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Shell-less culture of the chick embryo as a model system in the study of developmental neurobiology

A. Cevik Tufan [1, 2] ✦
Ilgaz Akdogan [3]
Esat Adiguzel [3]

[1] Pamukkale University, School of Medicine, Department of Histology and Embryology, Denizli, Turkey.
[2] Pamukkale University, Research Center for Genetic Engineering and Biotechnology, Denizli, Turkey.
[3] Pamukkale University, School of Medicine, Department of Anatomy, Denizli, Turkey.

✦ A. Cevik Tufan, M.D., Ph.D.
Assistant Professor,
Department of Histology and Embryology, and
Research Center for Genetic Engineering and Biotechnology,
Pamukkale University, School of Medicine, Kinikli Kampusu,
Morfoloji Binasi
20020 Kinikli, Denizli-Turkey
☎ 90-258-2134030/1395
90-258-2132874
✉ actufan@pamukkale.edu.tr

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Abstract

Experimental studies on animal models play essential roles in the development of preventive, diagnostic and therapeutic procedures for diseases in a wide spectrum of fields including neurological sciences. The goal of this study was to demonstrate the shell-less culture system of the chick embryo as a potential experimental model in the field of developmental neurobiology. We were able to observe and record the central nervous system development of a vertebrate embryo in an artificial experimental culture container starting from an early, three-vesicle brain stage up to a well-developed five-vesicle brain stage with an embryo survival rate of 100%. Thus, this model system has the potential to enhance our knowledge on molecular and developmental neurobiology at both basic and clinical science level.

Key words: [developmental neurobiology] [shell-less culture] [chick embryo]

Introduction

Pattern formation is the process by which embryonic cells form ordered tissues. Throughout the embryonic development, this process is mediated by specific molecular triggers that are governed by specialized organizing centers [1]. Central nervous system (CNS) of the chick embryo is an excellent model system to study the mechanisms underlying patterning of the vertebrate CNS during development.

Shell-less culture of the chick embryo is an embryo culture model, where the intact *in ovo* relationship between the embryo and the yolk sac/albumen is preserved outside the eggshell and shell membranes, i.e., in an artificial experimental culture container [2, 3]. Thus, it allows the direct observation of the developing vertebrate embryo and a variety of experimental manipulations during this development [3, 4].

In this study, we describe the technique of shell-less culture model system of the chick embryo and demonstrate the central nervous system development of a vertebrate embryo using this model system; starting from an early, three-vesicle brain stage up to a well-developed five-vesicle brain stage with an embryo survival rate of 100%.

Material and Method

Chicken embryos

Fertilized chicken eggs (the "Cock of Denizli" strain) were obtained from The Directorate of the Ministry of Agriculture and Village Affairs, The Cock of Denizli Production Unit and Hatchery, Denizli, Turkey.

Preparation of culture containers

Culture containers are consisted of clear, semipermeable polyethylene film (SERA-perfore brand stretch-film can be used) secured with elastic rubber bands in the mouth of a cylindrical plastic cup (diameter = 7 cm; height = 7 cm) covered with a sterile plastic Petri dish lid (Figure 1C) and presterilized under UV light for an hour.

Preparation of shell-less cultures

Shell-less cultures were prepared using the technique described by Hamamichi and Nishigori [3] with minor modifications (Figure 1). Briefly, fertilized chicken eggs were preincubated for 30–33 h at 37.5°C in a humidified egg incubator to bring them to stage 9 of Hamburger and Hamilton [5] (Figure 1A and B). Prior to explantation eggs were placed horizontally, sprayed with 70% ethanol and permitted to air-dry for 10 min

to reduce contamination from the egg surface and also to ensure that the embryo was properly positioned [6]. This also allowed the eggs to cool, reducing the occurrence of yolk ruptures on explantation [7]. The egg contents were then transferred under aseptic conditions (in a laminar flow hood) into the culture container by cracking the underside against an edge (Figure 1D and E). Only cultures with the blastodisc positioned to uppermost side of the yolk were used in the experiments. Each shell-less culture was then covered with a sterile plastic Petri dish lid, transferred to an incubator (EN400, Nuve Inc., Ankara, Turkey) at 37.5°C and saturation humidity, and incubated for the desired amount of time (Figure 1F). For the purposes of this study 96 h incubation, including the 30–33 h preincubation time, was enough to reach the well developed five-vesicle brain stage. Representative images from live embryos were photographed with a Nikon Coolpix 995 digital camera fitted to a Nikon SMZ 800 zoom stereomicroscope (Figure 2).

