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Evolution of the mechanical properties of a medical device regarding implantation time

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ABSTRACT

Background: Our study aimed at understanding the influence of healing time on the mechanical properties of meshes used in pelvic organ prolapse, once implanted in an animal model using the rat.
Methods: A standard polypropylene mesh was implanted in 42 rats in order to evaluate the mechanical properties of the implanted mesh. Explantation occurred at 1, 2, 3, 4 and 5 months and mechanical tests were performed. Each sample was mechanically evaluated by a uniaxial tensile test with a machine (BIOTENS). Biological tissues presented a nonlinear relation between stress and strain so it could be modeled by the 2 parameters C_0 and C_1 of a second-order Mooney-Rivlin law.
Results: The rigidity in small deformation might not be affected by healing time or the presence of the synthetic implant. On the contrary, changes seemed to occur on the stiffness in large deformation (C_1). The stiffness with the mesh composite changed with healing time. The “two-month implantation” rat group was significantly more rigid than the two control groups ($p_{control/2months} = 0{,}04$ and $p_{placebo/2months} = 0{,}04$). The 2- and 3-month healing groups were significantly more rigid than the 1-month healing group ($p^{1/2months} = 0{,}01$ and $p^{1/3months} = 0{,}003$). After 2 months, the mechanical properties seemed to stabilize ($p^{2/3months} = 0{,}44$, $p^{2/5months} = 0{,}16$ et $p^{3/5months} = 0{,}3$).
Conclusion: In order to evaluate the mechanical properties of an implanted mesh, the optimal time for explantation seems to be 2 months. Once this period is over, a more physiological mesh will be developed in order to be similar to native vaginal tissue once implanted and colonized by scar tissue.

Introduction

Success of surgical meshes in general surgery, combined with the high failure rates of traditional native tissue repair, has led gynecologic reconstructive surgeons to use mesh materials [1]. The use of synthetic meshes for the treatment of prolapse started in the 1990s. As the feasibility was confirmed by some early studies [2], the use of these synthetic meshes became quickly popular [3]. The use of prostheses brings about specific complications that are not without any consequences [4]. Because of these complications, several warnings from the Food and Drug Administration proved to be necessary [5]. We think that these complications are caused by unsuitability between the mechanical properties of the prolapse mesh and mechanical properties of the vaginal tissue because the first synthetic meshes were used for hernia repair [6]. The abdominal wall role is crucial: it protects and maintains the abdominal cavity. On the contrary, genital organs have a physiological large mobility, which is essential for the rectal, urinary and sexual functions [7]. A mismatch between the mechanical characteristics of the mesh and the pelvic tissue seems to contribute to the occurrence of specific complications with meshes [4]. Before commercialization, IUGA change to recommended the undertaking of in-vivo tests since meshes are still mostly evaluated ex-vivo, *i.e.* in a "dry" condition (in opposition with explanted meshes) [8]. Indeed, studies of dry meshes currently neglect the evolution of the mechanical properties of implanted mesh related to scar tissues after implantation. When the mesh is implanted, it forms a composite with scar tissue. The mesh should be designed according to the mechanical properties of the targeted, non-pathologic, tissues. Our study aimed at offering a new experimental protocol on rat model,

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studying the influence of healing time on the mechanical properties of the explants, in order to determine the necessary time for the explants (prosthesis and scar tissue) to have stable mechanical properties.

Materials and methods

Experimental animals

A total of 42 male Wistar rats were used in this study, which obtained ethics committee approval n° 3061. Animals were housed with a 12-h alternating light–dark cycle and provided free access to food and water. Seven groups of six rats were composed. Five groups were implanted with meshes and we created two other groups: a control group (6 rats) that received no surgery and a placebo group (6 rats) that received a surgery (dissection of the abdominal fascia but without implantation meshes). Mechanical tests were performed on raw mesh, before implantations started.

