REPORT

The Nanochannel Delivery System for Constant Testosterone Replacement Therapy

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ABSTRACT

Introduction. The goal of testosterone replacement is to provide long-term physiological supplementation at sufficient levels to mitigate the symptoms of hypogonadism.
Aim. The objective of this work is to determine if the implantable nanochannel delivery system (nDS) can present an alternative delivery strategy for the long-term sustained and constant release of testosterone.
Methods. A formulation of common testosterone esters (F1) was developed to enable nanochannel delivery of the low water soluble hormone. In vivo evaluation of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels by liquid chromatography/mass spectrometry and a multiplex assay, respectively, in castrated Sprague-Dawley rats implanted with nDS-F1 implants or polymeric pellets was performed over a 6-month period. The percent of testosterone concentrations observed that fell within the normal range of testosterone levels for each animal was calculated and used to compare the study groups.
Main Outcome Measures. Sustain release of testosterone in vivo for over 6 months.
Results. The subcutaneous release of F1 from nDS implants exhibited sustained in vivo release kinetics and attained stable clinically relevant plasma testosterone levels. Plasma LH and FSH levels were significantly diminished in nDS-F1 implant–treated animals, confirming biological activity of the released testosterone.
Conclusions. In conclusion, we demonstrate that nDS-F1 implants represents a novel approach for the treatment of male hypogonadism. Further studies will be performed in view of translating the technology to clinical use. **Ferrati S, Nicolov E, Zabre E, Geninatti T, Shirkey BA, Hudson L, Hosali S, Crawley M, Khera M, Palapattu G, and Grattoni A. The nanochannel delivery system for constant testosterone replacement therapy. J Sex Med 2015;12:1375–1380.**

Key Words. Testosterone; Androgen Deficiency; Sustained Release; Nanochannel Implants

Introduction

Hypogonadism in men is defined as below normal testosterone plasma levels associated with symptoms such as: loss of lean muscle mass, erectile dysfunction, loss of libido, weight gain, infertility, depressed mood, and osteoporosis [1]. Testosterone replacement therapy (TRT) is the standard of care currently used to treat hypogonadism [2]. Some of these therapies include: patches, gels, solutions, depot injections, and implantable polymeric pellets [3–6]. Although commonly used, these therapies still suffer from

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limitations that impact patient quality of life. For instance, gels, and patches, although easy to self-administer, require daily administration and can easily expose individuals in contact with the patient to excess amounts of testosterone. Injections can be used to overcome these limitations. However, the pharmacokinetic profile of injectable testosterone is not optimal given the initial burst in testosterone levels observed that is followed by a decrease to subtherapeutic levels before the next dose. Notably, polymeric implantable testosterone pellets slowly release testosterone over the course of several months; however, they exhibit typical polymer release kinetics that are initially too high and finally too low.

