New Perspectives of Penile Enhancement Surgery: Tissue Engineering with Biodegradable Scaffolds

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Objective: To evaluate in a multicenter, prospective study preliminary aesthetic and functional results of autologous ex-vivo tissue engineering for penile girth enhancement.

Methods: From July 1999 to January 2004, 204 men of mean age 26.77 (range 19–54 years) underwent this procedure. Indications for penile girth enhancement were penile dysmorphic disorder and previous failed surgery for penile girth enhancement. Fibroblast cells harvested from 1 cc of biopsied scrotal dermal tissue were expanded in culture until the total cell number of at least $2 \times 10^7$ was reached. Suspended cells in culture medium were then seeded on pretreated tube-shaped PLGA scaffolds and incubated for 24 hours. After penile degloving, scaffolds were shape adjusted and transplanted between dartos and Buck's fascia when the skin was compliant or under the neurovascular bundle when the skin was not compliant.

Results: A total of 84 randomly selected patients were followed 1 to 5 years postoperatively (median 24 months). The gain in girth ranged from 1.9 to 4.1 cm (mean 3.15 cm). Postoperative complications occurred as infection in three, penile skin pressure necrosis in two and seroma formation in five patients and were all treated conservatively. Surgical intervention was appraised by patients on a scale from 1 to 5 as follows: the best mark (5) was given by 44.05%, very good (4) by 36.90%, good (3) by 19.05% and only one patient gave the mark 2 judging general penile appearance as dissatisfactory; mean score was 4.25.

Conclusion: Autologous tissue engineering by using biodegradable scaffolds as a carrier is a new and safe therapeutic approach for penile girth enhancement.
1. Introduction

Following increased popularity of female cosmetic surgery, genital reconstructive surgery has successfully evolved during the past decade. Simultaneously, penile lengthening and widening gained tremendous interest in male population all around the world. Considerable improvement of penile aesthetic appearance and size is now possible although this surgery remains a challenge requiring strict attention to details and meticulous techniques [1].

The most common method so far employed for penile girth extension was lipofilling but with disappointing outcomes [2–5]. True corporeal body’s enlargement could be achieved by autologous tunica albuginea grafting with saphenous vein [6]. Wrapping the penile circumference outside Buck’s fascia with autologous dermal-fat grafts to obtain gain in penile girth was also reported [7,8]. Possible complications include temporary or permanent penile shortening, penile deformities, graft lost and unsightly donor site scars [1,5]. Synthetic materials like injectable materials (hydrogel) were used with moderate results [9].

The aim of our prospective study was to evaluate indications, feasibility, operating time, aesthetic and functional results and complications of autologous ex-vivo tissue engineering for penile girth enhancement (PGE).

2. Material and methods

Participants were thoroughly informed about the nature of the surgical procedure and written informed consent was obtained from all participants before surgery.

2.1. Patients and indications

Of the 204 patients operated for penile girth enhancement from July 1999 to January 2004 in Yeonsei-plus Urologic clinic in Seoul, Presidential Hospital of Russia in Moscow and University Hospital in Belgrade, 84 men who ranged in age from 20 to 50 years (mean age 28 years) were randomly selected to participate in this study. Among these, 70.24% presented with penile dysmorphic disorder while 25 patients (29.76%) had history of previously failed penile girth enlargement. Exclusion criteria were history of previous psychiatric morbidity, organic diseases (long lasting insulin-dependent Diabetes Mellitus, generalized atherosclerosis) and age less than 18 years. Physical measurements of penile length and mid-shaft penile circumference both in the flaccid and erect state were obtained and recorded pre and postoperatively. Preoperative and postoperative intracavernous injection of Prostaglandine E1 and Doppler ultrasound were used for assessment of erectile function (Table 1).

2.2. Methods

2.2.1. Cell harvest

After local infiltration of 1% of lidocaine solution, thumbnail sized, elliptical shaped skin incision was made and 0.5–1.0 cm³ of dermal tissue was harvested. Biopsied dermal tissue was washed to remove red blood cells in serum-free Dulbecco’s modified eagle’s medium (DMEM, Gibco, Maryland, USA) and minced into less than 1-mm pieces which were then distributed over three 100 × 15 mm Petri dishes. The sliced tissues were treated with collagenase type IV, (2 mg/ml) and incubated at 37 °C with 5% CO₂ incubator for 24 hours. Fibroblasts were suspended in culture media containing 5% of patient’s serum and expanded in culture until the total cell number of at least 2 × 10⁷ was reached. The time required is between 3 and 5 weeks depending upon individual patients (mean 27 days).