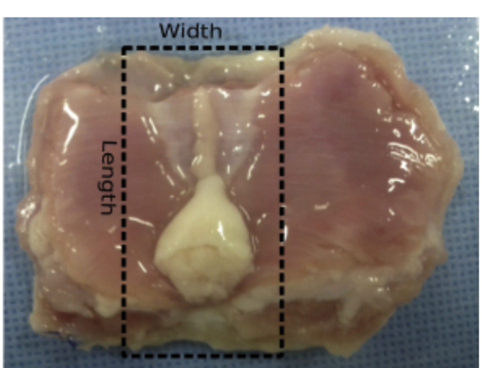
Mechanical tests

Prosthesis

For this study we used a Type I polypropylene monofilament prosthesis, measuring 80 μm in diameter (Amid Classification). This polypropylene monofilament mesh was macroporous because this remains the “gold standard” for pelvic prolapse mesh. The prosthesis we chose to implant had previously been the subject of a pre-clinical study and its choice was discussed between doctors from the *CHRU of Lille*, mechanical engineers, and textile manufacturers. No information on mechanical properties of the prosthesis can be disclosed for reasons of data confidentiality. Mechanical testing was performed on each collected specimen. The influence of healing time was studied: explants were taken off at zero, one, two, three, four and five months of healing. “Control group” rats were sacrificed from the beginning of our study on M0 (month zero). Drawing on a study by Röhrnbauer and Mazza [9], we modelled the explanted composite by a composite made of elastomer and our textile. The sample size was decreased from 20 cm long and 5 cm wide (standard testing dimension of a textile) to 3 cm long and 2 cm wide without any mechanical differences. Beyond those dimensions, the size of the sample is no longer representative (Picture 1). For that reason, we implanted 7-cm long and 5-cm wide prostheses, which took into account the above data.

Mechanical tests

Each sample was mechanically evaluated by a uniaxial tensile test with a machine (BIOTENS). We designed for biological tissue testing provided with a 100 N load cell. Displacement rate was set at 5 mm/min. The stress and strain were computed. Stress is the force per section unit: $\sigma = F/S_0$, with F effort in Newton (N) and S_0 the initial cross-section in mm^2. Strain is computed with the measure of displacement: $\lambda = 1+(L/L0)$, L is the current displacement and L0 the initial length between the grips. Biological tissues presented a non-linear relation between stress and strain (Fig. 1). Previous studies [9] showed that it can be modelled by the 2 parameters C_0 and C_1 of a second-order Mooney-Rivlin law. C_0 represents the rigidity in small deformation and C_1 the rigidity in large deformations. This study protocol has been used and approved on ewe tissues and on connective human tissue [10].



Picture 1. Localization of the prosthesis sample in the abdominal specimen.

Surgical technique

Implantation

After the rat was asleep, its abdomen was shaved. An injection with buprenorphine was given for local pain treatment. A median vertical abdominal cutaneous incision of 5 cm was performed. The abdominal fascia was then dissected sufficiently to place the mesh. The mesh was fixed to the abdominal fascia and muscles in the 4 corners with a single suture of a non-absorbable monofilament suture (Prolene 2/0, Ethicon, Johnson & Johnson®). The skin was sutured using single points of an absorbable suture (Polysorb 2-0, Covidien®).

Explantation

After induction box, the rat was sacrificed using a 2 mL lethal intracardiac injection of T61. The median incision was re-performed, and a dissection of the mesh-tissue complex was performed. After total liberation of the mesh from the skin tissue, a full-thickness resection of the abdominal wall (mesh + fascia + muscle + peritoneum) was performed. Thereafter, the resected tissue was immediately placed in physiologic water for transport to the place where the mechanical testing was performed.

Statistical analysis

The data from the explanted mesh-tissue composite were compared. A Kruskall-Wallis test followed by a post-hoc Mann-Whitney *U* test was performed with R. A p-value less than 0.05 was considered significant.

