# HIGHLY PURIFIED COLLAGEN COATING ENHANCES TISSUE ADHERENCE AND INTEGRATION PROPERTIES OF MONOFILAMENT POLYPROPYLENE MESHES

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## ABSTRACT

**Introduction:** Complications related to tissue integration of polypropylene implants used in the treatment of genital prolapses are relatively prevalent. Collagen, a biocompatible, less immunogenic material with modulating properties on the inflammatory process, may improve polypropylene integration. **Objective:** To study biomechanical and histologic effects of monofilament polypropylene mesh coated with purified collagen gel. **Material and Methods:** In 40 rats, a mesh fragment was implanted on one side of the abdominal wall (Group I). A similar fragment of collagen gel coated mesh was implanted at the other side of the abdominal wall (Group II). The animals were euthanized at 7, 30, 90 and 180 days after implant and their abdominal walls were excised for analysis. **Results:** On biomechanical study, it was observed that mesh adherence to neighboring tissues increased significantly in Group II ($p<0.05$). Acute ($p<0.001$), chronic ($p=0.004$) inflammatory responses and granulation tissue formation ($p=0.001$) was less intense in Group II in the early phase (7 and 14 days). Granulomatous inflammation and foreign body reaction was less significant at 7 days in Group II ($p=0.029$ and $p<0.001$). On birefringence analysis, higher mean brightness density was observed in the late phase in Group II meshes ($p=0.000$). **Conclusion:** Polypropylene mesh coated with purified collagen gel had increased adherence to tissues, promoted a less intense and lasting inflammatory response and triggered a greater organization and packing arrangement of collagen fibers in the late phase of implantation.

**KEYWORDS:** Genital dystopias; polypropylene mesh; purified collagen.

## INTRODUCTION

Genital prolapse repair in urologic and gynecologic practice presents a challenge. It is a common occurrence that has become more prevalent in view of the aging population. In prolapse treatment, an issue open to debate is when to indicate surgical correction and the use of meshes. Approximately 11% of the female population will undergo surgery to repair pelvic organ prolapse in her lifetime. Roughly 30% of these women will need reoperation, owing to prolapse recurrence within 4 years of surgery. These poor results are associated with traditional techniques using native tissue of worse quality [1].

The advantages of greater strength and durability, easy availability and versatility of synthetic mesh have been weighed against the increased risks of infection and erosion [2]. The use of prostheses permits a greater standardization of procedures, shorter surgical duration and a more rapid postoperative recovery. However, implications of the use of prostheses for pelvic floor reconstruction have still not been fully established. In particular, interactions with the bladder and rectum, as well as the potential consequences on sexual function have not been clearly addressed. Despite its wide acceptance and use, synthetic mesh has received minimal regulatory supervision. This is especially alarming now that the Food and Drug Administration issued an official public health advisory, citing a high rate of complications related to the use of mesh for genital dystopia treatment. Therefore, research on the impact of synthetic mesh on clinical results is extremely necessary. Studies begin in the laboratory, especially concerning newer meshes and designs where data is limited [3].

To obtain better surgical results, initial studies of coated meshes that are currently in development have been published. Meshes can be coated with collagen, titanium, bovine pericardium, or porcine intestinal submucosa [4, 5, 6, 7]. Initial tests in fibroblast culture show that these cells recognize and adhere to collagen fibers obtained from heterologous material (type I collagen obtained from bovine tendon). Such qualities support the hypothesis that type I purified collagen gel may be used as a framework in conjunction with polypropylene mesh. Collagen matrix processed from bovine tendon has been employed to treat some diseases, e.g. orthopedic lesions, axonal lesions, stress urinary incontinence and also genital prolapse [5, 8, 9].

