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COMPARISON OF BIOCOMPATIBILITY AND BIOSAFETY OF MESH IMPLANTS AND SUTURE MATERIAL IN VITRO

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Abstract

Treatment of genital prolapse and hernias, which occur in a third of elderly women, is often impossible without using mesh implants due to the lack of their own connective tissue structures. The mechanism of getting complications when using various implants and sutures is unclear and may be related to both the chemical structure of the material and the shape of grids and filaments. When the body adapts to the implant, the reaction of the connective tissue, nervous and immune systems is really important.

Purpose. Comparison of biocompatibility and biosafety of mesh implants and suture material on cultures of fibroblasts, nerve cells and cells of the immune system.

Material and methods. We studied in vitro the effect of the most common types of surgical m esh and suture material on cell cultures of fibroblasts, nerve cells and mouse splenocytes. The possibility of adhesion and proliferative activity of fibroblasts, metabolic activity of all cultures according to the MTT test were investigated.

Results. None of the materials were found to have adhesive properties to fibroblasts, nerve cells or splenocytes. Grids and suture material did not affect the proliferative activity of fibroblasts, and the metabolic activity of all studied cells were completely biocompatible.

Conclusion. The number of complications, the biocompatibility of surgical mesh and suture material have little to do with the material which they are made from and probably depend on their shape, surface, or mechanical properties.

Keywords: *biocompatibility, surgical mesh, suture material, cell culture*

Introduction

The widespread usage of mesh implants in hernia surgery and gynecology to correct genital prolapse is widely debated [1]. The only way to restore the integrity of the abdominal wall or pelvic floor weakness is to use surgical mesh [5]. Since every third elderly woman has some degree of genital prolapse, there is a great need for such operations [4]. The use of different types of mesh and suture material is arguable because ofsuch complications as infections, inflammation, erosion and rejection of the material [8, 13].

A variety of complications after using implants and a great number of different variants of mesh and suture material encourages the study of their biocompatibility, biosafety and mechanisms of their possible effects on the body [3, 7, 10].

Nowadays there are a sufficient number of studies on the dependence of the effectiveness of different grids and suture material and their biocompatibility, but the mechanisms of their influence and the response of individual cells have not been studied thoroughly enough [2, 10]. Once in the body, implants primarily interact with immune cells, fibroblasts that form connective tissue and nerve cells that innervate the postoperative area. The choice of mesh and suture material is relevant for every doctor who performs such operations. Preliminary studies of the biocompatibility of surgical mesh were performed on human fibroblasts, rat kidney cells or stem cells isolated from adipose tissue revealed inertness of materials, or stimulation of fibroblast growth [2, 9, 14, 15]. However, the stimulation of fibroblasts, in our opinion, can be assessed not only as accelerating healing, but also as the formation of a rougher scar. In addition, cell cultures were not evaluated for the response of the immune and nervous systems involved in tissue functioning.

Therefore, the purpose of the study was to compare the biocompatibility and biosafety of mesh implants and suture material on cultures of fibroblasts, nerve cells and cells of the immune system.

Materials and methods

We studied the biosafety and biocompatibility of the most common grids: polypropylene "Monomesh" ("Fiatos", Belarus), "Monomeshlightweight" ("Fiatos", Belarus), which differ in structure, "Polymesh" ("Fiatos", Belarus), which includes polyglycaprolactone; suture material polyester "Ti-cron" (Covidien, USA), lactomer "Polysorb" (Covidien, USA), polygluconate "Maxon" (Covidien, USA) polypropylene "Prolene" (Ethicone, USA), Catgut (Igar, Ukraine).When implanting a mesh or suture material, it interacts primarily with subcutaneous fibroblasts, nerve cells, and immunocytes. Thus, we selected the mouse embryo skin fibroblasts, mouse splenocytes, and mouse embryo brain cells as the culture test. The proliferative activity of fibroblasts, adhesion to suture material and metabolic activity of all cultures were also determined.

To prevent postoperative infections during contaminated or potentially contaminated surgical interventions, the meshes and suture material under study were immersed in a solution of an antibacterial drug (ceftriaxone 1 g was dissolved in 200 ml of 0.9% NaCl) before surgery.

To obtain cells, we used 35 fetuses of Balb / c mice at 20 days of gestation, obtained from 4 females aged 5 months and spleens from 5 males in Balb / c mice aged 5 months.

We isolated mouse fibroblasts of mouse embryos [12], nerve cells of the mouse brain [13] and mouse splenocytes [6] according to the previously described methods. Cells were cultured in DMEM medium with the addition of 10% fetal bovine serum in a CO_2 incubator (Thermo Fisher Scientific, USA) at 37 °C in an atmosphere with 5% CO_2.

