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NEEDLE-FREE DRUG DELIVERY SYSTEMS: A REVIEW

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ABSTRACT

This article will provide an overview of the three broad needle-free technologies: powder injection, depot (or projectile) injection, and liquid injection. This will cover only those technologies where the drug formulation itself is used to penetrate the skin via its mechanical energy. It will not describe any technology where a needle is used to puncture the skin, even if the needle is not visible to the patient or only the epidermis is punctured, such as mini-needles, microneedles, pen injectors, or autoinjectors. Also excluded are systems that ablate the skin mechanically or otherwise disrupt its chemical or mechanical structure to increase its permeability, such as laser ablation, microdermal ablation, electroporation, or iontophoresis. These are usually referred to as transdermal drug delivery, but can also be described as needle free.

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INTRODUCTION

The term “needle-free” has been used to describe a very wide array of drug delivery technologies, from those that do not have a needle but use electrophoresis to drive drugs through the skin, to those that use one or more very small needles, but needles nonetheless (often known as microneedle drug delivery). Needle-free drug delivery was first proposed as early as the mid-19th century¹ and was demonstrated to work many decades ago with one of the first major patents filed by Lockhart in the 1930s.² This avoids a multitude of disadvantages that are inherent in needle use:

- Risk of cross-contamination from needle-stick injury.
- Under- or overdosing resulting in poor injection technique of patients.
- Costs of sharps disposal.
- Needle phobia (up to 15% of people are clinically needle-phobic, and most people are apprehensive about receiving injections).
- Injection site pain.
- Poor compliance leading to long-term worsening of conditions.
- Increased costs due to patients visiting hospitals/physicians' offices for injections.

NEEDLE-FREE INJECTION TECHNOLOGY

Powder Needle-Free Injection

Powder needle-free injection relies on being able to formulate particles of a sufficient density and accelerating them to a sufficient velocity that they will penetrate the skin in large enough numbers to produce a therapeutic dose.

The developers succeeded by using a power source of compressed helium gas (the speed of sound in helium is about three times greater than in air, so much greater flow speeds are achievable without the complications of becoming supersonic) and using two different ways of formulating the drug. In the first, the drug (pure, or sometimes with excipients) is presented as hard particles of 10–50 μm in diameter, which have a density approximately the same as the crystalline drug. In the second, mostly used for vaccines, the drug is coated onto gold spheres of a few micrometers in diameter. The gold particles act as a vector for the vaccine,

penetrating the stratum corneum and then often the cell membrane, to enable the DNA to be taken up in the cell nucleus, for a DNA vaccine.

The drug is stored in a single-dose, disposable, “cassette,” which is aseptically assembled and consists of an annular support with the drug in the central space and a polymeric lid on either side. When the device is activated, the helium pressure is exerted on the lid on one side causing it to rupture, which in turn causes the pressure to be exerted on the other lid, rupturing it and carrying the drug particles forwards. The particles pass through a convergent–divergent nozzle, which accelerates them to a significant fraction of the speed of sound before they impact the skin. The particles that reflect off of the skin (due to insufficient momentum per unit area) are filtered out of the helium stream in an exhaust filter that also slows down the helium flow to reduce the noise generated by the device.

The device itself has been through a number of iterations, with the most recent appearing to be about 120mm long and about 30mm in diameter. It is a single-use, disposable device although designs of a reusable device with a replaceable helium canister and drug cassette have been developed.

It is difficult to accurately predict the proportion of a dose that is delivered into the epidermis since not many particles have sufficient momentum to travel through the epidermis into the dermis. Also, the maximum payload for a 20mm diameter “target area” of skin is about 2–3 mg. Therefore, this technology is best suited to those drugs with an effective dose of 1 mg or less, where accurate dose titration is not required and, ideally, where epidermal delivery is advantageous. Vaccine delivery fits all these criteria, DNA vaccines in particular. The epidermis has a very high concentration of antigen-presenting cells and is therefore an ideal site for administration of vaccines. PowderJect data would suggest that significantly lower doses of vaccine are required using their technology when compared with intramuscular injection for an equivalent immune response.^{3,4}

Another application that has been explored in detail with this technology is the delivery of local anesthetic to the skin and oral mucosa. The technology has demonstrated the ability to achieve consistent local anesthesia in both applications.³

Depot (or “Projectile”) Needle-Free Injection

In this very recent embodiment of the technology, the drug is formulated into a long, thin depot with sufficient mechanical robustness to transmit a driving force to a pointed tip. This tip may be formed from the formulation itself or it may be formed from a soluble inert material, e.g., a sugar.