Based on literature reports, the proposal to coat polypropylene mesh with purified collagen gel and modulate the initial phase of prosthesis-host integration, permitting better mesh incorporation into neighboring tissues, emerges as an evolution of surgical materials. The composition of type I collagen and its ultrastructural organization makes it favorable for platelet adherence and local primary hemostasis. Adhered platelets release fibroblast growth factor and stimulating angiogenesis factors [10, 11, 12]. It can be postulated that synthetic mesh coated with type I purified collagen gel may improve proliferative reactions of tissue repair, contributing to its biologic and functional adaptation. This experimental study in rats proposed to evaluate the effectiveness of coating polypropylene mesh commonly employed in genital dystopia treatment with a highly purified type I collagen gel by using biomechanical tests and histologic analysis (anatomopathologic and polarization microscopy).

## MATERIAL AND METHODS

The product used in this study was prepared with type I collagen obtained from bovine tendon. It consisted of two alpha-1 chains and one alpha-2 chain. The product was highly purified, according to previously

described technique, to eliminate the immunogenic telopeptide parts [10]. Collagen solubilization involved refrigerating the dissected tendons in an aqueous solution of 0.01% hydrochloric acid and 1 mg of pepsin/g of tissue for 24 hours. The collagen obtained was reconstituted by adding a 0.9% NaCl solution to a final concentration of 5%. The solution was then stabilized through extensive dialysis in distilled water for 5 to 7 days.

In this study, the meshes used were made of monofilament type I polypropylene – original weight: 85g/m², and were thus prepared for the following groups:

**Group I or PP** - Mesh measuring 20 x 10 mm.

**Group II or PP+C** - Mesh with same measurements, coated with collagen gel.

Meshes were sterilized with gamma rays (dose: 25KGy) to preserve the structural stability of collagen molecules [13].

The study was developed after approval from the Animal Experimentation Ethics Committee (CEEA-IB-UNICAMP) of the Institute of Biology of the *Universidade Estadual de Campinas,* according to protocol number 1972-1, following the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA).

**Surgical Technique.** The sample was composed of 40 Wistar female rats. Of these, 20 were allocated to histologic study and 20 were allocated to biomechanical study. After anesthesia with sodium Pentobarbital 3% (0.15mg/g), a 2-cm cross-sectional incision was made in the lower abdominal region. Mesh was implanted in the animal in a standardized manner on each side of the abdominal wall, separated by the linea alba, between the hypodermis and the anterior fascia of the abdominal musculature. Group I was located at the left side and Group II at the right side. The animals were divided into 4 subgroups, each with 10 animals, according to time elapsed from mesh implant to euthanasia: 07, 14, 90 and 180 days. In each subgroup, 5 rats were allocated to histologic study and the remaining five for biomechanical study. The rats were euthanized with a lethal dose of sodium Pentobarbital 3%. A median incision was then performed, separating the right from the left side of the abdomen, removing the abdominal wall en bloc.

**Biomechanical Study**. Assessment of tensile resistance was performed in an isolated abdominal wall fragment of 20 rats allocated for this purpose, using a previously published protocol [14, 15]. Tests were performed immediately after euthanasia and sample collection, making preservation unnecessary. Samples (rat abdominal wall) were prepared maintaining 2 mm of the proximal end of exposed mesh to fix to the upper staple of the tensiometer (Assay Universal Machine–LLOYD Instruments, model TA 500, Bognor Regis, United Kingdom). The distal portion of the abdominal wall, devoid of the mesh (containing only animal tissues), was fixed to the lower staple of the tensiometer. Samples were submitted to traction test, in which the upper staple was displaced upward with constant force (N) and velocity (2 mm/sec) until the mesh became detached from the tissue interface (Fig. 1).

Each test resulted in a graph that automatically extracted maximum load (N) results necessary for the displacement, determining mesh adherence to neighboring tissues. Automatic data acquisition was performed by using Nexygen 3.0 software, which exports files to the Microsoft Excel®.

