Electrospun nanofiber mesh with connective tissue growth factor and mesenchymal stem cells for pelvic floor repair: Long-term study

Sofie Husted Laursen1 | Signe Gellert Hansen2 | Mehmet Berat Taskin3 | Menglin Chen4 | Lise Wogensen5 | Jens Vinge Nygaard4 | Susanne Maigaard Axelsen1

1Department of Gynaecology and Obstetrics, Aarhus University Hospital, Aarhus, Denmark
2Department of Medicine, Soenderborg Hospital, Soenderborg, Denmark
3Leibniz Institute of Polymer Research Dresden, Max Bergmann Center of Biomaterials Dresden, Dresden, Germany
4Department of Biological and Chemical Engineering - Medical Biotechnology, Aarhus University, Aarhus, Denmark
5Deans Office Health, Aarhus University, Denmark

Correspondence
Sofie Husted Laursen, Department of Gynaecology and Obstetrics, Aarhus University Hospital, Aarhus, Denmark. Email: shlaursen@post.au.dk; laursen.sofiehusted@gmail.com

Abstract
Pelvic organ prolapse (POP) affects many women, with an estimated lifetime risk of surgical intervention of 18.7%. There is a need for alternative approaches as the use of synthetic nondegradable mesh was stopped due to severe adverse events, and as current methods for pelvic floor repair have high POP recurrence rates. Thus, we hypothesized that electrospun degradable meshes with stem cells and growth factor were safe and durable for the long term in elderly rats. In an abdominal repair model, electrospun polycaprolactone (PCL) meshes coated with connective tissue growth factor (CTGF)/PEG-fibrinogen (PF) and rat mesenchymal stem cells were implanted in elderly female rats and removed after in average 53 weeks (53-week group). Collagen amount and production were quantified by qPCR and Western blotting. Moreover, histological appearance and biomechanical properties were evaluated. Results were compared with previous results of young rats with identical mesh implanted for 24 weeks (24-week group). The 53-week group differed from the 24-week group in terms of (1) reduced collagen III, (2) strong reduction in foreign body response, and (3) altered histological appearance. We found comparable biomechanical properties, aside from higher, not significant, mean tissue stiffness in the 53-week group. Lastly, we identified mesh components 53 weeks after implantation. This study provides new insights into future POP repair in postmenopausal women by showing how CTGF/PF-coated electrospun PCL meshes with stem cells exhibit sufficient support, biocompatibility, and no mesh-related complications long term in an abdominal repair model in elderly rats.

KEYWORDS
biodegradable mesh, connective tissue growth factor, mesenchymal stem cells, pelvic floor repair, pelvic organ prolapse, tissue engineering

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2022 The Authors. Journal of Biomedical Materials Research Part B: Applied Biomaterials published by Wiley Periodicals LLC.
Pelvic organ prolapse (POP) is the descent of pelvic organs into or within the vagina. POP is a common condition, with incidences from 30.8% to 97.7% in 57- to 84-year-old women. The lifetime risk at 80 years of age for POP surgery (pelvic floor repair) is estimated at 18.7%. Unfortunately, pelvic floor repair has a risk for reoperation in the same compartment at 11.5%. Therefore, it is of great clinical importance to develop safe and long-lasting surgical procedures for pelvic floor repair. Furthermore, the demographic development of age will increase the need to reinforce weak tissue in elderly women with POP recurrence.

A wide range of surgical procedures are and have been used for pelvic floor repair. In 2010, more than one third of pelvic floor repair operations were mesh-based, using nondegradable biomaterials. In 2019, transvaginal, synthetic pelvic floor repair were removed from the American market by the Food and Drug Administration (FDA) due to lack of safety and effectiveness. Still, one to 3 years after surgery, a meta-analysis of 37 randomized controlled trials demonstrated a reoperation rate due to recurrent prolapse to be lower in mesh-based approaches than in native tissue repair. However, compared with the native tissue repair group, in total, more women in the mesh group were reoperated due to mesh extrusion in the vagina. Across studies, the risk of reintervention due to mesh complication is common. Altogether, long-term, mesh-based pelvic floor repair could have some advantages, especially in preventing recurrent prolapse. However, the level and severity of complications seen in several studies are unacceptable.

