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Research Paper

The Effects of System Parameters on *In Vivo* Injection Performance of a Needle-Free Injector in Human Volunteers

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Purpose. For a novel needle-free injection (NFI) system, the relationship between frequency of wet or incomplete injections and device-related factors and subject physiological variables was examined.
Materials and Methods. A total of 26 device configurations of a single-use pre-filled NFI system (Intraject®) were used to deliver a total of 3,211 subcutaneous injections into the abdomen of 302 healthy volunteers. Two validated methods were used to determine completeness of each injection (defined as ≥90% dose delivery). Skin-fold thickness, body mass index (BMI), Fitzpatrick skin type, sex, age, and injection site were noted for each volunteer.
Results. The proportion of complete injections ranged from 59–98% among the various combinations of device configurations. Two device parameters and two subject-related variables showed strong association with injection performance; Device gas mass (chamber pressure) and orifice size demonstrated statistically significant, independent effects, with increasing gas mass and larger orifice size associated with improved injection performance. BMI and site of injection on the abdomen also demonstrated statistically significant effects with increasing BMI and lateral rather than medial injection sites associated with better injections.
Conclusion. Both device-related factors and subject variables interact to mediate *in vivo* performance of a needle-free injector.

KEY WORDS: device and subject variables; *in vivo* performance; needle-free injection systems; safety and tolerability.

INTRODUCTION

Although needle-free transcutaneous delivery of drugs via injection into the subcutis has long been achievable (1–6), many needle-free injection (NFI) devices have suffered reliability limitations, in particular due to incomplete or so-called 'wet' injections whereby not all of the injectate is delivered successfully (7). In some cases the frequency of wet injections among marketed devices has been consistently 10–20% or even higher (7), and this inconsistency of penetration has been cited as a primary impediment to the progress of the development of needle-free injection (8). The frequency of sub-optimal performance may be explained to some extent by the fact that needle-free injectors have gained regulatory approval as devices, in which the requirement to establish meaningful reliability in the *in vivo* setting has been absent or satisfied with a paucity of data. With the advent of disposable, pre-filled NFI systems, much greater scrutiny will be placed on device performance because the devices will be regulated as a combination drug product, and thus the performance will be viewed by regulatory authorities as being inextricably linked to the safety and efficacy of the drug being delivered.

There are few reports describing whether the phenomenon of incomplete injection is due to some characteristic in the design of the device, the patient's use of the device, the inter-individual variations in the histomorphology of the skin, or perhaps the result of some complex interaction among all these factors. For example, a number of fundamental mechanical and biophysical properties of human skin relevant to transcutaneous drug administration have been studied including factors affecting the propagation of forces applied to the skin surface (9–11), stress, strain, and stiffness characteristics and their relationship to skin composition and content such as collagen and elastin (12), the effects of varying physicochemical environments on stress-strain variables (13), and the observation of racial differences apparent in various anatomical and physiological functions of the skin respondent to chemical insult (14). Despite the considerable respective efforts devoted to characterizing skin composition and properties and the separate engineering efforts expended on injection device design development, there has been little or no attention devoted to studying or optimizing the biology-device interface in the needle-free injection field.

We therefore sought to investigate this question further by modeling the relative contributions of device-related versus subject-specific factors in the performance of needle-free injection from data obtained during the evaluation of multiple configurations of the Intraject® needle-free device during the course of device optimization. The Intraject® device (currently in late-stage development by Zogenix Inc., Emeryville, CA, USA) is a pre-filled, disposable, single-use, needle-free