Results

In this chick embryo model system, we were able to observe and record the central nervous system development of a vertebrate embryo in an artificial experimental culture container starting from an early, three-vesicle brain stage, i.e., Hamburger-Hamilton (HH) stage 9 (Figure 3A), up to a well developed five-vesicle brain stage, i.e., HH stage 24 (Figure 3F), with an embryo survival rate of 100% (Figure 3A–I).

Furthermore, using this model system the neural tube development of a chick embryo was histologically studied and recorded starting again from the three-vesicle brain stage, i.e., Hamburger-Hamilton (HH) stage 9 (Figure 4).

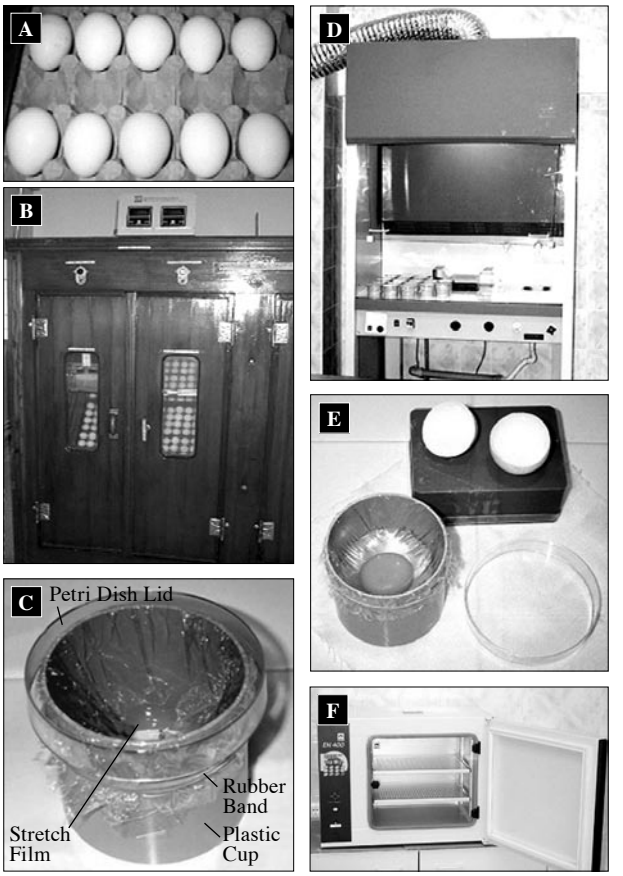

Figure 1. The sequence of events during the preparation of shell-less cultures of the chick embryo. **A:** Collection of fertilized eggs. **B:** Preincubation of fertilized eggs at 37.5°C in a humidified egg incubator. **C–E:** Explantation of embryos in to the presterilized culture containers at aseptic conditions. **F:** Incubation of shell-less cultures at 37.5°C in a humidified incubator.

Conclusions

Shell-less culture of the chick embryo is a cost efficient, simple, dependable, effective and reproducible model system to work with. One needs a minimal technical training to learn to use the technique. It allows the direct observation of the developing vertebrate embryo and a variety of experimental manipulations during this development. Thus, it allows studying the subdivisions