To study the adhesive properties of implants on 12-hole culture plates SPL (Korea) adhesive (Treated) we placed an implant with the size of 10 × 10 mm, or suture material with a total length of 30 mm, corresponding to the size of the well of the plate, and inoculated it with fibroblasts in culture medium at a concentration of 4×10^5 / cm^2, covered with glass for fixing. Adhesion was assessed after 3 days visually by phase contrast microscopy. To exclude cell adhesion to culture vessels, studies were repeated on non-adhesive (Non-treated) SPL culture plates (Korea). To assess the proliferative activity of the cell on the third day, the cells were removed from the plate with 0.25% trypsin solution, and counted. Cells cultured without suture material were used as controls.

To perform the MTT test on a 96-well flat-bottomed plate SPL (Korea) were placed fragments of implants 5 mm in diameter, or suture material with a total length of 10 mm corresponding to the size of the well of the plate. The test cells were seeded: fibroblasts at a concentration of 1×10^4 / well, or nerve cells at a concentration of 5×10^5 / well, or splenocytes at a concentration of 7×10^5 / well. Cells without adding implants or suture material were used as controls. The total amount of medium was equal to 100 µl / well. Cells were cultured for 24 hours, then 15 µl of MTT (Sigma, USA) was added to each well at a final concentration of 5 mg / ml, and incubated for 3 hours at 37 °C in an atmosphere of 5% CO_2.The medium was collected, formazan was dissolved 10% solution of sodium dodecyl sulfate in dimethyl sulfoxide. Absorption was measured on a SM600 plate spectrophotometer (Utrao, China) at a wavelength of 570 nm. Each study was performed on three different cultures and 8 wells of a 96-well plate.

Toup View V 3.7 soft ware was used for image processing. (Hangzhou Toup Tek Photonics Co. Ltd, Hangzhou, China). The Mann-Whitney U-test and the Kraskel-Wallis test were used to assess the significance of differences between comparison groups. Past V. 3.15 software (University of Oslo, Norway) was used for statistical calculations and data processing. The data represent the mean and standard deviations, the differences were considered significant at $p < 0.05$.

Results

When studying the adhesive properties of fibroblasts on implants or suture material on adhesive culture plates, we found out that all cells adhered to the plastic bottom of culture plates and did not remain on the material. After two days, the number of cells increased, they formed a monolayer, tightly adjacent to each other. At the points of contact of the grid with the culture surface, the morphology of the cells did not change, the cells did not adhere to the grids and suture material despite the tight contact (Fig. 1. A, B, C).

Since the literature describes the possibility of adhesion of mouse fibroblasts to polypropylene mesh [9], we assumed that all cells adhered to a more adhesive surface, which was a culture vessel. Thus, to exclude adhesion to the culture plate, the study was continued on low-adhesive plastic with fixation glass implants.

When culturing fibroblasts on non-adhesive culture plates in the presence of surgical mesh and suture material, the cells formed an unstable monolayer on the surface of the vials and partially separated, forming spheroids up to 100 µm in diameter, which floated freely (Fig. 1, D). The cells did not adhere to any of the materials, even in close contact, neither from spheroids nor from the surface of the vial (Fig. 1). The obtained data indicated the absence of adhesive properties of all studied grids, and their inertness to fibroblasts. The cell fixation was described [9] for the mesh which had thinner braided fibers of filaments about 10 micrometers thick, but less structured mesh shape; the fibers were close to each other, which may explain the fixation of cell mass on them, the authors did not describe the mesh coating. The samples which we studied had a filament thickness of 50-100 micrometers, a more structured grid, no coating. These characteristics explain their inertness and lack of adhesion and allow characterizing the material of the filaments, not the coating.

Studying the proliferative activity showed that the number of cells almost doubled in two days in all samples, but differed little regardless of introducing any material into the culture. The data in the samples with mesh or suture material were less homogeneous and had a larger error, but did not differ statistically from the control indicators. This can be explained by the mechanical effect of mesh and suture material on cell cultures and injury of individual cells during manipulation of culture vessels and shifts of mesh and suture material.

Studying the metabolic activity of fibroblasts in the presence of surgical nets or suture material proved that no suture material affected the metabolic activity of fibroblasts (Fig. 2. A, B).

When studying the nerve cells reaction to the mesh and suture material, we noted that nerve cells formed a monolayer when reseeding, which did not show adhesive properties in contact with the test material. Studying the metabolic activity of nerve cells by the MTT test proved that none of the studied materials affected the metabolic activity of

nerve cells, which did not differ statistically from the control and between different groups (Fig. 2. C).

