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The Chick Chorioallantoic Membrane as Test System for Biocompatible Materials*

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Summary. Biologic and non-biologic materials clinically used as hemostyptica, as vascular prostheses, or as temporary skin substitutes were implanted to the chick chorioallantoic membrane (CAM) during days 9–14 of incubation. The histological study revealed an intact CAM after application of cellulose gauze (Tabotamp). The fibrin tissue adhesive (Tissucol), the collagen sponge (Tachotop), or the gelatin sponge (Gelfoam) induced different amounts of connective tissue. Some fibroblasts were about to grow into the fibrin adhesive. Fibroblasts and capillaries filled the interstices of the collagen sponge poorly, and in the case of the gelatin sponge markedly. Neutrophils and giant cells of the foreign body type occurred more often in the gelatin sponge than in the collagen sponge. With regard to the non-biologic materials, expanded polytetrafluorethylene induced squamous metaplasia of the chorionic epithelium. Dacron caused ulceration with the onset of connective tissue ingrowth, based on a reaction of giant cells of the foreign body type. Polyurethane foam (SYSpur-derm) showed bleeding into the implant due to spikes of the material. Eosinophilic granulocytes were absent in all materials studied. The results are related to different clinical findings. The correlation allows consideration of the CAM as an in vivo model in screening materials for their biocompatibility and their connective tissue reaction.

Key words: Hemostyptica – Vascular prostheses – Skin substitute – In vivo test system – Chick chorioallantoic membrane

Introduction

There is an increasing need for appropriate biomaterials to substitute tissues or organs in humans. Once developed to substitute for human tissues, each biomaterial must be examined for possible responses due to toxicity, immunogenicity, cancerogenicity, or chemical interactions. Test systems obtained after

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short-term evaluate morphological and biochemical reactions of cells which are cocultured with the biomaterial under study [11]. Long-term investigations are conducted with the biomaterial implanted into mammals commonly used in the laboratory. Since the society for the prevention of cruelty to animals demands that experiments be reduced, alternatives to the in vivo approaches are urgently being sought.

The chick CAM, an embryonic organ of birds, is widely used as bioassay to test angiogenic factors released from a carrier material during its implantation on the CAM [13]. When different types of carrier materials have been studied by conducting the CAM technique, the histological response of the CAM is shown to depend on the type chosen [13]. This observation led to the following study. As it appears, the chick CAM, being easy in supply and handling, can replace organs of laboratory animals to a certain degree.

Material and Methods

Biomaterials

Biologic and non-biologic materials were chosen. The biologic materials are clinically applied as hemostyptica: oxidized and regenerated cellulose gauze (Tabotamp, Johnson & Johnson, Hamburg, FRG), highly concentrated fibrin as tissue adhesive (Tissucol, Immuno GmbH, Heidelberg, FRG), collagen sponge (Tachotop, Hormon-Chemie GmbH, München, FRG), and gelatin sponge (Gelfoam, Upjohn, Puurs, Belgium). The non-biologic materials serve either as vascular prostheses or as temporary skin substitutes: expanded polytetrafluorethylene [6] known as ePTFE prosthetic material (we used soft tissue patches, 2 mm thick, fibril length 17 μm, kindly supplied by Gore & Co. GmbH, Putzbrunn, FRG), a polyester [6], type polyethylene terephthalate and named Dacron (the vascular patch was a gift of Dr.-Ing. P. Stangier, Medizintechnik GmbH, Hamburg, FRG), and, finally, a bilaminar polyurethane foam (SYSpur-derm, Hartmann, Heidelberg, FRG).

Preparation and Procedure of Grafting

Fertilized chicken eggs bought at a local hatchery and incubated at 37.8°C and 60% relative humidity were prepared for implantation on day 2 of incubation as described [3, 13]. In brief, the CAM was displaced by aspiration of 1 ml albumin through the blunt pole of the egg. Thereafter, a 1.2 cm^2 window was cut out of the wide side of the egg shell. Silicon grease and a cover slip were used to seal the window. It was removed on day 9 of incubation to apply the different types of biomaterials.