2.2.2. Scaffold pretreatment and cell seeding

Dry poly(lacti-co-glycolic acid) scaffold (PLGA, Regen Biotech Inc., Sungnam, Korea) 50 mm in length, 30 mm in inner diameter and 3 mm in thickness, pore size 250 to 400 μm was hydrated by complete immersion into 75% ethanol solution in aseptic condition and kept in a refrigerator (4 °C) overnight (Fig. 1). The raw material of PLGA was purchased from Alkermes, Inc. (Cambridge, Massachusetts, USA). Ethanol solution was removed completely by repeated washing with
Sterile, pyrogen-free cold water, phosphate-buffered saline (Ca, Mg-free, pH 7.0) and serum-free culture medium.

Pretreated scaffolds were then seeded with approximately 20 million of fibroblasts and incubated for 24 hours on 37°C.

2.2.3. Implantation

Prophylactic antibiotic administration was systematically prescribed 24 hours before surgery. After subcoronal incision and penile degloving two cell seeded scaffolds were shape and positioned between tissues and sutures were used to ensure adequate hemostasis and transgluteal Between tissues and sutures were used to ensure adequate hemostasis and transgluteal. Ventral placement under the dartos fascia with penile eversion and degloving was performed in patients where non-compliant scrotal skin was present. The skin was brought together with sutures and 2 weeks after surgery. Patients were discharged in place for 7–10 days.

Table 1 – Sample characteristics of 84 patients according to indication for penile girth enhancement (PGE) and penile lengthening (mean value ± SD, range, cv)

<table>
<thead>
<tr>
<th>Primary indication</th>
<th>No (pts)</th>
<th>Age (years)</th>
<th>Operative stay (days)</th>
<th>Hospital stay (weeks)</th>
<th>Flaccid girth gain (cm)</th>
<th>Erect girth gain (cm)</th>
<th>Flaccid length gain (cm)</th>
<th>Erect length gain (cm)</th>
<th>Follow-up (months)</th>
<th>Complications (No pts)</th>
<th>Cell seeding</th>
<th>Prepubertal pubic hair, serum-free culture saline</th>
<th>Prepubertal pubic hair, serum-free culture saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary girth enhancement</td>
<td>44</td>
<td>88</td>
<td>28.16 ± 6.62</td>
<td>49.90 ± 2.90</td>
<td>27.47 ± 4.85</td>
<td>76.80 ± 8.06</td>
<td>2.40 ± 1.82</td>
<td>0.45 ± 3.35</td>
<td>0.31 ± 2.75</td>
<td>0.43 ± 21.91</td>
<td>9.56 ± 3</td>
<td>-</td>
<td>22.97</td>
</tr>
<tr>
<td>Previous surgery for PGE</td>
<td>25</td>
<td>60.77 ± 18.44</td>
<td>40-05</td>
<td>123 ± 0.45</td>
<td>3.17 ± 0.53</td>
<td>1.9-54</td>
<td>0.52 ± 3.17</td>
<td>0.53 ± 2.54</td>
<td>0.52 ± 2.24</td>
<td>0.29 ± 28.04</td>
<td>8.98 ± 7</td>
<td>23.17</td>
<td></td>
</tr>
<tr>
<td>Primary girth enhancement (PGE) and penile lengthening</td>
<td>15</td>
<td>60.77 ± 18.44</td>
<td>40-05</td>
<td>123 ± 0.45</td>
<td>3.17 ± 0.53</td>
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<td>0.52 ± 2.24</td>
<td>0.29 ± 28.04</td>
<td>8.98 ± 7</td>
<td>23.17</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>10.63 ± 10.63</td>
<td>19-54</td>
<td>40-95</td>
<td>1-5</td>
<td>1.9-54</td>
<td>0.52 ± 3.17</td>
<td>0.53 ± 2.54</td>
<td>0.52 ± 2.24</td>
<td>0.29 ± 28.04</td>
<td>8.98 ± 7</td>
<td>23.17</td>
<td>10.63</td>
</tr>
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</table>

twice a day (5–10 minutes) during the first six months to prevent temporary penile retraction. The clinical data concerning the final outcome and complications were obtained and recorded on control visits at 2 weeks, 1, 3, 6, 12 months after surgery and then yearly (Fig. 5).

