A comparative Histologic and Immunohistochemistry Evaluation Between Normal Aponeurotic Tissue, Fibrotic Aponeurotic Scars and Polypropylene Embedded Aponeurotic Scars

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Synthetic polypropylene materials are increasingly being used in surgery to repair parietal defects in perineal floor curing surgery, in genital prolapse and stress urinary incontinence. The tissue response to contact with these materials varies, and the inflammatory tissue response may be a prognostic marker of success in surgical interventions that involve contact between tissues and polypropylene materials.

Keywords: aponeurotic tissue, scars, inflammatory reaction, polypropylene

Used since 1950 to replace parietal defects initially only for complex inguinal hernia, by improving their characteristics, polypropylene materials have been introduced in the treatment of surgical gynecological and urological pathology.

The rate of relapse after use of prosthetic materials is significantly reduced, but there is a significant percentage of complications of which the most common are: local bleeding with hematoma formation, urinary tract and bladder trauma, erosions, intestinal perforations, urinary tract infections, vaginal expulsion, etc. [1-3].

The current trend is to find and produce ways to limit the inflammatory response and to increase the polypropylene tissue integration rate.

This would result in a significant decrease in local graft complications, complications that may be erosions, infections, excessive scarring, all of which impinging on a good outcome, especially through the risk of developing dyspareunia.

A material is considered as biocompatible when it does not harm nor create toxic reactions or systemic side effects [4].

In fact, no mesh has demonstrated a biocompatibility and integration rate in host tissues at optimal level, and in literature there are few studies that determine which of the multiple variants is the best method applicable for vaginal surgery.

Tissue responsiveness to mesh causes a cascade of healing, events involving clotting, inflammation, angiogenesis, fibroplasty, cellular matrix formation and cell and tissue contraction.

Biocompatibility is determined by the intensity of the foreign body reaction, and is expressed by the ability of the mesh and host tissue to resolve the tissue damage due to the implantation process.

Mesh-like features such as pore size, chemical composition, filament structure, amount of implanted material and biodegradability are characteristics that influence the processes of angiogenesis, inflammation, tissue neoformation, and may affect the healing process.

Inflammatory cells mobilized in the allograft implant site produce signaling molecules, molecules that will influence the tissue response to biomaterial [5].

The molecular response mechanisms of foreign body response body are poorly understood.

Macrophages that come in contact with the mesh surface are activated in an attempt to phagocytose the allograft fibers. They will merge and form FBGCs – foreign body giant cells.

The sequential secretion of cytokines, degrading enzymes and intermediate oxidative reactions of macrophages and FBGCs direct the inflammatory response and healing by influencing the behavior of other cell types like neutrophils (polymorphonuclear leukocytes [PMNs]), lymphocytes, fibroblasts and monocytes [6].

Recent laboratory studies highlight an in vivo gene expression involved in angiogenesis and modeling of the extracellular matrix after fitting prostheses used for anterolateral abdominal parietal defects including polypropylene, polyester, and polytetrafluoroethylene materials [7].

Experimental part

The study included histological parts as follows:

Healthy tissue - aponevrosis - histology base of the pathological anatomy section taken on different occasions for other indications. For the prosthetic material included in the aponeurosis, small fragments were taken during surgical interventions from patients who underwent recurrent surgical for recurrence or recovered material during initial correctional cure.

Scar tissue – collected from surgical cures and reinterventions for different pathologies.

All fragments of tissue and prosthetic material were collected with patients' informed consent.

We evaluated mesh biocompatibility by analyzing collagen density, fibroblast density, lymphocyte infiltrate and vascularization of a mesh embedded aponeurosis (a3), compared with a normal aponeurosis negative control (a2), and an aponeurotic fibrotic scar (a1)

Histologic and immunohistochemistry processing

Paraffin embedded sections were stained for collagen with the van Gieson method using standard protocols. IHC was performed for vimentin (fibroblasts and other mesenchymal cells) CD3, CD5 and CD20 (lymphocytes),

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CD31 (endothelial cells), and Ki67 (cell proliferation), following the manufacturers protocols.

Image acquisition and analysis

Five images at 100X and 10 images/cm^2 400X magnifications were obtained from each slide, using a Nikon Eclipse E200 microscope mounted with a Serioux cmos camera, under constant illumination settings.

Computer aided image analysis was performed in the FIJI distribution of ImageJ, under visual control of a pathologist.

Overall collagen (100X) and collagen fiber (400X) density was measured by background subs traction, followed by incremental brightness thresholding (Color Threshold function) until all visible collagen fibers were included in the selection.

Lymphocyte density was measured by incremental brightness thresholding followed by the Analyze Particles function, with surface area and sphericity parameters set at >100 pixels and 50-100, respectively.

Vimentin, CD31 and Ki67 staining was evaluated by incremental brightness thresholding and total surface measurement.