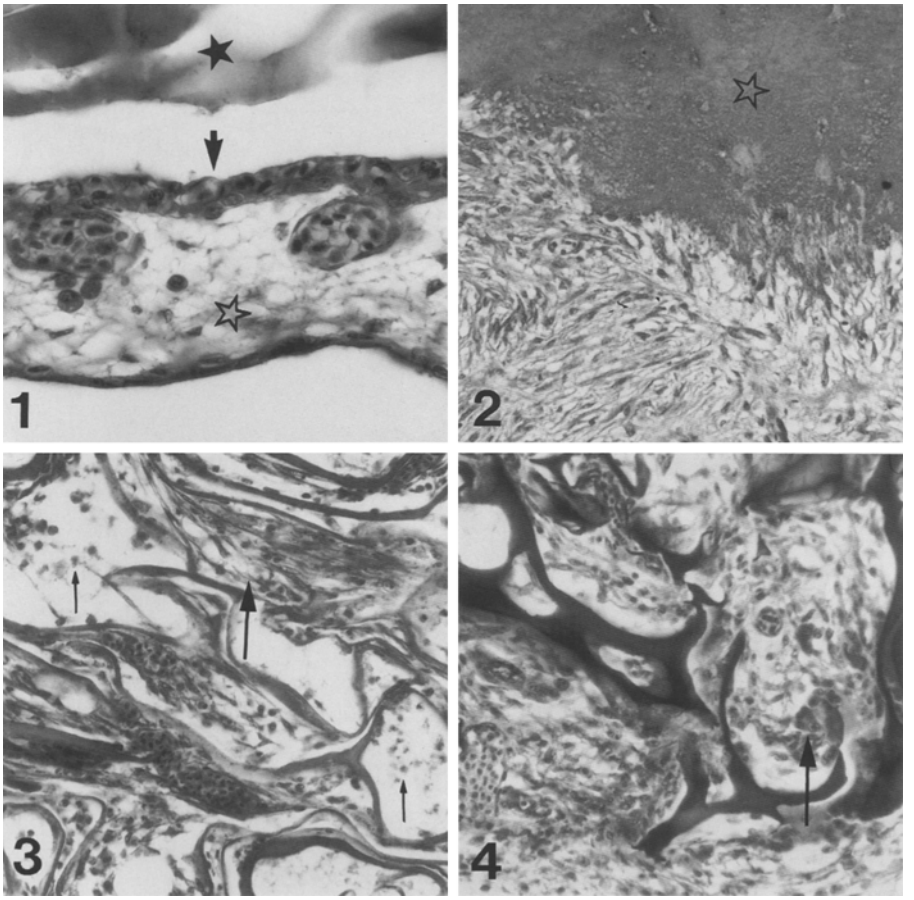
They were cut to 0.5–1.0 cm^2 patches and soaked in Eagle's minimal essential medium overnight, the fibrin clot excepted. After sealing the window, the incubation continued until day 14. At least five probes were conducted for each biomaterial studied.

Histology

The implants were fixed in situ with Bouin's solution for 30 min, removed and postfixed for another 12 to 24-h period. After processing according to conventional histological methods serial sections were stained with HE.