2.2.4. Instruments
A study instrument comprised short structured questionnaire, modified and adopted from a validated study for a long-term outcome evaluation in hypospadias surgery [10]. Answer options ranged from “dissatisfied” and “somewhat satisfied” to “completely satisfied”. Surgery was also judged on a scale from 1 to 5 with 5 being the best. Additionally, genital appraisal was assessed in the second part of questionnaire. Possible answer options for the patients-rated satisfaction with flaccid and erect penile girth were “satisfied” or “dissatisfied” while scores for satisfaction with general penile appearance ranged from 1 to 5 on the following scale: 1- very dissatisfactory, 2- dissatisfactory, 3-good, 4-very good and 5-excellent. (see Appendix). Data were analyzed using Students t-test (t-test for dependent samples), the $X^2$ test with Yets correction (Yates corrected Chi-square). A $p$ value of 0.5, 0.01 and 0.001 were considered statistically significant.

Fig. 3 – Scaffold implantation under neurovascular bundle in the case of non-compliant skin – lateral view.

3. Results
Of the 204 men operated, 84 patients with a mean age of $28.77 \pm 6.61$ at the last control were randomly selected to participate in the study. Participating subject did not differ significantly from non-responders regarding indications for surgery, mean age at the present study, mean number of operations and immediate postoperative complications so we assumed the sample representative.

Anesthesia was general in sixteen (20.12%), spinal cord in 25 (49.40%), epidural in 31 (36.90%) and local in 12(20.48%) patients. Of 59 (70.24%) patients with penile dysmorphic disorder, 44 (52.38%) underwent primary penile enlargement while 15 (17.86%) underwent combined release of penile ligaments and girth enlargement. In 25 patients, penile girth enlargement was performed after previously failed attempts. In 81.93% of cases, scaffolds were placed between darts and Buck’s fascia. In 18.07% of cases with noncompliant penile skin, scaffolds were placed under previously dissected neurovascular bundle. Mean operative time was $60.77 \pm 18.44$ min/ range $40–95$ min/ and significantly better for the patients with primary penile girth enhancement/ $43.91 \pm 2.40$ min/ comparing to those with previous surgery and combined penile girth enhancement and release of ligaments (80.84 $\pm$ 5.14 and 76.80 $\pm$ 8.06 min, respectively) ($p < 0.001$). Post-operatively, partial pressure necrosis of the skin occurred in two patients with a history of previous surgery and treated conservatively. Temporary seroma occurred in six patients who started with sexual intercourse earlier then advised (6 weeks after surgery) and treated successfully by repeated evacuations. Wound infections were identified in two patients and resolved after 2 weeks with local treatment. Mean hospital stay for the patients with primary penile girth enhancement and combined penile girth enhancement and release of ligaments was $1.82 \pm 0.45$ (range 1–3) and $1.53 \pm 0.52$ (range 1–2) days, respectively. For the patients with a previous enlargement surgery mean hospital stay was $2.96 \pm 1.06$ (range 1–5)/, significantly longer then for the other two groups ($p < 0.001$).

Mean follow-up was 24.67 months and ranged from 1 to 5 years. In total, mean value of flaccid and erect girth gain was $3.15 \pm 0.42$ (range from 1.9–4.1 cm) and $2.47 \pm 0.49$ (range from 1.8–3.0) respectively. In the group with combined penile girth enhancement and release of ligaments mean value of flaccid and erect length gain was $3.45 \pm 0.52$ (range from 2.1–4.5 cm) and $0.65 \pm 0.32$ (range from 0.5–1.0 cm), respectively. Among the different groups, the best results encountered were in a
group for primary penile girth enhancement with a mean flaccid girth gain of 3.35 ± 0.31 (range 2.3–4.1 cm), compared to those with a history of previous surgery and combined penile girth enhancement and release of ligaments 2.78 ± 0.24 (range 1.9–3.4 cm) and 3.17 ± 0.53 (range 2.5–4.1 cm), respectively (Table 1). Erectile function and penile sensitivity were not changed after surgery since there were no intraoperative damaging of the dorsal neurovascular bundle or other penile structures.