To compare measures between types of coating and time periods, the ANOVA for repeated measures with rank transformation was used. Tukey's test was used for multiple comparisons. A 5% significance level was adopted for statistical tests

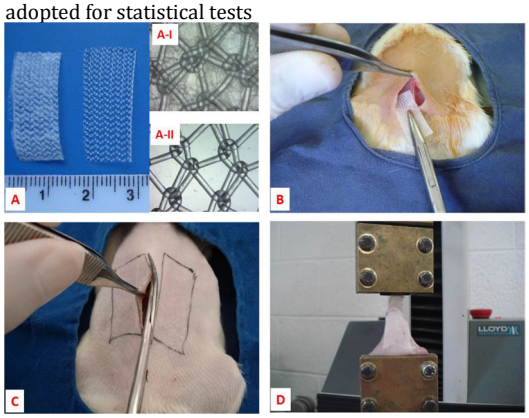


***Fig. 1. (A)*** *Monofilament type I polypropylene mesh coated with collagen (PP+C) and in natura (PP) – macroscopic view - Sony DSC W 70, 2.8-5.2 lens;* ***(A-I)****: Polypropylene mesh: coated with collagen and* ***(A-II)*** *– Uncoated, light microscopy, 4 x 0.65 - Primo Star (Carl Zeiss).* ***(B)*** *Mesh implant;* ***(C)*** *Specimen removal;* ***(D)*** *Tensiometer LLOYD TA 500, sample fixation to staples.*

**Histologic Study.** The samples were fixed in formalin10% for 24 hours and later transferred to a 70% alcohol solution where they remained for more 48 hours. Histologic sections were made, 5 µm in width. Four fragments of the material were placed on each slide. A set of slides was stained with Hematoxylin-Eosin (HE), for histologic study. For birefringence analysis, there were used a set of unstained slides, a set of slides stained with silver nitrate and another with Masson's Trichrome stain.

For histologic evaluation, a Zeiss Primo Star® microscope (Carl Zeiss Microscopy, Jena, Germany) was used. Image was acquired by using a Zeiss AxioCam ICC 1® camera. Image was processed and stored with the aid of AxioVision V 4.8.0.0 Software (Carl Zeiss Imaging Solutions®). Microscopy was used to assess the presence of acute (neutrophils), chronic and granulomatous inflammatory infiltrates. Samples were also analyzed as to the presence of granulation tissue and foreign body reaction. The intensity of the inflammatory process was classified as mild, moderate, or severe, according to previously established criteria [16, 17]:

(0) Absent: minor reaction, to a maximum of 5% of the cutting area

(1) Mild: reaction involving 5 to 25% of the cutting area

(2) Moderate: reaction involving 25 to 70% of the cutting area

(3) Severe: reaction compromising more than 70% of the cutting area

The ANOVA for repeated measures was used for comparisons, followed by Tukey's multiple comparison tests to compare the 4 time points in each situation. The contrast profile test was used to analyze both situations, at each time period. Variables were rank-transformed due to the absence of normal distribution and because they were considered in ordinal scale. A 5% significance level was adopted for statistical tests ($p<0.05$).

Polarization microscopy is a method that gives important information about the molecular ultrastructure and organization of cells and tissues. When quantified (birefringence intensity), it can reach high levels of accuracy, allowing the precise determination of molecular order, vibration direction and variations of the molecular aggregation and arrangement of collagen fibers. Birefringence measurements were taken with an Olympus BX51 polarization microscope (Olympus, Tokyo-Japan) with

Neofluar lens, using amplification objective (40/1-2.5X) to analyze collagen fibers. Images were analyzed using the Software Image Pro-Plus 6.0 System (Mediacybernetics, Inc; Bethesda, MD-USA).

Variation in collagen birefringence determined the variable of brightness intensity on polarization microscopy, which was assessed by an image analyzer and expressed in Gray scale Average (GA) values in pixels. Brightness tones varied from 50 to 255 pixels, with 255 corresponding to maximum brightness detected [18].