Results

The characteristic features of the CAM were maintained after implantation of the cellulose gauze (Fig. 1). On the other hand, when the fibrin clot, the colla-



Figs. 1–4. The different reactions of the chick CAM are shown after implantation of biologic substances during days 9–14 of incubation

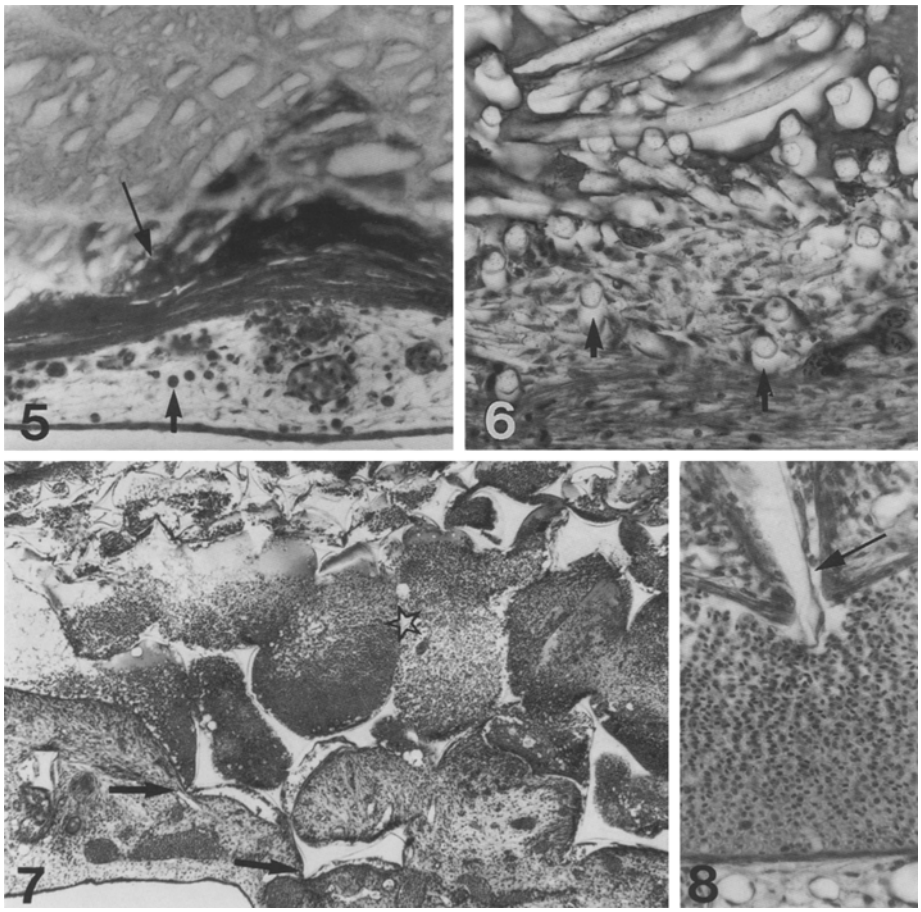
Fig. 1. The presence of a cellulose gauze (Tabotamp, *closed asterisk)* allows the capillary network of the chorionic epithelium *(arrow)* to be maintained. No inflammatory response appears in the mesoderm *(open asterisk).* × 420

Fig. 2. The fibrin clot (Tissucol) has not yet been organized *(asterisk),* but a dense connective tissue has developed in the mesoderm after ulceration of the chorionic epithelium. × 210

Fig. 3. Some interstices of the collagen sponge (Tachotop) contain macrophages and fibrin *(small arrow),* some show a loose array of connective tissue *(large arrow).* × 210

Fig. 4. All interstices of the gelatin sponge (Gelfoam) are filled by a well developed connective tissue, giant cells of the foreign body type are seen *(arrow).* × 210

gen, or the gelatin sponge were applied, the chorionic epithelium had disappeared (Figs. 2–4). The fibrin clot showed few signs of organization though fibroblasts, collagen fibers, and capillaries had strikingly increased in the mesoderm forcing the clot to protrude in a cap-like manner. The development of connective tissue elements was also noticed for the sponges of collagen or



Figs. 5–8. The different reactions of the chick CAM are demonstrated after implantation of non-biologic substances during days 9–14 of incubation

Fig. 5. The ePTFE implant induces a squamous metaplasia of the chorionic epithelium. Some epithelial cells are shed *(large arrow)*, and some inflammatory cells have invaded the mesenchym *(small arrow)*. × 210