3.1. Genital appraisal

Only one patient gave the mark 2 judging general penile appearance as dissatisfactory, while mean self-rated genital appraisal was 4.07 ± 0.71. Among the subjects in different groups, patients with a history of previous surgery tended to score genital appearance lower (3.88 ± 0.60) comparing to those with primary penile girth enhancement (4.20 ± 0.76) and penile girth enhancement and release of ligaments (4.00 ± 0.65), but without statistically significant differences. Participants in all groups were mostly satisfied with penile girth in flaccid and erects state (63 and 71%, respectively) without significant differences between the groups. Urologist-rated satisfaction with the flaccid and erect penile girth was slightly higher, given that they were satisfied with mentioned parameters in 67 and 73% of cases, respectively (Table 2).

3.2. Satisfaction with surgical results

Subjects who undergone penile girth enhancement after previous failed attempts scored surgical intervention slightly lower (4.04 ± 0.79). However, this difference was not statistically significant. In total, participating subjects appraised the surgical intervention as follows: the best mark 5 was given by 44.53%, 4 by 36.90%, and 19.05% of patients gave the mark 3 – “good”. When answer options ranged from “dissatisfied” and “somewhat satisfied” to “completely satisfied” 70% said that they were completely satisfied. Urologist-rated satisfaction with surgical intervention was “completely satisfied” in 73% of cases. Finally, only two (2.38%) patients wanted further surgical revision to improve accomplished results (Table 2).

4. Discussion

There are still ethical and medical dilemmas without uniform indication for penile enhancement surgery at present. The operative techniques and assessment of the results are currently not standardised in the literature with reports claiming exceedingly better results then generally possible. Several reports advocated different methods for penile girth enhancement [1–6]. Beside autologous lipofilling as the method most commonly employed,
autologous dermal-fat grafting was also reported [7,8]. Possible complications in this treatment option include graft lost, unsightly donor site scar, temporary or permanent penile shortening and penile deformities. A critical assessment of previous reports in the literature points out that reported disappointing results were mainly a consequence of the postoperative fat re-absorption. To reduce the chance of postoperative fat re-absorption, Asaadi and Haramis recommended irrigating the fat with a solution of 100 U of regular insulin to stabilise lipocyte membrane [11]. Yuksel investigated recently the local effect of insulin and growth factors on free fat graft survival. He found that long-term local delivery of growth factors have potential to increase fat graft survival [12]. Ayhan reported positive effect of β-blockers in increasing free fat graft survival in an animal study [13]. However, report by Chajchir showed no beneficial effect of insulin or centrifugation on adypocyte survival during their transplantation [14].

Tissue engineering in penile enhancement surgery is a new approach to fabricate a new functional tissue from autologous cells and consequent penile girth augmentation [15]. In this study, we applied a principle of transplanting autologous cells onto biodegradable scaffold that provides appropriate mechanical strength to induce three-dimensional growth of a new functional, autologous tissue [16–18]. We hypothesized that expanded cells harvested from the scrotum could migrate into the biodegradable and biocompatible scaffold, would form viable tissue and would start its degradation to support the growth of a completely normal tissue without inflammation.

We used macroporous, biodegradable PLGA (poly-lacti-co-glycolic acid) scaffolds whose porosity range between 85 and 90%. Average pore dimension
is between 250 to 400 μm which is adequate for migration and growth of fibroblasts who were naturally selected during cell proliferation period and whose diameter is about 10–15 μm. Earlier studies with non-woven mesh scaffolds have found that pore size, pore orientation, fiber structure and fiber diameter can influence cell behavior and tissue development [19,20]. The pores of PLGA scaffolds are interconnected to each other which provides adequate communication of growth factors, nutrients and oxygen as well as seeded cells migration and intracellular matrix and vascular regeneration.

The biocompatibility and degradative properties of PLGA scaffold were excellent and this finding confirms previous reports in the literature [21,22]. The degradation rate ranged from 6 to 16 weeks after pre-treatment with 75% ethanol. Histological examination six months after intervention demonstrated newly generated tissue which appeared viable with significant cell number, collagen content and ingrowth of capillaries.

Our clinical experience with this new treatment approach for penile girth enhancement showed its remarkable safety, reproducibility, superior cosmetic results, low morbidity and low incidence of postoperative complications comparing to previously established procedures [3–8]. Possibility of performing this procedure under local anaesthesia and short hospital stay (mean 2.11 days) are the points in favour for the low cost-effectiveness of this approach. Mean operative time was approximately one hour and final surgical outcome concerning penile girth gain was almost 3 cm. Erectile dysfunction or sensitivity deficiencies were not reported in immediate postoperative period or later. Except for the patients with seroma formation, all patients resumed their sexual activity 4–6 weeks after surgical intervention. The previous surgery often
limits the availability of compliant skin, so we assumed that extensive dissection and placement of the scaffold under the lifted neurovascular bundle would provide the most favourable conditions for successful outcome in these cases.

