnostic use.

Comparative performance of the VIA Extractor™ tissue disaggregator with gentleMACS™

Performance of the VIA Extractor™ tissue disaggregator in disaggregation of murine liver, lungs, kidneys and brain tissue was compared to semi-automated tissue disaggregation using the gentleMACS™ Dissociator. The gentleMACS™ method involves inserting the tissue sample in tubes with specifically designed stator and rotor that dissociate the tissue sample with an enzyme cocktail through a short blending action in contrast to a closed sample pouch with mild massaging of tissue using the VIA Extractor™ tissue disaggregator (Fig 2). The gentleMACS™ was used in accordance with the manufacturer's instructions. In order to ensure directly comparable performance between the two instruments, the enzyme cocktails recommended and supplied by Miltenyi Biotec were used for tissue dissociation in both instruments. Freshly dissected wild type mouse tissues were obtained from Charles River Laboratories. Experiments were conducted with three biological replicates, where each individual sample was dissociated using both instruments, allowing for sample pairing and like-for-like comparison. Two different sizes of tissues were dissociated to compare the efficiency of dissociation of standard sized organs and of biopsy sized tissue sample, which would be common where tissue has been derived from clinical samples. Following tissue dissociation, all cell suspensions were filtered through an appropriately sized cell strainer, pelleted by centrifugation at 300 xg and subjected to red blood cell lysis using the Red Blood Cell Lysis Solution (Miltenyi Biotec). Following cell resuspensions, cells were counted using the NucleoCounter™ NC-200™ automated cell counter.

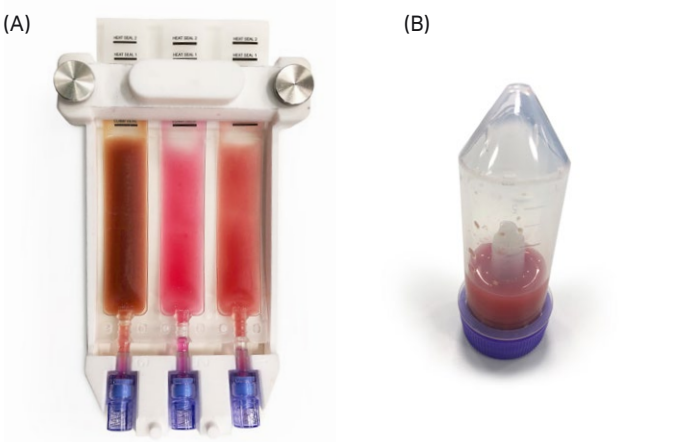

Fig 2. The VIA Extractor™ tissue disaggregator and gentleMACS™. (A) The VIA Extractor™ tissue disaggregator uses a gentle massaging of the tissue within a multicompartment pouch to disaggregate the tissue into single cells. Three different tissue samples are shown from a single experiment. (B) The gentleMACS™ system uses a rotor to physically blend the sample after enzyme incubation.

Mouse liver disaggregation

Mouse liver tissue was inserted into the Omics pouch with enzymes from the Liver Dissociation Kit (Miltenyi Biotec) and dissociated using the VIA Extractor™ tissue disaggregator at a constant (motor) speed of 200 rpm at 37°C for 10 minutes. A complete cell suspension was achieved in this time period, as demonstrated by the lack of visible tissue pieces remaining in the cell strainer following filtration (Fig 3). Mouse liver tissue was dissociated using the gentleMACS™ following manufacturer's instructions. Briefly, liver tissue was added to the C tube with the enzyme cocktail. The tube was placed on the gentleMACS™ and the defined program run (approx. 37 secs), following which the sample was incubated at 37°C for 30 minutes on the MACSmix™ Tube Rotor (Miltenyi Biotec). The tube was then placed on the gentleMACS™ and a second defined program was run to generate the final cell suspension.