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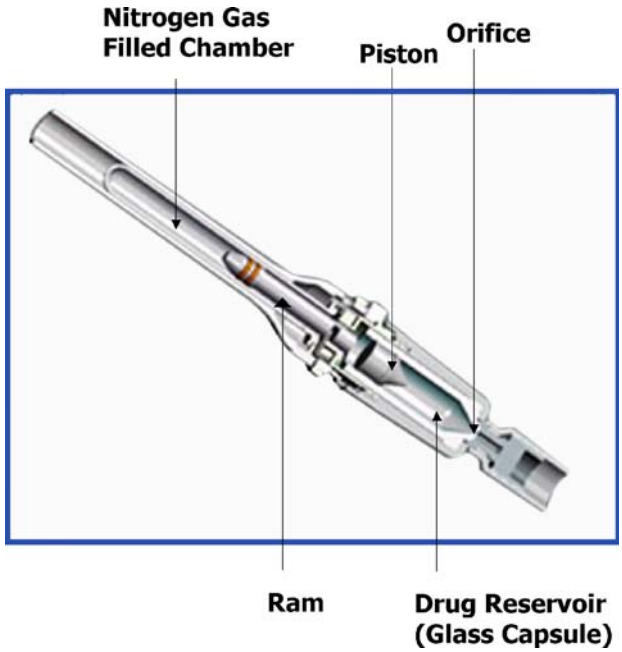

Fig. 1. Intraject® needle-free injection system.

injector, designed for delivering a fixed dose of liquid drug formulation (up to 0.5 mL) subcutaneously (Fig. 1).

Injection performance for a needle-free device is defined in terms of the frequency of wet injections in a sample of injections, a wet injection occurring when <90% of the injectate is delivered subcutaneously. Injection performance was evaluated across multiple configurations of the Intraject device, with configurations varying by gas mass (chamber pressure), orifice size, and piston material to arrive at the various configurations. A total of 26 different configurations were studied, along with a variety of biometric measurements of skin histomorphologic characteristics and other physical examination information.

METHODS AND MATERIALS

Device Configurations

Twenty-six device configurations were tested (Table I). Each configuration represented one combination of the following four factors (see Fig. 1 for device details): nitrogen chamber gas mass (proportional to chamber pressure) varied from 127–162 mg, orifice size varied from 0.295–0.435 mm diameter, and two different piston material compositions. Piston material composition P2 is stiffer than P1, potentially facilitating better energy transfer between the ram and the piston upon actuation. Not all possible device configuration combinations were evaluated based on previous experiments and clinical trials that provided insight into the expected system performance.

In Vitro Testing

Among other clinical release tests, device configurations were tested *in vitro* to assess the pressure profile in the glass capsule (formulation container) as a function of time. This was done in two ways, by affixing strain gauges to the glass capsule and measuring the strain as a function of time during actuation, and by simultaneously measuring the axial force exerted on a target by the liquid jet exiting the device as a function of time. A total of 25 trials were conducted for each configuration. Several metrics were assessed based on the average generated pressure profile for each configuration (a typical example of which is shown in Fig. 2). These metrics included Total Injection Time, Peak Pressure, and Pressure at 10% of the Injection Time. Delivered volume was measured gravimetrically for 15 of the 26 configurations (n=20 trials per configuration).

In Vivo Study

This was a double-blind, randomized study in 302 generally healthy male and female adults. Each subject received 12 subcutaneous injections of isotonic saline to the abdomen. Each injection was administered using a different device configuration (12 different configurations per subject) and injections were administered according to a randomized, balanced, incomplete block design so that the 26 configurations were distributed randomly among subjects and balanced across abdominal site (Fig. 3; note the two injection levels, one at the level of the naval, and one 25 mm below the naval, with six sites at each level, A–F).

Injections were delivered over two days, six per day, separated by an interval of a week. To administer the injection, the device is held perpendicular (90°) to the subject's skin and pressed, which actuates a trigger mechanism. This causes a small charge of compressed nitrogen to force a metal ram to accelerate forward until it impacts a plastic piston. The piston forces the liquid drug formulation through a small orifice in the opposite end of the drug container, which is in contact with the skin. The injectate penetrates the dermis and deposits into the subcutis.

In Vivo Performance Assessment

Assessment of the injection (successful or wet), sensation at the injection site, and injection site signs (bruising, erythema, bleeding) were made at the time of injection. Two additional measurements of injection site sensations and signs were made at 1- and 24-h post-injection. All device configurations appeared identical so that neither the patient nor the nurse administering the injections knew which configuration was being used.