Statistical analysis

Values are expressed as mean ± standard deviation per slide, calculated from the individual readings on each image. Statistical significance was calculated in Microsoft Excel by two tailed Student's t test. A value of $p < 0.05$ was considered significant.

Results and discussions

Overall collagen density, expressed as percentage of van Gieson stained area, was 78.23±7.6% for the mesh embedded aponeurosis, significantly higher (p=0.0014) compared with the negative control (61.59±2.7%), and significantly lower (p=0.0286) compared with the aponeurotic scar (89.22±2.1%) (fig. 1).

Collagen fiber density was 77.5±5.4% for the mesh embedded aponeurosis, significantly higher (p=0.0007) compared with the negative control (68.2±3.8%) and significantly lower (p=0.0013) compared with the aponeurotic scar (85.9±2.9%). The mesh granuloma presented the lowest collagen density, at 40.7±8.5%. (fig. 2).

Lymphocyte counts were *0* negative in the aponeurotic scar and negative control, and negative in the meshembedded aponeurosis (5, 13 and 0 lymphocytes/ cm^2 positive for CD_3, CD_5 and CD_{20}, respectively).

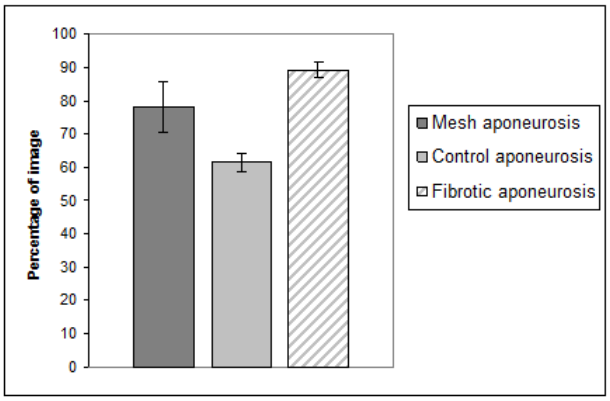

Fig. 1.

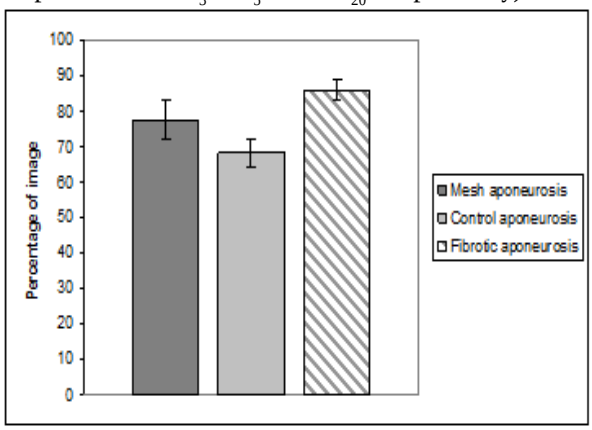

Fig. 2.

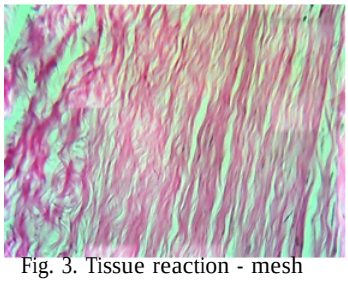

Fig. 3. Tissue reaction - mesh embedded mesh (Van Gieson stain)

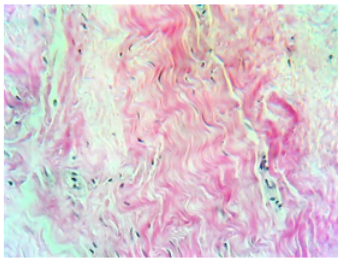

Fig. 4. Tissue reaction – Scared aponeurosis (Van Gieson stain)

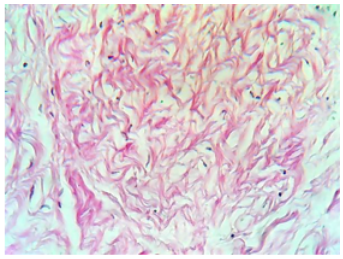

Fig. 5. Tissue reaction - Control aponeurosis (Van Gieson stain)

Vimentin staining was 5.63±2.41% for the mesh embedded aponeurosis, significantly higher than 2.58±1.26% for the negative control and 1.54±1.14% for the fibrotic aponeurosis (p=0.0002 and 0.004, respectively).