Following cell filtration through the cell strainers it is apparent that a greater proportion of tissue remains undissociated with the gentleMACS™ compared to the VIA Extractor™ tissue disaggregator (Fig 3). Cell viability and cell yield per mg of tissue using both instruments were similarly high (Fig 4A and 4B). A representative image from the NucleoCounter™ cell counter shows a fine complete single cell suspension using the VIA Extractor™ tissue disaggregator (Fig 4C). A histogram of cell size shows a higher peak in the number of cells at the desired cell size and less cellular debris using the VIA Extractor™ tissue disaggregator (Fig 4D).

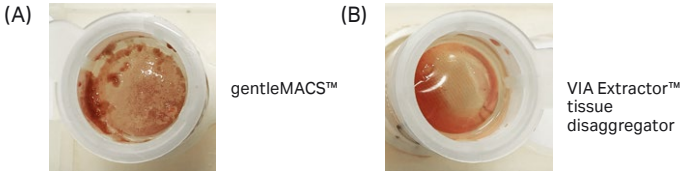

Fig 3. Mouse liver tissue was dissociated using the (A) gentleMACS™ or (B) VIA Extractor™ tissue disaggregator and cell suspensions prepared by filtering through 100 µm cell strainers. The VIA Extractor™ tissue disaggregator shows more complete digestion as indicated by fewer particulates remaining on the surface.

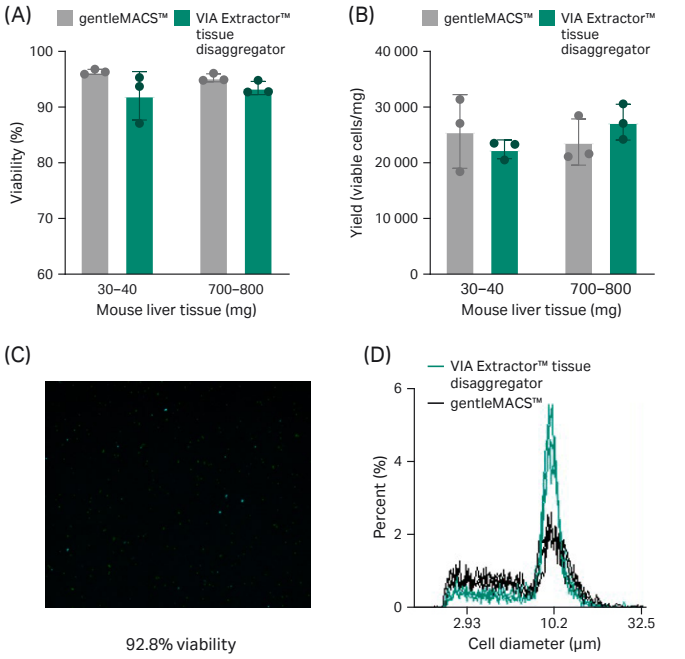

Fig 4. Small and large mouse liver tissues were dissociated using the VIA Extractor™ tissue disaggregator and gentleMACS™. Samples were matched so that tissue from the same liver was used in both instruments. Cells were counted in liver tissues following red blood cell lysis using the NucleoCounter™ NC-200™. (A) Average percentage cell viability. (B) Average yield of viable cells per mg of input tissue for dissociation. (C) A representative image from the NucleoCounter™ NC-200™ (Chemometec) of the counted cells. (D) Histogram showing the distribution of cells per cell diameter of all three 700–800 mg samples.

This data is based on a minimum of three independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Sovereign House, Chivers Way, Histon, Cambridge CB24 9BZ (R&D Laboratory) during June and July 2020 and is held at this location.

Mouse lung disaggregation

Mouse lung tissue was inserted into the Omics pouch with enzymes from the Lung Dissociation Kit (Miltenyi Biotec) and dissociated using the VIA Extractor™ tissue disaggregator at a constant speed of 200 rpm at 37°C for 35 minutes. Tissue from the same sample was dissociated using the gentleMACS™ following manufacturer's instructions, similar to that described for the mouse liver. Following cell filtration through the cell strainers it is apparent that a greater proportion of tissue remains undissociated with the gentleMACS™ compared to the VIA Extractor™ tissue disaggregator (Fig 5).

