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ORIGINAL ARTICLE

Male Health

Novel double-layer Silastic testicular prosthesis with controlled release of testosterone *in vitro*, and its effects on castrated rats

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Testicular prostheses have been used to deal with anorchia for nearly 80 years. Here, we evaluated a novel testicular prosthesis that can controllably release hormones to maintain physiological levels of testosterone *in vivo* for a long time. Silastic testicular prostheses with controlled release of testosterone (STPT) with different dosages of testosterone undecanoate (TU) were prepared and implanted into castrated Sprague-Dawley rats. TU oil was applied by oral administration to a separate group of castrated rats. Castrated untreated and sham-operated groups were used as controls. Serum samples from every group were collected to measure the levels of testosterone (T), follicle-stimulating hormone and luteinizing hormone (LH). Maximum intracavernous penile pressure (ICPmax) was recorded. The prostates and seminal vesicles were weighed and subjected to histology, and a terminal dexynucleotidyl transferase-mediated UTP nick end labeling (TUNEL) assay was used to evaluate apoptosis. Our results revealed that the weights of these tissues and the levels of T and LH showed significant statistical differences in the oral administration and TU replacement groups compared with the castrated group ($P < 0.05$). Compared with the sham-operated group, the ICPmax, histology and TUNEL staining for apoptosis, showed no significant differences in the hormone replacement groups implanted with medium and high doses of STPT. Our results suggested that this new STPT could release TU stably through its double semi-permeable membranes with excellent biocompatibility. The study provides a new approach for testosterone replacement therapy.

INTRODUCTION

Testes play essential roles in male reproduction. The absence of testes (anorchia) inevitably brings serious physical and mental distress to men, no matter whether the condition arises from congenital factors (e.g., vanishing testes syndrome or cryptorchidism) or acquired ones (e.g., torsion, trauma, or orchiectomy for testicular cancer or prostatic carcinoma). Implantation of testicular prostheses has been applied for treating anorchia for nearly 80 years.[1] Numerous materials, including vitallium, Lucite, glass marbles, plexiglass, Dacron, and polyethylene, have been used with limited success.[1–3]

Silastic and solid silicone rubber prostheses have been developed since the 1960s. Gel-filled silicone devices appeared in 1972 and became the standard form of prosthesis in 1988. However, in 1992, this treatment was halted by the Food and Drug Administration in the USA because of the risk of silicone leakage and migration. No subsequent evidence has been found to confirm any link between using testicular prostheses and connective tissue diseases.[4,5] Saline-filled prostheses were first used in the USA in 1995.[6] In China, a hollow Silastic testicular

prosthesis has been developed and used in patients with unilateral or bilateral anorchia.[7]

Implantation of a testicular prosthesis only deals with a patient's psychological issues, by imitating the appearance of the normal testes. In general, after receiving testicular prosthesis implantation, patients with congenital or acquired bilateral anorchia require persistent testosterone replacement therapy. Testosterone-based compounds and hormone replacement formulations have been widely used in clinics, such as oral testosterone, intramuscular injections, subcutaneous implants, transdermal patches, and buccal patches.[8–11] However, their pharmacokinetics need to be improved.[9,12] Oral and transdermal testosterone patches have relatively short durations and unstable effects.[13,14] Although intramuscular injections and subcutaneous implants have prolonged effects, they can cause pain and complications.[15,16]

This study aimed to develop a novel double-layer Silastic testicular prosthesis with controlled release of testosterone (STPT) under the standards of the Food and Drug Administration in China, and to explore the appropriate dose via an STPT as well as its safety and efficacy using a castrated rat model.

