Modulating the inflammatory response to provide the best environment for healing in the pelvic organ prolapse (POP) repair
A preliminary study using coated medical devices.

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Modulating the inflammatory response to provide the best environment for healing in the POP repair. A preliminary study using coated medical devices.

AIM: *We studied the inflammatory response in Phosphorylcholine (PC)-coated and uncoated meshes after 4 weeks of implantation in the subcutaneous tissue of the hypogastric region in six patients.*
MATERIAL AND METHODS: *Six patients underwent POP surgery using two different types of mesh. In three of them a PC-coated mesh was implanted and an uncoated one was implanted in the last three. A small part of the mesh has previously been cut with a standard size decided by the authors and was subsequently implanted in the same time of the pop repair in the subcutaneous fascia of the hypogastric region. After 4 weeks the small part of the mesh was explanted and tissue growth in the fishnet-like mesh was analyzed.*
RESULTS: *A typical foreign body response formed around the uncoated meshes. On the other hand there was a lack in the inflammatory response around the PC-coated mesh identifying less histiocytes, less giant cells and a thinner fibrous capsule formation.*
DISCUSSION: *PC polymers have demonstrated excellent biocompatibility and hemocompatibility. The adsorption of protein onto materials' surface and the trauma involved in surgery necessary for device implantation determines an inflammatory response. The ability of the PC coating to reduce the extent of nonspecific proteins, modulates the specific environment around the implant.*
CONCLUSION: *This preliminary study showed that modulating the inflammatory response we attempt to provide the best environment for healing.*

KEY WORDS: Inflammatory response, Pelvic organ prolapse, Phosphorylcholine-coated mesh

Introduction and Objectives

Pelvic Organ Prolapse (POP) results when the normal supporting structures of the vagina deteriorate. Patients experiencing symptoms associated with POP may be considering surgery as a treatment option. Whereas traditional surgery for POP using patients' existing tissue to reinforce the prolapsed organs has historically been associated with high rates of prolapse recurrence, synthetic (non-absorbable) and biological (absorbable) meshes were introduced into surgery as supporting materials in the surgical treatment. The use of the synthetic transvaginal mesh in the POP repair has spread widely and recent years have been marked by research for more suite mesh material for this type of surgery. Significant efforts were made in order to recognize new kits of meshes in order to minimize the adverse effects deriving from the mesh implant.

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Human species has developed a very complex and efficient set of mechanisms in order to detect and protect against any foreign body. Thus only few materials can be used without problems when placed within the hostile environment of the body. The chemistry research has focused on the improvement of the so called biomaterials by modulating the interface where tissue meet them. In the late 1970s the connection was first made between the cell membrane studies and biocompatibility [1].

One of these approaches was to modulate the tissue implant interface by coating the mesh of phosphorylcholine (PC) based polymers. The PC-coat was designed in order to reduce the foreign body response to the mesh. PC polymers are analogs of the phospholipids present on the outer leaflet of the red blood cell membranes and have shown in different studies to reduce protein adsorption [2,3] They have demonstrated excellent biocompatibility and hemocompatibility [4], so the body's ability to recognize the implants as foreign is reduced. This have been shown in the work of Defife et al [5] who have demonstrated the reduced adhesion of inflammatory cells on PC-polymers in vitro.

The improved biocompatibility of this polymer reducing proteins and lipids adsorption has proved to be highly successful in the healing response as well as the integration of the device in the body. After implantation of the mesh, fibroblast cells are often recruit to the site, stimulated by cytokines produced during the inflammatory response [6]. These cells adhere to the surface often forming a fibrous capsule around the device [7] the extend of which is largely dependent on the material's surface properties [8].

The objective of this paper is to show the inflammatory response in phosphorylcholine PC-coated and uncoated meshes after 4 weeks of implantation in the subcutaneous tissue in six patients who underwent POP repair.

Material and Methods

After receiving the ethical approval for our study, we performed a written informed consent for the patients we engaged. The patients did not differ significantly in age, degree of prolapse, associated medical problems and previous surgery. None of them referred chronic inflammatory disease of any type.