The depot is sterile and needs to either be manufactured aseptically or terminally sterilized. A simple way of connecting the sterile depot to the delivery device also needs to be developed, except if the entire delivery system is disposable.

The depot is driven into the skin with sufficient force to puncture the skin and to push it into the underlying fat. A typical depot might be about 1mm diameter and a few millimeters long. This limits the “payload” to a few milligrams, but this is more than adequate for many new therapeutic proteins and antibodies and even some small molecules. Dose titration cannot be achieved from a specific depot but some level of dose variation can be achieved by manufacturing depots of different lengths. It is also possible to target intradermal delivery to some extent by using a very short depot that is intended to reside in the dermis, which is usually about 2mm thick.⁵

The pressure to puncture the skin with a sharp-tipped punch, which the depot effectively represents, has been shown to be on the order of 3–8 Megapascals (MPa), depending on the area of the body and the individual.⁶ For a 1mm diameter depot, this requires a force of only a few Newtons (N). A delivery device, therefore, would need only a relatively small spring, assuming that there is an effective way of transmitting the spring energy directly into the depot.

Liquid Needle-Free Injection

Overview

As previously described, liquid needle-free injection was the first needle-free technology to be developed and has been the focus of the vast majority of companies working in the industry. Indeed, many millions of

needle-free injections have been successfully administered over the last 50 years, predominantly for mass vaccination campaigns of military personnel.^{7,8} This practice was banned in the mid-1980s when it was shown that it was possible, although extremely rare, for disease to be transmitted from one patient to another through the reusable needle-free injection equipment.⁹ Although a number of companies continue to develop needle-free systems for vaccine use, there has been a significant shift recently toward the delivery of both small molecule therapeutics and proteins using needle-free systems.¹⁰ Many of the technical requirements are similar across all these areas, but there are some differences which are addressed below. The key to achieving a successful injection with a needle-free system is to understand the necessary mechanics for the consistent penetration of skin and fat with a liquid jet, without causing unnecessary trauma to the tissues and to the molecule being delivered. Only recently have detailed studies of the fluid mechanics of needle-free injection appeared.^{6,11} The fluid mechanics conditions necessary for a consistent targeted needle-free injection are addressed in the following section.

Puncturing the skin

The fundamental technological requirement of a needle-free injection system is that it consistently punctures the skin and delivers the liquid into the desired tissue, most commonly the subcutaneous layer, but may also be the dermis or intramuscular tissues. A column of liquid is analogous to a blunt punch and thus requires about twice the pressure to puncture the skin than would be required by a sharp-tipped punch or a solid needle. Depending on the site of injection and the mechanical properties of the skin for an individual, this pressure is about 12–15MPa for a jet of about 0.4mm in diameter. This pressure is significantly higher for smaller jets.^{6,12} Any successful needle-free technology needs to achieve this minimum pressure with enough excess to allow for dissipation over its shelf life and for interpatient variability, and rapidly enough so that drug is not wasted by being delivered from the device before the skin is actually punctured.

Drilling the fat

Even once the skin has been punctured, this does not guarantee a successful needle-free injection. There are several technical conditions that must be satisfied to ensure all the fluid is delivered into the subcutaneous layer.

The first condition is that the orifice of the device remains correctly positioned over the hole in the skin. This is known as registration. If the device slips along the skin during the injection, then the liquid jet could cut the skin if the pressure is still high enough to cause a puncture. This is clearly undesirable and was an issue with early needle-free technologies. On the other hand, if the pressure has dropped below skin puncture threshold, as is probable for reasons explained below, then an incomplete injection will result. This problem can be substantially avoided by designing the device so that it is actuated by pushing it against the skin, as opposed to implementing a separate trigger, by ensuring the injection is complete in less time than human reaction time (around 100 msec), and by not using a too small orifice. A larger orifice, for example, will be more tolerant of 50 mm of registration “slip,” than a very small one.

The second condition is that the fluid pressure must remain high enough to keep the hole in the skin open and avoid the elastic forces that tend to cause resealing. This pressure is on the order of a few megapascals, and so premature resealing is only likely to occur for devices that exhibit a substantial pressure reduction near the end of the injection.