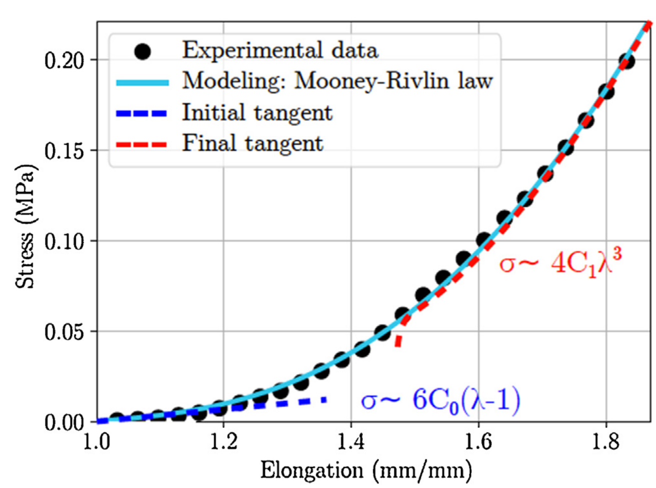


Fig. 1. Illustration of the bilinear behaviour of a biological tissue (example shown with a sample of rat abdominal wall).

Results

We observed four deaths in our study (9,52%) and mesh exposure concerned 5/28 (17,85%) rats and we were not able to get information on the two deceased rats because the animal facility got rid of them before we could observe their abdominal wall (Table 1).

The abdominal wall was tested for each group with meshes. However, we were at least able to test four samples in each group, except for the group with implantation at four months in which we could only test two samples. The reason was that it made it impossible to extract mechanical data from the prosthesis as is it was either too retracted or too exposed, or even because of the death of the animal. So, we could not present the four months implantation results because of the small number of animals in this group (two rats).

The results of the mechanical tests are summarized in Table 2.

Firstly, we did not find significant differences in the C_0 coefficients at 1, 2, 3, 5 months in control or placebo groups. The rigidity in small deformation might not be affected by healing time or the presence of the synthetic implant. On the contrary, the stiffness in large deformation (C_1) is affected by healing time. The influence of healing tissue was studied in 2 control groups. There was no significant difference between control and placebo groups for C_0 and C_1 coefficient ($p_{control/placebo}$ = 0.93 and $p_{control/placebo}$ = 0.58). Then we noted $p^{i/jmonths}$ the p-value comparing the group months of healing to the j months group. The 2- and 3-months healing groups were significantly more rigid than the 1-month healing group ($p^{1/2months}$ = 0,01 and $p^{1/3months}$ = 0.003). After 2 months, the mechanical properties seemed to stabilize ($p^{2/3months}$ = 0.44, $p^{2/5months}$ = 0.16 et $p^{3/5months}$ = 0.3) (Fig. 2).

Discussion

Our study provides considerable information on a minimum implantation period of 2 months before being able to determine the mechanical properties.

Animal models

Several animal models can be used for the study of genital prolapse: rats, mice, pig, rabbits, sheep and nonhuman primates. The aim of our study was to investigate the mechanical behaviour in-vivo of the prosthesis made of scar tissue. We were able to demonstrate that it was necessary to work with a minimal prosthesis size of 3 x 2 cm in order to obtain significant mechanical data [11]. Hence, it was not appropriate to use pig, ewe or non-human primates. Rat, mice or rabbit were the possibilities left to us. The rat is an animal model that presents several advantages, on the economical (low cost of purchase) and practical (small, storage, maintenance and sacrifice) levels as well as from and from the point of view of durability [12]. We therefore chose the rat for all the reasons above-mentioned but also because it is a widely used animal model for the understanding of genital prolapse [13].

Table 1
Clinical complications.

Explantation time	Number of rats	Clinical complications :		
		Mesh exposition	Death	Shrinkage
1 month	6	**1**	0	**1**
2 months	6	0	0	**2**
3 months	6	**1**	0	**1**
4 months	6	**3**	**1**	0
5 months	6	0	**1**	**1**
Control Group	6	0	**1**	0
Placebo	6	0	**1**	0
Total	42	5/28	4/42	5/28
Results (%)		17,85**%**	9,52**%**	17,85**%**

Table 2
Evolution of C_0 and C_1 according to the time of implantation.

