Brief Communication:
An in vitro Culturing Model for Rabbit Dural Cells

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Abstract. The objectives of this study were (a) to construct an in vitro model of rabbit dural healing, (b) to test the influence of collagen, laminin, and poly-L-lysine on the migration and proliferation of dural cells, and (c) to study the healing mechanism of duraplasty. Rabbit dural pieces (1.5 cm x 1.5 cm) were perforated in their central part with a 2 mm punch to mimic a dural defect. The dural pieces were cultured in 24-well plates that had been coated with collagen, laminin, or poly-L-lysine, and the influence of different extracellular matrices on migration and proliferation of dural cells was observed. Cells were subcultured on slides for immunocytochemistry to study their characteristics; dural healing was observed by scanning electron microscopy. The results demonstrated that only the dural pieces that were cultured on collagen-coated wells showed migration of cells into the central defect after a period of 8 to 10 days and that healing of the dural defect occurred by 13 to 15 days. The cultured dural cells stained strongly positive with an antibody to vimentin, but negative with an antibody to factor VIII. New collagen fibers were observed in the dural defects. This report demonstrates that an in vitro model for dural healing was successfully constructed in collagen-coated wells; the results implicate cellular migration of fibroblasts from the dural defect margin as an important mechanism of wound healing following duraplasty.

Keywords: duraplasty, fibroblast culture, extracellular matrix

Introduction

After surgical resection of the dura mater for any reason, the remaining dural defect needs to be repaired to protect the central nervous system from contamination. Various dural substitutes, including autologous grafts, allogenic transplants, and synthetic biomaterials have been evaluated for dural closure during the years since 1926, when Dandy used a transplant of fascia lata for the first successful repair of a dural lesion [1,2]. Autografts such as fascia lata or deep temporal fascia are commonly used and widely accepted. Disadvantages of these grafts include limitations in the size of the fascia that can be harvested and the need for secondary surgical procedures. Alloplasts, however, may cause adverse reactions and may not form a watertight closure [3]. Studies of many tissues and materials for duraplasty indicate that a satisfactory solution is still missing [1,2].

Histologically, the dura mater is composed of a rich network of collagen fibers with intermingled fibroblasts. Experimental testing of new materials has been performed in only a few animal studies, with the animals sacrificed after a defined period for macroscopic and histological examination at the site of dural repair [4]. Limited information is available in the literature on the mechanisms of the healing process after duraplasty. The process of...
wound healing after dural repair using a degradable transplant is believed to involve replacement of the graft by endogenous tissue. It has been proposed that the transplant acts as a scaffold that is broken down by enzymatic and cellular processes and is ultimately replaced with connective tissue [5]. Non-degradable synthetic biomaterials are covered with a thin tissue layer during the healing process [6].

This study devised an in vitro model for culture of rabbit dural cells that can be used (a) to analyze the cellular healing processes of dural defects, (b) to study the influence of collagen, laminin, and poly-L-lysine on the migration and proliferation of dural cells, and (c) to test various dural substitutes in vitro.

Materials and Methods

**Dura preparation.** New Zealand rabbits (age 2 mo, body wt 1.6 to 1.8 kg) were the experimental animals. The experimental protocols were reviewed and approved by the Zhongshan University Animal Institute. General anesthesia was induced by sodium pentobarbital (3% w/v; iv) and the scalp skin was shaved. A median scalp flap was created and craniotomy was performed under sterile conditions. The dura mater was harvested by detachment from the surface of the brain and was washed repeatedly to remove blood. Dural pieces (1.5 cm x 1.5 cm) were created and perforated with a needle to form a central hole (2 mm diameter) that constituted a dural defect.

**Dura culture.** The dural pieces were cultured in 24-well plates (Hyclone, USA) or on slides in quadruperm units (Viva Science, Germany). The plates and slides had been coated with 5 µg/cm² collagen (Sigma, USA), 2 µg/cm² laminin (Sigma), or 2 µg/cm² poly-L-lysine (Sigma). The standard culture medium was Dulbecco’s minimal essential medium (Gibco, Scotland) with the addition of 200 mM α-glutamine (Gibco), 100 U/ml penicillin (Gibco), 100 µg/ml streptomycin (Gibco), and fetal calf serum (Gibco) to a final concentration of 10% (v/v). Culturing was performed in a moist atmosphere of 5% CO₂ at 37°C; the culture medium was changed every 2 days. The central perforations were analyzed for cellular ingrowth from the dural borders every day, using a light microscope. The cells in the defect were enumerated daily.