The third condition is that the initial pulse of fluid is able to drill a deep enough channel into the fat to enable the remainder of the dose to be dissipated away from the hole in the skin. Otherwise, the fluid pressure immediately inside the skin is equal to that in the device nozzle and there is no longer enough “driving force” to push the fluid into the skin, making it unlikely that the injectate will continue to go through the skin and will instead remain on the skin surface. This possibility is overcome by designing an appropriate initial pulse of fluid having sufficient energy to enable delivery of the design dose in the time and through the orifice size that have been chosen. This quantum of energy will vary

considerably according to orifice diameter and drug dose.

Finally, the fourth condition is that the pressure drops sufficiently quickly and to an extent to where the fluid cannot penetrate the muscle fascia and result in an intramuscular injection. Some individuals have a subcutaneous layer that is only a few millimeters thick and so the muscle fascia is almost certain to be impacted by the liquid jet and if the pressure of the liquid does not drop substantially in the first few milliseconds, possibly even the first millisecond in very thin people, then an intramuscular injection will result. This will consequently result in a significantly different pharmacokinetic profile and may also result in a significantly more painful injection. These conditions are summarized in Fig. 1.

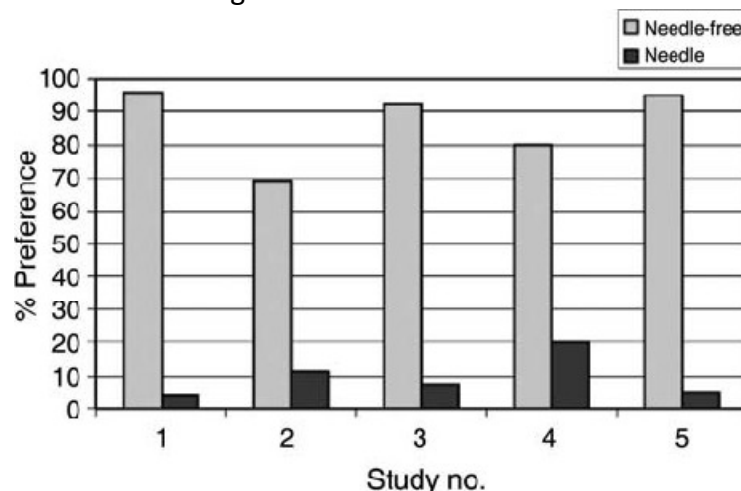


Fig.1 Summary of preference between needle-free delivery (Intraject, Aradigm Corporation) and needle-based delivery (courtesy of Aradigm Corporation). Volunteers were asked which system they would prefer if they had to self-inject regularly at home.

Power sources

There are a wide variety of power sources available to designers of needle-free injection technology. There has been a recent trend which seems likely to continue of making devices self-contained and easily portable, obviating some of the originally used power sources such as compressors, large tanks of compressed gas, large batteries, and foot-powered devices.

Clinical data and acceptability

Much of the discussion in this article has focused on the physics of drug delivery into or through the skin. However, clinical efficacy is also critically important, since this is the true purpose of any injection system. Achieving the desired clinical outcome depends on three key factors:

- Ensuring that the correct dose of the drug is delivered into the target tissue.
- Ensuring that the drug is not adversely affected by being delivered through a tiny orifice at very high pressure.

- Ensuring that the pharmacokinetics of the drug are appropriate to provide the intended therapeutic effect.

Table 1 summarizes an incomplete list of drugs for which there exist clinical data from needle-free administration, all of which demonstrated broadly similar performance between the needle-free system and the needle system.

Table 1 Reports of needle-free injection containing clinical data

| Drug | References | Comments |
|------------------------------|-------------------|---|
| Insulin | 13-15 | Liquid delivery |
| Insulin | 16 | Powder delivery |
| Human growth hormone | 17-19 | Some variability in the pharmacokinetics between the studies |
| Erythropoietin | 20 | |
| Lidocaine | 20-23 | |
| Monoclonal antibodies | 24 | |
| Vaccines | 25-30 | Various vaccines have been studied, including hepatitis A and B, yellow fever, influenza, measles, mumps, rubella, diphtheria, tetanus, typhoid, and tuberculosis |
| Alpha interferon | 31 | |
| Gamma interferon | 32 | |
| Morphine | 33 | |
| Triamcinolone | 34 | |
| DNA vaccines | 35,36 | |
| Low molecular weight heparin | 37 | |
| Orgalutran (GnRH) | 38 | |

Recent published data from both Intraject_ (Aradigm Corporation) and MiniJect_ (Biovalve) have shown that these reliability issues are no longer experienced and that patients prefer needle-free injections even more strongly, typically in the 85–95% range compared to needle-based injection. This was partly because patients experienced less sensation but also because of the huge ease of use benefits resulting from a well-designed, easy to use, disposable needle-free device (Figs. 1).^{39,40}

CURRENT NEEDLE-FREE INJECTION TECHNOLOGIES

The needle-free injection field has evolved enormously in recent years. Companies have come and gone, taking with them some technologies. This section summarizes the technologies and the companies behind them that are currently active in the field. A review of some of the more prevalent technologies was published in 2002.⁴¹

Algorx

Algorx has the rights to develop the PowderJect needle-free injection technology in all areas outside of DNA vaccines. The device is helium powered.