Observations always began with the longest axis of collagen fiber at 45 degrees from the azimuth of the electric vector of polarized light. Then areas with high fiber definition were selected. Each section was analyzed in at least 6 points.

The results obtained in both groups were analyzed by the Mann-Whitney nonparametric test. The rejection level of the null hypothesis was set at 0.005.

## RESULTS

There were no deaths among the animals. During the observation period, no signs suggestive of systemic compromise or related complications specifically due to the procedure were present. None of the animals exhibited extrusion of the polypropylene mesh implanted.

### Biomechanical Study

Before the animals received mesh implantation, a comparative traction test of meshes from groups I and II was performed, showing that collagen gel applied to monofilament polypropylene mesh did not alter its physical characteristics.

The maximum load (N) was significant greater in meshes coated with collagen gel (PP+C) compared to uncoated meshes at all time periods (Table 1).

### Anatomopathologic Study

**Acute inflammatory infiltrate** was graded, based on the presence of neutrophil groups and edema. It was more intense in animals submitted to mesh implant uncoated with collagen gel, euthanized after 7 and 14 days ($p<0.001$). In contrast, at 90 and 180 days, there was no significant difference between groups.

On analysis of **granulation tissue,** only fibroblast proliferation and neovascular formation were evaluated. At 7 days, fibroblasts adherent to implant collagen were visualized, corresponding to the proliferative phase of the remodeling inflammation process. Adherence of collagen to the polypropylene mesh was clearly detected. Newly formed collagen fibers that coated polypropylene mesh were observed. There were mononuclear cells, lymphocytes, histiocytes and newly formed vessels (Fig. 2A). Animals showed a significant difference after 7 and 14 days. Granulation tissue formation was less intense in group II animals ($p=0.001$). In contrast, at 90 and 180 days, no significant difference was observed between groups.

The presence of **foreign body reaction** was based on granuloma formation and the appearance of foreign body giant cells. The reaction was less intense in group II animals ($p<0.001$) euthanized 7 to 14 days since implantation. In animals euthanized at 90 and 180 days, there were no differences between groups. **Granulomas** are small collections of modified macrophages termed epithelioid cells surrounded by lymphocytes and giant cells. Granuloma formation was less intense in animals submitted to polypropylene mesh implant coated with collagen gel ($p=0.029$) and euthanized after 7 days. In contrast, in animals euthanized at 14, 90 and 180 days, no significant differences were observed.

**Chronic inflammation** was evaluated as to the presence of macrophages, lymphocytes and histiocytes, in addition to the presence of vascular and fibroblast proliferation and fibrosis. Significant differences were observed between meshes in euthanized animals after 7 and 14 days, regarding the intensity of chronic inflammatory process. Inflammation was less intense in the group with meshes coated with collagen gel ($p=0.004$). In contrast, at 90 and 180 days, no significant difference in chronic inflammation was observed between groups.

### Polarization Microscopy

Initially, morphologic analysis of structures in slides stained by Masson's Trichrome method and impregnated with silver was performed. Differences in color intensity, degree of aggregation and distribution of collagen fibers were observed. At seven days after implant, it was observed that fibers were less dense (Fig. 2B).

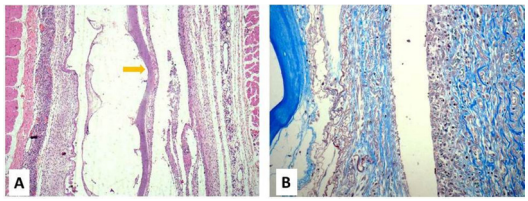


***Fig. 2. (A)*** *Micrograph of mesh implant coated with collagen 7 days after implant, in which a basophil band is observed, indicating collagen adherent to mesh (arrow). Mesh filaments are not present because of removal during material processing. The remaining free spaces are artifacts caused by differences in biomechanical properties during microtome sectioning. Fibroblasts, vessels and mononuclear cells are observed. PP+C mesh, HE 4x1.* ***(B)*** *PP+C mesh seven days after implant. On the left side of the slide, collagen for implant was stained in dark blue. On the right, separate non-packed newly formed fibers are observed in blue. Masson's Trichrome stain at 7 days, 20x.*