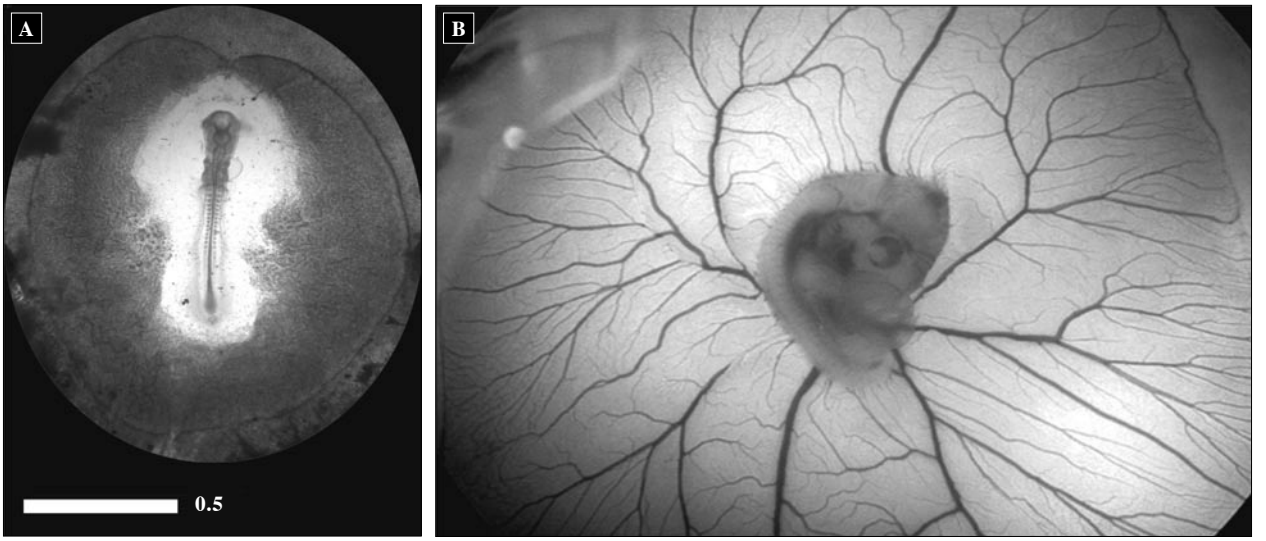

Figure 2. Representative images of whole embryos in shell-less culture. **A:** HH stage 12 chick embryo (45–49 h of incubation). **B:** HH stage 24 chick embryo (96 h of incubation). Bar = 0.5 cm for both panels.

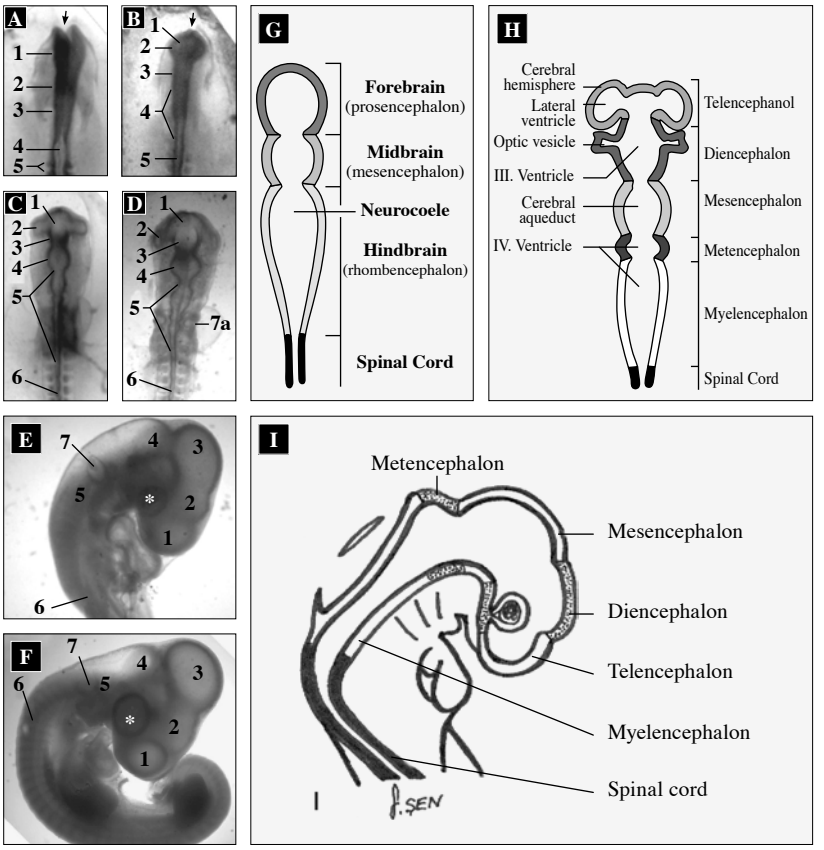

Figure 3. Developing structures of the chick central nervous system. **A:** HH stage 9 chick embryo (30–33 h of incubation); arrow, anterior neuropore. *(**1:** prosencephalon, **2:** mesencephalon, **3:** rhombencephalon, **4:** notochord, **5:** somites)* **B:** HH stage 10 chick embryo (33–38 h of incubation) arrow, anterior neuropore. *(**1:** prosencephalon, **2:** optic vesicle, **3:** mesencephalon, **4:** rhombencephalon, **5:** spinal cord)* **C:** HH stage 11 chick embryo (40–45 h of incubation) **D:** HH stage 12 chick embryo (45–49 h of incubation). *(For both C and D **1:** prosencephalon; **2:** optic vesicle, **3:** infundibulum, **4:** mesencephalon, **5:** rhombencephalon, **6:** spinal cord, **7:** auditory pit)* **E:** HH stage 17/18 chick embryo (60–69 h of incubation). **F:** HH stage 24 chick embryo (96 h of incubation). *(For both E and F **1:** telencephalon, **2:** diencephalon, **3:** mesencephalon, **4:** metencephalon, **5:** myelencephalon, **6:** spinal cord, **7:** otic capsule, ***:** optic cup and lens)* **G:** Schematic representation of a three-vesicle chick brain. **H–I:** Schematic representation of five-vesicle chick brain at HH stage 11–12 (40–49 h of incubation) **(H)** and at HH stage 17–24 (60–96 h of incubation) **(I)**. Panels **(G-I)** are adapted from "Developmental Biology Online" web site: http://www.uoguelph.ca/zoology/devobio/dbindex.htm and modified for the purposes of this manuscript.

