

Isolation and Growth of Adult Mouse Dorsal Root Ganglia Neurons

Seong-il Lee* and Joel Levine

Department of Neurobiology and Behavior, Stonybrook University, Stonybrook, USA

*For correspondence: seongil.lee@stonybrook.edu

[Abstract] Adult dorsal root ganglia neurons are among the few adult neuronal cell types that can be purified and grown relatively easily in dissociated cell culture. Here we describe a procedure for the isolation and growth of dissociated adult mouse DRG neurons using Percoll gradients and a chemically defined medium. These cultures can be used for morphological, biochemical and electrophysiological studies of neuronal growth and function.

Materials and Reagents

1. 6 to 9 weeks old female mouse
2. Poly-L-lysine hydrobromide (100 µg/ml in water) (Sigma-Aldrich, catalog number: P1524)
3. Laminin (1 mg/ml) (Thermo Fisher Scientific, Invitrogen™, catalog number: 23017-015)
4. Collagenase from Clostridium histolyticum (10 mg/ml in Ca²⁺, Mg²⁺ free PBS) (Sigma-Aldrich, catalog number: C-9891)
5. Dispase II (10 mg/ml in Ca²⁺, Mg²⁺ free PBS) (Roche Diagnostics, catalog number: 04-942-078-001)
6. Deoxyribonuclease I from bovine pancreas (4 mg/ml in PBS) (Sigma-Aldrich, catalog number: DN25)
7. Leibovitz's L-15 Medium (Thermo Fisher Scientific, Gibco™, catalog number: 11415-064)
8. Neurobasal®-A Medium (Thermo Fisher Scientific, Gibco™, catalog number: 10888-022)
9. B-27® Supplement (50x), serum free (Thermo Fisher Scientific, Invitrogen™, catalog number: 17504-044)
10. Percoll® (Sigma-Aldrich, catalog number: P1644)
11. Hank's Balanced Salt Solution (HBSS, calcium, magnesium, no phenol red) (Thermo Fisher Scientific, Gibco™, catalog number: 1425092)
12. 15 mm Glass coverslips (Neuvitro, catalog number: GG-15)
13. Pasteur pipette
14. Falcon® 35 mm Not TC-Treated Easy-Grip Style Bacteriological Petri Dish, 20/Pack, 500/Case, Sterile (Corning Incorporated, Falcon®, catalog number: 351008)

15. Falcon® 60 mm x 15 mm Not TC-Treated Treated Bacteriological Petri Dish, 20/Pack, 500/Case (Corning Incorporated, Falcon®, catalog number: 351007)
16. Falcon® 14 ml Round Bottom High Clarity PP Test Tube, Graduated, with Snap Cap, Sterile, 25/Pack, 500/Case (Corning Incorporated, Falcon®, catalog number: 352059)
17. Falcon® 14 ml Round Bottom PP Test Tube, without Cap, Sterile, 125/Pack, 1000/Case (Corning Incorporated, Falcon®, catalog number: 352018)

Equipment

1. #10 scalpel blade (Fine Science Tool, catalog number: 10010-00) (Figure 1)
2. Standard scalpel handle #3 (Fine Science Tool, catalog number: 10003-12) (Figure 1)
3. Spring Scissors-Angled to Side (Fine Science Tool, catalog number: 15006-09) (Figure 1)
4. Small curved scissors (Fine Science Tool, catalog number: 14095-11) (Figure 1)
5. Standard Pattern Forceps (Fine Science Tool, catalog number: 11000-12) (Figure 1)
6. Dumont #5 forceps (Fine Science Tool, catalog number: 11252-20) (Figure 1)
7. Dissecting microscope
8. Swinging bucket rotor (Sorvall instrument, model: HB-4)
9. Centrifuge (Sorvall instrument, model: RC5 plus)

