

ELECTROSPUN NANOFIBER MESH WITH FIBROBLAST GROWTH FACTOR AND STEM CELLS FOR PELVIC ORGAN PROLAPSE REPAIR – AN IN VIVO STUDY IN RATS

Hypothesis / aims of study

An in vivo study was conducted to evaluate the cellular and biomechanical properties of six long-term biodegradable polycaprolactone (PCL) meshes. The six meshes, comprising basic fibroblast growth factor (bFGF) and connective tissue growth factor (CTGF) including rat mesenchymal stem cells (rMSC), were tested in a full-thickness abdominal wall defect model in rats to mimic the weakened vaginal wall in women with pelvic organ prolapse (POP). The study was based on the hypothesis that bFGF-loaded PCL-meshes enhance collagen formation through direct stimulation of fibroblasts (1). Furthermore, that CTGF will direct the differentiation of rMSC into fibroblasts thus improving the regenerative response in vivo (2).

Study design, materials and methods

Randomly, 84 rats were divided into three groups with two arms in each group. The groups were: (1) Hollow fiber PCL-meshes with bFGF; (2) Solid fiber PCL-meshes with bFGF; (3) Solid fiber PCL-meshes with CTGF and rMSC. bFGF in dosages of 4.8 ug and 48 ug, respectively, was loaded in the core of the hollow fiber meshes. Solid fiber meshes were embedded in either 10 ug bFGF or 9 ug CTGF; 2.5×10^6 rMSCs were cultured, expanded and seeded on the sterile CTGF-coated solid fiber mesh (*figure 1*). The meshes consisted of biodegradable PCL fibers fabricated by electrospinning, where a mesh of hollow and solid fibrous nanostructure were spun, respectively.

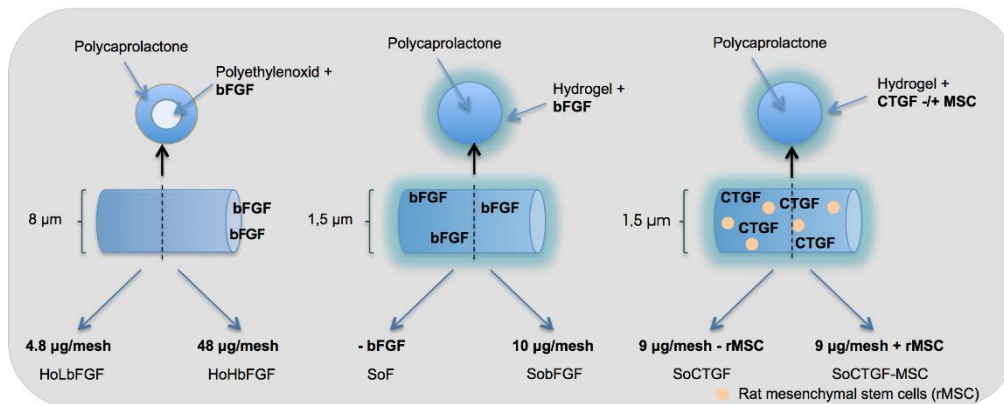


Figure 1. Six long-term biodegradable PCL-meshes.

HoLbFGF: Hollow fiber 4.8 ug bFGF; HoHbFGF: Hollow fiber 48 ug bFGF; SoF: Solid fiber without bFGF; SobFGF: Solid fiber 10 ug bFGF; SoCTGF: Solid fiber 9 ug CTGF; SoCTGF-MSC: Solid fiber 9 ug CTGF + rMSC.

A full-thickness part of the abdominal muscle-fascia layer was resected en bloc followed by implantation of a mesh covering the defect and 0.5 cm of the surrounding tissue. After eight and 24 weeks, seven rats from each arm were euthanized. The mesh area including one cm adjacent tissue was resected and divided into four sections for subsequent analysis.

Evaluation at week eight and 24 included quantitative polymerase chain reaction (q-PCR) for mRNA-expression of collagen-I and -III, Western blot for protein assessment of collagen-I and -III and Hydroxyproline quantification for total collagen content. Histologically, the sections were stained with Masson's trichrome, blinded and evaluated to quantify the presence of giant cells, mesh thickness and collagen amount, individually ranked between none (0) and abundant (4). Biomechanical properties of the explanted mesh were tested by uniaxial mechanical testing.