**Immunocytochemistry.** Dural pieces were cultured on collagen-coated slides. After a sufficient quantity of cells had migrated from each piece of dura, the slides were rinsed with 0.01 M phosphate-buffered saline (PBS, pH 7.4), fixed with 10% formaldehyde for 10 min, washed thoroughly with PBS, and then soaked in 3% aqueous H₂O₂ for 30 min to block endogenous peroxidase activity. The slides were incubated with 10% goat serum for 30 min at 37°C, followed by overnight incubation with primary antibodies at 4°C. The primary antibodies were anti-vimentin and anti-factor VIII (DAKO, Germany) at dilutions of 1:200 (v/v), which gave good staining of control tissues. The slides were washed 3 times with PBS (5 min each) and incubated successively at 37°C with goat-anti-mouse secondary antibodies (20 min) and streptavidin-biotin peroxidase solution (20 min) (DAKO). As a control, the primary antibodies were replaced with 0.01 M PBS. Finally, 3,3′-diaminobenzidine (DAB) solution (DAKO) was used for visualization.

**Scanning electron microscopy.** Dural pieces with central perforations that had been cultured for specified numbers of days were examined by scanning electron microscopy. The specimens were rinsed with PBS (pH 7.4) and fixed with 0.8% paraformaldehyde and 0.8% glutaraldehyde for 3 days. After additional fixation in 1% OsO₄ for 1 hr, the specimens were dehydrated in a graded series of ethanol solutions, followed by 100% methanol. The specimens were mounted on aluminum stubs, gold sputtered, and viewed under a JEOL-300 scanning electron microscope (Japan) to visualize cell migration into the defect and cell proliferation on the specified days.

Results

Dural pieces that were cultured on collagen-coated wells showed migration of cells into the central defect after a period of 8 to 10 days. No cell migration occurred in the dural pieces that were cultured on poly-L-lysine-coated or laminin-coated surfaces. The migrated cells initially appeared to be slender and elongated; they changed to an ellipsoid appearance when there was confluent growth. After culture for 13 to 15 days, the defects of dura were completely covered by rapidly proliferated dural cells (Figs. 1, 2).

Immunocytochemical study of dural cells that migrated into the defect showed strongly positive cytoplasmic staining with the antibody against vimentin (Fig. 3). However, the dural cells gave negative staining with the antibody against factor VIII. The cellular components of dura mater are mainly fibroblasts and endothelial cells; vimentin expression is characteristic of cells derived from mesenchyme (ie, fibroblasts and endothelial cells). Thus, the negative staining reaction for factor VIII, which is an indicator for endothelial cells, suggests that the dural cells that migrated into the defect were probably fibroblasts.

Examinations by scanning electron microscopy showed complete closure of the dural defect following culture on collagen-coated wells. Newly synthesized collagen fibers became visible on the surfaces of the dural cells (Figs. 4, 5).
Fig. 1. Photomicrograph that shows dural cells (arrow) migrating from the margin of the dura on day 8 of in vitro culture (x100).

Fig. 2. Photomicrograph that shows a dural defect that was completely covered by dural cells on day 14 of in vitro culture (x40).

Fig. 3. Immunocytochemical reaction shows positive cytoplasmic staining of cultured dural cells with an antibody against vimentin (DAB chromogen, x200).

Fig. 4. Scanning electron photomicrograph shows that the dural defect became covered by dural cells (D = dura; C = cells; x750).

Fig. 5. Scanning electron photomicrograph shows newly synthesized collagen fibers on the surface of dural cells that have covered the dural defect (Co = collagen; x2000).

Discussion

Dural reconstruction, or duraplasty, is an important aspect of neurosurgery and the repair of dural defects is critical to separate the intracranial and extracranial spaces and to prevent CSF leakage. Its history dates from 1893 when Beach used a gold foil to prevent meningoencephalic adhesions and 1895 when Abbe used a rubber laminate for the reconstitution of a dural defect [1,7]. Since that time, more than 40 materials, mostly collagenic connective tissues or synthetic materials, have been used to reconstruct dura mater [8,9].
Limited information is available in the literature on the mechanism of healing after duraplasty. Many authors agree that the healing of dural wounds is a result of fibroblastic proliferation and the formation of connective tissue fibers induced by the dural margin or adjacent tissues [10].

Our study was conducted to construct an in vitro culture model of rabbit dural cells that could be used to analyze the healing processes of dural defects. We found that the dural cells were fibroblasts and that fibroblasts were the main functional cells in the process of dural healing in vitro, which is in accordance with previous finding of fibroblasts within remodeled dural transplants in animal studies [10,11]. Collagen, an important extracellular matrix, was needed for cellular migration of dural cells from the defect margin in our study. This finding is consistent with clinical experience and observations in experimental animals that collagen substitutes show high success rates in duraplasty and that collagen is a potent chemotactic component for fibroblasts [12,13]. Therefore, our observations support the use of collagenic dural substitutes as an ideal choice.

Our results indicate that cellular migration from the dural defect margin is an important mechanism of wound healing after duraplasty. Proliferation of fibroblasts and synthesis of new collagen fibers leads to the eventual healing of dural defects. Fibroblastic cellular proliferation after dural repair using a collagenic transplant is believed to promote graft replacement and to result in a tight and stable dural repair [5]. Our cell culture model provides an experimental system to study the specific factors and conditions that influence cellular migration from dural borders.

References