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Received: 05 May 2015; Revised: 25 June 2015; Accepted: 11 January 2016

MATERIALS AND METHODS

Manufacture of STPT

Powdered testosterone undecanoate (TU) (Zizhu Pharmaceutical Corp., Beijing, China) and medical-grade silicone rubber (SILBIONE® MM 71791/70 U, Dow Corning Corp., Michigan, USA) were mixed in a rubber mixing machine (JTC-752A, Zhanjiang Machine Factory, Guangdong Province, China). The mixture was configured into the drug-delivery cores (diameter 9.5–11.5 mm, length 12.5–14.5 mm) in a pressure-forming machine (140°C; 12 min; 9.5 MPa; XLB-D400-400 East Machine Ltd., Zhejiang Province, China). Using the rubber-mixing machine, medical-grade methyl-vinyl-polysiloxane (Right Fortune Industrial Ltd., China), a reinforcing agent and a firming agent were mixed to form viscose, around which drug cores were pressed and dried in a drying cabinet for 24 h. Then, the second layer for controlled release was assembled, pressed and dried again (**Figure 1a**). Three different doses of TU in STPTs: 10 mg (white), 20 mg (yellow), and 30 mg (red) were designed. All STPTs were sterilized with ethylene oxide (**Figure 1b**).

Assay of testicular prosthesis for in vitro drug delivery

STPTs were immersed in 100 ml ultrapure water, and oscillated in a constant temperature water bath (37.0 ± 0.5°C; 60 oscillations per min; amplitude 3 cm; HZS-H Donglian Electronic Tech, Ltd., China). The water was replaced every 24 h and the level of TU was assayed using high-performance liquid chromatography.[17]

Implantation of testicular prostheses in Sprague-Dawley (SD) rats

The experiment was approved by the Ethics Committee of Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine. A 12-week-old male SD rats (*n* = 60) were divided randomly into six equal groups (AdomlySTPTs were implanted into the scrotal sacs of castrated rats in Groups A, B, and C. The white STPTs were implanted into Group A, yellow into Group B, and red into Group C. Corn oil (0.75–1.0 mg, 2 mg kg⁻¹) containing TU was given by oral gavage to the castrated rats in Group D at 8 a.m. daily. The control groups consisted of rats that underwent castration with no hormone replacement (Group E) and sham operations (Group F). At weeks 0, 4, and 8 in the trial, blood was collected from the tail veins to measure the levels of testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The tissues were retrieved, and all rats were euthanized by cervical dislocation at the end of the experiment, for morphological analyses.

Castration

Rats were anesthetized with 1% intraperitoneal chloral hydrate and placed supine for surgical castration. A midline scrotal incision was made, and the abdominal wall was retracted. Both testes were manipulated from the abdominal cavity to the incision of scrotum. The vas deferens and associated vasculature were identified and ligated separately and the testes were removed.

Histological and microscopic analyses

The prostates and seminal vesicles were cut off and weighed after rats were euthanized at the end of each experiment. Hematoxylin and eosin (HE) staining (right side of the prostates and seminal vesicles) and terminal dexynucleotidyl transferase-mediated UTP nick end labeling (TUNEL) apoptosis assays (left side of the prostates and seminal vesicles) were performed using paraffin wax sections to evaluate morphology and apoptosis, respectively. The heights of epithelial cells and area of the glandular lumen were measured and analyzed using an optical digital imaging system.[18] The apoptosis index (AI = number of apoptotic cells/ total cells × 100) was also calculated. HE stained prostatic tissues were also examined.

Functional studies

At the eighth week, rats from every group were anesthetized (intraperitoneal injection of 30 mg kg⁻¹ chloral hydrate). The abdominal cavity was opened and the major pelvic ganglion was located behind the prostate, then the penile cavernous nerve (CN) was isolated. The CN was subjected to electrical field stimulation (EFS) to elicit increased penile intracavernous pressure (ICP). Electrical stimulation (5 v for 5 ms, frequency 20 Hz, time 60 s) was applied to the CN, and the curves of ICP over time were recorded using a multi-purpose recorder attached to the pressure sensor (Power lab/8 sp, AD Instruments, Sydney, Australia).

Serum hormone assays

Blood samples from tail veins were collected from 08:00 to 09:00 and the serum was prepared by centrifugation. T, FSH, and LH were assayed using double antibody sandwich enzyme-linked immunosorbent assays (ELISAs).[19] Optical density (OD) was used to measure absorbance at 450 nm with each ELISA, and the concentrations of hormones were calculated from standard curves.

Statistical analysis

All data are shown as the mean ± standard deviation using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Normality of data distribution was confirmed using the Shapiro–Wilk test. Statistically significant differences were defined as $P < 0.05$. Multiple comparisons were analyzed by one-way analysis of variance.