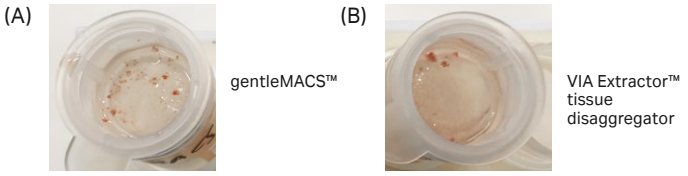

Fig 5. Mouse lung tissue was dissociated using the (A) gentleMACS™ or (B) VIA Extractor™ tissue disaggregator and cell suspensions prepared by filtering through 70 µm cell strainers. The VIA Extractor™ tissue disaggregator shows more complete digestion as indicated by fewer particulates remaining on the surface.

The viability of dissociated biopsy sized lung tissue was variable using the gentleMACS™, but consistently above 80% using the VIA Extractor™ tissue disaggregator. The viability of lung tissue between 110–160 mg is significantly better using the VIA Extractor™ tissue disaggregator (Fig 6A). The yield of viable cells is higher using the VIA Extractor™ tissue disaggregator compared to the gentleMACS™ (Fig 6B). Representative image from cell counter shows the cell suspension using the VIA Extractor™ tissue disaggregator (Fig 6C). Histogram of cell size shows a similar profile of cells extracted using both instruments (Fig 6D).

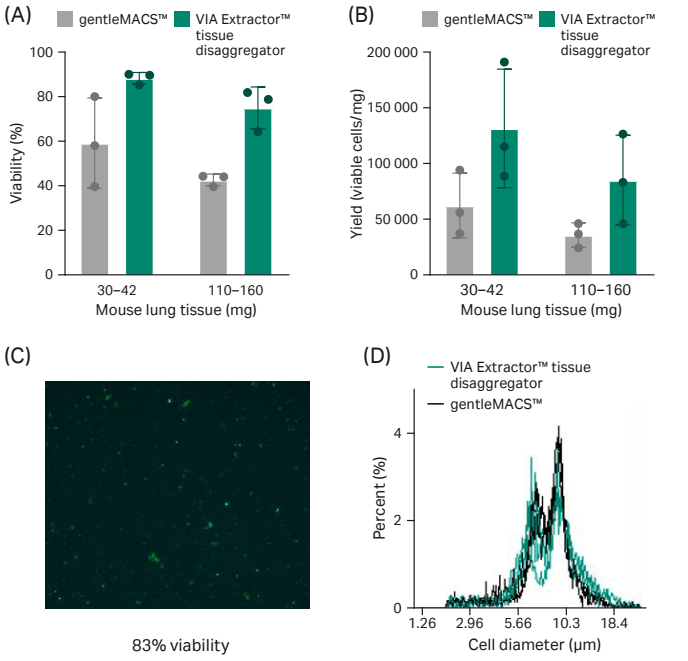

Fig 6. Small and large mouse lung tissues were dissociated using the VIA Extractor™ tisue disaggregator and gentleMACS™. Samples were matched so that tissue from the same lung was used in both instruments. Cells were counted liver tissues following red blood cell lysis using the NucleoCounter™ NC-200™. (A) Average percentage cell viability. (B) Average yield of viable cells per mg of input tissue for dissociation. (C) A representative image from the NucleoCounter™ NC-200™ of the counted cells. (D) Histogram showing the distribution of cells per cell diameter of all three 110–160 mg samples.

Mouse kidney disaggregation

For mouse kidney dissociation, the Multi Tissue Dissociation Kit 2 (Miltenyi Biotec) was prepared and used according to the protocol from Miltenyi Biotec. The overall protocol is similar for both VIA Extractor™ tissue disaggregator and gentleMACS™ as described for the mouse liver. The use of the VIA Extractor™ tissue disaggregator is quicker (overall 10 minutes dissociation time) compared to the gentleMACS™ (overall 32 minutes dissociation time). Kidney tissue was dissociated into a complete cell suspension using the VIA Extractor™ tissue disaggregator: however, visible clumps of tissue remained in the cell strainer after using the gentleMACS™ (Fig 7).

