Implantation of Dkk-1-soaked Beads into the Neural Tube of Chicken Embryos

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[Abstract] Chick embryos are known to be a powerful system to test gene function due to the "*in vivo*" accessibility, short time for results retrieval and the possibility to perform a large number of experiments. Synthetic micro-beads soaked in morphogenetic signals or receptor inhibitors can be implanted in selective embryo regions at precise developmental stages activating or blocking different signaling pathways. Here, we describe the manipulation of Wnt signaling pathway using Dkk-1-soaked micro-beads, implanted *in ovo* in the anterior part of the developing neural tube of chicken embryos; specifically at the prospective zona limitans intrathalamica at stage HH10.

Materials and Reagents

1. Fertilized chick (Gallus gallus)
2. Recombinant mouse Dkk-1 (R&D Systems, catalog number: 1765-DK)
3. Heparin Acrylic beads (Sigma-Aldrich, catalog number: H5263)
4. Bovine Serum Albumine (BSA) (Sigma-Aldrich, catalog number: A2153)
5. Pelikan Drawing Ink Black
6. Silikon Peroxid (IDEX Health & Science, catalog number: SC0083ST)
7. Tungsten wire (0.380 mm, 10 FT) (World Precision Instruments, catalog number: TGW1510)
8. Grid with concentric circles (NE42-21 mm) (Graticules LTD, Tonbridge Kent)
9. Penicillin-Streptomycin (10,000 U/ml) (Gibco, catalog number: 15140-122)
10. NaCl (Sigma-Aldrich, catalog number: S-3014)
11. KCl (Sigma-Aldrich, catalog number: P-9541)
12. $CaCl_2 \cdot 2H_2O$ (Prolabo, catalog number: 22317297)
13. $NaH_2PO_4 \cdot 2H_2O$ (Scharlab, catalog number: SO0334)
14. $MgCl_2 \cdot 6H_2O$ (Prolabo, catalog number: 27778293)
15. D(+) glucose anhydre (Prolabo, catalog number: 24370294)
16. 10x Phosphate-buffer saline (PBS) (see Recipes)
17. 4% Paraformaldehyde (PFA) from 20% PFA (see Recipes)
18. PBS/0.1% BSA (see Recipes)

19. Tyrodes buffer (see Recipes)
20. 0.5% Bicarbonate (see Recipes)
21. 0.5% Glucose (see Recipes)
22. Tyrodes supplemented (see Recipes)

Equipment

1. 37 °C, 5% CO_2 forced-air incubator (Novital, model: Covatutto 120-4V)
2. Dissecting microscope (Leica Microsystems, model: MS5)
3. Butane burner (Butsir)
4. Glass micropipet (homemade)
5. Pasteur pipets (Deltalab, catalog number: 200001)
6. Silicon tube (IDEX Health & Science, catalog number: SC0083ST)

Procedure

1. Fertilized chick (Gallus gallus) was incubated at 37 °C in a forced-air incubator. Chick embryos were developed until stage HH10 according to Hamburger and Hamilton, 1951.
2. Glass micropipets were prepared using a Bunsen burner. One side of the micropipet was introduced into a silicon tube that was used to aspirate solution while picking up each bead (Figure 1A-D). Tungsten wire was joined to a Pasteur pipet to handle easily (Figure 1E).

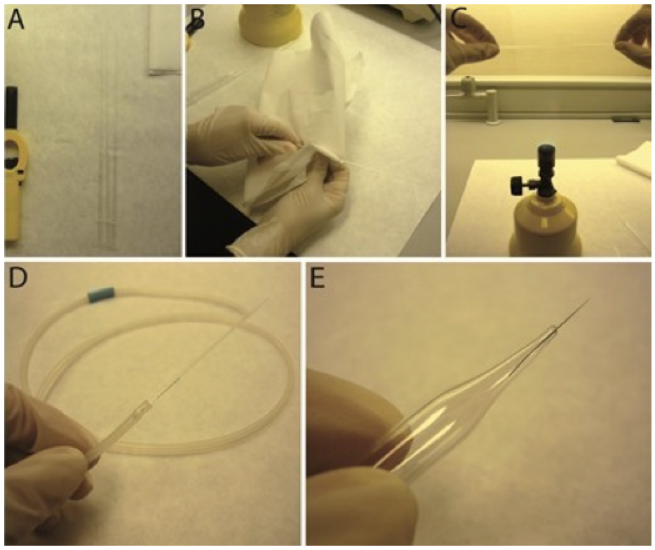
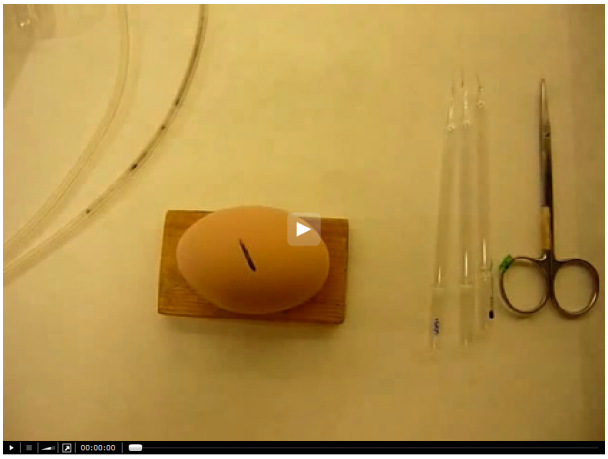


Figure 1. Micropipets preparation. C-D. Tight part of Pasteur pipets was used to prepare micropipets. E. Wide part was used as a handle for the tungsten wire.