Fig. 6. The Dacron implant has ulcerated the chorionic epithelium without the appearance of a marked number of inflammatory cells in the mesoderm. Dacron fibers *(arrows)* are already surrounded by connective tissue. × 210

Fig. 7. The polyurethane foam (SYSpur-derm) has increased in size due to striking hemorrhages into its interstices *(asterisk)*. The spikes of the material are noticed close to blood vessels *(arrows)*. × 85

Fig. 8. A spike of the polyurethane foam (SYSpur-derm) has pierced the wall of a blood vessel *(arrow)*. × 210

gelatin, this time with ingrowth into the sponges. Visual assessment indicated a lower degree of connective tissue formation in the interstices of the collagen sponge as compared to the gelatin one. Fewer macrophages and neutrophils or giant cells of the foreign body type became apparent in the collagen sponge when compared to the gelatin sponge.

The ePFTE implants caused a squamous metaplasia of the chorionic epithelium with a slight edema of the mesoderm (Fig. 5). Dacron, however, led to an ulcerative reaction of the CAM with a moderate response of the mesoderm (Fig. 6). In addition, connective tissue and capillaries had increased and were beginning to invade the Dacron meshes, their fibers often being surrounded by giant cells of the foreign body type. The apparent changes observed for the polyurethane foam were hemorrhages into the interstices of this biomaterial apart from a moderate increase in connective tissue and its ingrowth into the implant (Figs. 7, 8). The hemorrhages seemed to be due to spikes of the material piercing adjacent blood vessels.

Discussion

The chick CAM shows different morphological responses to various implants tested in this study. There is the maintenance of the chorionic epithelium with its capillary network as is the case for the cellulose gauze (Tabotamp). Squamous metaplasia occurs by ePTFE. A simple squamous metaplasia is reported for metacrylate polymers, the material from which soft eye contact lenses are made [13]. All three responses of the CAM indicate a high biocompatibility of the above mentioned materials. In fact, when they are applied, good clinical results are obtained [8, 9].

The chorionic epithelium is ulcerated by other types of implants: the collagen sponge (Tachotop), the gelatin sponge (Gelfoam), the fibrin tissue glue (Tissucol), or the polyurethane foam (SYSpurderm). The observed ulcerative response speaks for a low biocompatibility. Since each implant induces ulcerative changes with different amount of connective tissue formed, it likely expresses various degrees of compliance between graft and host as does the presence of inflammatory cells or of cells of the foreign body type. The amount of connective tissue induced may influence the healing potential of biomaterials. According to the actual knowledge obtained by clinical performances, Tabotamp ranks first as the material with 7–20 days of persistence in the organism (information given by Johnson & Johnson). It is followed by Tachotop, being absorbed within 3–6 weeks without essential hyperplasia of the connective tissue [10]. Gelfoam persists even longer and can lead to adhesion [5]. Tissucol is absorbed within days or weeks depending on the concentration used. A low dose provokes a mild connective tissue reaction in the case of, e.g., healing of a cut nerve. A high dose is applied when a parenchymatous organ has ruptured with diffuse bleeding, and a scar with a high burst strength is needed [2].

The maintenance of the chorionic epithelium or its ulcerative response probably demonstrates the absence or presence of a toxic interaction between graft

and host. An immunologic response is excluded to a small degree because eosinophilic granulocytes, messengers of a histoincompatibility process, are lacking. However, this morphological observation confirms reports that neither collagen sponges nor fibrin tissue adhesives are likely to induce immunologic reactions in animals or humans [1, 2, 10]. Reactions with invading eosinophils can take place in 14-day-old chicken after grafting a rat kidney to the CAM [14] in accordance with maturation of the chick immune system at this point in age [12].