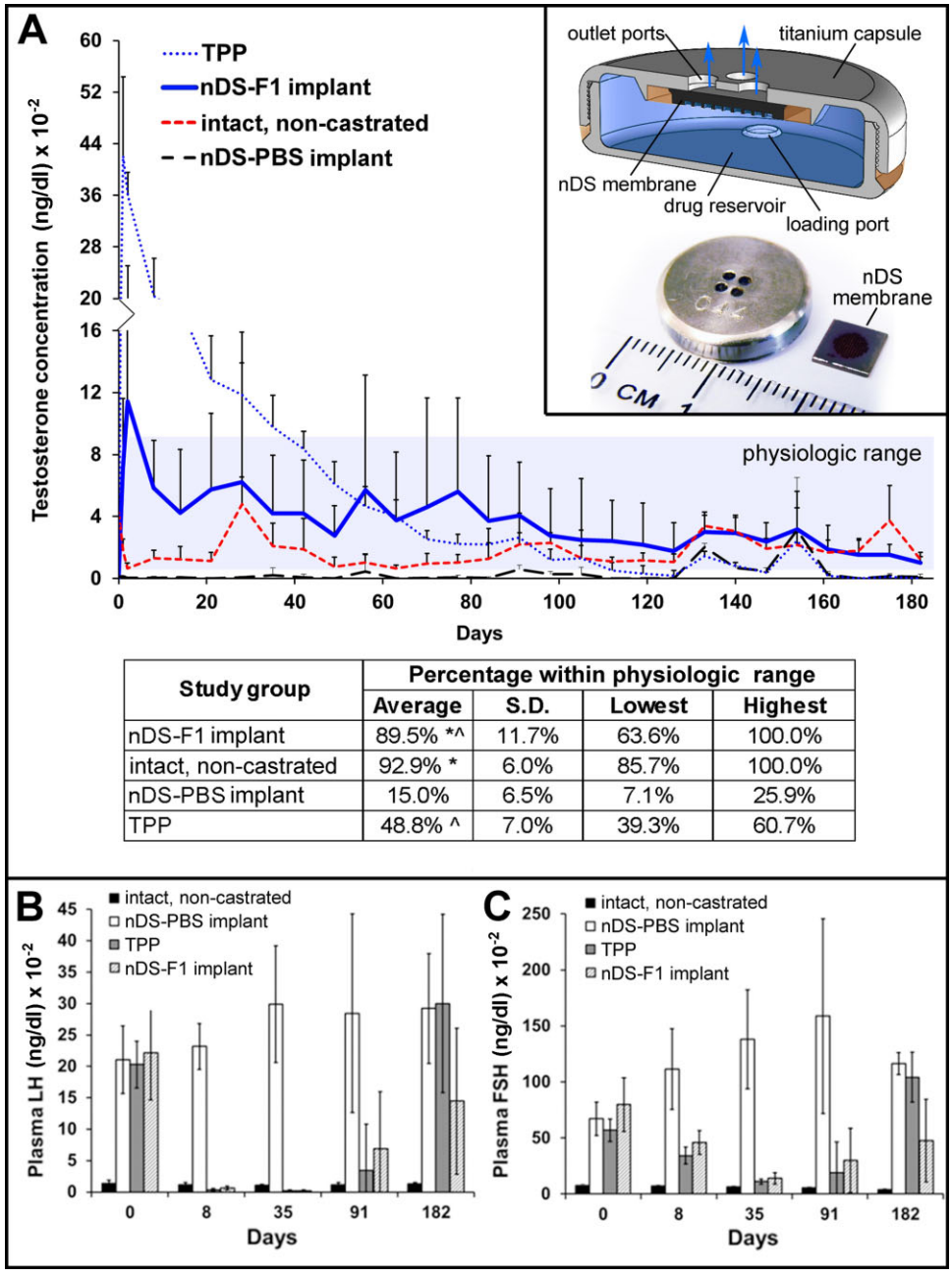
A delivery system capable of providing constant, long-term, and sustained testosterone release would markedly improve treatment options for postpubertal males suffering from androgen deficiency leading to hypogonadism. In prior work, we used our nanochannel delivery system (nDS) [7,8] to achieve long-term and sustained release of several drugs including letrozole, growth hormone, leuprolide, and interferon gamma, and nanoparticles [9] among others. Extensive description and characterization of the device can be found on previous publications [10]. Briefly, the platform leverages a silicon membrane presenting $10^5 \sim 10^7$ identical, geometrically defined nanochannels that control the rate of drug release by physico-electrostatic confinement [11,12]. The constant release is achieved by tailoring the nanochannel size to each specific drug molecule to allow passive constrain of molecular diffusion thus achieving constant release rates. By altering the number of nanochannels, nDS can be customized to vary the dose according to target daily delivery rates. The membrane is attached to an implantable titanium or polyether ether ketone plastic reservoir with medical grade UV cured epoxy [7]. The device does not require moving components or power source for its operation. It does not suffer from decaying release profiles as it is not affected by the amount of drug loaded in the reservoir up to ~90% of the released amount. The device can be easily used for the release of a broad spectrum of drugs, rendering it a potential flexible and versatile tool for clinical applications [7].

In the study reported here, we optimized the nDS for the long-term and sustained release of testosterone formulations in preclinical models. The goal was to restore rat's physiological systemic concentrations of testosterone in castrated animals.