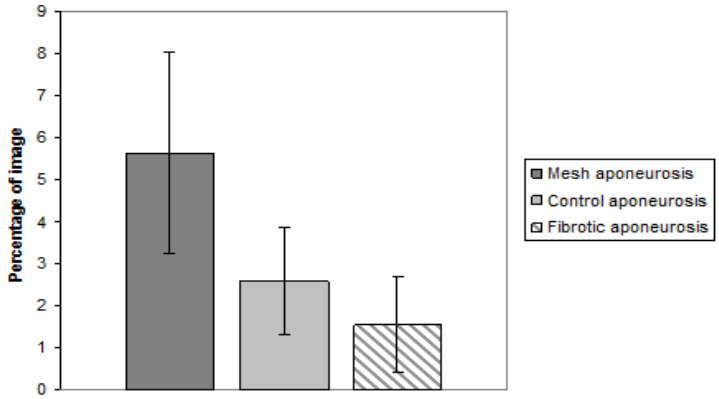

Fig. 6.

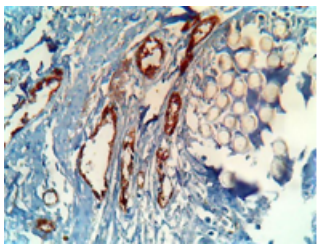

Fig. 7 CD31 reaction in embedded aponeurosis

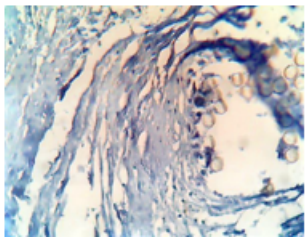

Fig. 8. CD20 reaction in mesh embedded aponeurosis

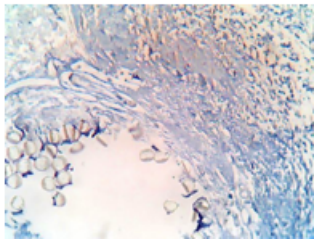

Fig. 9. CD3 mesh embedded aponeurosis

CD31 staining showed no significant difference in vascularization, with values of 3.72±1.76% for the mesh embedded aponeurosis, 2.05±1.42% for the negative control and 3.23±1.47% for the fibrotic aponeurosis.

Ki67 was *0* negative in all cases.

Treatment of abdominal parietal defects, pelvic floor defects, and urinary stress incontinence currently requires the use of allografts mesh. Most commonly used are polypropylene, due to its biochemical, physical characteristics as well as the availability and ease with which can be used in surgical practice.

The local evolution of contact between mesh and tissue through inflammatory mechanisms and local apoptosis as an initial mechanism, and subsequently by reforming the extracellular matrix with evolution towards embedding tissue in tissue involves complex mechanisms.

Biochemical, histopathological, immunohistochemical and genetic studies highlight the existence of local reactions and the existence of an interaction between these changes and the occurrence of postoperative complications.

Our study examined the amount of collagen related to 3 situations: tissue with the presence of prosthetic material, healthy tissue-negative control and scar tissue.

There is an overall collagen density at elevated levels for tissue in contact with alloplastic materials, but the inflammatory response values of collagen proliferation are lower in tissues that have integrated polypropylene compared to scar tissue of another nature.

The density of higher collagen fibers in tests in contact with the urinary band compared to healthy tissues is explained by the absence of a local inflammatory process due to surgical intervention and the fitting of foreign material.

Of note, however, is the presence of an exacerbated inflammatory reaction for scar tissue that did not involve banding, such as the overall collagen density, collagen fiber density and inflammatory responses in the presence of CD31, CD5, CD3 and CD20 as well as by inflammatory reactions highlighted by Van Gieson staining and protein dosing Vimentin (used as a marker of mesenchymal-derived cells or cells undergoing an epithelial-to-mesenchymal transition) are higher than in and polypropylene embedded aponeurotic scars.

The existence of an inflammatory reaction of tissues in contact with foreign material is a natural one, the study reveals as a novelty factor a much higher inflammatory response for scar tissue of another nature.

In other words, although the polypropylene prosthetic material is foreign, and the foreign body reaction is predictable, it seems that healing with only its own tissue substrate requires a more intense inflammatory recurrence with more intense scarring.

The explanation could be the tissue requirement for correcting the parietal defect, a material that in a tissue cure involves local or neighboring tissues with already modified tissue structure (causing the defect), the healing process, the collagen proliferation and implicitly the inflammatory response required being higher. [8, 9]

The polypropylene allograft is the most widely used in the past 20 years due to its resistance, stability and low tissue reaction [10]. The existence of a prosthetic material with the characteristics of polypropylene that replaces the defect, causes a lower inflammatory process than pure tissue healing.

Local inflammatory reaction is an important factor in the evolution to local complications (erosions, infections, excessive scarring, dyspareunia), the use of polypropylene in the treatment of urinary stress incontinence is a method that by the histological, biochemical and substitution advantages of the response as well as due to reduced rate of evolution towards complications recommends this surgical approach to other surgical approach.

Conclusions

Polypropylene used in the treatment of urinary stress incontinence causes lower histological, immuno-histochemical and inflammatory changes than scar tissue lacking in foreign material, which is an important factor for local evolution without complications and implicitly reducing the risk of relapse.

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