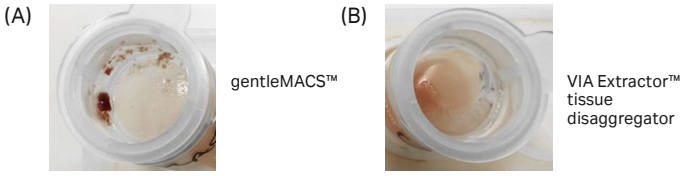

Fig 7. Mouse kidney tissue was dissociated using the (A) gentleMACS™ or (B) VIA Extractor™ tissue disaggregator and cell suspensions prepared by filtering through 70 µm cell strainers. The VIA Extractor™ tissue disaggregator shows more complete digestion as indicated by fewer particulates remaining on the surface.

Resulting cell viability is equally high when using the VIA Extractor™ tissue disaggregator to gentleMACS™ (Fig 8A), however the yield of viable cells is higher using the VIA Extractor™ tissue disaggregator for both tissue sizes (Fig 8B). Representative image from cell counter shows the cell suspension using the VIA Extractor™ tissue disaggregator (Fig 8C). Histogram of cell size shows a higher peak in the number of cells at the desired cell size suggesting more efficient dissociation using the VIA Extractor™ tissue disaggregator (Fig 8D).

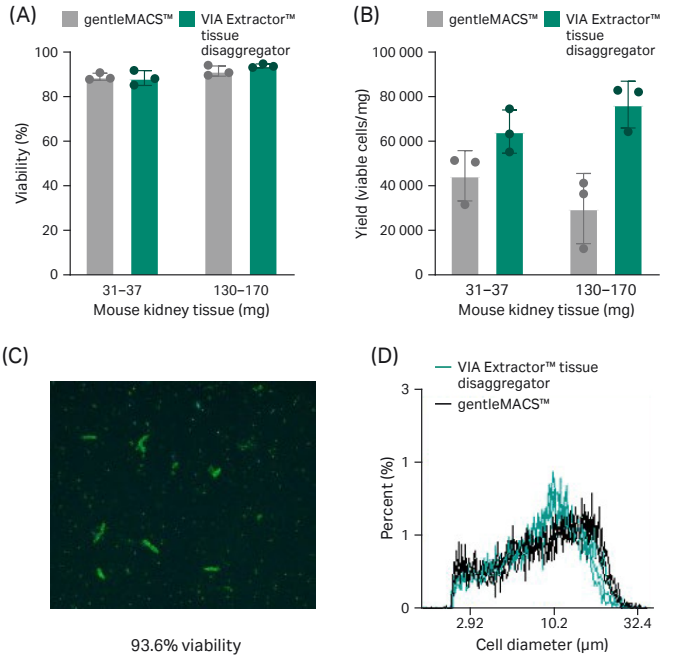

Fig 8. Small and large mouse kidney tissues were dissociated using the VIA Extractor™ tisue disaggregator and gentleMACS™. Samples were matched so that tissue from the same mouse was used in both instruments. Following red blood cell lysis, cells were counted using the NucleoCounter™ NC-200™. (A) Average percentage cell viability. (B) Average yield of viable cells per mg of input tissue for dissociation. (C) A representative image from the NucleoCounter™ NC-200™ of the counted cells. (D) Histogram showing the distribution of cells per cell diameter of all three 130–170 mg samples.

