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Amnion as a prosthetic material in congenital defects
An experimental study in rats

Abstract In pediatric surgery, amniotic membranes taken from autologous placenta are occasionally used as an implant in cases of large ventral abdominal clefts. The questions arise, which part of this organ should be used and how to use it in the recipient organism. Amniotic membranes consist anatomically of amnion and chorion, which are of fetal origin, and maternal decidua. In our experimental studies, we used the fetal parts of the amniotic membrane as an implant in a standardized rat model and investigated the utilization and possible foreign-body reaction (FBR) induced. Fifteen, 30, and 90 days after implantation the macroscopic appearance, light microscopy, and immunohistology of the specimens were examined. Adhesions to parenchymal organs and omentum were present irrespective of the side facing the abdominal cavity. Amnion induced a rapid FBR that diminished with time. Chorion and parts of the amnion were resorbed within the examined period after infiltration with recipient cells and neovascularization. Our studies have shown that for best results, only amnion in its anatomical definition and parts of the chorion should be preferred as an implant.

Keywords Fetal membrane • Amnion • Exomphalose • Child

Introduction

In clinical practice, congenital defects can often only be closed with the help of prosthetic materials. The results of different clinical and experimental studies are conflicting, and direct comparison is very difficult. The amniotic membranes (AmM) is a biological prosthetic material used by surgeons since the beginning of the century [8] that has become more popular in recent years [4, 5]. Unfortunately, the previous publications did not differentiate which parts of this anatomic entity were implanted. In our study we standardized the implant according to the anatomic structure of the AmM.

Figure 1 schematically demonstrates the placenta and AmM. The placenta is attached to the uterus and is covered by chorion (Ch) and amnion (Am). The placental mass consists of the decidua (De), which is maternal tissue, in contrast to Ch and Am, which are fetal tissues. All three layers expand like a balloon to form the amniotic cavity; De and Ch are reduced to a thin membrane called the reflected Am. Based on this structure, we developed a standardized animal model to examine utilization of the amniotic membranes in the recipient organism.

Materials and methods

After cesarean sections, we received human placentas and dissected free an 8 x 8-cm piece of the reflected AmM under sterile conditions. This was placed in a balanced electrolyte solution (Jonosteril-P crud II) and transported to the laboratory. The specimen was placed on a plastic foil with the De facing the observer, moistened with the above solution. With damp, soft gauze, the De was washed away mechanically. Figure 2 shows schematically the histologic structure of the implant. After birth there is a physiological separation of De and Ch [13] so that the separation in the laboratory occurs easily at the desired layer. Trypan-blue staining showed cell vitality of over 90%. The AmM was implanted within 30 min after harvesting.

In this animal model the anterior surface of the liver was used as the implantation site, as the liver surface is anatomically defined, so that the tissue reaction to the liver and the abdominal cavity could be examined selectively. With the animals under anesthesia with intraperitoneal barbiturate injection a laparotomy was performed in the supine position and the left inferior lobe of the liver was exposed. A 1 x 1-cm piece of the prepared AmM was sutured at all four edges with 7-0 prolene on the liver surface. In the first group of 9 animals the AmM was placed with the fetal side, the Am epithelium, on the liver surface. In the second group of 9 the maternal side, the Ch, was placed on the liver. Fifteen, 30, and 90 days after implantation 3 animals per subgroup were killed and the liver was perfused with 2.5% glutaraldehyde.

The site of implantation was inspected macroscopically, giving special attention to adhesions to neighboring structures in the abdomi-
inal cavity. The implant surface facing the abdominal cavity was divided into four quadrants. An adhesion index (AI) was established by applying a mathematical equation according to the number of quadrants involved. In addition, histologic slides (30–40 μ) from three different parts of each specimen were prepared for light microscopy and stained with methylene blue and hemalum.

The same numbers of animals were operated upon in the same way, however, perfusion was performed with physiological saline solution. These unfixed samples were deep-frozen immediately after extraction and the same number of slides as above was used for immunohistologic examinations. Four monoclonal antibodies were used for identification of T-cells, granulocytes, macrophages, and peritoneal macrophages as an indicator of foreign body reaction (FBR), and one monoclonal antibody for staining basement membrane, indicating neovascularization. According to staining intensity, a classification with four grades was established. Grade 0 was defined as no reaction, i.e., the same result as the controls, and grades 1, 2, and 3 stained progressively to the absolute positive control. This evaluation was performed for every identified layer (liver, reaction layer, Am, Ch, omentum) of each histologic slide.

Results

Macroscopic appearance

Adhesions and fusion with the liver were present in both groups by 15 days after implantation. On the abdominal side, the omentum was fixed to parts of the implant. Figure 3 presents the AI graphically. In cases where Am epithelium was facing the abdominal cavity adhesions were somewhat fewer, although a statistical significance could not be proven. In the 90-day groups there were less adhesions.

Microscopic appearance

In the group with the Am epithelium placed on the liver, Glisson’s capsule had resolved 15 days after implantation and a reaction layer developed between liver and implant containing macrophages and lymphocytes. The Am consisted of the Am epithelium with polygonal, flattened cells followed by a compact fibroblast layer (CFL). The Ch marked the border to the abdominal cavity with adhesions to the omentum (Fig. 4). Thirty days after implantation, the reaction layer between implant and liver was still present. The Am epithelium and CFL were unaltered, however, the Ch was present in a reduced form. Ninety days after implantation the reaction layer was thinner; the Am epithelium could not be identified and the CFL was the only unaltered structure.

In the group with the Ch placed on the liver, Glisson’s capsule also resolved within the first 15 days. A reaction layer was present in this group as well between liver and implant, followed by the Ch. The Am epithelium and CFL