

## An Experimental Study: Does Topically Applied Sildenafil Citrate (Sildegra®) Have an Effect on the Preservation of the Viability of the Graft in Full-Thickness Tubed Free Skin Grafts?

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**Aim:** In this study we investigated the effectiveness of topically applied sildenafil citrate in increasing graft viability.

**Methods:** The study included 15 rats (5 rats each in control, sham, and study groups). Physiological saline (1 ml) was given to the sham group and sildenafil citrate solution (0.5 mg/kg/day in 1 ml saline) to the study group. One week later the grafts were evaluated by histopathological investigation.

**Results:** The feeding tube in the control and sham groups had totally melted due to xylol. Widespread neutrophil leukocyte infiltration, a thin full-thickness free tubed graft in the periphery, widespread neutrophil and inflammation zones lower down and widespread necrotic foci were present at the site of the tube. However, in the sildenafil group, a widespread foreign body reaction was observed. There was an increase in the granulation tissue. Inflammation, necrosis, and fibroblastic reactions were present. In contrast to the control and sham groups, new vessel formations were seen.

**Conclusion:** It was observed that topical sildenafil citrate application had no effect on the increase in viability of free tubed grafts.

**Key Words:** Free tube, full-thickness graft, sildenafil citrate, viability

### DeneySEL Bir Çalışma: Topikal Uygulanan Sildenafil Sitratinin (Sildegra®) Full Thickness Tüp Oluşturulmuş Serbest Cilt Greftlerinin Canlılığının Korunmasında Bir Etkisi var mıdır?

**Amaç:** Bu çalışmada, greftlerin canlılığını arttıracak şekilde topikal uygulanan sildenafil sitratinin etkinliğini araştırdık.

**Yöntemler:** Çalışmamızda 15 rat kullandık. Kontrol grubunu 5 rat, sham grubunu 5 ve çalışma grubunu 5 rat oluşturdu. Sham grubundaki ratlara her gün 1 ml serum fizyolojik verilirken, çalışma grubundaki ratlara 1 ml 0,5 mg/kg/gün sildenafil sitrat çözeltisi verildi. Bir hafta sonra greftler histopatolojik olarak değerlendirildi.

**Bulgular:** Kontrol ve sham grubundaki beslenme tüpleri ksilolün etkisi ile erimişti. Tüp bölgesinde yaygın nötrofil infiltrasyonu, periferde doğru incelen full thickness greft, yaygın nötrofil ve inflamasyon alanları ve yaygın nekrotik alanlar izlendi. Ancak sildenafil grubunda yaygın olarak yabancı cisim reaksiyonu gözlemlendi. Kontrol ve sham gruplarından farklı olarak granülasyon dokusunda artış, inflamasyon, nekrozis, fibroblastik reaksiyon ve yeni damar oluşumları gözlemlendi.

**Sonuç:** Topikal sildenafil sitrat uygulamasının serbest tüp greftlerin canlılığını arttırmadığı gözlemlendi.

**Anahtar Sözcükler:** Canlılık, full thickness greft, serbest tüp, sildenafil sitrat

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#### Introduction

Today, tubed free skin grafts are used urogenital system surgery for urethral stenosis, urethral traumas, hypospadias and for the correction of defects which arise due to cancers (1). It has been stated that mucosa of the cheek and bladder, skin from the genital and extragenital region, post-auricular tissue, and tissues from tunica vaginalis, peritonea and labium majus have been used with this aim as full-thickness tubed free skin graft (1-7). The success rates of tubed free skin grafts in urethral surgery show variability according to the region from which the grafts were taken, the blood

circulation of the area to which the grafts are applied, the location and the structure. Different success rates, ranging from 0 to 100%, have been stated in the literature (2,5,8-12). As a result, investigations continue today directed to topical agents, surgical techniques and materials to enhance the success rate. Unfortunately, to date, no effective topical agent for the preservation of graft viability has been found.

After having experimentally formed tubed free skin graft from the abdominal region of rats, we topically applied sildenafil citrate with the aim to continue the viability of the grafts. The results in the study group were evaluated histopathologically and compared with the control group. In our prior study, we observed successful results when we topically applied sildenafil to the flaps before we had planned the study (13). Given the successful outcome, we decided to explore whether sildenafil could be useful in tubed free skin grafts, and this hypothesis guided our study.