Comparative performance of the VIA Extractor™ tissue disaggregator with manual tissue dissociation

Traditionally, solid biological tissues are dissociated into single cell suspension by dissection of the tissue into small pieces (2–4 mm) using scissors and scalpels, followed by long incubation periods in enzyme cocktails to release the cells. This technique is time consuming, labor intensive and less reproducible. Tissue dissociation was compared using three independent mouse organs, with half the material used for each technique. Following tissue dissociation, all cell suspensions were filtered through an appropriately sized cell strainer, pelleted by centrifugation at 300 xg and subject to red blood cell lysis using the Red Blood Cell Lysis Solution (Miltenyi Biotec). Following cell resuspensions, cells were counted using the NucleoCounter™ NC-200™ automated cell counter.

Mouse liver disaggregation

For manual disaggregation, mouse liver tissue (400–500 mg) was minced using scalpel and scissors, washed and incubated at 37°C on rotation with 0.1% Collagenase A for one hour. For comparison, mouse liver tissue (400–500 mg) was disaggregated by placing in the Omics pouch with 0.1% Collagenase A and running the VIA Extractor™ tissue disaggregator for 10 minutes at 200 rpm at 37°C. The residual tissue in the cell strainer following manual tissue disaggregation is indicative of the less efficient process, whereas a complete cell suspension is achieved with VIA Extractor™ tissue disaggregator in a fraction of the time (Fig 9A). The viability of cells is higher using the VIA Extractor™ tissue disaggregator as is the yield of viable cells (Figure 9B and 9C). The cell suspension as a result of VIA Extractor™ tissue disaggregator disaggregation compared to manual has a larger number of cells at the desired size and fewer small particles, suggesting a purer sample with reduced cell damage (Fig 9D).

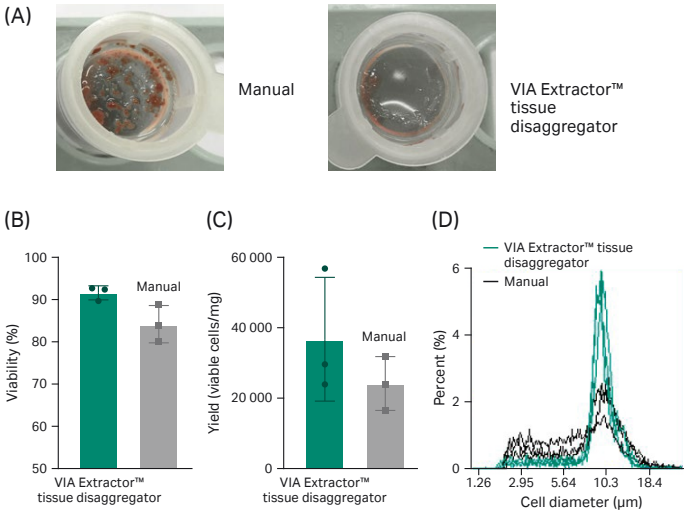

Fig 9. Three independent wild type mouse livers were halved and dissociated using either the VIA Extractor™ tissue disaggregator or traditional manual method as described above, and cell suspensions were prepared by filtering through 70 µm cell strainers. (A) The VIA Extractor™ tissue disaggregator shows more complete digestion as indicated by fewer particulates remaining on the surface. Panels B, C, and D show data derived from the automated cell counter. (B) Average percentage cell viability. (C) Average yield of viable cells per mg of input tissue for dissociation. Dots show individual values. Bar shows mean of three samples. Error bars show standard deviation. (D) Histogram showing the diameter of individual cells for each sample. It can be seen that there is less debris from the VIA Extractor™ tissue disaggregator method as indicated by lower signal of particles below 8 µm.

Mouse lung disaggregation

For manual disaggregation, mouse lung tissue (100–140 mg) was minced using scalpel and scissors, washed and incubated at 37°C on rotation with 0.3% Collagenase IV for 45 minutes. For comparison, mouse lung tissue (100–140 mg) was disaggregated by placing in the Omics pouch with 0.3% Collagenase IV and running the VIA Extractor™ tissue disaggregator for 35 minutes at 200 rpm at 37°C. Again, the lung tissue was not fully dissociated into a single cell suspension using the manual technique, as is seen by the residue in the cell strainer (Fig 10A). The cell viability and yield are much better using the VIA Extractor™ tissue disaggregator (Fig 10B and 10C). There are more particles of small diameter when dissociating with the manual process compared to VIA Extractor™ tissue disaggregator, indicating the VIA Extractor™ tissue disaggregator is a gentler process resulting in less debris (Figure 10D).