Results

After 24 weeks, the hollow fiber meshes were fully degraded both macroscopically and microscopically causing herniation of the mesh area. On the contrary, the solid fiber meshes were intact after 24 weeks providing biomechanical reinforcement to the weakened abdominal wall. The PCL-meshes were associated with multiple complications, such as localized abscess formation and hematoma, except from the solid fiber PCL-mesh with rMSC, where no complications were observed neither at eight nor at 24 weeks.

Students *t*-test revealed a significantly higher protein amount and mRNA expression of collagen-I and -III after 24 weeks for the hollow fiber meshes with 4,8 ug bFGF compared to 48 ug bFGF ($p < .05$) (*figure 2*).

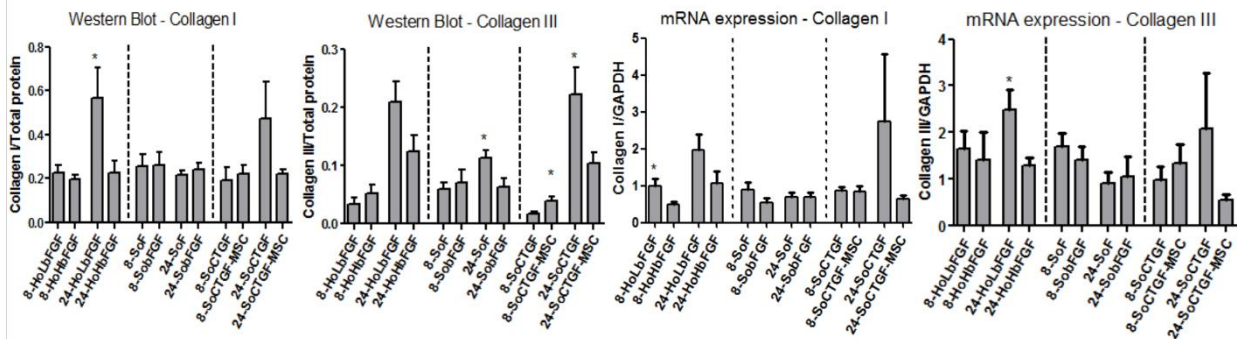


Figure 2. Bar graphs for protein amount and mRNA expression of collagen-I and III.
* $p < .05$.

Analysis of variance (ANOVA) with Dunnett’s test showed a significant difference between the solid fiber PCL-mesh with rMSC and muscle-fascia with respect to ultimate tensile strength after eight weeks ($p < .05$). Furthermore, the solid fiber PCL-mesh with rMSC showed a significantly higher stiffness compared to the solid fiber PCL-mesh without rMSC after 8 weeks ($p < .01$).

Interpretation of results

The hollow fiber meshes were fully degraded after 24 weeks resulting in herniation of the mesh area indicating an undesirable degradation rate of the hollow PCL-fibers compared to neo-tissue formation. The herniation tendency following implantation of hollow fiber PCL-meshes was also demonstrated by Glindtvd et al. (3). The mechanical tests demonstrated lower ultimate tensile strength and stiffness for the hollow fiber meshes compared to solid fiber meshes. This suggests, that hollow fiber PCL-meshes do not meet the criteria for the optimal tissue-engineering strategy in the treatment of POP, because the degradation rate is too fast. Differently, SoCTGF+rMSC showed higher stiffness compared to the corresponding mesh without rMSC and muscle-fascia. Western blot and q-PCR analysis detected higher amounts of collagen-I and -III in HoLbFGF compared to HoHbFGF after 24 weeks thus demonstrating that collagen synthesis is not dependent on a high dosage of bFGF. Interestingly, none of the rats in SoCTGF+rMSC developed complications at eight or 24 weeks whereas the corresponding PCL-mesh, SoCTGF, was associated with severe abscess formation. This indicates a beneficial effect on the inflammatory response due to rMSCs thus reducing the risk of abscess formation through non-explored mechanisms of action. In the present study, a higher protein amount of collagen III was detected for SoCTGF+rMSC compared to SoCTGF after 8 weeks. At 24 weeks, the difference was reversed. This could imply an initial differential stimulus of the rMSCs and hence an increased collagen formation as described by Jangö et al. (2).

Concluding message

SoCTGF+rMSC is a potential alternative to conventional POP-repair because of the lack of complications and because of biomechanical reinforcement of the weakened abdominal wall, which is a preferable feature from a clinical safety point of view. Furthermore, we found no dose-response relationship between growth factor-dose and collagen neoformation.

References

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Disclosures

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