RESULTS

TU assay in vitro

The peak release of TU *in vitro* appeared on day 2. After day 7, the total daily amounts of TU released from the three types of STPT were 100 μg (white), 200 μg (yellow) and 300 μg (red) daily and these stable releases lasted for 5 weeks (**Figure 1c**).

Testicular prostheses in SD rats

During the 8 weeks after implantation, 59 rats survived and one in Group D died on the fourteenth day because of trauma from the gavage. Mild hematuria was found in three rats postoperatively, caused by intraoperative traction to the bladder, but this disappeared 1 week later. No local swellings in the scrotum, or wound dehiscence were found. At the end of week 8, no abscesses or granulomata were found around the prostheses.

The prostate and seminal vesicle weights, and levels of T and LH showed significant differences ($P < 0.05$) between the oral TU administration and TU replacement Groups A, B, and C, compared with the castrated control Group E (**Figure 2**). Serum T levels and prostate and seminal vesicle weights in the STPT-implanted Groups (B and C, dose >20 mg) were significantly greater than those in the oral administration group (**Table 1**).

ICP induced by EFS showed that an erectile response could not be induced in the castrated control Group E. Compared with the normal control Group F, the maximum ICP (ICPmax) values of the STPT implant Group A and oral administration Group D were significantly different ($P < 0.05$) while the ICPmax values of the STPT implanted Groups B and C (TU dose >20 mg) showed no significant differences (**Figure 3**).

Image analysis of the prostatic epithelial cell height and luminal area showed significant differences ($P < 0.05$) in the STPT implanted Groups B and C (TU dose >20 mg) compared with the oral administration Group D (**Figure 4a** and **4b**).

TUNEL staining of the prostate and seminal vesicle tissues showed that the numbers of apoptotic cells were significantly greater ($P < 0.05$) in the testosterone replacement Groups A, B, C, and D, compared with those from the castrated control Group E. The AI decreased significantly ($P < 0.05$) in the STPT implanted Groups B and C (TU dose >20 mg) than in the oral administration Group D (**Figure 4c** and **4d**).

DISCUSSION

Testosterone plays an important role in men's growth and development, and low testosterone levels can impair their health. Testosterone deficiency syndrome has adverse effects on many organs and systems, including bone mineral density, physical agility, sexual functions, and emotions.[20,21] Furthermore, testosterone deficiency increases the risk of cardiovascular disease.[22]

Many studies have shown that men benefit from hormone replacement therapy when they have hypogonadism.[23] Testosterone replacement therapy can change the composition of the body, improve strength, and correct mood disorders including poor sexual desire and function.[24] It can also increase energy, reduce insulin resistance,[25] and reduce the amount of fatty tissue[26] and the risk of cardiovascular disease. Testosterone compounds and hormone replacement
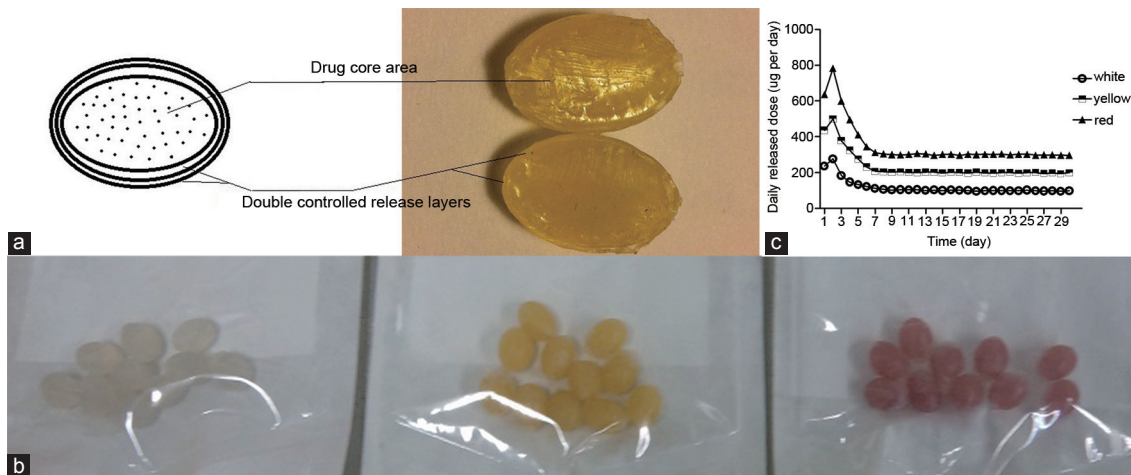