**Materials and Methods**

The experimental study was carried out after obtaining the approval of the local Ethics Committee, and utilized 15 rats in the animal laboratories of Ankara Training and Research Hospital. The rats were allocated randomly into three groups, control, sham and study, each consisting of five rats. A 15 x 5 mm full-thickness tubed free skin graft was formed from the skin tissue of the abdominal region in all rats, allowing for the insertion of a silicone 5 F feeding tube (Figure 1). A number of small holes were made in the feeding tube. The formed graft was placed on another part of the abdomen in such a way that a part of the feeding tube was left outside (Figure 2).

No topical agent was applied to the rats in the control group. In the sham group, saline (1 ml/kg/day) and in study group, sildenafil citrate (Sildegra®, Fako) solutions were given to the rats by a catheter. The sildenafil citrate solution was prepared by mixing the sterile and lyophilized powder form of 50 mg sildenafil citrate in saline. This solution was applied at a final concentration of 0.5 mg/kg/day in 1 ml of saline for one week (14).

A week later, the rats were sacrificed and the status of the grafts was compared histopathologically. The histopathological evaluation was carried out with hematoxylin-eosin staining under a light microscope.



Figure 1. A full-thickness tubed free skin graft.

**Analysis of vessel density**

At high magnification (X40), four fields with the highest visible blood-vessel density were counted in two randomly chosen sections for each animal after blinding the slide identity. All vessels were included regardless of size. A total of 80 fields in 20 randomly chosen sections were analyzed for each group.

**Statistical analysis**

The statistical data were analyzed using Stastical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 13.0 for Windows. All values were expressed as mean ± SD. The differences between means of vascular density were analyzed using ANOVA test. P values less than 0.05 were considered to indicate statistical significance.



Figure 2. The graft placed on another part of the abdomen.

**Results**

In histopathological evaluation, the changes occurring in the sildenafil group showed similarities with those in control and sham groups. The feeding tube in the control and sham groups had completely melted away due to xylol. A widespread neutrophil leukocyte infiltration, a

thin full-thickness tubed free skin graft in the periphery, widespread neutrophil and inflammation zones lower down and widespread necrosis foci were present at the site of the tube (Figure 3a, 3b). In the study group, the feeding tube had also melted away due to xylol. The histopathologic features were: a widespread foreign body

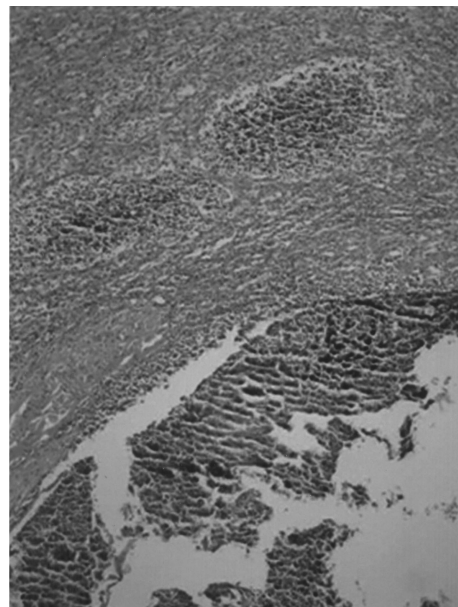
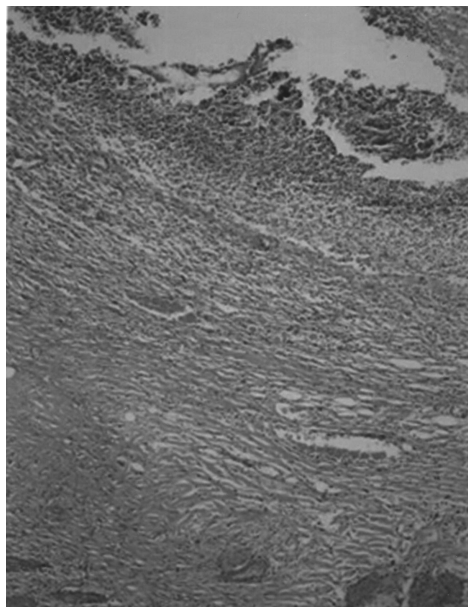


Figure 3a-b. The control and sham groups (H&E X100).

reaction, increased granulation tissue, increased inflammation, necrosis, and fibroblastic reaction. However, in contrast with the control and sham groups, formation of new vessels was present in the study group (Figure 4). There was no significant difference between the three groups according to mean vascular density values (Table 1).