Each injection was visually inspected using a validated 5-point scale (Visual Assessment Score [VAS]) to estimate the degree of completeness of the injection by estimating the amount of fluid, if any, not injected, as follows:

0 = 100% splash-back of injectate, no hole in the epidermis
1 = Hole in the epidermis but very little, if any penetration of injectate
2 = Some penetration of injectate (5 to <90%)
3 = 90–94% penetration of injectate
4 = ≥95% penetration of injectate

A second method of precisely quantifying uninjected fluid was used by measuring the net change in the weight of a piece of filter paper placed at the injection site after injection

Table I. Injection Performance of Intraject Devices

Orifice Diameter (mm)	Piston Type	Gas Mass (mg)				
		127	134	141	150	162
0.295	P1	101		100		100
		59%		**76%**		**90%**
	P2					
0.340	P1	149	149	150	150	100
		66%	**77%**	**87%**	**85%**	**94%**
	P2	100	100	98	100	
		74%	**91%**	**92%**	**92%**	
0.385	P1	202	202	199	200	100
		71%	**79%**	**82%**	**89%**	**94%**
	P2	100	100	99	100	
		81%	**93%**	**94%**	**91%**	
0.435	P1	100	99	101	101	102
		69%	**79%**	**89%**	**94%**	**98%**
	P2					

In each cell of the table, the top number is the total number of injections, and the bottom number is the percentage of complete injections. Complete injection was >90% injectate delivered.

to absorb remaining fluid, if any. A combination of the two methods was used to rate injection success.

A successful injection was defined as a VAS of 3 or 4, combined with increase in weight of the filter paper of less than or equal to 50 mg. If the filter paper had a significant amount of blood staining, than only the VAS was used. A wet injection was defined as a VAS of 0, 1, or 2 or an increase in weight of the filter paper of greater than 50 mg.

Sensation at the injection site was assessed immediately after each injection and at 60-min and at 24-h after the last injection using a categorical pain scale (0=no pain to 10=worst imaginable pain). Injection site signs were assessed in a similar manner by measuring the diameter in millimeters of any erythema, and/or bruising at the injection site. Whether or not blood was present was also recorded.

Fitzpatrick Skin Type scores were obtained by means of a self-reported questionnaire. Subjects' height, weight, abdominal caliper skin fold thickness (SFT), heart rate, and sitting blood pressure were also recorded.

Statistical Analysis

An analysis of variance (ANOVA) model was used to estimate the effect of each experimental factor on the proportion of successful injections primary endpoint. The following untransformed factors were included as main effects in the overall model: gas pressure, orifice size, shock absorber, subject age, BMI, SFT, Fitzpatrick skin type, injection site, injection day, and subject gender. Interactions between variables were also investigated. Statistical significance was reached when $p<0.05$. Results are presented in a standard

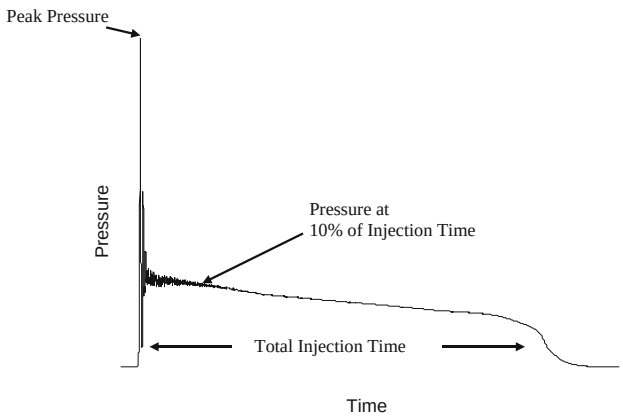

Fig. 2. Pressure profile of the formulation inside the drug reservoir as a function of time following actuation of the Intraject Needle-free Injection System. Total injection time is approximately 50 ms.