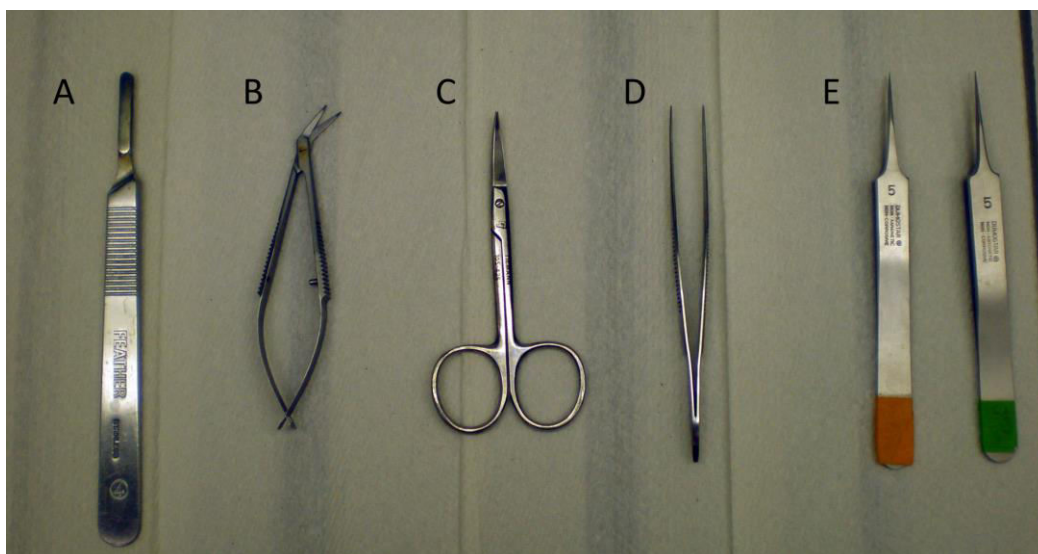


Figure 1. The dissection tools for mouse dissection and DRG isolation. A. Standard scalpel handle #3; B. Spring scissors; C. Small curved scissors; D. Standard forceps; E. Dumont #5 forceps.

Procedure

- A. Preparation of coverslips for DRG neuronal culture

1. The day before the dissection, place 1 sterilized glass coverslip into a 35 mm Petri-dish. Cover with 250 μ l of poly-L-lysine (PLL) (100 μ g/ml in water) and incubate at 37 °C overnight. (Tissue culture wells can also be used here.)
2. The day of dissection, aspirate off the PLL solution from each coverslip and add 250 μ l of sterile phosphate-buffered saline (PBS) on the coverslip. Repeat this 3 times. After third wash, leave the PBS solution on the coverslip until the laminin solution is ready at room temperature.
3. Thaw laminin aliquot slowly on ice and dilute it to 2~5 μ g/ml in PBS. Remove PBS from the coverslip and add 200 μ l of the diluted laminin onto the PLL-coated coverslip.
4. Incubate the coverslips at 37 °C at least 2 h before plating cells.

Note: Other proteins of interest, such as BSA, chondroitin sulfate proteoglycans, Nogo and myelin extract can be added to the laminin solution. Laminin is required for extensive growth of DRG neurites.

B. Dissection and isolation of the dorsal root ganglion

1. Euthanize a 6 to 9 weeks old female mouse via CO₂ inhalation. Shave the back and wash the skin with 70% ethanol (Figure 2A).