Six patient with POP of the anterior and apical vaginal compartment underwent transvaginal surgery. The same surgical procedure was performed by the same surgeon. All patients had prophylactic treatment with antibiotics and usual non-steroidal anti-inflammatory drugs were used for the pain at the first day after surgery. None of them had treatment with cortisone drugs neither before nor after surgery.

The meshes used in all patients had the same pore size and the same mechanical properties such as resistance, pliability, elasticity and ductile qualities. The type of tissue structure was woven and the type of fiber used was a multifilament one. The only difference between the meshes was that three of them were PC-coated. A small part of the mesh was previously tailored by the surgeon from the principal one in a rectangular fashion during the time of surgery. It's size was decided arbitrarily from the authors (1cm x 1cm). After the transvaginal time was completed, a small incision was performed in the subcutaneous fascia in the right hypogastric region. The small part of mesh was implanted and steri strips was used instead of the suture to close the skin. The patients were discharged on the fourth day after surgery. After 4 weeks all the patients underwent a local anesthesia in the site of the hypogastric region where the small part of mesh was implanted. The small part of mesh with the surrounding tissue was explanted and the tissue was fixed in a usual buffered formaldeide solution.

Transverse sections were cut through the explanted part taking care not to remove the fibrous capsule. These sections were stained using standard hematoxylin and eosin. Color images of the histology slides were taken using a light microscopy vision. Fibrous capsule was measured using scion image analysis software. Point selected for measuring the capsule thickness were three random locations making a total of six readings. The average thickness was calculated later. The area showing the cellular infiltrate around implants including the fibrous capsule was selected for the inflammatory measurements. Quantifications was performed by counting particles at a set threshold of 80. Each sample measurement was reported three times and the average count of all three readings was the mean inflammatory cell count sample.

Results

Some of the histology slides are reported respectively (Figg. 1, 2, 3). A normal healing course was observed for the hypogastric implants and for the transvaginal site. After the mesh explant the cellular infiltrate was selected for inflammatory measurements taking care of the areas presenting the fibrous capsule. In order to have no influence from the pathologist we did not reveal her the PC-coated explants. The inflammatory response of the uncoated mesh is shown in Fig. 1. There is a typical foreign body response around the uncoated mesh with histiocytes, giant cells and fibrous capsule formation. The measurements demonstrated a fibrous capsule thickness varying from 1,6 mm till 2.07mm. The inflammatory response of the PC-coated mesh is shown in Figg. 2 and 3. The pathologist confirmed us a lack in the inflammatory response identifying less histiocytes, less giant cells and a thinner fibrous capsule formation. The measurements demonstrated a fibrous capsule thickness varying from 0,29 mm- 1,09 mm of fibrous capsule thickness in the PC-coated meshes. In the PC-devices there were more eosinophils and granulocytes cells and neoangiogenesis resulted more vigorous.

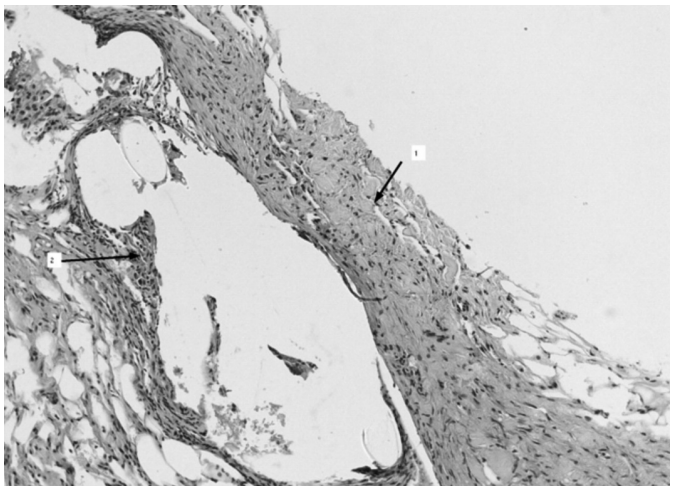

Fig. 1: Light micrographs of hematoxylin and eosin stained sections on PC-coated implant. (1) arrow neoangiogenesis. (2) Giant cell.