Material and Methods

Nanochannel membranes were attached to Gr2 titanium reservoirs (NanoMedical Systems, Austin, TX, USA) with medical grade epoxy (OG116-31, Epoxy Technology, Inc., Billerica, MA, USA) and cured by UV light. Nanochannel membrane and assembly are described in detail elsewhere [13]. Testosterone formulation (~500 mg) or phosphate buffer control were loaded into the device. The amounts loaded were calculated for release treatments expected to last for about 6 months. The in vivo study was carried out according to the approved protocol (AUP-0411-0018) from the Institutional Animal Care and Use Committee (IACUC) of The Houston Methodist Research Institute. Castrated male Sprague-Dawley (SD) rats (Harlan Laboratories, Indianapolis, IN, USA) were randomized into three groups (n = 6–12): (i) nDS implant loaded with a testosterone esters formulation (nDS-F1 implants); (ii) implant loaded with phosphate buffered saline 1X, pH 7.2 (nDS-PBS implants); and (iii) 25 mg Testopel® testosterone polymeric pellets (TPPs). Intact, noncastrated rats were used as controls (n = 6). Nanochannel implants and degradable pellets were subcutaneously inserted in the dorsum of anesthetized animals. Standard postsurgical animal care was provided for up to 48 h postsurgery. Blood samples were collected at different time points from the saphenous vein. Plasma was separated at $5{,}000 \times g$ for 10 minutes.

Plasma testosterone concentrations were measured with mass spectrometry with an optimized protocol described in detail elsewhere [14]. For each animal, data were analyzed by calculating the percentage of observed concentrations that fell within the normal range of testosterone levels in plasma for rats and comparing the study groups (Figure 1A, table). The normal physiological range for rats was based on data from seven previously published studies [15,16]. Some of these studies reported the minimum and maximum plasma level values. For those studies in which the minimum and maximum values were not indicated, the values were calculated by subtracting/adding two standard deviations to the reported mean. The minimum (50 ng/dL) and the maximum (950 ng/dL) were used to define the normal testosterone range for rats. Each measured concentration was categorized as within or not within the normal range. The percentage of observations within range was calculated for each animal throughout all the time points and averaged across the animals in each study group



Study group	Percentage within physiologic range			
	Average	S.D.	Lowest	Highest
nDS-F1 implant	89.5% *^	11.7%	63.6%	100.0%
intact, non-castrated	92.9% *	6.0%	85.7%	100.0%
nDS-PBS implant	15.0%	6.5%	7.1%	25.9%
TPP	48.8% ^	7.0%	39.3%	60.7%

Figure 1 Picture: nDS membrane and titanium implants, and 3D rendering of the implant structure; Graph: Testosterone concentration in plasma from noncastrated rats (control, n = 6), and castrated rats implanted with either: nDS-PBS implants (negative control, n = 6); nDS-F1 implants (n = 14); or testosterone polymeric pellets (TPP) (n = 6). Values were corrected for animal weight differences during the experiment. Note that the *y*-axis is noncontinuous, and the scale in the bottom section has been modified for clarity. The blue-shaded region represents normal testosterone physiologic range in plasma in rats. Table: Summary statistics of the percentage of observations in each study group where testosterone was in the normal range (0.5×10^2 to 9.5×10^2 ng/dl). Average, lowest and highest percentages are reported. *t*-Tests were used to compare the percentage of observations in normal range between study groups. Averages followed by "*" are significantly different and by "∧" are not significantly different ($P = 0.05$) (SD: standard deviation) (A). Plasma LH and FSH levels obtained from all animal groups. Some castrated rats had LH beyond the range of the standard curve, hence, they were considered to be at the highest standard concentration (30×10^2 ng/dl) (B–C). Error bars represent the standard deviation (A–C).