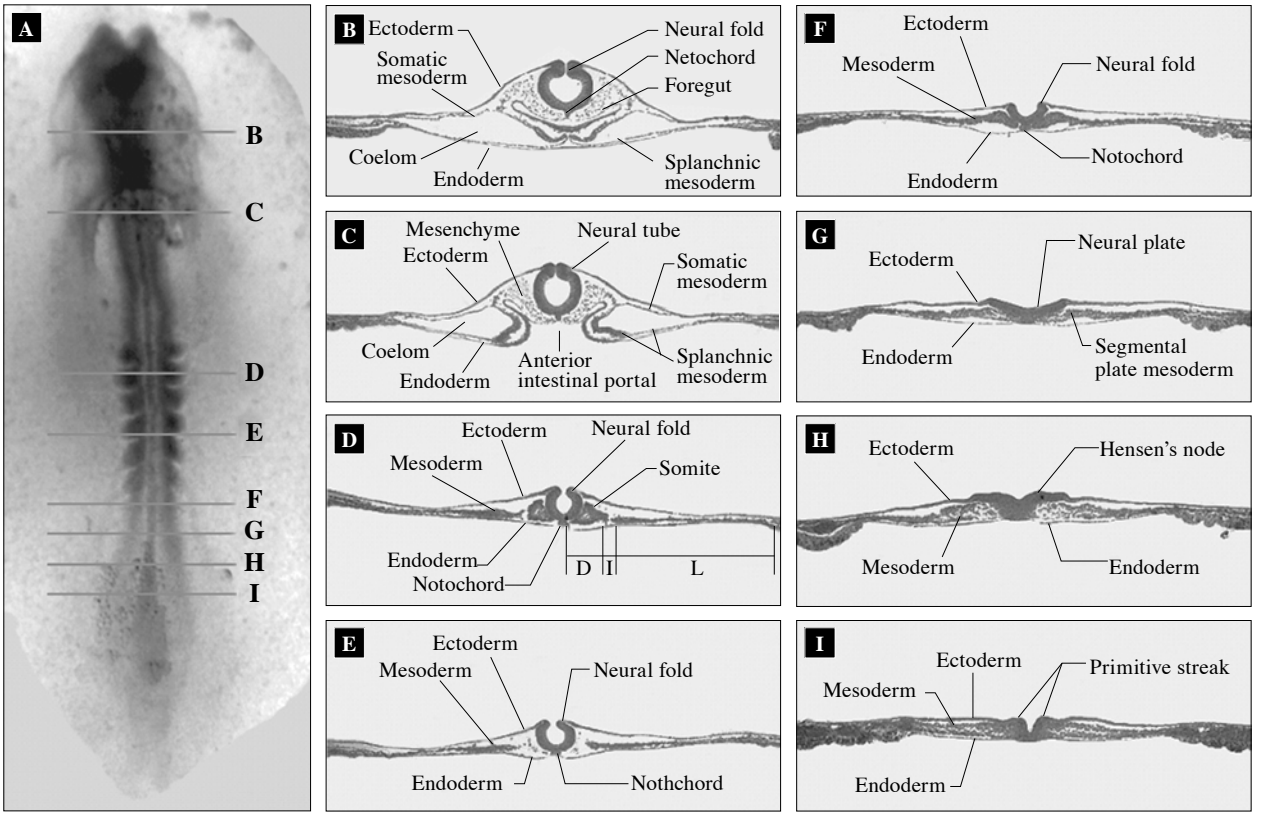

Figure 4. Neural tube development of a HH stage 9 chick embryo. **A:** Ventral view of a HH stage 9 chick embryo. Levels of cross sections shown in panels **B–I:** are indicated with the corresponding letter. **D:** *(**D:** dorsal mesoderm, **I:** intermediate mesoderm, **L:** lateral plate mesoderm)*. Panels (B–I) are adapted from "Developmental Biology Online" web site: http://www.uoguelph.ca/zoology/devobio/dbindex.htm and modified for the purposes of this manuscript.

and associated structures of the developing neural tube.

This model system recently started at Pamukkale University Research Center for Genetic Engineering and Biotechnology, Department of Anatomy and Department of Histology and Embryology laboratories, has the potential to enhance our knowledge on molecular and developmental neurobiology at both basic and clinical science level.

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