New approaches have intended to improve the performance and safety of meshes by using other materials. Because mesh-related complications are the biggest drawback of synthetic, nondegradable meshes, degradable meshes could be an alternative. One material being explored for degradable meshes is polycaprolactone (PCL). PCL can be formed into extracellular matrix (ECM)-like micro/nanofibers using the electrospinning technique. These electrospun meshes can be engineered to have mechanical properties comparable to pelvic connective tissue, and in in vitro studies, vaginal fibroblasts from POP patients adhered to electrospun PCL matrices, showed better survival, and produced an ECM on these electrospun PCL matrices. Furthermore, hydrogels with their hydrophilic three-dimensional networks also mimic ECM structure, and hydrogels made of PEG-fibrinogen (PF) enable cell attachment and spreading. However, alone, PF hydrogels have low mechanical strength, and as they degrade rapidly, they only offer mechanical support initially. On the contrary, PCL has a slower degradation time. Therefore, combing degradable materials, electrospinning, and specifically designed hydrogels might offer the scaffold and support needed in pelvic floor repair.

POP tissue is a dysfunctional tissue in several ways. It is biomechanically different from non-POP tissue, which emphasizes the need for external support. Moreover, studies have found a state of acquired fibroblast dysfunction, as well as a net reduction in collagen content. Therefore, to improve the outcome of pelvic floor repair, the process of tissue repair might need to be facilitated, which could be done by adding growth factors and stem cells to the meshes. The use of meshes with mesenchymal stem cells (MSCs) is known to aid the initial tissue formation by increasing neovascularization, cellular infiltration, and collagen deposition, as well as having an anti-inflammatory effect.

The positive immunomodulator effect of stem cells seems important to develop safe and long-lasting surgical procedures for pelvic floor repair. Furthermore, the demographic development of age will increase the need to reinforce weak tissue in elderly women with POP recurrence. In elderly women, two other essential success criteria are weight gain and acquired fibroblast dysfunction, as well as a net reduction in collagen content.

The effects of CTGF and stem cells were further tested in vivo using an immunodeficient mice model. Here, CTGF and MSCs, delivered using the same construct as in our study (CTGF/PF-coated electrospun PCL mesh), reinforced fibrogenesis and angiogenesis compared with cell-free scaffolds 6 weeks after implantation. Later, Hansen et al. showed in a rat abdominal repair model that the group with CTGF/PF-coated electrospun PCL meshes with MSCs had no complications 24 weeks after surgery. Altogether, the use of stem cells and growth factor in mesh-based tissue repair could offer an alternative to today's pelvic floor repair methods.

The main success criterion for POP repair is long durability. To our knowledge, no long-term outcomes of meshes with stem cells (and growth factor) have yet been evaluated. Therefore, in this study, we will evaluate if the zero complication rate after 24 weeks found by Hansen et al. for CTGF/PF-coated electrospun PCL meshes with MSCs continues long term, in the present study defined as 53 weeks. In elderly women, two other essential success criteria are effectiveness and safeness. Therefore, we studied the long-term outcome in rats older than 24 weeks, as the mean age for women undergoing primary POP surgery is around 60 years, and heavy rats, as one of the several physiological changes in aging women is weight gain.

We compare the results of mesh implantation in this elderly group of rats with a “historical control group” in terms of previously published data from Hansen et al., who found promising results using the same type of mesh for 24 weeks in a group of young rats.

2 MATERIALS AND METHODS

2.1 Animals and ethics

Eight female Wistar rats (Taconic Biosciences, Denmark) were housed in cages in a controlled environment. Caretaking and animal experiment facilities were provided by the Animal Facility, Institute of Biomedicine, Aarhus University, Denmark. Procedures of this study were approved by the Danish Animal Inspectorate (permission no. 2016-15-0201-01057), and their guidelines for use and care of laboratory animals were followed. During the study period, the rats were placed in cages with hamster wheel 30 min twice weekly in an attempt to prolong their life span.