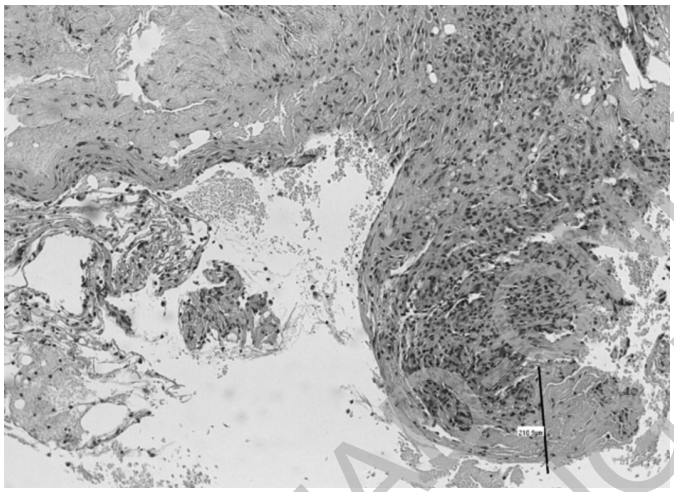

Fig. 2: Light micrograph of hematoxylin and eosin stained section showing fibrous capsule thickness in uncoated mesh.

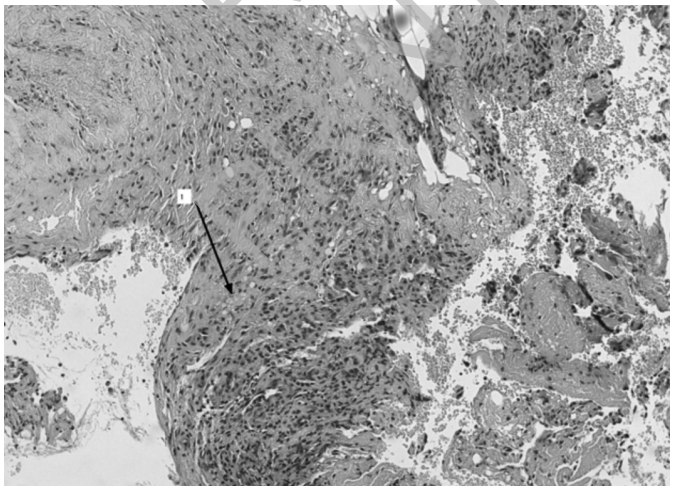

Fig. 3: Light micrograph of hematoxylin and eosin stained section showing giant cell in a pc-coated mesh.

Discussion

Over time in response to an increasing demand in the surgical community, surgical mesh kits continue to evolve adding new insertion tools, tissue fixation anchors, absorbable and biologic materials. The introduction of biomaterials and their use in clinical practice is in continuous evolution. Furthermore the developments in tissue engineering are promising, from cell cultures to the in-vitro prefabrication of composite tissues [9]. Quite interestingly results are obtained in using synthetic polymers for a suitable cell adhesion and growth support [10].

We tried to identify the inflammatory response to PC-coated meshes and uncoated devices currently used for POP repair. PC polymers have demonstrated excellent biocompatibility and hemocompatibility [4]. One of the first responses to take place following implantation of a medical device is protein adsorption onto the surface of the material [11-13] .The adsorption of these proteins seems to be dependent on the surface properties of the material [14,15]. Once adsorbed proteins denature and expose binding domains [16] which are recognized by receptors located on the surface of cells. As a consequence of the adsorption of protein onto materials' surface and the trauma involved in surgery necessary for device implantation an inflammatory response is initiated. Proteins that contact surfaces modified by PC polymers have shown to maintain their natural conformation and do not adhere irreversibly [17] it is therefore surning that a lower inflammatory response may be expected as adhesion of platelets and macrophages are also reduced. This is somewhat supported by the work of Defife et al [5] who have demonstrated the reduced adhesion of inflammatory cells on PC-based polymers in vitro. Even there were included only six patients, the observation from the entire study was that in all assays performed showed fewer inflammatory cells adhered to the PC-coated samples compared with the uncoated controls. The well-reported ability of the PC coating to reduce the extent of non-specific proteins.