(average %). The lowest and highest percentage for each study group was also reported. *t*-Tests were used to compare the percentage of observations in normal range between nDS-F1 implant and TPP groups, and nDS-F1 implant and intact groups. Analysis of luteinizing hormone (LH) and follicle stimulating hormone (FSH) plasma levels was performed at the Ligand Core (University of Virginia, VA, USA) with the Millipore Multiplex Endocrine Assay (Millipore, Billerica, MA, USA). At the end of the study, a necropsy was performed removing the implant and collecting tissue for histology. Tissue sections were collected from the implanted site (skin layer above exit port or in proximity of the implanted pellet) and from a nonimplanted site (least 1 cm away from the implantation site). Histological analysis including sample preparation and scoring was performed in the Pathology Core at the Houston Methodist Research Institute.

Results

To enable the otherwise poorly soluble testosterone to be delivered by nDS within the aqueous subcutaneous in vivo environment, a testosterone ester formulation (F1) was developed with an excipient content lower than 20%, and the release of F1 from nDS implants and bioactivity was studied in vivo. Titanium implants (Figure 1A, picture) assembled with the optimally sized nanochannel membrane, as determined through in vitro testing, were loaded with F1. PBS loaded nDS implants were used as negative controls, whereas TPPs, the conventional treatment, were used as positive control. Devices and TPPs were subcutaneously implanted in the dorsum of 2-month-old male castrated SD rats. All animals were treated per the approved protocol (AUP-0411-0018) from the IACUC of the Houston Methodist Research Institute. Testosterone levels were monitored on days 0, 1, 2, 8 and then weekly throughout the experiment by measuring testosterone concentration in plasma samples by LC/MS. Figure 1A reports the plot of plasma concentrations vs. time. Total testosterone levels in castrated rats implanted with nDS-F1 implants were constant and sustained for up to 182 days (Figure 1A, graph) with an average concentration of testosterone of 380 ng/dL ± 493. Noncastrated rats showed an average testosterone concentration of 180 ng/dL ± 164, whereas nDS-PBS implants, used as negative control, showed testosterone levels always close or equal to zero.

At the end of the study, tissue samples from implanted animals were processed for histopathology. Samples taken both at the exit port of the implant and 1 cm away from the implant site were analyzed by a blinded pathologist and scored for fibrosis and inflammation. Fibrosis and mild inflammation were found near the exit ports in the tissue of all animals implanted with nDS-F1 (Figure 2, table and C) and near TPP implantation site (Figure 2, table) as compared with normal tissues (Figure 2, table and B).

The levels of LH and FSH determined in plasma by a multiplex assay, indicated that the testosterone released through nDS was still biologically active [2,17]. All castrated rats showed baseline levels of LH and FSH several folds higher than noncastrated rats (Figure 1B, C); with an average of 2,120 (±500.38) ng/dL vs. 138 (±49) ng/dL for LH

A

Study group	Inflammation[1]				Fibrosis[1]			
	1 cm away[2]		proximal[2]		1 cm away[2]		proximal[2]	
	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
nDS-F1 implant	0.2	0.4	1.4	1.1	1.4	1.3	3.2	0.8
intact, non-castrated	0	0	n/a	n/a	0.8	0.8	n/a	n/a
nDS-PBS implant	0	0	0.8	1.2	0.7	0.8	2.5	1.2
TPP	n/a	n/a	0.8	1.3	n/a	n/a	2.7	1.8