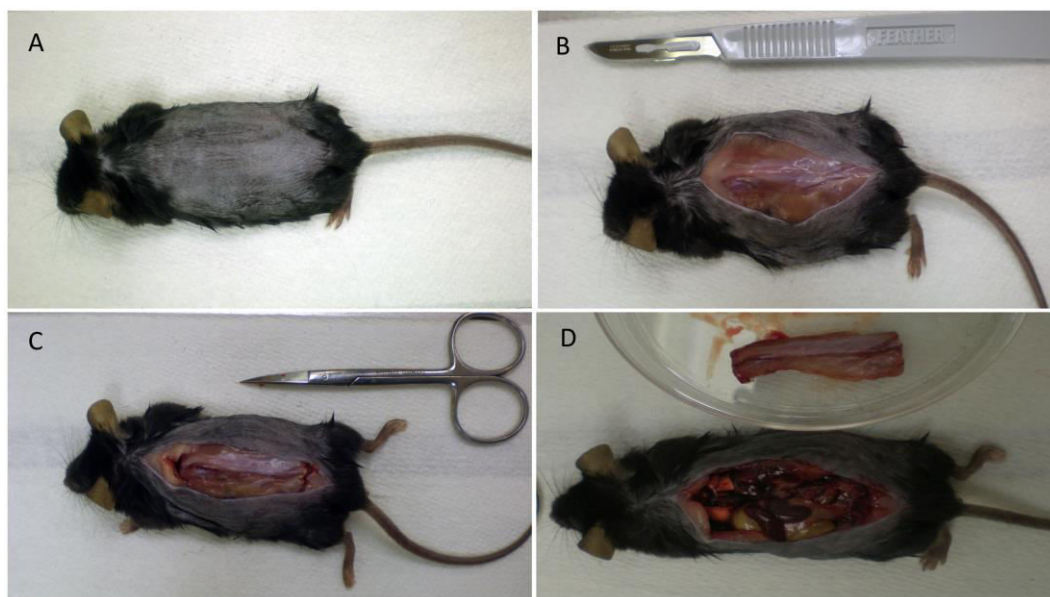


Figure 2. Mouse dissection. A. Shave the back and wash the skin with 70% ethanol; B. Make a midline incision along the back with a scalpel; C. Cut through the lumbar end of the spine; D. Cut the cervical end of the spinal cord and remove to a 60 mm petri dish.

2. Make a midline incision along the back with a scalpel and deflect the skin laterally (Figure 2B). Using a small curved scissors cut through the lumbar end of the spine (Figure 2C). Hold and lift up the incised lumbar part with a forceps and cut through the ribs and bones along both sides. Cut off any attached connective tissues attached to

the sides and underneath the spine (Figure 2D). Using a small sharp scissors, cut the cervical end of the spinal cord and remove to a 60 mm petri dish. Carefully remove the ribs, muscle and connective tissues as much as possible. Cut the cleaned spinal column into three pieces (Figure 3A and B).

3. Put one blade of spring scissors between dorsal side of vertebral column and spinal canal and carefully cut through vertebral column. Repeat this along the ventral side and pull apart the right and left halves of vertebral column (Figure 3C and D).
4. Place the spine pieces into L15 media at room temperature (RT). Remove the spinal cord using a fine forceps (#5 Dumont). Under a dissecting microscope, locate the DRGs which sit in small cavities along the lateral vertebral column. Place the tips of a #5 forceps underneath the DRG, pluck it out and transfer it to the petri-dish containing L15 media.
5. Using fine forceps, remove any fibrous structures surrounding the DRGs and cut off any attached nerve roots. Transfer the DRGs into 15 ml tube filled with L15 media.