	Median	Q1	Q3	Q3-Q1	mean	SD	95 % CI
C0 (MPa)							
One month	0,006	0,004	0,008	0,004	0,004	0,002	0,003200667
Two months	0,005	0,003	0,008	0,005	0,005	0,003	0,004157788
Three months	0,008	0,004	0,01	0,006	0,007	0,004	0,003695811
Four months	0,005	0,004	0,006	0,002	0,005	0,001	0,001600333
Five months	0,006	0,006	0,007	0,001	0,006	0,002	0,00392
Control	0,004	0,002	0,005	0,003	0,004	0,002	0,002095328
Placebo	0,004	0,001	0,007	0,006	0,005	0,003	0,00339482
C1 (MPa)							
One month	0,016	0,015	0,023	0,008	0,02	0,008	0,012802666
Two months	0,032	0,029	0,037	0,008	0,036	0,012	0,016631151
Three months	0,039	0,031	0,052	0,021	0,046	0,024	0,022174869
Four months	0,032	0,03	0,034	0,004	0,032	0,004	0,011087434
Five months	0,058	0,048	0,064	0,016	0,05	0,015	0,0294
Control	0,023	0,018	0,03	0,012	0,027	0,015	0,015714961
Placebo	0,02	0,016	0,029	0,013	0,022	0,01	0,011316065

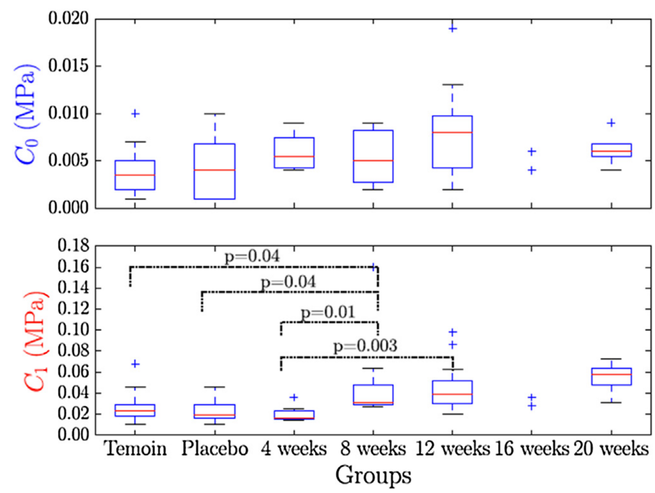


Fig. 2. Representation of the median rigidity (interquartile range) in small (C_0) and large deformation (C_1) for control groups and different healing time (4, 8, 12, 20 weeks). Temoin = Control.

Healing time

Healing process is a phenomenon that occurs over several weeks and that intercedes with various factors. Indeed, after the "acute" phase, there comes a chronic phase in which the definitive scar tissue appears. These different phases involve crucial components for the formation of a definitive scar tissue in response to a foreign material [14,15]. We think it is essential, in a research protocol for the design of a new prosthetic material, to take account of the healing process, and therefore the in-vivo evolution of the prosthesis, to retrieve the mechanical properties. The study on the animal model appears therefore an essential step before the commercialization of new prosthetic implants, in order to prevent withdrawal of some devices by laboratories after successive recalls from the Food and Drug Administration [8]. This is all the more important because the prosthesis used in genital prolapse corrective surgeries are nowadays the subject of extreme lobbying that can sometimes lead to undermine the use of these materials in some cases.

The comparison of our data with data from the literature is difficult for several reasons. On the one hand, there is a large heterogeneity among animal models used. It is therefore more difficult to provide relevant results when we compare mechanical properties between two different animal models. Currently, there is no comparative data on the mechanism (cellular, histological, immunohistochemical) involved in the healing process between the various animal models used.

On the other hand, our study relies on a biomechanical law (Mooney-Rivling Law), which is not used by the other teams in this particular field. Previous studies published in literature on human pelvic tissues have highlighted that pelvic tissues (bladder, rectum, vagina, ligament) could behave according to that law. Hence, our study was based on this law and the comparison between coefficient C_0 and C_1. In light of these elements, it is therefore more difficult and less relevant to compare our data to the one used by the other teams.