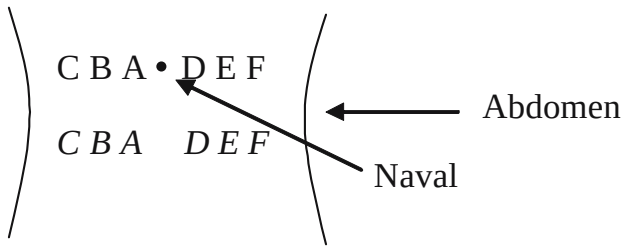

Fig. 3. Abdominal injection sites. *Top row* indicates sites injected during first clinical injection visit (*aligned with the naval*) and *bottom row* (*italics*) indicates sites injected during second clinical injection visit.

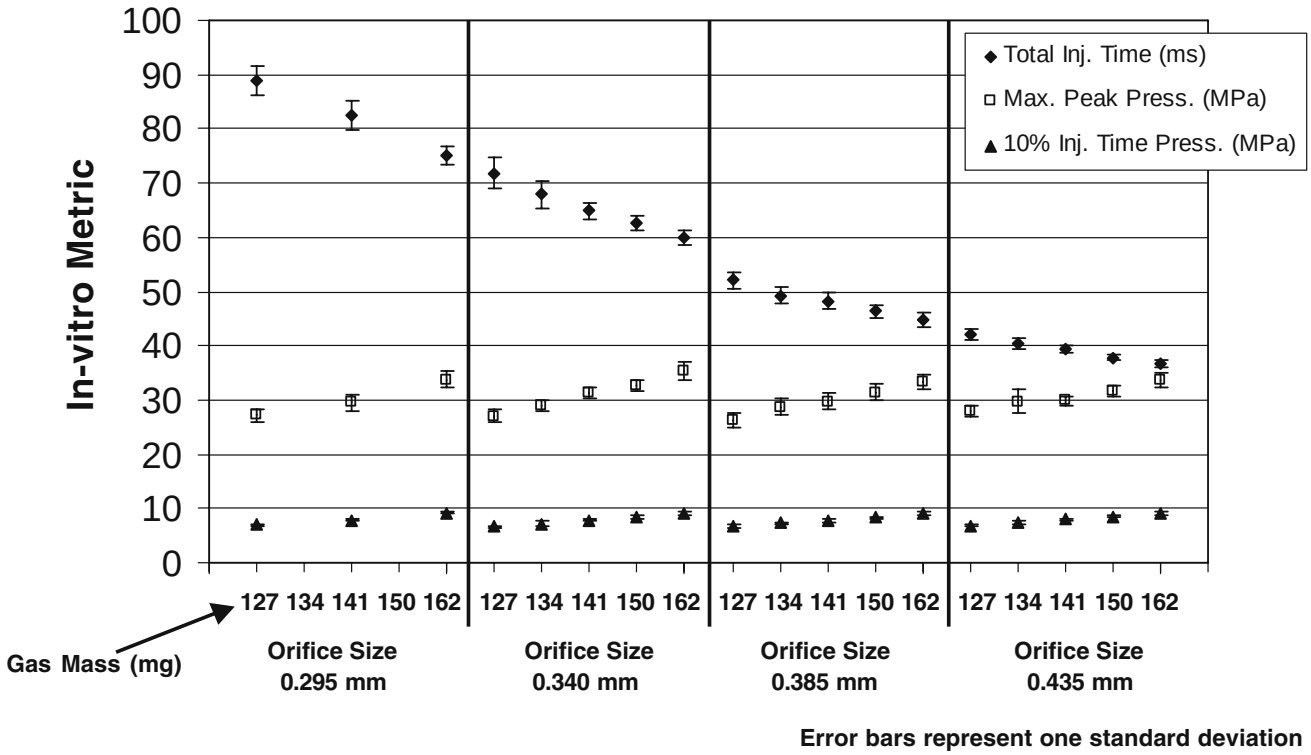

Fig. 4. *In-vitro* metrics for Intraject configurations with the P1 piston. Each data point represents the average of 25 trials using the indicated gas mass and orifice size. The units for each metric are indicated in the legend, and all metrics refer to the same *y*-axis.