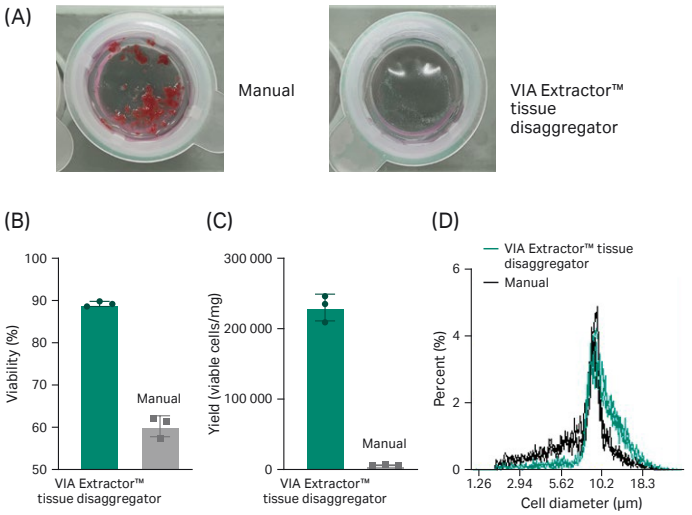

Fig 10. Three independent wild type mouse lungs were halved and dissociated using either the VIA Extractor™ tissue disaggregator or traditional mincing using scalpel blades and scissors (manual). Cell suspensions were prepared by filtering through 70 µm cell strainers. (A) The VIA Extractor™ tissue disaggregator shows more complete digestion as indicated by fewer particulates remaining on the surface. Panels B, C, and D show data derived from the automated cell counter. (B) Average percentage cell viability. (C) Average yield of viable cells per mg of input tissue for dissociation. Dots show individual values. Bar shows mean of three samples. Error bars show standard deviation. (D) Histogram showing the diameter of individual cells for each sample. It can be seen that there is less debris from the VIA Extractor™ tissue disaggregator method as indicated by lower signal of particles below 8 µm.

Mouse kidney disaggregation

Mouse kidney tissue (240–300 mg) was minced using scalpel and scissors, washed and incubated at 37°C on rotation with 0.1% Collagenase IV for 30 minutes. A like sample was disaggregated by placing in the Omics pouch with 0.1% Collagenase IV and running the VIA Extractor™ tissue disaggregator for 10 minutes at 200 rpm at 37°C. The residual tissue in the cell strainer following cell filtration of the manual disaggregation incicates incomplete tissue dissociation (Fig 11A). Cell viability and yields are higher with the VIA Extractor™ tissue disaggregator (Fig 11B and 11C). With this tissue there are equal amount of small cell particles in the sample with both techniques (Fig 11D).

