Amniotic membrane as a bioprosthesis: Is it a permanent material?

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Human amniotic membrane has been used as a bioprosthesis for years. However, the fate of amniotic membrane in the host body in long term is still obscure. We postulated that amniotic membrane is a permanent structure which does not induce foreign body reaction when used a bioprosthesis in the host organism. To test this hypothesis, pieces of human amniotic membrane were implanted to liver surfaces of rats. Monthly histological examinations showed that, amniotic membrane remained intact without inducing any foreign body reaction for len months. We concluded that, human amniotic membrane can be used confidently as a successful surgical tissue. [Turk J Med Res 1994; 12(5): 224-227]

Key **Words:** Amniotic membrane, Bioprosthesis

Human amniotic membrane has been utilized in clinical and experimental studies as a bioprosthesis for years. It has been used in bladder reconstruction, in bile duct repair, in preventing pelvic adhesions after surgery, as a biologic wound dressing in burn and xeroderma pigmentosum patients, and as supportive tissue in abdominal wall defects (1-4). It was also used as an intestinal patch for neomucosal growth in an experimental study, suggesting its future use in neonates (5). Previously we applied amniotic membrane over lacerated rat kidneys and spleens, and observed better wound healing compared to controls (6,7). Most of these studies with encouraging results regarding the amniotic membrane have been evaluated short term consequences. The present study was predominantly aimed at examining the condition of amniotic membrane in the recipient body as a longterm bioprosthesis.

MATERIALS AND METHODS

Twenty male outbred rats weighing 200-225 g were used in the experiment. They were housed in stainless-steel cages (four in one cage) under constant temperature (20°C) and allowed rat chow and tap water ad libitum.

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Fresh human placentae were obtained from the Obstetrics Department of the same hospital where they were collected at the time of delivery from mothers with no history of premature rupture of the membranes. Meconium-stained or postmature placentaes were discarded. Amniotic membrane was peeled away from the ebion and cotyledins by blunt finger dissection leaving chorion aside. The amniotic membrane was rinsed with sodium hypochlorite solution and prepared according to Dino's method {8). It was stored for 24 hours at 4°C in glass bottles containing sterile saline solution with amikacinsulphate (500 mg/l) in it. Membranes were cultured prior to use and found to be sterile.

Rats were anesthetized using 3% solution of chloral hydrate given 1 ml/100 g of body weight intraperitoneally. A median laparotomy was made on each animal, and the left lobe of the liver was exposed. A 3x3 cm patch of amniotic membrane was adhered to the anterior surface of the liver using three separate simple 5/0 atroumatic catgut sutures, with the mesenchymal layer of the amnion facing the liver capsule.

Starting from the first postoperative month, two rats were sacrified monthly for ten consecutive months. Swabs for aerobic cultures were taken from hepatic surfaces of sacrified animals. Pieces of the liver where amniotic membranes were attached were examined histologically under light microscopy using standard techniques.

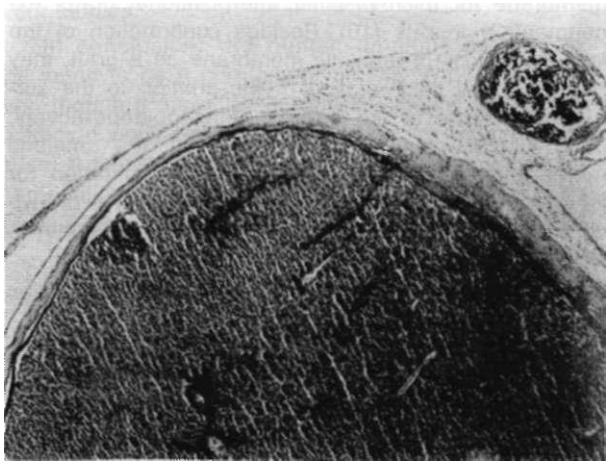

Fig 1a. Histologie appearance of the amniotic membrane at first month. The mesenchymal layer of the amniotic membrane shows close adherence to the liver surface (HEx44).

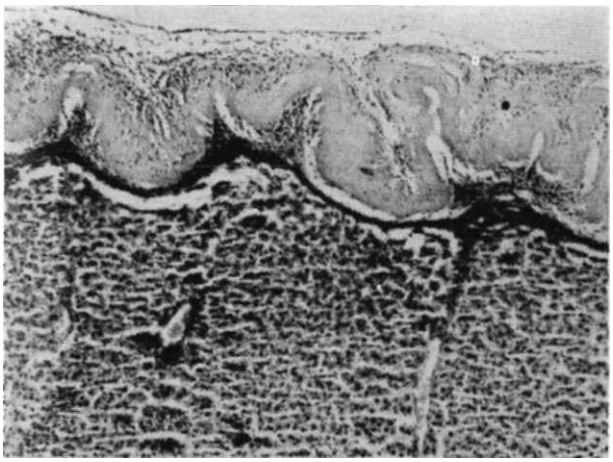

Fig 1b. Mesenchymal proliferation and loss of cuboid epithelium noted at the second month (HEx110).