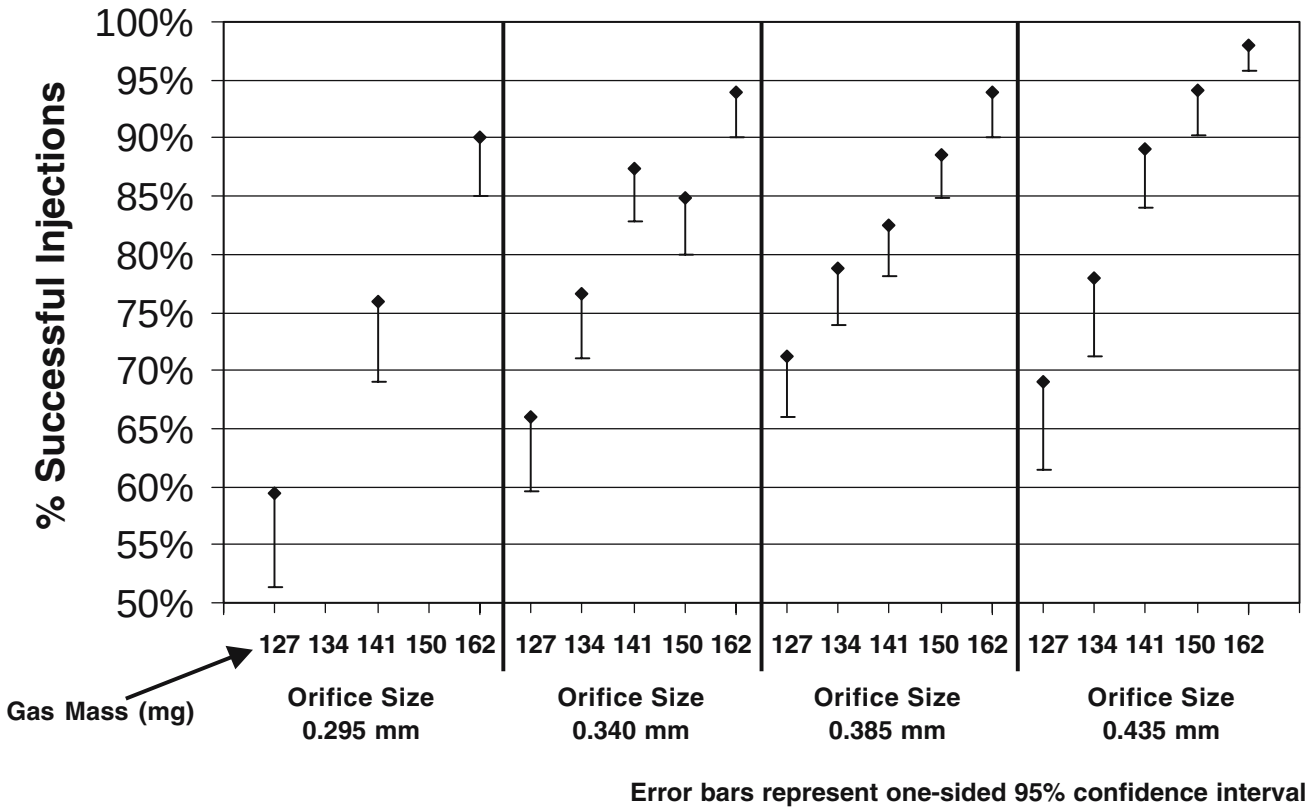

Fig. 5. Effect of orifice size and gas mass on Intraject injection performance for configurations with the P1 piston.

Table II. Analysis of Variance: Effect of Device and Subject Variables on Injection Performance

Variable	DF	*F*-Value	*P*-Value	Comment
Orifice	1	15.13	<0.001	Positive correlation
Gas Mass	1	193.26	<0.001	Positive correlation
Age	1	1.57	0.210	
Body Mass Index	1	41.45	<0.001	Positive correlation
Skin-fold Thickness	1	0.04	0.844	
Skin Type	1	0.00	0.966	
Injection Site	5	13.45	<0.001	Lateral sites better than medial
Injection Day	1	10.35	0.001	Second day better than first
Sex	1	0.12	0.726	
Error	3,197			

ANOVA summary table and descriptive statistics are shown for individual factors of interest and displayed graphically.