The sample of mesh implanted in the subcutaneous tissue was previously prepared. The size of the sample was arbitrarily chosen but in our practice we usually modify the principal mesh.

The timing of explant was decided after consulting data already existing in literature. We have found different study which compare PC-coated materials inflammatory cell response in vivo and in vitro. Goreish et al reported that PC coating offers a simple surface treatment that is incredibly resistant to the adhesion of inflammatory cells in vitro [18]. The "in vivo" response was studied at different timing. According to the literature data we have chosen a 4 weeks time implantation. Granulation tissue which is formed from blood vessels and fibroblasts a few days after injury functions to restore the damaged tissue (with parenchymal cells of the same type). If this reconstruction has not taken place then connective tissue as

fibrous encapsulation formation occurs isolating the implant from the surrounding environment. Fibrous capsules with variable thickness were observed. The capsule thickness was slightly less around tissue implanted with PC coated meshes. It is known that capsule formation can continue over extended periods and is dependent on factors such as the site of implantation and the chronic effects of the implant material on inflammation. Indeed, a progressive increase in fibrous capsule thickness for a period up to 20 months after subcutaneous implantations in rats has been reported [12]. Yet the observation from this study indicates that despite the known biocompatibility of the control out of the three implant materials studied, the coated mesh with the PC polymer performed better than the uncoated ones in terms of inflammatory response and fibrous capsule formation after 4 weeks of implantation.

Conclusions

Preliminary data shows that PC-coating polymers can reduce the inflammatory cell interaction with the implants contributing to its long term stability. Perhaps a lack of a vigorous inflammatory response against the implanted mesh can reduce the risk of erosion during the time. On the other hand we should investigate if a reduced fibrous capsule formation around an implanted mesh may contribute in the recurrence of the pelvic organ prolapse. Further data are necessary for a better understanding of the long-term effects of PC-coated meshes in the pop repair.
As awards from lawsuits against reputed manufacturers became known and some companies (eg. Ethicon, Bard) decided to no longer market their mesh products, the light of the recent FDA release dated march 27 2013, on sling tape for SUI (19) and emerging new scientific data. It's evident that in this atmosphere meshes are located in an area of uncertainty [20]. Thus it's our intention of providing a "silent" material which does not trigger an unaffordable host-tissue reaction.

Riassunto

La risposta infiammatoria svolge un ruolo cardine sia nella riparazione tissutale che nelle complicanze mesh-correlate della chirurgia trans-vaginale. L'utilizzo dei biomateriali grazie alla loro capacità biomimetica può modulare la risposta infiammatoria nel tempo. Sei pazienti sono state sottoposte alla correzione del POP per via transvaginale utilizzando due tipi di mesh. Una rivestita con la PC e l'altra no. Una volta completata la riparazione transvaginale una piccola parte della mesh originale precedentemente ritagliata con delle dimensioni standard è stata impiantata nella fascia sottocutanea della regione ipogastrica. Dopo 4 settimane in anestesia locale è stata espiantata la parte di mesh dalla tasca sottocutanea della regione ipogastrica ed il tessuto che lo inglobava è stato analizzato.
La risposta infiammatoria attorno alla mesh rivestita da fosforilcolina risultava ridotta rispetto alla mesh non rivestita attorno alla quale si era formata una tipica risposta da corpo estraneo. Su questi risultati si può affermare che modulando la risposta infiammatoria a lungo termine si possono ridurre le complicanze dovute ad una risposta infiammatoria abnorme e si propone il loro utilizzo per verifiche in casistiche più ampie.

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