To our knowledge, there is only few data in the literature on the impact of healing time on mechanical properties of a mesh in pelvic organ prolapse on rats or on other animal models. Our study is an important source of information because it is necessary to wait a minimum of two months before extracting prosthetic explants in order to study their mechanical properties. There is no comparable study to ours in this area; however, we could find in the literature some studies focusing on explants rigidity at various implantation times, without specific mentions of the healing process.

Hernandez Gascon et al. [16] with rabbit model demonstrated that prosthesis rigidity was more important after 180 days of implantation compared to 3 months and 2 weeks of implantation without observing any statistically significant differences between 3 and 6 months. Nevertheless, the rigidity of the meshes was still evolving after 6 months. Melman et al. [17], on pig, did not observe any statistically significant differences concerning rigidity after implantation of two meshes for 1, 3 and 5 months. In Ulrich et al. [18], explant rigidity was not the same according to the various times of implantation, with a more important rigidity at 3 months than at one week, and a bi-linear curve at 3 months that is corroborated in our study. This data was also highlighted in Konstantinovic et al. [19] who studied the evolution of mechanical properties of prostheses at 1 week, 2 weeks, one month and 4 months. Finally, O Sullivan et al. [20] also determined a 3 months integration period of the prosthesis in the abdominal wall of the rat. To ensure that 2 months are required, we extended the healing durations up to 5 months. We think that our animal model, which is different from other animal models, can explain our results.

The overall of the studies highlights that explant rigidity increases with time, without showing any statistically significant differences. Our study therefore brings complementary information on the study of mechanical properties of explants in the rat. Hence, in regard of the overall results, we can estimate that it is necessary to wait a minimum of two months before extracting prosthesis to study its mechanical properties.

Complications

Regarding the rate of exposure of the prostheses on the animal model, it is estimated of 16.66% in our study. We have very little literature data on the rate of exposure for rats. This element is not mentioned in most of studies using the rat as an animal model. This can be due to a too short implantation time (less than 3 months) in the various research protocols [21–23]. Regarding other models, we notice very heterogeneous rates of exposure between 10%–30% for De Tayrac et al., Manodoro et al. or Endo et al. [24,25]. This heterogeneous rate of exposure certainly depends on the type of prosthesis implanted, the implementation location and finally the animal model used. We can see that we have, with this animal model, a fairly satisfactory rate of exposure with respect to the rates reported in the literature; however this rate of exposure shall be carefully interpreted because it would need to be realized individually, in every implantation group, in order to determine a significant difference.

There are two main shortcomings in our study. On the one hand, the small number of animals within the groups can limit the interpretation of our results. We were limited in the number of animals for this preliminary study because the project contains three other trial groups with a limited number of animals that we had to respect for ethical reasons. On the other hand, we did not make any histological descriptive study to explain this increase and then stabilization of the- rigidity of implants. We cannot establish any reason for the gradual increase. It was already the case in other studies.

Conclusion

A minimum period of two months seems necessary in order to retrieve the mechanical properties for the design of prosthetic devices. This period takes account of the healing process, resulting in the integration by the host of this foreign material. The data we provide in this article corroborate those from the literature regarding minimum time of implantation 2 to 3 months that is necessary for mechanical properties of prostheses to stabilize. Such minimum time of implantation to be fulfilled before any study of the mechanical properties of prostheses essential because it would undoubtedly make it possible to design more physiological medical devices (taking into account the process of healing particular and thus reduce the risks of complications related to these prostheses.

Declaration of Competing Interest

Michel Cossson: Disclosures for teaching sessions, expert meetings and round table : FRESENIUS, ALLERGAN, BOSTON SCIENTIFIC.

Guillaume Doucède, Annie Morch, Pauline Lecomte-Grosbras, Mathias Brieu and Chrystèle Rubod : None

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