Figure 1: (**a**) Structure of an STPT. (**b**) The three types of STPT in this experiment. (**c**) The daily release rate of TU was stable for all three types of SPTP.
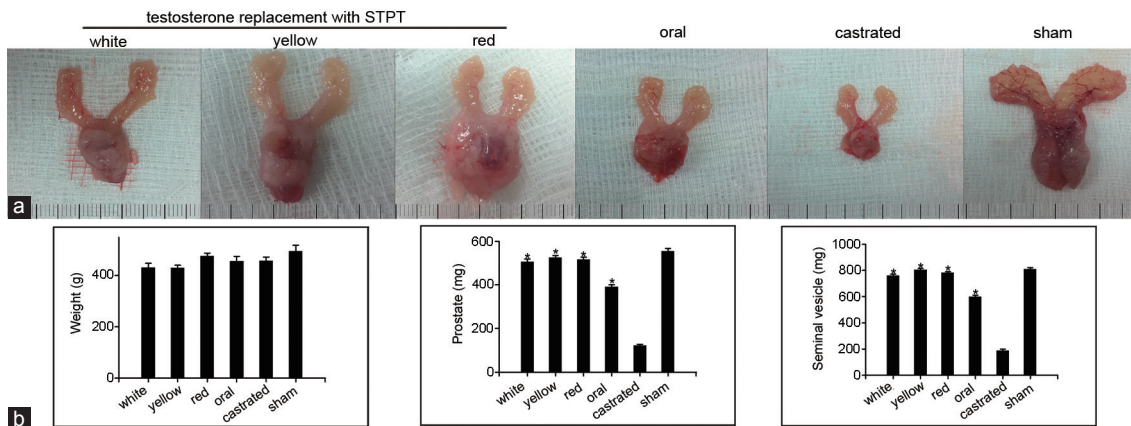

Figure 2: (**a**) Gross morphology of the rats prostates and seminal vesicles. (**b**) Comparison of the weights of the body, prostates and seminal vesicles in the castrated SD rats. *$P < 0.05$ compared with the castrated control Group E.

Table 1: Serum T, FSH, and LH levels in s.d. rats in the six experimental groups

Group	T (µg l⁻¹)			FSH (ng l⁻¹)			LH (ng l⁻¹)		
	0 week	4 weeks	8 weeks	0 week	4 weeks	8 weeks	0 week	4 weeks	8 weeks
A	3.33±0.76	1.71±0.67ᵃ	2.28±0.62ᵃ	3.51±0.21	3.42±0.74	3.57±0.39	4.56±0.55	5.65±0.76ᵃ	6.44±0.87ᵃ
B	3.25±0.76	2.86±0.81ᵇ	2.89±0.97ᵇ	3.47±0.42	3.58±0.35	3.46±0.74	4.68±0.39	4.71±0.43ᵇ	4.73±0.81ᵇ
C	3.28±0.52	2.96±0.61ᵇ	3.01±1.13ᵇ	3.62±0.61	3.75±0.58	3.67±0.38	4.61±0.57	4.68±0.56ᵇ	4.73±0.73ᵇ
D	3.36±0.63	1.45±1.83ᵃ	1.61±1.47ᵃ	3.55±0.31	3.59±0.53	3.65±0.47	4.46±0.34	5.78±1.26ᵃ	6.64±1.42ᵃ
E	3.29±0.91	0.32±0.11ᵃ	0.07±0.13ᵃ	3.48±0.53	3.51±0.78	3.84±0.82	4.53±0.48	7.28±0.78ᵃ	8.41±1.28ᵃ
F	3.33±0.56	3.45±0.82	3.41±0.81	3.53±0.45	3.47±0.51	3.56±0.61	4.69±0.38	4.75±0.48	4.62±0.33