Splenocytes, which are non-adhesive cells when sown on a culture plate, did not form a monolayer, but settled to the bottom without changing the spherical shape. Upon contact with the grids or suture material morphologically, the cells did not change. Studying the metabolic activity of splenocytes by the MTT test, we noted that none of the studied materials significantly affects the metabolic activity of splenocytes (Fig. 2. D).

The data obtained in this way indicate the inertness of the studied grids and suture material on mouse fibroblasts, nerve cells and splenocytes in the in vitro system. It means that at the cellular level the grids and suture material do not to cause complications and are biologically safe. A review of the literature revealed the possibility of grids to change the metabolic and proliferative characteristics of native cells isolated from rat kidneys and the NRK-49F line, especially in the degradation of material [14], which may limit their use in lowered kidneys. In this case, fibroblasts, elements of the nervous and immune systems are most important in wound healing and scar formation in any operation, not just the kidneys.

The literature sources described different number of complications that occurred when using different grids and suture material [1, 3, 4, 5, 8] which made it the main reason for the discussion about the feasibility of their usage. The study eliminates the effect of the described material on the cells of complications and suggests the need to look for the causes of complications in the shape of the mesh, thickness and shape of the fibers that can injure the tissue during implantation or suturing.

Conclusions

The study of biocompatibility of surgical mesh and suture material containing polypropylene, polyglycaprolactone, lactomer, polygluconate, catgut showed that they did not have the adhesive properties to fibroblasts, nerve cells and splenocytes, and they did not change the metabolic activity of these cells and proliferative activity of fibroblasts. Different number of complications, or different biocompatibility of surgical mesh and suture material may probably relate to their shape, surface characteristics, or mechanical properties that affect the surrounding tissues in close contact, rather than the material which they are made from.

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The authors declsre that there are no conflicts of interest.

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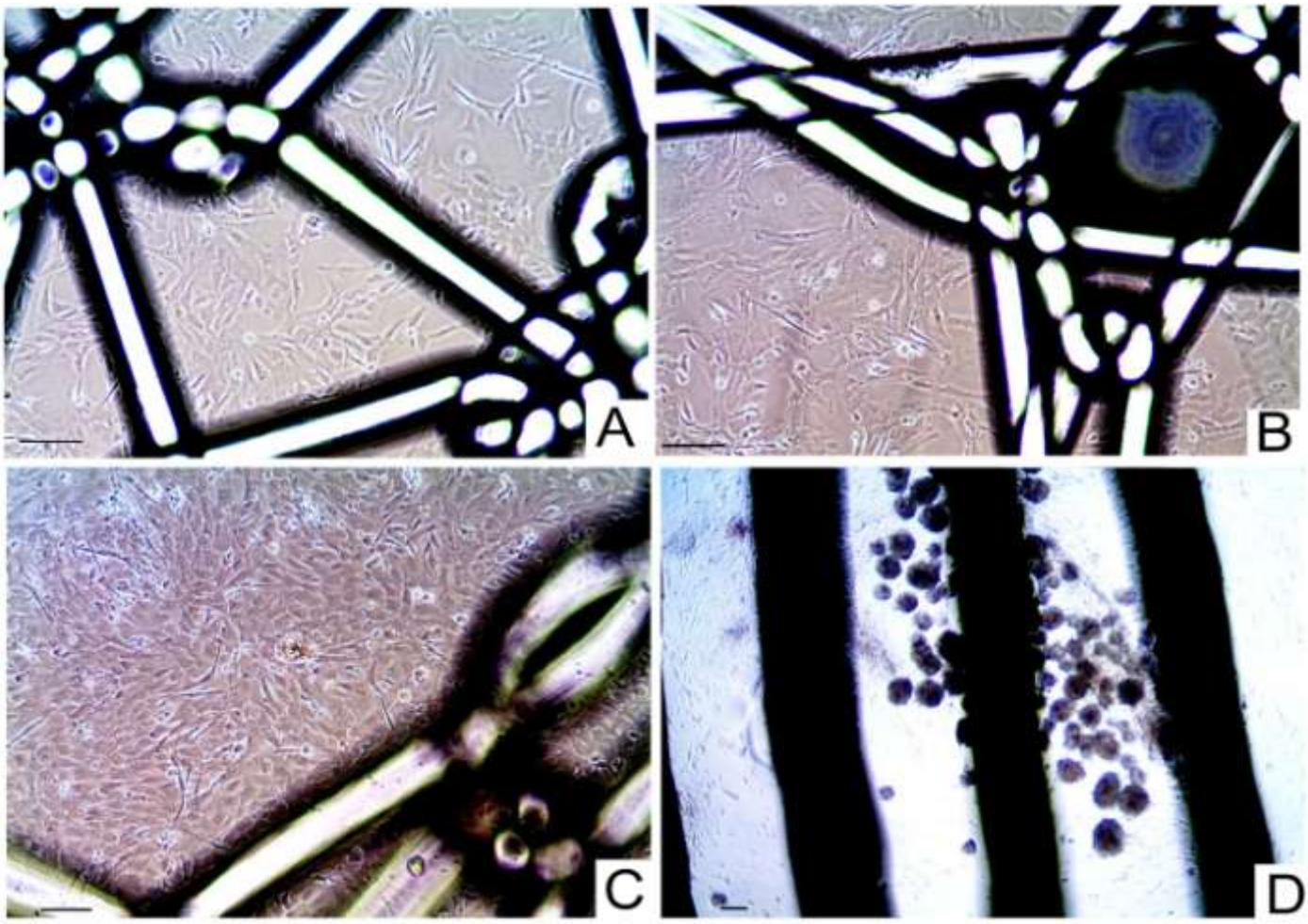