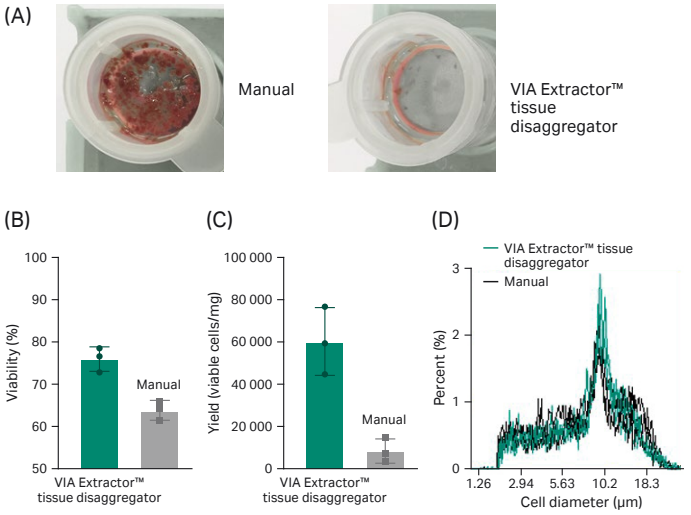

Fig 11. Three independent wild type mouse kidney pairs were dissociated using either the VIA Extractor™ tissue disaggregator or traditional mincing using scalpel blades and scissors (manual). Cell suspensions were prepared by filtering through 70 µm cell strainers. (A) The VIA Extractor™ tissue disaggregator shows more complete digestion as indicated by fewer particulates remaining on the surface. Panels B, C and D show data derived from the automated cell counter. (B) Average percentage cell viability. (C) Average yield of viable cells per mg of input tissue for dissociation. Dots show individual values. Bar shows mean of three samples. Error bars show standard deviation. (D) Histogram shows the diameter of individual cells for each sample.

This data is based on a minimum of three independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Sovereign House, Chivers Way, Histon, Cambridge CB24 9BZ (R&D Laboratory) during June and July 2020 and is held at this location.

Selecting a method for difficult tissues — Example: mouse brain

Brain tissue is a highly sensitive and difficult tissue from which to get viable cells after dissociation. In order to standardize this disaggregation technique to achieve the best results in downstream applications such a flow cytometry and single cell sequencing, automated mechanical methods of tissue dissociation combined with enzymatic digestion were compared.

For mouse brain (hypothalamus) dissociation, five C57BL6 mice were dissected and the hypothalamus carefully isolated. The tissues were kept in ice cold buffers and on ice to maintain the cell viability. These samples were weighed and combined. The samples were dissociated using the VIA Extractor™ tissue disaggregator method (Table 1). The Adult Brain Dissociation Kit (Miltenyi Biotec) was used and enzyme mixes were prepared according to manufacturer's protocol.

Hypothalamus samples were inserted into the Omics pouch with the help of Omics applicator. Enzyme mix from the Adult Brain Dissociation Kit (Miltenyi Biotec) were added to the compartment using luer-lock syringe and dissociated using the VIA Extractor™ tissue disaggregator at a constant speed of 200 rpm at 37°C for five minutes (Fig 12A and 12B). A complete cell suspension was achieved in this time period, as demonstrated by the lack of visible tissue pieces remaining in the cell strainer following filtration (Fig 12C).

Table 1. Amount of tissue used along with other parameters used for the dissociation

Mouse	VIA Extractor™ tissue disaggregator
Hypothalamus	56.1 mg Sample 1
Hypothalamus	105.8 mg Sample 2
Enzyme Mix	2 mL gentleMACS™ enzymes
Speed of Extractor (RPM)	200
Time to complete digestion (mins)	5
Temperature	37°C