2.2 Study design

At an age of 46 weeks, eight female rats had a CTGF/PF-coated electrospun PCL mesh with MSCs implanted. Long-term outcome was
evaluated by comparing with a “historical control group” from a previous study, the SoCTGF-rMSC group from Hansen et al., where younger female rats had the same type of mesh for 24 weeks (24-week group).26

2.3 | Mesh composition

The scaffolds were fabricated according to the earlier works.15,28 Briefly, fiber meshes were fabricated via electrospinning of 12% (w/v) PCL (80 kDa) solution prepared in chloroform/ethanol (v/v). The solution was pumped through a metallic 20 g blunt needle with a flow speed of 8 ml/h, and the extruded polymer solution was collected on the grounded metallic mandrel by applying 20 kV. Obtained scaffolds consisted of randomly distributed fibers with an average diameter of 1.61 ± 0.13 μm.

Each PCL fiber scaffold was then infiltrated with poly(ethylene glycol)-fibrinogen (PF) hydrogel precursor containing 0.1% photoinitiator (made of 10% w/v Irgacure®2959) and 9 μg CTGF. The hydrogel precursor was crosslinked via 10 min of exposure to UV light (365 nm, 4–5 W/cm²). Freshly purchased rat MSCs (Lonza, Switzerland) was cultured and expanded following the company protocols and finally seeded (3rd passage) on the PCL-PF hydrogel coated constructs at a density of 2.5 x 10⁶ cell per scaffold. Cells were further cultured on constructs for 3 days prior to the animal implantation.

2.4 | Surgical procedure

Rats were anesthetized with 2% Isoflurane® mask inhalation. A 5-cm abdominal midline incision was made, followed by subcutaneous blunt dissection, to loosen the skin from the muscle. A full-thickness portion of the abdominal muscle-fascia measuring 1.5 x 3.0 cm was resected lateral to the left rectus muscle. A mesh measuring 2.5 x 4.0 cm was placed directly above, covering the defect and 0.5 cm of the surrounding tissue, and fixated with continuously, absorbable sutures (Novosyn® 3/0). The skin was closed with running, subcuticular absorbable sutures (Novosyn® 3/0). Subcutaneous analgesia and antibiotic prophylaxis were administered 3 days postoperatively.26

2.5 | Euthanasia and tissue sampling

Thrice weekly, the rats were examined for herniation, hematoma, and general well-being. The animals were sacrificed at week 50–60 after mesh implantation. Meshes and 1 cm of adjacent tissue were evacuated en bloc and divided into four sections for further analysis (gene expression, protein content, histology, and biomechanical testing). During the study period, one animal died at age 71.2 weeks, and another animal was sacrificed at age 96.5 weeks; both animals were included in the study. Two animals were sacrificed at age 80.4 weeks and 100.4 weeks due to large tumor growth. Unfortunately, we were unable to remove the meshes, and the two rats were excluded from study.

2.6 | Biomechanical testing

Biomechanical uniaxial tensile testing was performed to evaluate the stiffness and strength of the tissue. We followed our previous methodology.26 Samples were cut into 4-mm-wide strips, and their average thickness was calculated. Afterward, the strips were inserted into clamps modified with emery cloth to secure the grip and mounted with an interclamp distance of 10 mm in a mechanical testing machine (Zwick/Roell Z0.5) with a 100 N load cell. During testing, the strips were kept moistened with 50 mM of Tris/HCl buffer. The clamps moved with a constant speed of 0.333 mm/s. Load (N) and elongation (mm) were recorded until strip failure. The ruptured strip specimens were collected for analysis of total collagen content. The rationale for conducting a uniaxial tensile test is that it mimics the failure mode realized during herniation. From the test data, the ultimate tensile strength (UTS), strain at UTS, and Young’s modulus were calculated.

2.7 | Gene expression and protein content

Collagen I and collagen III gene expression and protein amount were measured by real-time qPCR and Western blotting, respectively.26

2.8 | Histopathology

The tissue samples for histological evaluation were fixed with 4% formalin and embedded in paraffin. The paraffin blocks were cut into sections, and two serial sections from each rat were harvested: one with hematoxylin and eosin (HE) and one with Masson’s trichrome (MT). Images of the specimens were made by the Pathological Institute, Aarhus University Hospital, and the images were viewed with NDP.view 2 software.

The number of foreign body giant cells (FBGCs), the degree of vascularization, the amount of collagen, and mesh remnants were evaluated by microscopy. Specimens from the two groups were blinded prior to evaluation. For each specimen, 10 nonoverlapping areas at 200x magnification were evaluated for FBGCs, as well as blood vessels by counting in HE stains. Moreover, mesh remnant and integration were evaluated at 100x magnification in the HE stains. The amount of collagen was evaluated in the MT stains in five nonoverlapping areas at 50x magnification by estimating the percentage of collagen. Afterward, the general histological appearance of the specimens was examined.