Figure 1. Surgical mesh and suture material with fibroblast culture.
A –Monomesh, B –Monomesh lightweight, C –Polymesh, D – Polyester - spheroids from fibroblasts.
Scale lines of 100 microns.

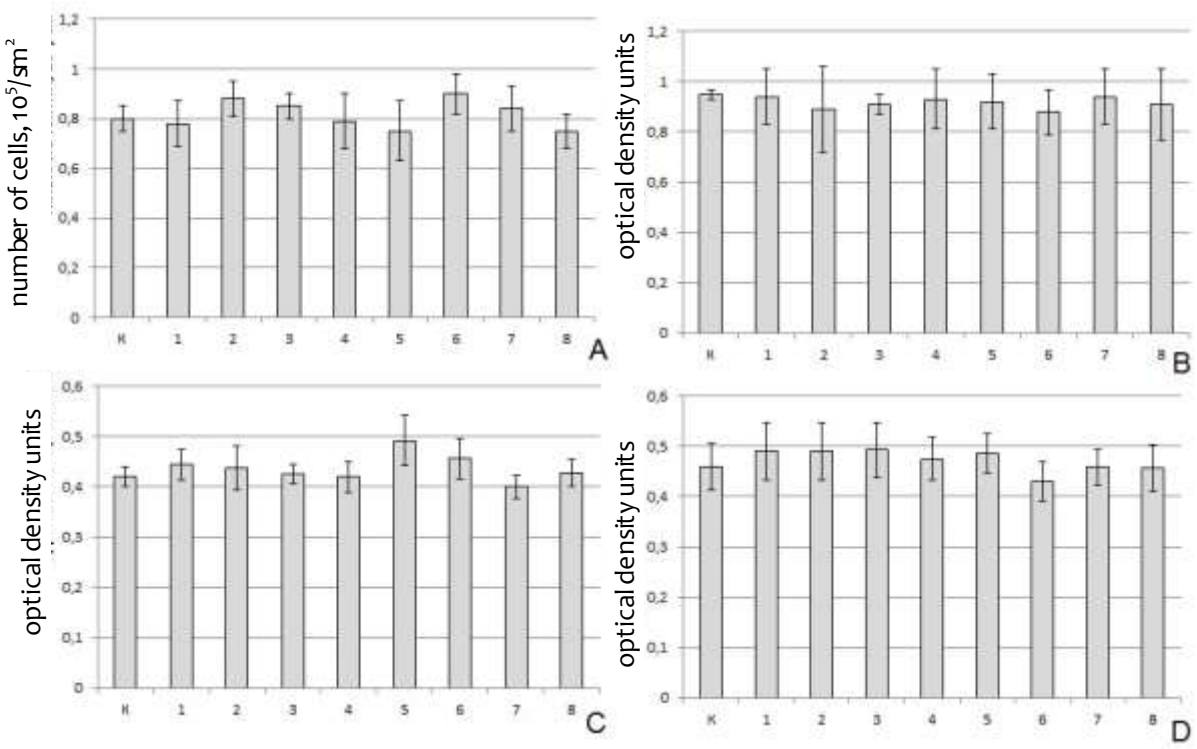

Figure 2. Characteristics of fibroblasts, neurocytes and splenocytes after cultivation with surgical mesh and suture material.

A –proliferative activity of fibroblasts, B – MTT test with fibroblasts, C – MTT test with nerve cells, C – D – MTT test with splenocytes. K – control, 1 – "Monomesh" ("Fiatos", Belarus), 2 – "Monomeshlightweight" ("Fiatos", Belarus), 3 – "Polymesh" ("Fiatos", Belarus), 4 – "Ti-cron»(Covidien, USA), 5 –«Polysorb»(Covidien, USA), 6 – «Maxon»(Covidien, USA), 7 –«Prolene»(Ethicone, USA), 8 – Catgut (Igar, Ukraine).