RESULTS

None of the animals died during the experimental period. Aerobic cultures of hepatic surfaces were all sterile.

Amniotic membranes were not visible macroscopically after the second month, but a slight whitish color change was observed instead, which persisted until the tenth month of implantation.

Histopathologic examination:

At the first month, the mesenchymal layer of the amniotic membrane showed a close adherence to the liver surface. The cuboid epithelium of the amnion was still intact and appeared to be viable. There was no adherence to surrounding structures, no foreign body reaction or inflammation was noted. Neovascularization was not observed (Fig 1 a).

At the second month, the most significant feature was mesenchymal proliferation and the loss of cuboid epithelium. However, the thickness of the membrane which was reduced to a hyalinized image was protected. Amniotic membrane was no longer viable, but was still intact. Again there was no inflammation (Fig 1b).

After the third month, the number of the mesenchymal cells decreased gradually (Fig 1c). Up to ten months, the hyalinized appearance of the amnion did not change, and some mesenchymal cells still could be seen between the amniotic membrane and the liver capsule. In all specimens, the membrane was intact, and there were no inflammatory reaction. Regardless of that there was a foreign body reaction with mononuclear cell infiltration and giant cell formation against the catgut suture material even at the end of ten months (Fig 1d).

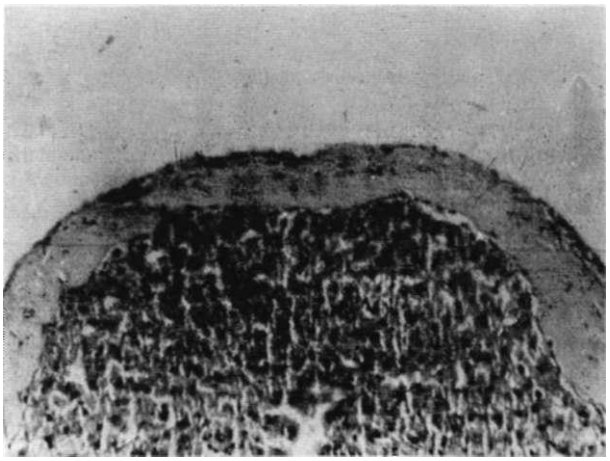

Fig 1c. A decrease in the number of mesenchymal cells of the amniotic membrane was observed at the third month (HEx220).

DISCUSSION

Human amnion possesses several favorable characteristics such as its low antigenicity, stable biological properties, permeability, availability, storability, and cost-effectiveness that all make it attractive as a bioprosthesis. Amniotic membrane derives its nutrients by diffusion, thus it has a higher likelihood of surviving free-tissue engraftment. Moreover, at least one study group have suggested the presence of a weak antibiotic-like substance in amniotic fluid which may be elaborated by the amniotic membrane and serves to reduce infection (3).

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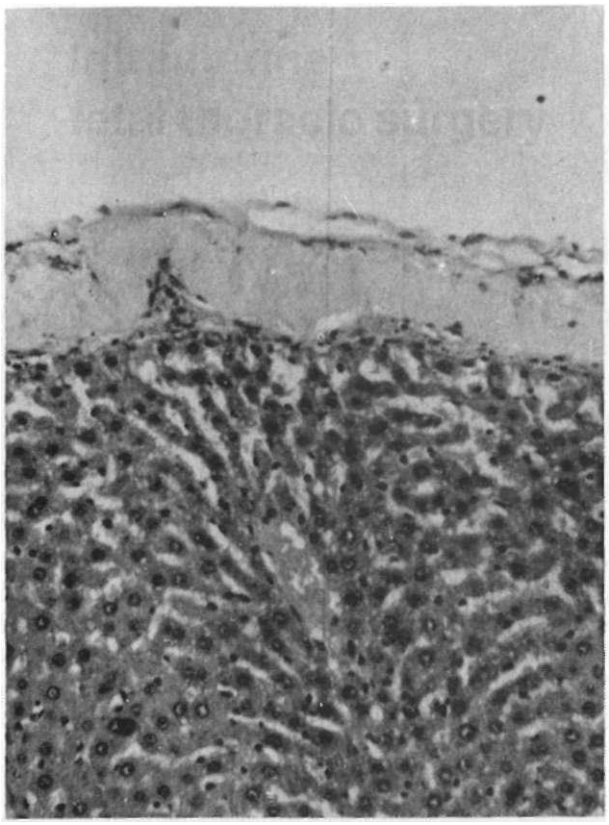

Fig 1d. Histologic appearance of the amniotic membrane at the tenth month (HEx200).

induced renal, and splenic lacerations in rats (6,7). We applied amniotic membrane on the surface of the injured organs as a splinting material. In both studies amniotic membrane yielded better wound healing compared to controls. It was also easy to handle, it adhered to the surface of the organs on which it was applied, it gave an effective control of hemorrhage, it did not induce a foreign body reaction or inflammation, and it was cheaper than any other commercial splinting product.