2.9 | Data analysis and statistics

Data are shown as mean ± SEM. An unpaired t-test was used to compare protein amount, mRNA expression, and the histological evaluation between the 53-week group and the 24-week group. One-way ANOVA was used to compare the biomechanical data for the 53-week group with the 24-week group and muscle fascia.
Statistical analysis was done in GraphPad prism 9, p values <.05 were considered statistically significant.

3 | RESULTS

Six rats with an average life span of 99.2 ± 14.3 weeks were included. They had an average end weight of 357.8 ± 27.6 g and an average time of mesh implantation of 52.7 ± 14.2 (≈53 weeks). Several rats developed tumors during the study period (Table 1). None of the rats developed herniation during the study period. Data on the 24-week group from the study by Hansen et al. are also presented in Table 1.

3.1 | mRNA expressions and protein amount

No significant differences in the mRNA expression of collagen I and collagen III or protein amount of collagen I were observed (Figure 1).

| TABLE 1 | Information on life span, weight, time with mesh, and complications for the 53-week and 24-week groups |
|------------------|-----------------|-----------------|-----------------|
| Life span (weeks) | Weight (g) | Time with mesh (weeks) | Complications |
| 53-week group    |                |  |                      |
| Age at mesh implantation: 46 weeks |  |  |                      |
| 106.9            | 367            | 60.3 | Tumor growth related to stomach |
| 71.1             | 335            | 24.9 | Died at week 71 |
| 106.9            | 330            | 60.4 | - |
| 106.9            | 402            | 60.3 | Reoperated at week 80 due to large tumor at one of mammary papillae |
| 106.9            | 341            | 60.4 | Tumor growth at one of mammary papillae |
| 96.6             | 372            | 50.1 | - |
| Average          | 99.2 ± 14.4    | 357.8 ± 27.6 | 52.7 ± 14.2 |
| 24-week group*   |  |  |                      |
| Age at mesh implantation: 13 weeks |  |  |                      |
| 37.1             | 344            | 24.1 | - |
| 37.2             | 309            | 24.2 | - |
| 37.2             | 310            | 24.2 | - |
| 37.2             | 288            | 24.2 | - |
| 37.1             | 292            | 24.1 | - |
| 37.1             | 310            | 24.1 | - |
| 37.1             | 273            | 24.1 | - |
| Average          | 37.1 ± 0.05    | 303.7 ± 22.6 | 24.1 ± 0.05 |

*Data on the 24-week group are from the study by Hansen et al.

(A) mRNA expression: Collagen I (B) mRNA expression: Collagen III (C) Western blot: Collagen I (D) Western blot: Collagen III

Figure 1. Collagen I and collagen III. mRNA expression of collagen I (A) and collagen III (B) relative to GAPDH expression, and protein amounts of collagen I (C) and collagen III (D) as ratio of total protein. *p < .05. Data on the 24-week group is from the study by Hansen et al.
FIGURE 2  Histological assessment. (A,B) From the 53-week group showing; (A) HE stain; foreign body giant cells (FBGCs) marked with arrows, blood vessels marked with ‘V’, and (B) MT stain; estimated percentage of collagen shown. (C) Amount of FBGCs evaluated as an average amount at ×200 magnification. (D) Degree of vascularization evaluated as average amount of blood vessels at ×200 magnification. (E) Collagen content evaluated as percentage of collagen. ****p < .0001. Evaluated specimens of the 24-week group is from the study by Hansen et al.26

FIGURE 3  Main histological categories; high foreign body giant cell (FBGC) response, low FBGC response, high vascularization and abscess formation, and their appearance in the two groups. Images of high FBGC response and abscess formation from the 24-week group, and images of low FBGC response and high vascularization from the 53-week group. Evaluated specimens of the 24-week group is from the study by Hansen et al.26

<table>
<thead>
<tr>
<th></th>
<th>24 weeks</th>
<th>53 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>High FBGC response</td>
<td>7/7 = 100%</td>
<td>3/6 = 50%</td>
</tr>
<tr>
<td>Low FBGC response</td>
<td>7/7 = 100%</td>
<td>6/6 = 100%</td>
</tr>
<tr>
<td>High vascularization</td>
<td>0/7 = 0%</td>
<td>2/6 = 33.3%</td>
</tr>
<tr>
<td>Abscess formation</td>
<td>1/7 = 14.3%</td>
<td>0/0 = 0%</td>
</tr>
</tbody>
</table>
However, the 53-week group exhibited a significant lower amount of collagen III protein compared to the 24-week group ($p < .05$) (Figure 1).