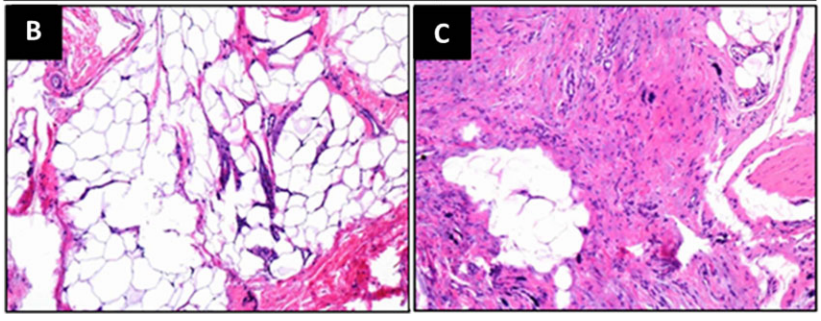


Figure 2 Histopathology results: Histology scoring of tissue collected from noncastrated rats and castrated rats implanted with either: nDS-F1 implants or nDS-PBS implants or testosterone polymeric pellets (TPPs) (SD: standard deviation). [1]Scale: Inflammation: 0, none; 1, mild; 2, moderate; 3, marked. Fibrosis: 0, 0%; 1, 25%; 2, 50%; 3, 75%; 4, 100%. [2]Tissue samples collected from the skin layer proximal to the implant and at least 1 cm away from the implant were paraffin embedded and then stained for hematoxylin and eosin stain (H&E) (A). H&E staining micrographs: Subcutaneous tissue collected from noncastrated control animal (B) and from skin layer near nDS-F1 implants exit port (C).

and 6,800 (±1,869) ng/dL vs. 750 (±117) ng/dL for FSH. Castrated animals implanted with PBS also displayed significantly elevated levels of LH (2,770 ± 1,062 ng/dL) and FSH (13,120 ± 6,540 ng/dL), for all the analyzed time points (8, 35, 91, and 182 days). In contrast, in animals treated with nDS-F1 implants and TPP, LH, and FSH levels decreased on day 8 and reached physiological levels (similar to noncastrated rats) by day 35. Additionally, both LH and FSH levels remained low up to day 91 in animals implanted with nDS-F1 and lower than in animals implanted with nDS-PBS and TPP until the end of the experiment (day 182).

Discussion

Although the average concentration of testosterone in castrated rats implanted with nDS-F1 implants (380 ng/dL ± 493) was distinguishably higher than the average concentration obtained in intact, noncastrated rats (180 ng/dL ± 164), these groups were not statistically different when the percentage of observations that fell within the range of normal testosterone levels were compared by *t*-test ($P = 0.05$) (Figure 1A, table). This indicates that the levels of testosterone released from nDS implants in castrated rats were within the physiological levels. As expected, castrated rats implanted with nDS-PBS implants, scored the lowest percentage of observations within the range of normal testosterone levels (Figure 1A, graph and table).

The observed fibrotic encapsulation and inflammation near the implants and TPP (Figure 2) are consistent with those found in literature for other implantable devices [18]. In addition, despite the fibrotic encapsulation, testosterone release was not negatively affected. This, combined with no adverse immune reaction to the implants or abnormal wound healing, confirmed the biocompatibility and safety for the use of the device in vivo, for long-term treatments. In the context of their clinical use, it is foreseeable that the devices will have transcutaneous refill capabilities to minimize repeated implantation and explanation procedures.

LH and FSH levels (Figure 1B, C), two biological indicators of testosterone function, correlate well with the constantly sustained testosterone plasma concentrations observed in nDS-F1 implant animals throughout the experiment and the testosterone concentration decrease in TPP animals after day 65 (Figure 1A).

Conclusion

In conclusion, we demonstrate that a testosterone formulation (F1) can be successfully delivered via our nDS implant in a constant manner for up to 6 months. These findings suggest that our nDS platform may mitigate several key problems with current TRT modalities and hence might represent an attractive alternative strategy for the long-term treatment of male hypogonadism. In this context, nDS implants may provide the optimal platform technology for sustaining tight constant levels of testosterone in the plasma while avoiding the peak and trough in testosterone release associated with currently adopted delivery modalities. The implant may improve patient compliance and quality of life by minimizing the need for frequent dosing and plasma level adjustments. Additionally, the nDS implant represents a flexible technology that can be adapted for the controlled long-term delivery [19] of a broad spectrum of therapeutics for chronic pathologies. A limit of the system is represented by the need of device explanation which, however, could be overcome by adopting transcutaneous refilling protocols.

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Conflict of Interest: The authors L.H., S.H., M.C., M.K., G.P., and A.G. disclose a financial interest in NanoMedical Systems, Inc. M.K. also discloses a financial interest in Merck, Lilly, Auxilium, and Meda. All other authors disclose no competing financial interest.

Statement of Authorship

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