The fate of amnion following its engraftment to a different body has not been investigated extensively. Scudamore et al studied human amniotic membrane as a bioprosthesis for bile duct reconstruction (2). They stated that, amniotic membrane had been absorbed at six weeks after repair, although amniotic membrane can be identified in the histologic picture given in their published report. The end of the "gradual resorption" of the amniotic membrane that they postulate was not clear enough. Boltenberg et al suggested a model for studying growth and function of human placenta and amniotic membrane grafted to nude mice (9). They showed that histological structure of the placenta and production of placental proteins and hormones had been maintained for five weeks. Despite the disappearance of amniotic membrane in 3 weeks in one of the mice, the histological picture was identical with that of the original tissue, with no reduction in size, even 35 days after transplantation in the remaining 4 animals. Gray et al used human amniotic membrane for microvascular interpositional grafts experimental'y in rats (10). Besides confirmation of the low antigenicity of amniotic membrane as a graft, they observed well incorporation of the amnion to the surrounding tissue at the three months postoperatively. They also mention in their report that, the amniotic membrane grafts have been under investigation for long-term follow-up of 6 to 24 months. However, we could not trace any published report regarding that in a review of the literature.

Fishman et al studied fresh placental membranes in bladder reconstruction in dogs (1). In the absence of significant residual amnion at histological examination at six months, they concluded admitting amniotic membrane as biodegradable. This finding was not confirmed in our study, since we showed amniotic membrane histologically intact even at ten months. The discrepancy between the results of two study may be due to the difference in methods. Since the aims of two study groups were based on their prospected clinical applications, amniotic membrane pieces were placed in different locations. Aiming to use amniotic membrane as a splinting material for ruptured solid organs, we placed it over the liver substance intraperitoneally, whereas Fishman et al left amniotic membrane in close proximity to perivesical connective tissue, a well-known vascular area. This may account for the relatively rapid degradation of amnion, although no sign of inflammation was noted in that study as well.

Trelford, with his associates, has probably been one of the most accomplished researchers in the amniotic membrane field (3). He performed a number of painstaking investigations including both experimental and clinical applications of amnion. He showed in sheep experiments that the amniotic membrane (devoid of chorion) was taken as a permanent graft when implanted subcutaneously as an autograft into its own newborn infant. Amnion was clearly visible at nine months in histological sections (11). On the other hand, allografts of the amniotic membrane, although found to be identical to the autografts initially, were reduced to hyalinized images by day 20 to 30. These data was confirmed by our findings in the first and second month after engraftment, although human amniotic membrane was implanted as a xenograft in our study. Since we are dealing with the application of amniotic membrane as a splinting material or bioprosthesis, the "take" phenomenon was not required nor proposed in our study. The long-term permanance of amniotic membrane without inducing inflammation gives no harm, but an advantage with regards to this purpose.

The reason for the low antigenicity of amniotic membrane, though subjected to a number of studies, is still unknown. The common observation of most of the investigators is that, the chorion provokes neovascularization and inflammatory reaction in the host tis-

sue, which eventually causes a rejection phenomenon (3). This is why we preferred amnion without chorion in our studies.

In conclusion, the human amniotic membrane was found to be a permanent material in host tissues. Even though it loses its viability, it remains intact with no froeign body reaction at least for ten months. Because of these properties, amniotic membrane can be used confidently as a successful bioprosthesis.

Bioprotez olarak amnion zarı: Kalıcı bir yapı mıdır?

İnsan amnionu uzun yıllardır bioprotez olarak kullanılmaktadır. Buna karşın, amnionun konakçı bedenindeki uzun süreli geleceği halen belli değildir. Amnionun konakçı organizmada bioprotez olarak kullanıldığı zaman herhangi bir yabancı cisim reaksiyonu oluşturmadan kalıcı bir yapı olarak korunabileceği görüşü ileri sürülmektedir. Bu hipotezi doğrulayabilmek için sıçanların karaciğer yüzeylerine insan amniotik membıan parçaları implante edilmiştir. Aylık olarak yapılan histolojik incelemeler sonucunda, amnionun 10 ay boyunca herhangi bir yabancı cisim reaksiyonu oluşturmada, ı konulduğu alanda sağlam olarak yaşayabilirliğini koruduğu belirlenmiştir. Sonuçta; insan amnionunun cerrahi doku olarak başarıyla kullanılabileceği kanısına varılmıştır. [TurkJMedRes 1994, 12(5): 224-227]

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