**Discussion**

The idea that tubed free skin grafts could be used in the reconstruction of the urethra was first put forward at the beginning of the 1900s. Although Humby started using full-thickness skin grafts from the anterior surface of the arm in 1941, these were not widely used until 1961 when Devine and Horton re-started their application (5,14,15). Today, however, tubed free skin

grafts have an important place in reconstructive surgery of the urethra.

Skin grafts are prepared in two forms, as split-thickness and full-thickness. While split-thickness includes differing thicknesses of the dermis, full-thickness skin grafts include the entire dermis layers, i.e. sweat and fat glands, hair follicles and capillaries (16). Cheek and bladder mucosa, genital or non-genital region skin and post-auricular skin were used as tubed free skin grafts, and different success rates were obtained (3,12,15). Since grafts taken from extragenital regions increase the morbidity, preputium (5) and other genital organ grafts are preferred as skin grafts. These grafts are used in urethral surgery especially for urethral diseases, where free tube is formed with the aim of reconstruction, such as in stenosis, traumatic defects, congenital defects and cancer (1). The use of free tube grafts has several

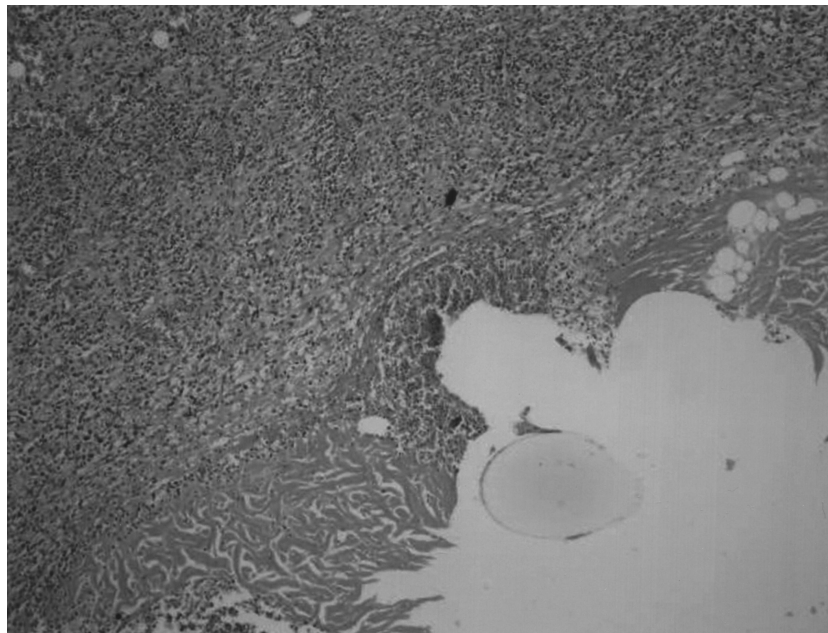


Figure 4. Sildenafil citrate group (H&E X100).

Table 1. Comparison of mean vascular density values

	Sham group	Control group	Sildenafil group*
Mean vascular density	76.17 ± 7.077	76.21 ± 7.865	76.27 ± 8.041

\* p>0.05 for comparison of sildenafil group with sham and control groups (ANOVA)

complications, such as hair growth, graft contraction, stenosis, stone formation and diverticula (16). Despite the fact that there are a number of new tissues which can be used as tubed free skin grafts in urethral surgery, these still have advantages and disadvantages, such as the specific technique of removing the graft, the long period of time spent in hospital and the morbidity of the donor or the site from which the graft was taken (1).

The success rates of free tube skin grafts used in urethral reconstruction differ to a considerable degree (17). In a retrospective analysis carried out in our clinic, in which cheek or bladder mucosa, preputium or post-auricular skin was used, the general success rate of tubed free skin grafts was found to be approximately 50% (12).

Bladder mucosa is still being used as full-thickness graft, but its use is limited because of the difficulties of obtaining the graft. The results, however, are thought to be more satisfactory due to the graft's origin from the urinary system (2).