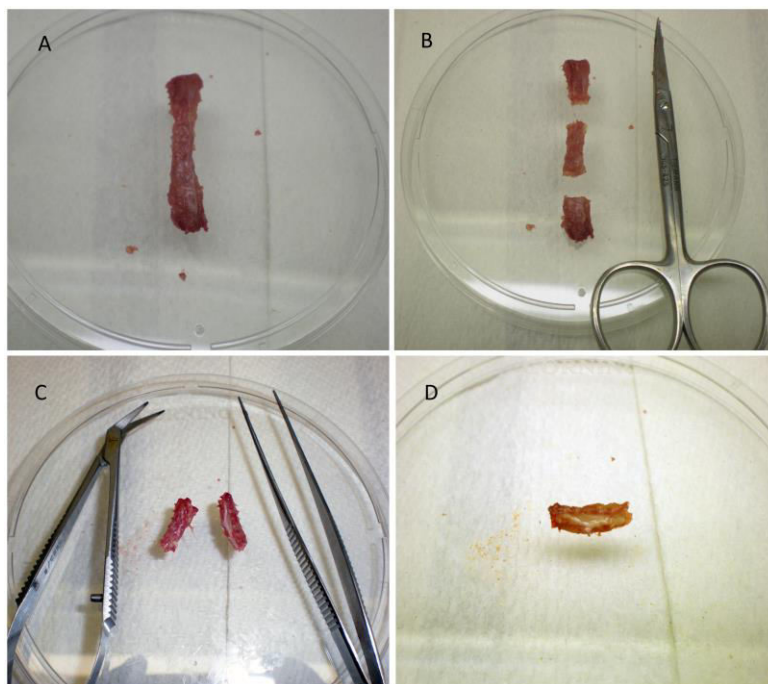


Figure 3. Spine dissection. A. Remove the ribs, muscle and connective tissues from a spine column; B. Cut the cleaned spinal column into three pieces; C. Cut through the vertebral column from dorsal to ventral side; D. Pull apart the right and left halves of vertebral column.

6. Centrifuge it at 200 x *g* for 2 min. Remove the media and replace with 1 ml of L15 media. Add 200 μ l each of collagenase and Dispase II solution. Gently mix the suspension and incubate at 37 °C for 20 min.
7. Remove the enzyme solution carefully and add 2 ml of L15.

8. Triturate the solution with fire-polished glass Pasteur pipette, add 25 μ l of DNaseI and incubate the cell suspension at 37 °C for 20 min.
9. Repeat the trituration again and add 5 ml of L15.
10. Spin down the cells at 200 x g for 5 min and remove the supernatant and resuspend the cell pellet in 5 ml of L15.
11. Repeat this step one more time and resuspend the cell pellet in 2 ml of L15 media.

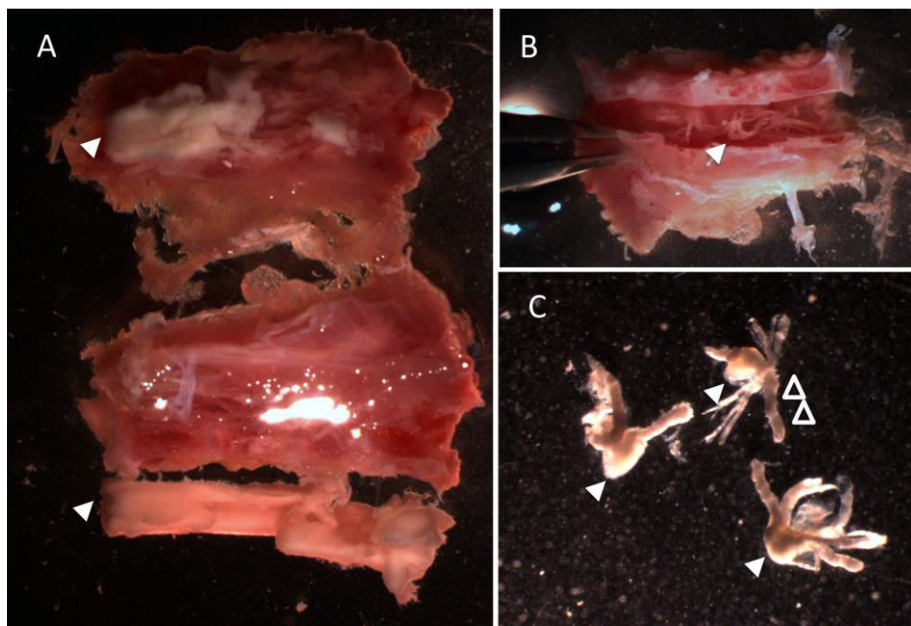


Figure 4. Isolation of DRGs. A. Dissected vertebral column, Arrow and Arrow head; spinal cord; B. Vertebral column after spinal cord was removed. Arrowheads; DRGs sitting in the spine; C. Arrowheads; Isolated DRGs, Open Arrow heads: DRG fibers.