Group A: castrated and implanted with white STPTs (10 mg); Group B: castrated and implanted with yellow STPTs (20 mg); Group C: castrated and implanted with red STPTs (30 mg); Group D: oral gavage after being castrated; Group E: castrated control group; Group F: sham-operated control group. ᵃ$P<0.05$ compared with castrated control group E; ᵇ$P<0.05$ for the androgen replacement Groups B and C compared with the oral medication Group D. STPTs: silastic testicular prostheses with controlled release of testosterones; T: testosterone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; s.d.: standard deviation
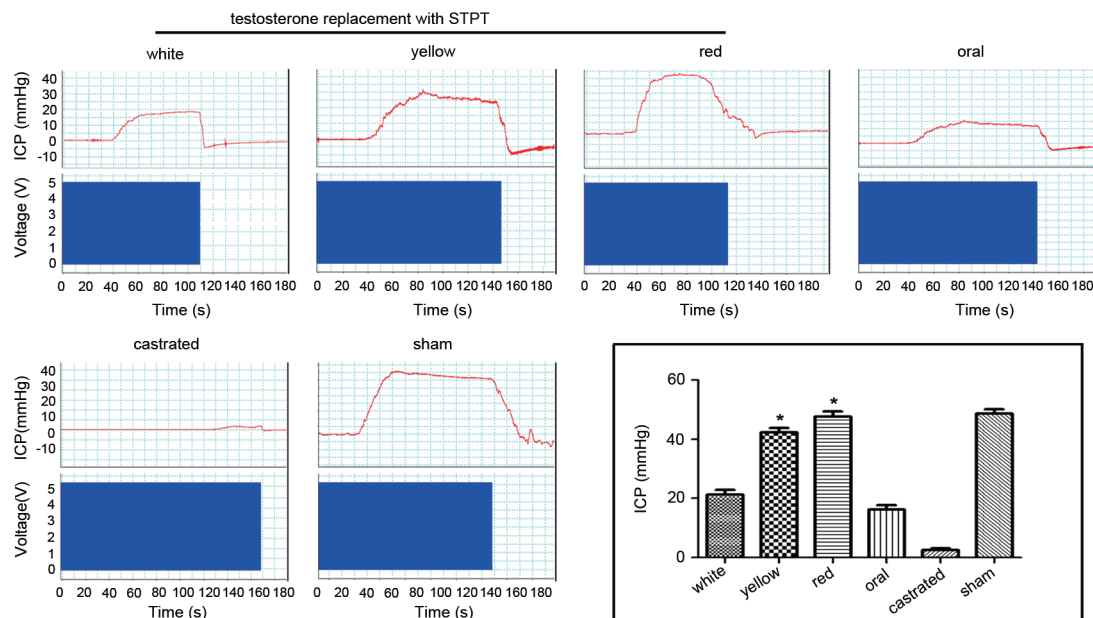
Figure 3: The comparison of electrical stimulation of cavernous nerves for detecting the ICPmax. $P > 0.05$ (not significant) compared with the sham-operated Group F.
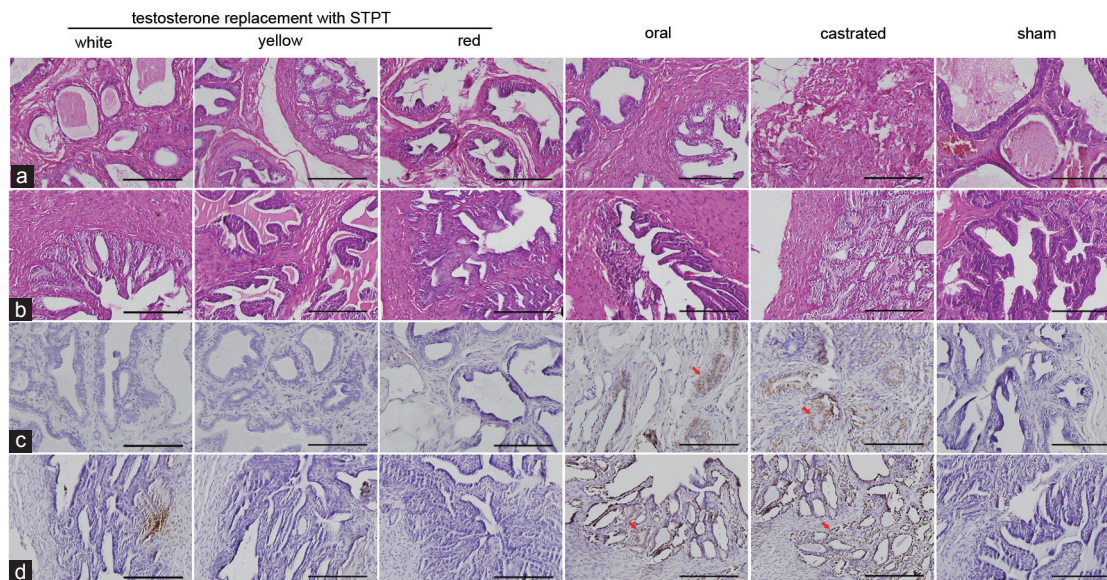