Because it is easily obtained and has tissue characteristics like resistance to infection and skin diseases, a thin epithelium, which contains a high number of elastic fibers and a thin lamina propria, is more easily applied in urethral surgery (3,18). If any tissue is to be used as a graft and is too thick, it can rapidly become destructed when vascularization begins. If it is too thin, contraction may occur, and if taken from a well-vascularized bed, it may rapidly vascularize (5).

In the literature, it has been found that there is no substance or method used for the preservation of the viability of the tubed free skin grafts. In a study carried out by Gürdal et al. (19), honey was used under the graft to preserve the graft viability and a more rapid healing was obtained. When the antimicrobial effect of honey together with the chemical debridement effect, the anti-edema, epithelization and granulization accelerating effects and the regulatory effects of nutrition of the wound were all brought forward, they supported the idea that honey was effective on infections and other wounds. They have observed that grafts to which honey was applied healed in a shorter time in comparison to normal grafts.

In our study, we implanted the tubed free skin graft prepared from the abdominal wall again to the abdominal region. However, we do not know whether the tubed

free skin graft prepared from the abdominal region and the transfer (to the same region) would produce the same result in penile tissue or urethral region environment (20). Therefore, in this study, we attempted to create a urethral environment by inserting a catheter in the graft to simulate a urethra.

Sildenafil citrate is a specific inhibitor of type 5 cyclic guanosine monophosphate (cGMP)-specific phosphodiesterases (PDE). It was used as an antianginal drug in the 1980's and due to an unexpected side effect caused erection in males. It increases the nitric oxide level and nitric oxide in turn increases the cGMP level within the cell. The concentration of cGMP is regulated by guanylate-cyclase, which is responsible for its synthesis, and by PDEs, which are responsible for its destruction. The enzyme PDE5 is found in platelets and in the smooth muscles of the vessels. Sildenafil citrate increases the concentration of cGMP within the cell by inhibiting PDEs. This increase loosens the smooth muscles of the vessels, causes vasodilatation and increases blood flow (13,20-22). In another study, it has been shown in vivo that it causes the dilation of peripheric arteries and veins and prevents the formation of thrombi by platelets (23).

Graft necrosis in reconstructive surgery is a serious problem and is dependent on ischemia or on the lack of nourishment. Hematoma or serious swelling should not develop under the graft, otherwise, the graft cannot be nourished and necrosis will develop (24). In one of our studies (13), when 0.5 mg/kg of sildenafil citrate was placed under the flap, flap viability was observed to be higher when compared with the control group. Ulusoy et al. (25) obtained nearly 100% viability in flaps in which sildenafil was used with fibrin adhesives. Therefore, we decided to apply sildenafil citrate to tubed free skin grafts. However, our results were not as encouraging as the results observed with the flaps. An increase in inflammation and foreign body reaction was observed in all the grafts and necrosis had developed, and the results were similar with those seen in the control and sham groups. In contrast to the control and sham groups, new vessel formations and fibroblastic activity were observed in the sildenafil group, and these events coincided with the third phase of graft healing. However, probably due to the poor results of the first two out of the three phases required to increase the success of skin grafts, we think that the necessary results were not obtained in the final phase.

In conclusion, although the results obtained with flaps using topical sildenafil citrate alone or together with fibrin adhesives in an attempt to increase the viability of flaps were nearly perfect, topically applied sildenafil in an attempt to increase our success in the free tube used in urethral surgery did not achieve the viability of tube

grafts. When compared with the control and sham groups, our results were similar, and necrosis had developed in all the grafts. We concluded that topically applied sildenafil citrate, in contrast to results observed with flaps, was not effective in the achievement and preservation of tubed free skin graft viability.