At 14 days after implantation, collagen adherent to mesh was no longer observed. After 90 and 180 days, silver impregnation revealed that connective tissue involving the polypropylene filaments had higher collagen fiber density than implants uncoated with collagen. Collagen bundles impregnated with silver adjacent to coated meshes revealed typical fibril structures that were better visualized than in uncoated implants (Fig. 3).

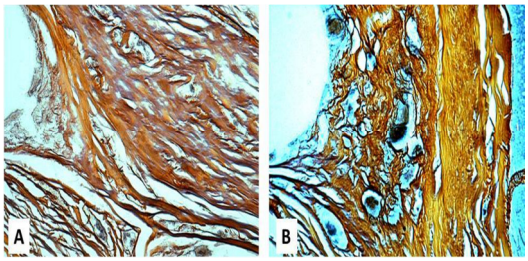


***Fig. 3.*** *Image of section impregnated with silver 90 days after implantation. PP* ***(A)*** *and PP+ C* ***(B)****, in which the packing arrangement of collagen fibers is noticed. Ag, 40x1*

Measurements of birefringence brightness were performed in unstained slides, since collagen bands immersed in water have higher optical retardation [11, 19, 20]. N = 4.362 measurements, involving 435.779 $\mu m^2$ were made, constituting the maximum measurement obtained in all material available [11, 12, 20]. Table 2 shows the descriptive statistics (median values) of

birefringence in both groups expressed in pixels in the study period.

Birefringence of newly formed collagen fibers at seven days after implantation was higher in fibers surrounding uncoated meshes (p=0.000). In this period,

**Table 1**. Biomechanical Study. Maximum load in the rupture (values expressed in Newtons-N).

| Time | N | Material | Mean | SD | Minimum | Median | Maximum | *p*-value |
|---|---|---|---|---|---|---|---|---|
| **7 days** | 5 | PP+C | 12.079 | 0.881 | 10.861 | 12.170 | 13.322 | **0.0016** |
| | | PP | 9.695 | 1.477 | 8.149 | 9.588 | 11.780 | |
| **14 days** | 5 | PP+C | 18.772 | 0.795 | 17.731 | 18.909 | 19.861 | **0.0039** |
| | | PP | 15.951 | 0.893 | 14.896 | 16.315 | 16.981 | |
| **90 days** | 5 | PP+C | 25.155 | 1.416 | 23.244 | 25.120 | 27.124 | **0.0009** |
| | | PP | 22.105 | 1.123 | 20.987 | 21.836 | 23.896 | |
| **180 days** | 5 | PP+C | 25.108 | 1.326 | 23.205 | 25.497 | 26.574 | **0.0029** |
| | | PP | 22.641 | 1.025 | 21.193 | 22.6759 | 23.869 | |

PP: Polypropylene mesh; PP+C: Polypropylene mesh coated with collagen ; SD: Standard deviation

**Table 2.** Birefringence the collagen fibers in meshes coated and meshes without highly purified collagen gel

| Time | Group | n | median values | *p*-value* |
|---|---|---|---|---|
| **7 days** | PP | 554 | 110,15 | 0.000 |
| | PP+ C | 554 | 81,0 | |
| **14 days** | PP | 598 | 102,34 | 0.000 |
| | PP+ C | 598 | 115.66 | |
| **90 days** | PP | 515 | 87,59 | 0.000 |
| | PP+ C | 515 | 94,78 | |
| **180 days** | PP | 514 | 84,00 | 0.000 |
| | PP+ C | 514 | 89.66 | |

n = number of measures of Gray Average. * Mann Whitney Test

there was intense inflammatory reaction and reabsorption of collagen complexed with polypropylene mesh, and a smaller amount of newly formed collagen in group II.