### 3.2 Histology

We saw a significant difference in the FBGC response between the two groups. Here, we saw a reduced amount of FBGCs in the 53-week group ($p < .0001$) compared with the 24-week group, but no significant difference was found for vascularization or collagen amount (Figure 2). Despite a large difference between the groups in mean vascularization, the result was insignificant due to a very high vascularization in two of specimens in the 53-week group. Subtraction of the two specimens with high vascularization from the 53-week group gave similar mean vascularization between the groups (24-week group: 3.7, 53-week group: 3.2, $p > .05$).

![Figure 4](https://example.com/figure4.png)  
**Figure 4** Evaluation of mesh remnants. Representative images of thick and thin cell dense and cell poor areas, mesh fibers in cell dense area, and dissolved mesh. All images from the 53-weeks specimens.

![Figure 5](https://example.com/figure5.png)  
**Figure 5** (A) Stress–strain curves. (B) Ultimate tensile strength (UTS). (C) Strain at UTS. (D) Young’s modulus. *$p < .05$; **$p < .01$; ****$p < .0001$. Data on the 24-week group is from the study by Hansen et al. [26].
The histological appearance was categorized into four groups: High FBGC response, low FBGC response, high vascularization, and abscess formation (Figure 3). All specimens in both groups exhibited areas of low FBGC response. High FBGC response, vascularization, and abscess formation differed between the groups. Areas of high FBGC response were present in all 24-week specimens but only in 50% of the 53-week specimens. High vascularization was absent in the 24-week specimens but was found in 33.3% (two specimens) of the 53-week specimens. Only in the 24-week group did we find abscess formation. Here, we found an abscess of 4 × 1.5 mm in one of the specimens.

The histological mesh evaluation differed between the two groups. In all specimens, it was possible to identify mesh fibers within the cell dense tissue, but the cell dense area appeared narrower, and the central cell poor area seemed larger in the 53-week group compared with the 24-week group (Figure 4). Moreover, the meshes seemed more dissolved and more torn apart in 53-week group than in the 24-week group. Altogether, the total cell amount (cell dense area) seemed lower in the old rats exposing the mesh (cell poor area), which was partly degraded.

### 3.3 Biomechanical properties

The biomechanical testing showed that ultimate tensile strength (UTS) in the 53-week group was not significantly different from the 24-week group (Figure 5). For both the 24- and 53-week groups, strain at UTS was significantly lower than muscle fascia ($p < .0001$). Young’s modulus, representing the tissue stiffness, was significantly higher in the 53-week group than muscle fascia ($p < .01$). Although not significant, the mean Young’s modulus in the 53-week group was higher than in the 24-week group.

### 4 DISCUSSION

This study demonstrates how electrospun nanofiber meshes with CTGF and MSCs provide sufficient long-term support without mesh-related complications. Overall, the group of elderly and heavy rats, the 53-week group, showed (1) a decrease in collagen III, (2) a strong reduction in foreign body giant cells response, (3) more exposed and partly degraded mesh, (4) altered histological appearance, and (5) higher tissue stiffness when compared with the “historical control group” of young and lighter rats, the 24-week group.

We saw a significant decrease in collagen III in the 53-week group compared with the 24-week group. As we have no baseline level of collagen III to aligning; this finding could be due to either an increased amount in the 24-week group or a decreased amount in the 53-week group. Several possible explanations for this exist.

The finding could indicate a fundamental difference in young and old rat tissue after surgery, showing that old tissue has reduced regenerative potential. However, due to the two groups’ different timelines, it is impossible to finally conclude. The age difference could also contribute to the difference. The 53-week group had an average age of 99 weeks versus 37 weeks in the 24-week group, and collagen is known to change with age. Moreover, collagen III is known to be part of the initial wound healing process. The amount of collagen increases postoperatively; one study found the maximum level of collagen III serum markers 3 weeks after surgery. If such an elevation persists, it could explain the higher amount of collagen III seen in the 24-week group compared with the 53-week group. Lastly, it is known that abscesses are enclosed by collagen-rich capsules. We found abscess formation in one of the 24-week specimens, so it seems reasonable that abscess formation in some of the samples could explain an increased collagen III in the 24-week group.