Figure 4: (**a**) HE staining of the right side prostatic tissue in SD rats. (**b**) HE staining of the right sided seminal vesicle tissue in SD rats. Scale bars = 100 µm. (**c**) The left prostate subjected to TUNEL staining (apoptotic cells are indicated by red arrows). (**d**) The left seminal vesicle subjected to TUNEL staining (apoptotic cells are indicated by red arrows). Scale bars = 100 µm.

formulations have been widely used in clinical settings, such as oral doses of TU, intramuscular injections, subcutaneous implants, and transdermal and buccal patches. Furthermore, it is now a consensus that patients who suffer from bilateral anorchia should receive implants of testicular prostheses and testosterone replacement therapy.[27–29]

The first subcutaneous testosterone implantations were applied clinically in 1937. As small sterile tablets with high purity, they were implanted without any accessories. A prospective, randomized, crossover design clinical trial showed that such pellets (6 × 100 mg) gave better results in maintaining the patients' serum T level than intramuscular injections of testosterone heptanoic acid (250 mg) every 2 weeks or oral TU (120 mg per day). In addition, a high acceptance ratio was noted because of the need for implant surgery 2–4 times a year.[30] Shortcomings of subcutaneous implant pellets were also noted: an implant procedure is required, and extrusion of the pellet can cause adverse effects such as bleeding, inflammation, infection, and subcutaneous fibrosis.[31]

Several studies have demonstrated that Silastic-based materials might serve as ideal drug-releasing carriers. They have increasingly become a popular topic of study in biological and medical research.[20,32] We have used Silastic as a carrier for testosterone release systems and first made it into a long-term controlled-release testicular prosthesis in 2010. In our previous study, a Silastic carrier

was combined with an antiandrogen drug (fluorine amine) to produce a testicular prosthesis, which could release the drug in a sustained manner. Drug delivery began to stabilize after 12 days, releasing 1230 µg fluorine amine per day on average. In later animal experiments, slow-release testicular prostheses could stably release these drugs *in vitro*, which inhibited the growth of subcutaneous prostatic cancer cells in immunodeficient nude mice. Based on that study, we tried to produce a testicular prosthesis that could release TU controllably. At first, one layer of Silastic membrane was designed and the data showed that the release of TU was too much to maintain the plasma concentration for a long time. We also tried to increase the thickness of the Silastic layer, but the result was unsatisfactory. Finally, double-layers of Silastic membrane were added in our latest type of testicular prosthesis, which made the drug release smoother and more durable.