The only good functional results available to assess objectively delayed final outcome of penile enhancement surgery including patients satisfaction with achieved surgical results is the series of Austoni [6,23]. He reported true increase in volume of the corpora cavernosa by bilateral saphenous grafting of tunica albuginea with excellent patient’s satisfaction and no complications. However, this technique enables penile girth augmentation only in erect penis with reported postoperative increase in diameter that ranged from 1.1 up to 2.1 cm. In the present study, minimal flaccid girth gain encountered was 1.9 cm and ranged up to 4 cm for the majority of patients, even in the group with previous surgery. To address this controversy in the outcome, the present data showed no association between penile enlargement surgery and penile shortening. Importantly, in the cases of combined penile girth enlargement and release of ligaments, placement of the scaffold proved to be suitable to prevent ligament reattachment and consequent penile shortening. Penopubic ligamentolysis provides apparent but not real lengthening since penile structures remain unchanged. Thus, any gain in penile length is noticeable in flaccid but minimal in erect state. Vacuum device was advocated 5–10 minutes twice a day due to tendency for temporary penile retraction during scaffold degradation, which occurs one to three months postoperatively. Thus, there were no cases of permanent penile retraction. Consequently, this technique could be most reliably applied for successful penile girth augmentation even after previously failed attempts. Moreover, this technique could be repeated also one year after primary enlargement in order to gain additional girth. In some patients [6] new procedure to increase girth gain is required as a consequence of penile dysmorphic disorder.

The study population comprised mainly young men who had received psychiatric counselling related to dissatisfaction with body image attributed to a small penis. They were referred to our institutions as patients with penile dysmorphic disorder. More then two thirds of 84 patients, claimed to be completely satisfied with achieved surgical results while almost 80% of patients scored surgical intervention as “excellent” and “very good” (44.05 and 36.90%, respectively). There was also high agreement between patient’s and surgeon’s satisfaction with accomplished results.

In total, mean value of self-scored genital appraisal was “very good” while two-third of patients were satisfied with penile girth in flaccid and erect state (63.09 and 69.09%, respectively). According to presented data, it would seem that this approach is a straightforward and useful method to reduce negative psychological impact of the low genital appraisal in this men population.

Although our scaffold consisted of biodegradable synthetic polymer, possible limitations of this concept could be the absence of biologic recognition. As an approach toward the incorporation of cell-recognition domains into these materials, copolymers with amino acids have been synthesized [13]. Other biodegradable synthetic polymers, including poly (anhydrides) and poly (ortho-esters), can also be used to fabricate scaffolds for genitourinary-tissue engineering with controlled properties [14]. However, taken together, this approach represents potentially important improvement in the field of genital reconstructive surgery.

5. Conclusion

Autologous tissue engineering by using biodegradable scaffolds as a carrier is a new and safe therapeutic approach for penile girth enhancement. The preliminary experience with this procedure showed significantly lower complication rate then previously established procedures. Other advantages are simplicity, low morbidity, reduced operative time and preliminary good results. The outcome of this study points out the necessity for its expanded clinical applicability in the future.

Appendix A. Questionnaire of patients with penile girth enhancement

Are you satisfied with your body image?
How many times were you operated on?
How would you describe yours satisfaction with surgical results: “dissatisfied”, “somewhat satisfied” or “completely satisfied”?
If you are not satisfied, what is the major problem/penile appearance, penile size, etc.? On a five point scale with 5 being the best estimate the result of surgical intervention/5-excellent, 4-very good, 3-good, 2-dissatisfactory 1-very dissatisfactory?
Would you consider another surgical intervention to solve apparent problems?
Did you have problems in achieving erection during first sexual intercourse after intervention?
Did you ever have problems with erection after intervention?
Did you have painful erection after intervention?
Did you have sensitivity problems after intervention?
Are you satisfied with a penile appearance?
On a 5 point scale with 5 being the best, estimate the penile appearance/5-excellent, 4- very good, 3-good, etc. . . ?
Are you satisfied with a flaccid penile girth size?
Are you satisfied with a penile girth in the state of erection?

References