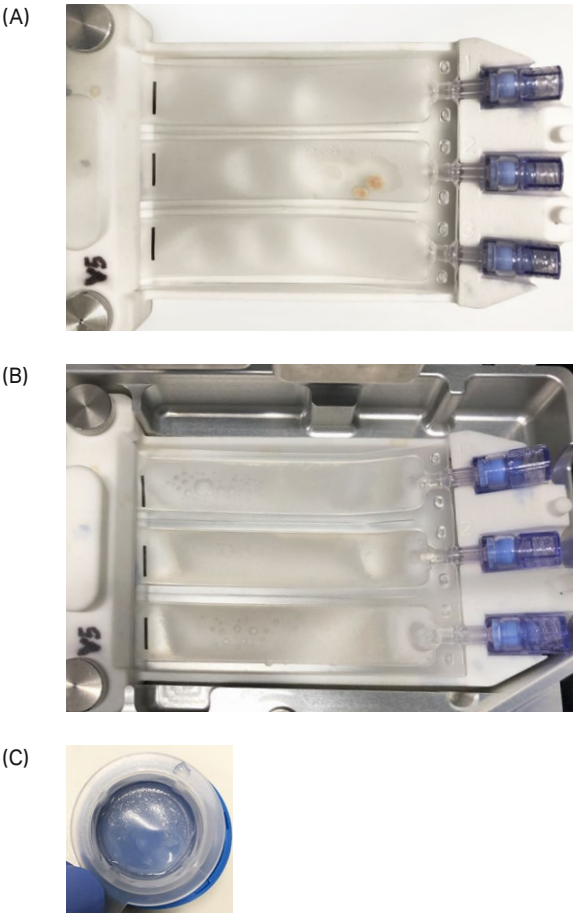

Fig 12. Preparation for murine brain dissociation using VIA Extractor™ tissue disaggregator. C57BL6 mice were dissected to isolate the hypothalamus from the rest of the brain tissue. Samples were combined from littermate controls to achieve enough material for cell isolation. (A) Assembly of Omics pouch and Omics clamp before tissue disaggregation. (B) Assembly after tissue disaggregation. (C) Cells were then passed over pre wet 70 µm cell strainers to filter out any undigested tissue material and debris. Please note that the hypothalamus sample was dissociated along with other brain samples from which the data is not included here.

Cell counting was performed using TC20™ Cell Counter (Bio-Rad) along with manual counting by Hemocytometer. Cell viability and cell yield for each sample dissociated using both instruments are shown in Table 2.

Table 2. Cell viability and yield. Cells were counted using automated TC20™ Cell Counter and compared with Haemocytometer manual counting. Trypan blue was mixed with the cell suspension in 1:1 concentration and 10 µL of sample was used in each method to count the viable cells. Cells were obtained using VIA Extractor™ tissue disaggregator

Sample	Description	Total count cells/mL	Live count Bio-Rad TC20™ cells/mL	% viability Bio-Rad TC20™ cells/mL	Haemocytometer counting cells/mL
1	VIA Extractor™ tissue disaggregator Hypo	1.83×10^7	1.37×10^7	75%	1.28×10^7
2	VIA Extractor™ tissue disaggregator Hypo	1.59×10^7	1.10×10^7	70%	1.59×10^7

Cell viability is extremely important when investigating biological pathways or transcription profiles in cells derived from brain tissue dissociation: therefore, a method that offers low impact dissociation and maximizes cell health is desirable. From this experiment, it is clear that for sensitive cells such as those in the brain, using the VIA Extractor™ tissue disaggregator for disaggregation of brain tissue provides consistently high yields with significant increase in percentage of viable cells. Additionally, dissociation was achieved in a very short time period.

This data is based on two independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally and according to manufacturers' protocol and recommendations. Data was collected at University of East Anglia, Norwich Research Park, Norwich NR4 7TJ (R&D Laboratory) August 2020 and is held at this location.

Conclusion

Solid tissue dissociation using the VIA Extractor™ tissue disaggregator is a simple and efficient technique that generates viable cells in suspension for downstream analysis. The mild tissue massage offers low impact tissue dissociation to maintain cells in the best possible condition. The data described shows that the VIA Extractor™ tissue disaggregator produces higher single cell yields and viability percentages with less cell debris than other methods. With disaggregation taking as little as 10 minutes, the VIA Extractor™ tissue disaggregator provides the speed needed to minimize any stress-induced effects due to the processing that can interfere with downstream analysis.

Ordering information

Product	Pack size	Product code
Omics bundle (VIA Freeze™ Uno controlled-rate freezer, VIA Extractor™ tissue disaggregator, and Omics clamp)	1 of each item	29517120
Omics clamp	1	29509355
Omics pouch, 3-sample	Pack of 10	29726921
Omics applicator	Pack of 60	29509359

Related products	Pack size	Product code
GenomiPhi™ Single Cell DNA Amplification Kit	100 reactions	29108039
	25 reactions	29108107
Sera-Mag™ Select size selection and PCR clean-up reagent	5 mL	29343045
	60 mL	29343052
	450 mL	29343057
Sera-Mag™ Oligo (dT) magnetic beads	1 mL	38152103011150
	15 mL	38152103010250
	100 mL	38152103010350

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