C. Purification of DRG neurons by using Percoll gradients

1. Add 1 ml of 10x Hank's Balanced Salt Solution (HBSS) to 9 ml of Percoll and mix thoroughly to make a 90% working solution.
2. Dilute 90% Percoll in 1x HBSS to make stock solutions of 30 and 60% Percoll.
3. Place 3 ml of 60% Percoll in 14 ml round bottom tube and carefully overlay with 3 ml of 30% Percoll. It is important not to disturb the interface between the 2 concentrations of Percoll.
4. Place 1 ml of the cell suspension over the Percoll gradient solution being careful to not disturb the interface of Percoll.
5. Centrifuge the tubes at 800 x g for 20 min at 4 °C using a swinging bucket rotor.
6. After centrifugation, the cloudy layer at the interface of the two Percoll solutions contains the DRG neurons (Figures 5 and 6). Gently remove and discard the first 3 ml from the top which contains mainly myelin and Schwann cell debris. Transfer the next 2 ml containing the neurons into 15 ml conical tube.
7. Fill up the 15 ml tube with L15 media. Centrifuge it at 200 x g for 10 min.

8. Remove the supernatant and suspend the cell pellet in 1 ml of Neurobasal-A supplemented with B-27.
9. Count cell number. We usually obtain about 10,000 viable DRG neurons from a single mouse which is sufficient for 8-10 15 mm round coverslips with a proper density for morphological studies such as neurite length, polarization, *etc.*



Figure 5. An example of Percoll gradient. Arrowhead; Schwann cell layer; Arrow; DRGs layer.

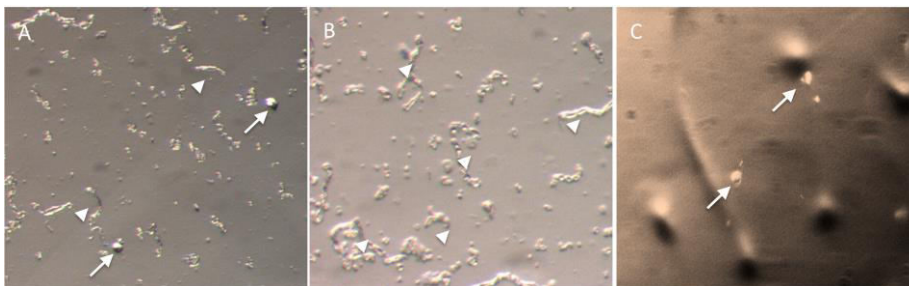


Figure 6. Isolation of neuronal cell by Percoll gradient. A. The microscopic image of cell suspension before Percoll gradient; B. The microscopic image of cell suspension acquired from top layer after Percoll gradient; C. The microscopic image of cell suspension acquired from middle layer after Percoll gradient. Arrow; DRG neurons; Arrow head; Schwann cells.

10. Wash the laminin-coated coverslips with PBS three times. After aspirating off the third wash, immediately plate 150 μ l of cell suspension onto each coverslip. Incubate the cultures at 37 °C for 1 h and then feed an additional 1 ml of Neurobasal-A supplemented with B-27 into each 35 mm petri-dish containing the coverslip.
11. After about 24 h, extensive neurite outgrowth should be observed (Figure 7). Keep monitoring the growth. For neurite growth experiments, we usually fix the culture 48 h after plating.

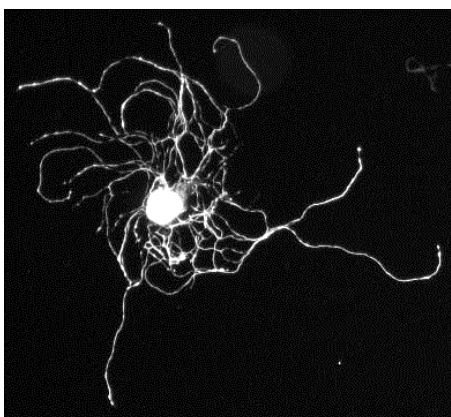


Figure 7. A representative picture of DRG neurons *in vitro* culture. Dissociated DRG neurons from adult mice were grown on coverslips coated with 2 $\mu\text{g}/\text{ml}$ of laminin. After 48 h, the cultures were fixed and immunostained with anti-TUJ antibody.

Acknowledgments

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References

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