In vivo study, three dosage forms of STPT were developed and implanted into castrated SD rats. These STPTs showed excellent histocompatibility, which was fully illustrated by no death, no infection or prosthetic rejection, and no obvious abnormalities in cardiovascular or other tissues surrounding the prosthesis. Medium and high dosages of TU (>20 mg) implanted in the SD rats could maintain their physiological serum T levels for 8 weeks. In addition, the serum T, FSH, and LH levels, and the gonadal weights were not significantly different from the normal control group. No changes in serum sex hormone levels were detected after day 7, which suggested that these STPTs released TU smoothly and effectively. The ICP test and cell apoptosis measured by TUNEL staining showed normal penile function and cell morphology, respectively. Moreover, we also observed that the oral treatment group was inferior to the medium-dose STPT Groups (B and C), in terms of the levels of serum sex hormones, gonadal weight, ICP test and AI although the oral drug delivery dose (2 mg kg^{-1}) was much greater than that released from the medium dose STPT (0.4 mg per day). Thus, the controlled-release drug delivery mode was better than the oral one. There are several possible reasons. It was hard to ensure that all of the TU given by gavage was absorbed completely by the rats in Group D. Moreover, the serum concentrations of hormones in this group varied widely between individuals, meaning that there were significant individual differences in bioavailability with oral drug delivery. Therefore, oral drug delivery is not recommended unless other methods are not available.

The results confirmed that the STPTs could release TU *in vivo* feasibly, safely, and efficaciously. A single STPT could maintain the endocrine requirements of SD rats for more than 4 weeks. STPTs trialed here could afford a new drug delivery system for androgen replacement therapy and provide new ideas for drug treatment. Better than the subcutaneous pellets products, these novel STPTs could satisfy the cosmetic need of the patients with anorchia and maintain a normal serum testosterone levels at the same time. Because of the limited volume, we could not deliver the whole life dosage of TU in two STPTs. A new type of STPT with a hollow middle core is to be designed in our laboratory. Using this new type of STPT, we should be able to achieve "one implant for whole life" by re-injecting TU into the hollow core regularly.

Of course, these new STPTs still have some problems before clinical trials could be contemplated. Thus, drug withdrawal problems remain to be solved to avoid side-effects. Furthermore, the normal circadian rhythm of testosterone release is another question to be solved. Our next goal is to develop better STPTs, which could regulate the dose of TU to realize a normal circadian rhythm without removing the testicular prosthesis.

CONCLUSIONS

Bilateral anorchia impairs the physical and mental health of patients. They require the implantation of testicular prostheses or continuous exogenous hormone replacement therapy. Our study proved that these STPTs can release TU safely and efficaciously *in vivo* and *in vitro*.

AUTHOR CONTRIBUTIONS

HXC, YN, and ZL conceived and designed the study. HHS took part in producing the STPTs. SY, YN, and MM performed the literature searches, surgery on the rats and extracted data. HXC, SY, RHT, and YFL performed the statistical analysis and wrote the manuscript. WQG, WLX, and ZL guided the study. All authors have read and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declared that they have no competing interests associated with this study.

REFERENCES

1 Girdansky J, Newman HF. Use of a vitallium testicular implant. *Am J Surg* 1941; 52: 514.
2 Rea CE. The use of testicular prosthesis made of lucite with a note concerning the size of the testis at different ages. *J Urol* 1943; 49.
3 Hazzard CT. The development of a new testicular prosthesis. *J Urol* 1953; 70: 959–60.
4 Barrett DM, O'Sullivan DC, Malizia AA, Reiman HM, Abell-Aleff PC. Particle shedding and migration from silicone genitourinary prosthetic devices. *J Urol* 1991; 146: 319–22.
5 Peters W, Keystone E, Snow K, Rubin L, Smith D. Is there a relationship between autoantibodies and silicone-gel implants? *Ann Plast Surg* 1994; 32: 1–5.
6 Turek PJ, Master VA. Safety and effectiveness of a new saline filled testicular prosthesis. *J Urol* 2004; 172: 1427–30.
7 Ning Y, Cai Z, Chen H, Ping P, Li P, *et al*. Development and clinical application of a new testicular prosthesis. *Asian J Androl* 2011; 13: 903–4.
8 Amano T, Imao T, Takemae K, Iwamoto T, Yamakawa K, *et al*. Profile of serum testosterone levels after application of testosterone ointment (glowmin) and its clinical efficacy in late-onset hypogonadism patients. *J Sex Med* 2008; 5: 1727–36.
9 Jockenhovel F. Testosterone therapy – What, when and to whom? *Aging Male* 2004; 7: 319–24.
10 Salehian B, Wang C, Alexander G, Davidson T, McDonald V, *et al*. Pharmacokinetics, bioefficacy, and safety of sublingual testosterone cyclodextrin in hypogonadal men: comparison to testosterone enanthate – A clinical research center study. *J Clin Endocrinol Metab* 1995; 80: 3567–75.
11 Gooren LJ. Advances in testosterone replacement therapy. *Front Horm Res* 2009; 37: 32–51.
12 Jockenhovel F. Testosterone supplementation: what and how to give. *Aging Male* 2003; 6: 200.
13 Darby E, Anawalt BD. Male hypogonadism: an update on diagnosis and treatment. *Treat Endocrinol* 2005; 4: 293–309.
14 Mazer NA, Shifren JL. Transdermal testosterone for women: a new physiological approach for androgen therapy. *Obstet Gynecol Surv* 2003; 58: 489.
15 Sartorius G, Fennell C, Spasevska S, Turner L, Conway AJ, *et al*. Factors influencing time course of pain after depot oil intramuscular injection of testosterone undecanoate. *Asian J Androl* 2010; 12: 227–33.
16 Cavender RK, Fairall M. Subcutaneous testosterone pellet implant (Testopel®) therapy for men with testosterone deficiency syndrome: A single-site retrospective safety analysis. *J Sex Med* 2009; 6: 3177–92.
17 Pylypiw HM Jr., Grether MT. Rapid high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in foods. *J Chromatogr A* 2000; 883: 299–304.
18 Sysko LR, Davis MA. From image to data using common image-processing techniques. *Curr Protoc Cytom* 2010; Chapter 12: 12.21.1–12.21.17.
19 Wheeler MJ. Assays for LH, FSH, and prolactin. *Methods Mol Biol* 2006; 324: 109–24.