The histological evaluation revealed several interesting results. We found a much lower FBGC count in the 53-week group than in the 24-week group. A FBGC response is a known host reaction to biomaterials, it degrades some types of biomaterials, and it can exist lifelong. Moreover, long-term foreign body response in women after POP repair is associated with mesh complications in terms of mesh exposure and pain. The reduction in FBGC response in the present study indicates long-term biocompatibility in elderly rats and is possibly associated with reduced complications, both critical for future clinical use.

Furthermore, we still saw mesh fibers in the 53-week group, although the mesh appeared more dissolved and torn apart than in the 24-week group. In vivo degradation of PCL is highly variable and depends on several factors as the molecular weight, the architecture of the mesh, and the implantation environment. Our PCL-based mesh type degradation time is more than 53 weeks. It is necessary to evaluate the meshes in vivo for more than 53 weeks to observe outcomes with fully degraded meshes.

Moreover, we found that the total cell amount was lower in the 53-week group compared with the 24-week group. There could be many different explanations for this. It could be due to the reduced foreign tissue response, hence a reduced number of reactive cells. Otherwise, it could be due to completion of tissue regeneration, hence a reduced number of regenerative cells. Contrary, it could also represent tissue degeneration, hence loss of connective tissue cells. Cell-specific analyses are needed to conclude on this matter. Intuitively, one would want the mesh to be replaced by new tissue as the PCL degrade over time. However, as none of the 53-week rats developed herniation, the observed mesh histology provided sufficient support at the time of evaluation.

Lastly, regarding the histology, in two of the 53-week rats, we found a high degree of vascularization. In a review of mesh with stem cells for soft tissue repair, neovascularization was the most prevalent effect. As MSCs are known to be heterogenic both between and within isolates this might explain the difference in high vascularisation. Otherwise, the high vascularization could also represent granulation tissue, which could be part of a foreign body reaction to biomaterials during tissue healing. Granulation tissue is described to precede connective tissue proliferation. The two animals with high vascularization were both sacrificed earlier than the other animals in the 53-week group. Therefore, this finding might show a transitory
state of tissue healing. Yet, none in the 24-week group showed this high degree of vascularization. The young age of the 24-week group could be an explanation as tissue healing is known to slow down in elderly individuals.\textsuperscript{40} This indicates a fundamental difference in tissue regeneration in young and old rat tissue, highlighting the importance of evaluating surgical provides for elderly woman in elderly animal models.

The biomechanical properties of the 53-week group were comparable to the 24-week group. Although not significant, the mean Young's modulus in the 53-week group was higher than the mean in the 24-week group. The difference was insignificant due to a high variance in the 24-week group. We need inclusion of more rats for clear results regarding Young's modulus. Moreover, the 53-week group was different from muscle fascia with lower strain at UTS, meaning that it starts to deform or break at a lower strain, and with higher Young's modulus, showing that tissue is stiffer than muscle fascia.

The maintained tissue stiffness of the 53-week group compared with the 24-week group must be explained by changes in the ECM as the mesh appeared more dissolved in the 53-week group than in the 24-week group. Likewise, the increased tissue stiffness compared with muscle fascia, could besides the mesh properties, also be due to ECM changes. The maintained and increased stiffness could represent fibrotic tissue, as this displays higher tissue stiffness. Increased stiffness of tissue is explained by accumulation and crosslinking of ECM components.\textsuperscript{41} In particular, collagen I and III are deposited during fibrosis.\textsuperscript{42} However, we found comparable collagen I levels and reduced collagen III levels in the long-term group. Therefore, the maintained stiffness between the two groups is probably due to increased crosslinking of ECM proteins. Different types of crosslinking are known to happen both due to aging, chronic inflammation and in diseases.\textsuperscript{42} From a tissue regeneration point of view, fibrosis is unwanted as it can lead to tissue dysfunction.