At 14, 90 and 180 days after implant, it was observed that birefringence measurement of newly formed collagen fibers was higher for group II fibers (p=0.000) (Fig. 4). These findings support the hypothesis that collagen complexed with mesh exerts a remodeling action on the course of mesenchymal repair, involving polypropylene mesh. We considered that a more ordered coating of collagen fibers determined an increase in the birefringence of group II tissue. Values of higher brightness intensity suggest improvement in ordered compaction, making the tissue more resistant.

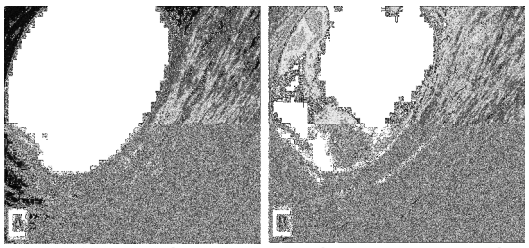

***Fig. 4. (A)*** *Unstained section immersed in water (PP+C 90 days after implantation), showing filament cut perpendicularly, involved by collagen fibers with various brightness intensities. Birefringence is revealed by brightness contrasted with a dark background.* ***(B)*** *Same image, after compensation, in which collagen fibers appear dark. Thin fibers are observed in different directions. In this case, the optical retardation of birefringence was 50 nm. 20x1.*

## DISCUSSION

This study hypothesized that the presence of collagen complexed with polypropylene mesh elicited a mesenchymal response, remodeling connective tissue response. The theory was based on findings that collagen molecules are self-assembled, constructing a supramolecular ultrastructure with records resulting from the order of its molecular residues and molecular configuration [10, 11, 12].

Thus, this experiment was based on reports that type I collagen, and even other types of collagen, contain amino acid residues that have cellular receptors. A frequently cited sequence such as arginine-glycine-glutamic acid, RGD, is bound to cell membrane receptors, triggering cytoplasmic modifications by actin, activating gene activities or even releasing physiologically active molecules. This occurs with platelets bound to collagen that rupture and release fibroblast growth factor and vasoactive substances. Binding of platelets to collagen has been used for clinical testing of platelet binding [10, 11, 12]. In the present case, it was observed that vascular proliferation was more intense in implants containing collagen, in addition to signs of early collagen remodeling.

The acquisition of collagen gel used in this study was based on the principle of spontaneous self-aggregation of macromolecules, which ensures a typical supramolecular organization, when fragments of original molecules are submitted to certain physical and chemical conditions. Regarding collagen, attempts at ordered fibrillogenesis arising from the solubilized molecule, were previously described. However, a structure similar to the original was not obtained [21]. Based on this principle, Vidal in 1995, first described the acquisition of molecules with supramolecular arrangement similar to tendon collagen, following prolonged dialysis with distilled water. In this process, water molecules formed hydrogen bridges with collagen molecules, facilitating fibril movement and reorganization of a helicoid structure similar to tendon fibril structure. The author also observed that early interruption of dialysis resulted in fiber bundles with irregular diameter and orientation [10]. In this same study, the author objectively showed that elasticity is directly proportional to duration of collagen dialysis. Elasticity reaches its peak between five and seven days.

To design and execute this research study, increased biocompatibility of propylene mesh would be achieved when tissue interface showed: higher values of Maximum Load in the biomechanical study; reduction in the intensity and persistence of acute and chronic inflammatory process, in addition to formation of

fibrosis, granuloma and foreign body reaction in the anatomopathologic study; and increase in collagen fiber deposition and improved collagen supramolecular organization, assessed by anisotropic properties (polarization microscopy).