The hemorrhages into SYSpur-derm, the polyurethane foam, after its application to the CAM are obviously related to the spikes of the biomaterial. They puncture blood vessels. This new finding explains why extensive hemorrhages occur in the foam after its application on experimentally induced skin burns of pigs [7]. These hemorrhages probably stimulate an even capillary sprouting in the wound area, and, thus, condition it for a successful skin transplantation.

An evident ingrowth of fibroblasts and capillaries into Dacron patches is seen after implantation to the chick CAM. This is not the case for implants using ePTFE. This finding reflects clinical findings. Though different results are available for animals [4, 15], vascular prostheses made of ePTFE show a limited invasion of vascularized granulation tissue in humans [8, 9]. Additionally, the healing rates of ePTFE appear to be lower than those of Dacron prostheses which adhere strongly to the surrounding tissue within 1 month.

The described CAM method gives important preliminary data on graft/cell interactions within a short period of time. This in vivo system is easier in handling than a recently reported in vitro test which examines the penetration of fibroblasts into vascular prosthetic material [8]. However, the induced model of the chick CAM does not substitute experiments with biomaterials to solve problems on thrombogenicity, biodegradation, infection, or cancerogenicity occurring in mammals.

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References

1. Adelmann-Grill BC, Otto K (1987) Immunological safety evaluation of a hemostatic agent and wound dressing made of horse collagen fibrils. Arzneimittelforschung 37:802–805
2. Cotta H, Braun A (1982) Fibrinkleber in Orthopädie und Traumatologie. Thieme, Stuttgart New York
3. Hamburger V (1966) A manual of experimental embryology. The University of Chicago Press, Chicago, pp 143–158
4. Hanel KC, McCabe C, Abbott WM, Fallon J, Megerman J (1982) Current PTFE grafts. A biochemical, scanning electron, and light microscope evaluation. Ann Surg 195:456–463
5. Langnickel R, Salenbauch N, Hörnle S, Jung A (1978) Experimentelle Untersuchungen zur quantitativen Resorption von drei Gelatineschwammpräparaten in der Bulla des Meerschweinchens. Arch Otorhinolaryngol 221:243–254

6. King MW, Guidoin RG, Gunasekera KR, Gosselin C (1983) Designing polyester vascular prostheses for the future. Med Progr Technol 9:217–226
7. Malinke PF, Rose E, Riedeberger J (1980) Vergleichende Untersuchungen am synthetischen temporären Hautersatz (Synthograft) "SYSpur-derm" und "Epigard". Zentralbl Chir 105:145–153
8. Mathisen SR, Coan DE, Sauvage LR, Wu HD, Wechezak AR, Goff SG (1987) A rapid in vitro test of the in vivo healing potential of vascular prostheses. J Biomed Mater Res 21:1081–1091
9. Rahlf G, Urban P, Bohle RM (1986) Morphology of healing in vascular prostheses. Thorac Cardiovasc Surg 34:43–48
10. Remberger K, Hübner G (1979) Experimentelle Untersuchungen über Zell- und Gewebereaktion nach Implantation von xenogenem Kollagenschaum. Res Exp Med (Berl) 175:67–79
11. Schmalz G (1981) Die Gewebeverträglichkeit zahnärztlicher Materialien. Möglichkeit einer standardisierten Prüfung in der Zellkultur. Thieme, Stuttgart New York
12. Solomon JB (1971) Foetal and neonatal immunology. In: Solomon JB (ed) Frontiers of biology, vol 20. North Holland Publishing Co, Amsterdam, pp 203–233
13. Spanel-Borowski K, Schnapper U, Heymer B (1988) The applicability of the chick chorioallantoic membrane assay in the assessment of angiogenic factors. Biomed Res 9: 253–260
14. Takenaka K, Kholoussy AM, Yang Y, Kodellas L, Matsumota T (1985) The healing of thin-walled expanded polytetrafluorethylene vascular graft. Vasc Surg 19:383–389

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