Tissue dysfunction could be through the concept of stress shielding, where stiff tissue results in tissue degeneration in adjacent less stiff tissue.\textsuperscript{43} In our study, this could result in herniation next to the mesh area. As herniation was absent in the 53-week group the level of stiffness was tolerated long-term. However, as stress shielding is an ongoing process, it can progress over time. Altogether, the biomechanical changes seen could indicate overly extensive scarring, which can cause tissue dysfunction long term. However, biomechanical testing of human vaginal tissue reported similar or higher tissue stiffness compared with the 53-week group.\textsuperscript{44,45} Therefore, this mesh type might not be too stiff for human pelvic floor repair.

Several rats developed tumors during the study period. As we had no long-term control group, this raises a concern for a possible carcinogenic effect of meshes with stem cells and growth factor. However, spontaneous tumors are a common finding in elderly laboratory rats, with studies reporting incidences of 25%-57%, a great part of being mammary tumors, as in the present study.\textsuperscript{46}

Long-term culturing of stem cells can produce genomic instability, and in vitro tumor transformation has been detected in some studies.\textsuperscript{47} The MSCs used in this study were used from an early passage.\textsuperscript{15} Recently, it has become evident that MSCs can have pro-tumorigenic effects, such as promoting tumor growth and metastasis.\textsuperscript{48} However, a study only found stem cells to exhibit metastatic effects when mixed with tumor cells and not when injected in tissue nearby tumors.\textsuperscript{49} In our study, the tumor did not present near the mesh site. Therefore, the observed tumors seemed to be independent of the stem cells. This is supported by another study on stem cells where implanted stem cells did not migrate.\textsuperscript{22}

Besides MSCs, CTGF was added to the implant. CTGF stimulates differentiation of MSCs to fibroblasts but is also associated with tumor development, both inhibitory and acceleratory.\textsuperscript{50,51} Therefore, CTGF could have potentiated the tumor development seen in this study. However, in a previous in vitro study, the CTGF release happened only initially, and CTGF would have to leak into the circulatory system and be distributed in other tissues in sufficient amounts to aid tumor growth.\textsuperscript{52} Altogether, it seems unlikely that the tumor development in our study should be due to stem cells or CTGF. Repeating the study with a long-term control group could enlighten this issue.

The strengths of the present study are the use of elderly, female rats, and long-term evaluation. The female rats had the meshes implanted at an older age, and they kept the meshes for a longer time than investigated in previous studies, making this study more comparable to human pelvic floor repair. Moreover, the high weight of the rats mimics the average weight gain in women around and after menopause.\textsuperscript{52} A limitation of the present study is the small study group. Inclusion of more rats would have made this study more powerful. As the 53-week group was heterogenic in age and weight, it would have been interesting to evaluate whether weight or age correlate with outcome measures. However, due to the small sample size, a subgroup analysis was not statistical reliable. Moreover, uncertainties arise when comparing data from two separate studies, as the data from the two groups were generated at different timepoints. Especially, the histological evaluations without an internal control are uncertain. In addition, the 53-week group exercised twice weekly whereas the 24-week group did not, which may as well have affected the outcome.

Furthermore, the rat abdominal repair model differs from POP in women, and we need evaluation in a large animal with a real POP pathology. This would also enable evaluation of the mesh even more long-term than the present study. As the PCL-based mesh evaluated in this study were only partly degraded after 53 weeks, it is important to evaluate the outcome years after implantation. Moreover, a control group undergoing sham operation would be useful in evaluating the long-term safety of meshes with stem cells and growth factor.

### 5 CONCLUSION

As no previous study has investigated biodegradable meshes with growth factor and stem cells long-term in elderly rats, this study provides new insights into future POP repair in elderly and post-menopausal women. This study shows that CTGF/PF-coated electrospun PCL meshes with MSCs provide sufficient support and biocompatibility without inducing long-term mesh-related complications. Our
study group consisted of rats older and heavier than rats in previous studies. This mimics elderly, post-menopausal women, the major group to undergo pelvic floor repair, making this study highly relevant for future pelvic floor repair research. Prior to introduction into human clinical practice, further long-term intervention studies in a large animal model are pivotal.

ACKNOWLEDGMENTS
We are grateful to Kristina Bang Christensen and Lotte Arentoft for their technical support.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Sofie Husted Laursen https://orcid.org/0000-0002-5805-1535

REFERENCES