## References

1. Chen F, Yoo JJ, Atala A. Experimental and clinical experience using tissue regeneration for urethral reconstruction. *World J Urol* 2000; 18: 67-70.
2. Demirbilek S, Yalçınkaya F, Atayurt H. Bladder mucosa in posterior urethral repair. *Int Urol Nephrol* 1997; 29: 59-62.
3. Burger RA, Müller SC, El-Damanhoury H, Tschakaloff A, Riedmiller H, Hohenfellner R. The buccal mucosal graft for urethral reconstruction: a preliminary report. *J Urol* 1992; 147: 662-664.
4. Mundy AR. The use of grafts and flaps for substitution urethroplasty, in *Urethral Surgery, ESU Organized Course at the 52<sup>nd</sup> DGU Congress, 2000*; pp. 55-56.
5. Atala A, Guzman L, Retik AB. A novel inert collagen matrix for hypospadias repair. *J Urol* 1999; 162: 1148-1151.
6. Devine CJ Jr, Horton CE. Hypospadias repair. *J Urol* 1977; 118: 188-192.
7. Hendren WH, Crooks KK. Tubed free skin graft for construction of male urethra. *J Urol* 1980; 123: 858-860.
8. Lopez JA, Valle J, Timon A, Blasco B, Ambroj C, Murillo C et al. Use of autologous buccal mucosal graft for urethral surgery in males. *Eur Urol* 1996; 29: 227-230.
9. Kane CJ, Tarman GJ, Summerton DJ, Buchmann CE, Ward JF, O'Reilly KJ et al. Multi-institutional experience with buccal mucosa onlay urethroplasty for bulbar urethral reconstruction. *J Urol* 2002; 167: 1314-1317.
10. Verit A, Darçın OT, Yeni E, Unal D, Ozardali I, Karatas OF. Neointimised ringed polytetrafluoroethylene tubing graft as prosthesis in the canine urethra: a preliminary experimental study. *Turk J Urol* 2003; 29: 241-244.
11. Khoury AE, Olson ME, McLorie GA, Churchill BM. Urethral replacement with tunica vaginalis: a pilot study. *J Urol* 1989; 142: 628-633.
12. Germiyanoglu C, Özkardeş H, Kurt Ü, Erol D. Hipospadiyas onarımında serbest tüp greft üretroplastisi yöntemi (in Turkish). *Cerrahi Tıp Bülteni* 1993; 2: 108-110.
13. Ayyıldız A, Uysal A, Kocer U, Karaaslan O, Huri E, Germiyanoglu C et al. The efficacy of sildenafil citrate on flap viability: an experimental study in rats. *Scand J Plast Recons Surg Hand Surg* 2005; 39: 204-208.
14. Humby G. A one-stage operation for hypospadias. *Br J Surg* 1941; 29: 84-92.
15. Devine JC, Horton CE. A one-stage hypospadias repair. *J Urol* 1961; 85: 166-170.
16. Brannan W, Ochsner MG, Fuselier HA, Goodlet JS. Free full thickness skin graft urethroplasty for urethral stricture: experience with 66 patients. *J Urol* 1976; 115: 677-680.
17. Vyas PR, Roth DR, Perlmutter AD. Experience with free grafts in urethral reconstruction. *J Urol* 1987; 137: 471-474.
18. Heinke T, Gerharz EW, Bonfig R, Riedmiller H. Ventral onlay urethroplasty using buccal mucosa for complex stricture repair. *Urology* 2003; 61: 1004-1007.
19. Gürdal M, Kireççi S, Piriñçi N, Sakız D, Karaman MI. The effect of natural honey on wound healing in graft and flap treatment. *Turk J Urol* 2003; 29: 245-249.
20. Şimşek F. Editör yorumu: Greft ve flep tedavisinde doğal balın yara iyileşmesindeki etkisi (in Turkish). *Turk J Urol* 2003; 29: 248-249.
21. Brock G. Sildenafil citrate (Viagra®). *Drugs Today* 2000; 36: 125-134.
22. Kendirci M, Miroğlu C. Oral tedaviler: sildenafil. In: Kadioğlu A, Başar M, Semerci B, Orhan İ, Açı R, Yaman MÖ, Çayan S, Usta MF, Kendirci M, editors. *Erkek ve Kadın Cinsel Sağlığı* (in Turkish) Istanbul: Türk Androloji Derneği; 2004. pp. 187-206.
23. Li Z, Xi X, Gu M, Feil R, Ye RD, Eigenthaler M et al. A stimulatory role for cGMP- dependent protein kinase in platelet activation. *Cell* 2003; 112: 77-86.
24. Michelle JP, Steven CH, Robert AH. Basic techniques and principles in plastic surgery. In: Aston SJ, Beasley RW, Thorne CHM, editors. *Plastic Surgery*. New York: Lippincott-Raven; 1997. pp: 13-25.
25. Ulusoy MG, Uysal A, Kocer U, Karaaslan O, Cuzdan SS, Ayyıldız A et al. Improved flap viability with site-specific delivery of sildenafil citrate using fibrin glue. *Ann Plast Surg* 2005; 55: 292-296.