3. Use forceps to selective heparin acrylic beads based on their size. A grid inserted in one ocular of the operating microscope was used to select beads of ~40 µm. Afterwards, beads were rinsed in PBS and then soaked in a solution of 25 µg/ml of Dkk-1 protein in PBS/0.1% BSA or in PBS overnight (o/n) at 4 °C.
4. An opening in the shell was made with scissors. To counterstained the embryos *in ovo*, inject Indian ink (diluted 1:1 in Tyrode supplemented) under the blastoderm by a glass micropipet made with a Bunsen burner. The vitelline membrane that covers the embryo is slipped out with tungsten wire on the spot where microsurgery was to be performed (see Video 1).

Video 1. Counterstaining of chicken embryos *in ovo*



5. A grid with concentric circles was inserted in one ocular of the operating microscope. The working magnification used during microsurgery was 40x. This grid was positioned in relation to the embryo: the vertical axis followed the embryo ventral midline; the transversal axis was positioned by crossing the optic-diencephalic angle at both sides (Figure 2A) (Garcia-Lopez *et al*., 2004).
6. Afterwards, the soaked-beads were cut to obtain half-beads using forceps. Then they were rinsed in PBS several times and one soaked half-bead implanted in the right side of the neural tube of embryos (Figure 2B). For control experiments, beads were soaked in PBS in the same manner.

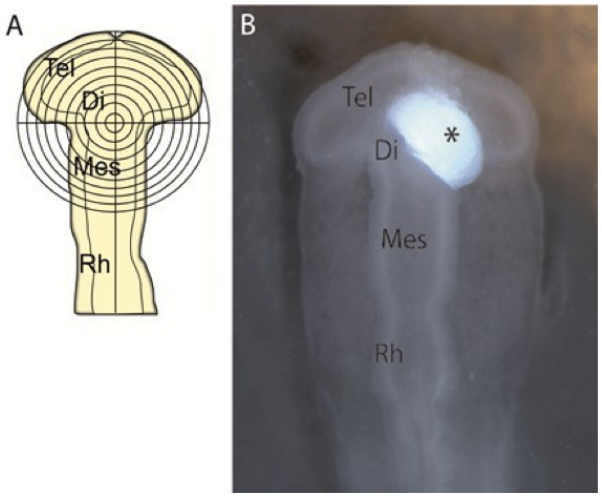


Figure 2. Bead-implanting procedure. A. Schematic representation of the grid positioned in relation to the embryo. B. Dkk-1 (black asterisk) or PBS-soaked beads were implanted into the prospective zona limitans intrathalamica of chick embryos at HH10. Abbreviations: Di, diencephalon; Mes, mesencephalon; Rh, rhombencephalon; Tel, telencephalon.

7. When the operation was completed, the opening in the eggshell was sealed with a piece of tape and incubated in horizontal position until the stage selected for fixation.

Recipes

1. 10x PBS– phosphate-buffer saline
   Mix 80 g of NaCl with 2 g of KCl, 14.4 g of Na_2HPO_4, 2.4 g of KH_2PO_4
   Adjust pH to 7.4 with NaOH
   Add dH_2O to 1,000 ml
   Filter sterilize (0.2 µm)
   Stored at RT
2. 20% PFA- paraformaldehyde
   Mix 600 ml of preheated 1x PBS at 65 °C with 200 g of PFA
   Add PBS to 1,000 ml
   Adjust pH to 7.4 with NaOH
   Filter sterilize (0.2 µm)
   Stored at -20 °C
3. PBS/0.1% BSA
   Mix 0.1 g of BSA with 100 ml of 1x PBS
4. Tyrodes buffer
   4 g NaCl

0.1 g KCl

0.13 g $CaCl_2 \cdot 2H_2O$

0.028 g $NaH_2PO_4 \cdot 2H_2O$

0.022 g $MgCl_2 \cdot 6H_2O$

Add dH_2O to 300 ml, sterilize and stored at 4 °C

5. 0.5% Bicarbonate

   0.5 g sodium hydrogen carbonate $NaHCO_3$

   Add dH_2O to 100 ml, sterilize and stored at 4 °C

6. 0.5% Glucose

   0.5 g D(+) glucose anhydre

   Add dH_2O to 100 ml, sterilize and stored at 4 °C

7. Tyrodes supplemented

   30 ml Tyrodes buffer

   10 ml 0.5% Glucose

   10 ml 0.5% Bicarbonate

   500 µl 10,000 U/ml penicillin-streptomycin

Acknowledgments

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