20 Wu Y, Zhu L, Zhou G, Jiang H. Experimental study on construction of tissue-engineered testicular prosthesis *in situ* on rabbit. *J Tissue Eng Reconstruct Surg* 2011; 7: 136–8.

21 Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, *et al.* Testosterone therapy in adult men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2006; 91: 1995–2010.

22 Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, *et al.* Effect of testosterone treatment on bone mineral density in men over 65 years of age. *J Clin Endocrinol Metab* 1999; 84: 1966–72.

23 Liu PY, Swerdloff RS, Veldhuis JD. The rationales, efficacy and safety of androgen therapy in older men: future research and current practice recommendations. *J Clin Endocrinol Metab* 2004; 89: 4789–96.

24 Rosenthal BD, May NR, Metro MJ, Harkaway RC, Ginsberg PC. Ajunctive use of AndroGel (testosterone gel) with sildenafil to treat erectile dysfunction in men with acquired androgen deficiency syndrome after failure using sildenafil alone. *Urology* 2006; 67: 571–4.

25 Smith MR, Lee H, Nathan DM. Insulin sensitivity during combined androgen blockade for prostate cancer. *J Clin Endocrinol Metab* 2006; 91: 1305–8.

26 Kupelian V, Page ST, Araujo AB, Travison TG, Bremner WJ, *et al.* Low sex hormone-binding globulin, total testosterone, and symptomatic androgen deficiency are associated with development of the metabolic syndrome in nonobese men. *J Clin Endocrinol* 2006; 91: 843–50.

27 Bodiwala D, Summerton DJ, Terry TR. Testicular prostheses: development and modern usage. *Ann R Coll Surg Engl* 2007; 89: 349–53.

28 Schiffman A, Straker N. The psychological role of the testicles. A case report of a 9-year-old boy with agenesis of the testicles. *J Am Acad Child Psychiatry* 1979; 18: 521.

29 Beer M, Kay R. Testicular prostheses. *Urol Clin North Am* 1989; 16: 133.

30 Conway AJ, Boylan LM, Howe C, Ross G, Handelsman DJ. Randomized clinical trial of testosterone replacement therapy in hypogonadal men. *J Androl* 1988; 11: 247–64.

31 Handelsman DJ, Mackey MA, Howe C, Turner L, Conway AJ. An analysis of testosterone implants for androgen replacement therapy. *Clin Endocrinol* 1997; 47: 311–16.

32 Mel T, Sharon S. Silicone elastomers for healthcare tubing. *China Med Devices Inf* 2008; 8: 4–6.