RESULTS

All device configurations were tested *in-vitro*. The metrics calculated from the injection profile testing, (Total Injection Time, Maximum Peak Pressure, and 10% Injection Time Pressure), are shown in Fig. 4. Mean delivered volume ranged between 0.494–0.510 ml across all configurations tested, with individual values ranging between 0.488–0.518 ml. The nominal injection volume was 0.500 ml.

A total of 3,211 injections were administered. Two subjects withdrew from the study after the first set of injections for personal reasons not related to the trial. There were no serious adverse events. Injection performance for the device configurations tested is shown in Table I and Fig. 5. The overall rate of successful injection (>90% of injectate penetration) ranged from 59–98% among the 26 device configurations.

Two device parameters and two subject-related variables showed strong association with injection performance in addition to an observed period effect (Table II). Both device gas mass (chamber pressure) and orifice size demonstrated statistically significant, independent effects (F=193, df=1, p<0.001; F=15.1, df=1, p<0.001, respectively) showing a positive relationship to injection performance, with increasing gas mass and a larger orifice size both associated with improved injection performance (Fig. 5).

Body mass index (BMI) and site of injection also demonstrated independent, statistically significant effects (p<0.001), with increasing BMI having a positive relationship to injection performance and lateral injection sites being better than medial injection sites (F=13.45, df=5, p<0.001). BMI shows significantly lower injection performance for low BMI values (Fig. 6). A significant period effect was evident (F=10.35, df=1, p=0.001) with improved injection performance noted on the second day of injection.

Secondary endpoints of sensation, bleeding, and bruising at the injection site indicated that all configurations were well