Experimental models similar to that proposed by the present study have been used to evaluate graft and prosthesis integration. The choice of mesh implant in the subcutaneous rat tissue is commonly observed in the literature and proved to be quite adequate for the present study [22, 23, 24]. We also considered that the model described was appropriate for research purposes, since it was a paired study and the animal was its own control. Furthermore, the effects of individual variability in the process of mesh integration could be adequately controlled.

The biomechanical properties of different types of mesh are usually stated in their kits by manufacturers. However, mesh behavior in the recipient bed is still unknown. In the literature, there are various studies addressing these properties [3, 25, 26]. Nevertheless, those studies have analyzed material separately or in material restored after experimentation, not at the implant site, as proposed in the present study. After publication of an unedited methodology by our laboratory [14, 15], an experimental model intended to analyze biomechanical mesh properties in the tissue interface became known. The model assessed mesh adherence to neighboring tissues and was applied in this study. Based on our data one can infer that mesh coated with purified collagen gel will probably have lower mobility when implanted. It is postulated that micro movements in the tissue interface can determine a chronic inflammatory process and predispose to integration defects, including implant extrusion and exposure. Since patients are encouraged to walk soon after surgery, an initial appropriate primary tissue fixation could lead to a better clinical result.

An association between collagen and polypropylene mesh as a protective factor for permanent material, reducing the tendency towards encapsulation and peri-implantation inflammatory reaction efficient at decreasing mesh exposure was successfully demonstrated in experimental studies [6, 7]. Another important characteristic of the presented collagen gel is its capacity to be rapidly and completely degraded after implantation [7]. In a previous study, it was demonstrated that the integration process of polypropylene mesh extends to later phases [17]. However, since the aim of the current experience was to assess the behavior of purified collagen gel that is replaced in the initial phases, the use of an initial study group was justified (7$^{th}$ postop). This allowed us to monitor the replacement of gel complexed with mesh by native collagen [12]. Confirming the study hypothesis, we observed that collagen gel complexed with polypropylene mesh was rapidly removed from the implantation site. Thus, it acted as a mold for new collagen formation, modulating subsequent phases of tissue repair and preventing the development of granulomatous process surrounding the polypropylene. Provenzano and Vanderby, stated that the mechanical behavior of connective tissue is determined by collagen composition and organization. Immature tissue is more disorganized than mature tissue, i.e. collagen fibers are disordered with several proteoglycan bridges among them. Mature tissue is more organized, continuous, has few crosslinking, resulting in greater resistance [27]. Huiling et al., showed that the more organized the collagen fibers, i.e. more oriented, the higher the birefringence and the greater the resistance [28]. The characteristics of collagen deposition observed in the present study by birefringence analysis, permit us to affirm that purified collagen gel was replaced mostly by collagen newly formed by the host.

We also observed that purified collagen gel associated with polypropylene triggered the formation of scarring tissue which did not tend to encapsulate and minimized the formation of foreign body giant cells. Furthermore, a tendency towards an earlier resolution of the local inflammatory process, absence of necrosis and local infection observed in this association, could contribute to an effective decrease in local exudation and vaginal mesh exposure. We considered that such biological behavior added potential advantages to the use of collagen gel, qualifying this proposal for future clinical studies.

Thus, the search for hybrid materials, i.e. implants that have absorbable or definitive components emerge in the medical literature as an alternative to minimize the complications associated with the use of synthetic mesh.

## CONCLUSION

In conclusion, monofilament polypropylene mesh coated with purified collagen gel, when implanted in the tissue interface of the abdominal wall of adult rats increased adherence of the mesh to neighboring tissues; caused a less intense and persistent lymphocyte, plasma cell and granulomatous reaction; and promoted a higher birefringence level of collagen fibers, reflecting improved molecular organization of newly formed collagen and a positive remodeling action in mesenchyme repair involving polypropylene mesh.

## CONFLICT OF INTEREST

None declared


## ACKNOWLEDGMENTS

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