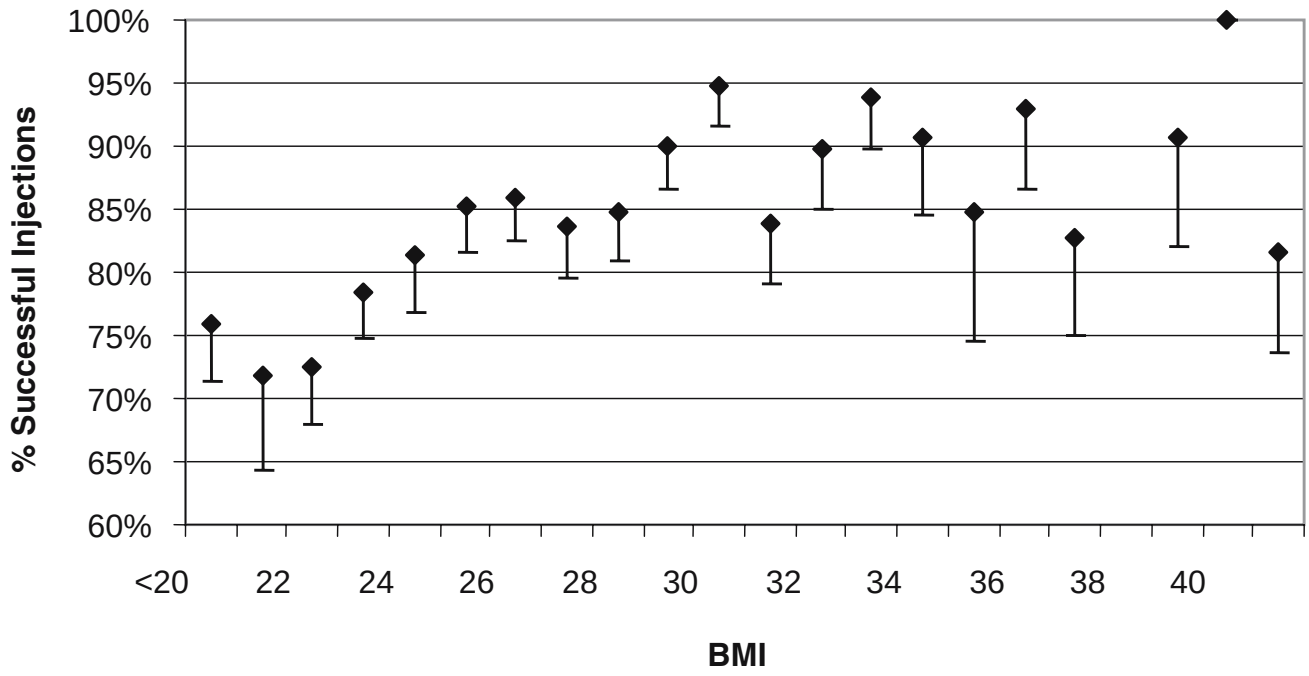

Fig. 6. Injection performance as a function of subject body mass index, across all device configurations tested. *Error bars* represent one-sided 95% confidence interval.

tolerated with mean sensation scores for the different configurations ranging from 1.2 to 1.9 units (possible range, 0 to 10). The overall incidence of blood observed at the injection site was ≤4% (mean was <2% for the entire study) for all configurations, except for one outlying configuration (150 mg gas mass, 0.435 mm orifice diameter, P1 piston) for which blood was noted at the injection site in 9% of cases. There is no clear explanation for this outlier.

The incidence of bruising observed for all configurations (obtained at 24 h post-injection) ranged from 0 to 5%, with no discernible pattern across device configurations. Bruising occurred for <2% of the entire 3,211 injections; 15 of the 26 configurations (58%) caused bruising in 1% or fewer of the injections.

DISCUSSION

It is apparent from this study that both device-related factors and subject variables interact to mediate injection performance. Nitrogen gas mass, and hence glass capsule pressure during injection, appears to exert a strong influence on the likelihood of a wet injection occurring. This is perhaps intuitive since a device that ejects fluid at a relatively low pressure is less prone to penetrate skin than one ejecting fluid at a substantially higher pressure (Fig. 5).

Work by Shergold (15) and Schramm and Mitragotri (8) have shown that a larger orifice size requires a lower pressure to penetrate skin owing to the mechanism of skin puncture. This would therefore enable a device with a larger rather than smaller orifice to have a higher proportion of successful injections at constant gas mass, a principle clearly reflected in these data. The principal of 'jet power' as a determinant of dermal delivery penetration and dispersion of injectate has been described as a combined function of nozzle diameter and jet velocity (16).

Selection of the site of injection appears to be an important factor in the success of needle-free injection delivery. When administered to the abdomen, abdominal sites lateral and distal to the navel (C and F in this study) are superior to sites medial and proximal to the navel (sites A and D). This is consistent with work by Jansen (17) who demonstrated that human abdominal skin is up to 40% thicker near the navel than in the lateral regions of the abdomen, with thinner skin favoring better injection success.

We have found substantiation for anecdotal evidence that has been accumulating for some time that suggests that individuals with little subcutaneous fat, and hence low skin fold thickness and BMI, are more difficult to inject successfully. This could be due to the increase of pressure in the fat under the skin during the course of injection as a result of the fluid accumulating at the injection site. A subcutaneous increase in pressure could eventually lead to a point of equilibration, where the pressure in the fluid under the skin is equal to that produced by the liquid jet outside the skin. This would result in a complete loss of motive force for the remainder of the injection, forcing the remainder of the fluid to be deposited on the skin surface, rather than in subcutaneous fat.

It follows that a higher fluid pressure, resulting from a higher loaded device gas mass, would decrease the likelihood of this occurring since higher fluid pressure creates a deeper hole and a larger subcutaneous space for deposition of the injectate. The relationships among device parameters, injectate deposition, and skin drilling are confirmed by previous work showing that hole depth is significantly related to drug delivery by jet injections. For a given jet velocity and nozzle diameter, hole depth increases with increasing volume up to an asymptotic limit and decreases as a function of increasing uniaxial Young's modulus of skin (18). A deeper hole and a larger subcutaneous space would thus lead to a lower incidence of incomplete injection and little or no fluid remaining on the skin. In fact a lower incidence of incomplete injection is observed for configurations with increasing gas mass, as hypothesized.

No significant relationship was found between injection performance and subjects' Fitzpatrick score, sex, or age. This suggests that the device is likely to perform similarly across different demographic populations, a finding borne out by an earlier unpublished report,[1] which showed 99% successful injection rates across a wide range of ethnic types, ages groups (18–80 years of age), and in both males and females. This is despite clear differences in skin composition and skin physiology (e.g., differences in intracellular cohesion, lipid content, sebum expression, elasticity, hydration of the stratum corneum, lipidization) which have been reported for varying ethnic and age groups (14).

In the present study, injection performance was significantly better on the second day than on the first day of injection. It is important to mention that one group of patients had their second set of injections before a second group had their first set, therefore the observed period effect is likely more attributable to the entire cohort rather than to study-specific factors. However, three possible explanations include: (1) a 'training' effect e.g., the nurse who administered all the injections was somehow improving her technique over time. This seems unlikely, partly because the device is extremely simple to use and partly because the improvement was observed even in the last group of patients, by which time the nurse had already delivered over 2,500 injections; (2) the properties of the skin at the site of the second set of injections, which was 1 in. (25 mm) below the first injection, were different. Although there are data to suggest that the skin is thinner further from the naval as previously discussed, it seems unlikely that such a small distance would contribute independently to a significant difference in performance, across all the sites which were spread over 10 in. (250 mm) across the abdomen; (3) the subjects were psychologically more comfortable with the injection system and procedure at the second visit and perhaps this had some effect on skin properties (e.g., through changes in neuromuscular tone), thus enhancing the ability of the liquid jet to penetrate the skin. Discussions with the study staff, including the nurse who administered all the injections, suggested that the subjects were substantially more relaxed on their second visit.

The eventual explanation could, of course, be any combination of these and other reasons, but it suggests that it is good clinical practice to ensure that subjects are as comfortable with the device and the procedure as possible, and to use the optimum injection sites.

In terms of injection site sequelae, all configurations seem to be approximately equally well tolerated. The

[1] Data on file at Zogenix Inc.

sensation scores are consistently very low, with the mean score for all configurations <2, and the bruising level, at <2% across the entire study, is less than or equivalent to that seen with needle-based injection, according to anecdotal data.

The best-performing configuration demonstrated ≥98% successful rate of injection, while the three worst showed 59, 66, and 71%. These three configurations all contained the lowest gas mass used in the study. This suggests that, if there are 'randomly' occurring wet injections, they are very infrequent. It also suggests that device performance within each group is likely to be highly reproducible, otherwise the observed performance would evidence more variability. This assertion is corroborated by *in vitro* data from individual device configurations (Fig. 4). Key variables, such as overall injection time, were found to have a standard deviation of <4%.

CONCLUSIONS

Optimizing needle-free injector performance depends on understanding and implementing several key design principles. Our evaluation of needle-free injection performance suggests that: (1) device factors play a large part in mediating the occurrence of wet injections. Identifying the proper combination of gas mass and orifice size is essential for optimizing device performance. Optimum device design appears to depend on a higher, rather than lower gas fill, and a larger, rather than smaller, orifice diameter. Such a designed device can yield >95% successful injections over broad subject demographics, and injection sites; (2) there is little or no association with Fitzpatrick skin type or the subject's sex or age, suggesting that the Intraject System is likely to be appropriate for a very broad range of subject groups. There is a relationship between thicker skin, thinner subcutaneous layers, and a higher incidence of wet injections. Avoiding injection sites with thicker skin further ensures consistent success; (3) various configurations were all well tolerated, with low sensation scores and little or no bruising or bleeding evident. Such sequelae have historically been issues with needle-free devices; (4) the various configurations were mechanically robust, with no glass capsule failures occurring during the study; (5) it is prudent to ensure that subjects are familiar with needle-free devices before giving injection to ensure consistent results.

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