Antares

Formerly Medi-Ject, Antares has over 20 years of experience in the industry and continues to develop patient-filled, reusable devices for a broad range of applications, particularly those requiring frequent, chronic dosing. Their devices are powered by metal springs.

Aradigm

Aradigm has recently acquired the Intraject technology, first developed by Weston Medical. This is a single use, prefilled, disposable system, powered by compressed nitrogen gas. It is principally suited for acute use or infrequent chronic dosing, where dose titration is not needed or is done in discrete steps.

BioJect

BioJect has been in the field for many years and their Biojector and Vitaject products are well known in the industry. Their devices are patient-filled, reusable devices, powered by either compressed carbon dioxide or by metal springs. In addition, they have the Iject, a prefilled, disposable device in development.

BioValve

BioValve has developed the MiniJect, a disposable device that enables the patient to fill it easily from a separate vial. It is powered by a proprietary chemical gas generation system.

Caretek Medical

As described above, Caretek is pioneering the “projectile” depot needle-free delivery system that is currently in development.

Cross-Ject

Cross-Ject has developed a system based on airbag gas generation technology that claims to be prefilled and disposable.

National Medical Products

National Medical Products (NMP) developed a disposable needle-free injector, the J-Tip, powered by a charge of carbon dioxide gas.

PowderMed

PowderMed is a recently-formed company to whom Chiron have assigned the rights to the prior Powder-Ject powder needle-free technology discussed earlier, apart from those rights held by Algorx.

Visionary Medical Products

The PenJet system, developed by VMPC, is powered by compressed gas and uses a prefilled, polycarbonate cartridge. It is a single-use, disposable device.

CONCLUSIONS

The basic principles underlying the use of drugs to penetrate the skin, whether the drug is in liquid, powder, or depot form, have been established for many years and are relatively simple engineering concepts. needle-free injection can give effective injections for a wide range of drugs and bioequivalent to a needle and syringe, results in less pain, and is very strongly preferred by patients. The devices are, in some cases, far easier to use than even a prefilled syringe, are much easier to dispose of, and give less variability from injection to injection than a needle-based injection administered by a trained healthcare professional. There is a wide range of devices available to suit different applications. Some are ideally suited to chronic injections of varying doses of insulin several times a day, others are more suited to weekly dosing of the same

dose of a therapeutic protein or even a monoclonal antibody. Still others will be capable of conveniently reconstituting lyophilized drug formulations in a much more patient-friendly manner than current techniques. Some of these devices can deliver liquids more viscous than could ever be delivered using even the largest of needles.

Needle-free devices have demonstrated consistent delivery to the epidermis, the dermis, the subcutaneous layer, and the intramuscular space. While questions remain over the ability of this technology to target the dermis or the muscle across a very wide range of subject morphologies, published data suggest that the delivery is at least as good as that achieved with a needle which remains the gold standard for all parenteral injections.

REFERENCES

1. Be'clard, F. Pre'sentation de l'injecteur de Galante, Se'ancedu 18 de'cembre 1866, Pre'sidence de M. Bouchardat. Bull.Acad. Impe'riale Me'd. (Fr.) 1866, 32, 321–327.
2. Lockhart, M. U.S. Patent No. 69,199, March 16, 1936.
3. Burkoth, T.L.; Bellhouse, B.J.; Hewson, G.; Longridge, D.J.; Muddle, A.G.; Sarphie, D.F. Transdermal and transmucosal powdered drug delivery. Crit. Rev. Ther. Drug Carrier Syst. 1999, 16, 331–384.
4. Degano, P.; Sarphie, D.F.; Bangham, C.R.M. Intradermal DNA immunization of mice against influenza A virus using the novel PowderJect(r) system. Vaccine 1998, 16, 394–398.
5. Potter, C. Caretek medical device. Management Forum Conference on Needle-Free Injection Systems and Auto- Injectors. Management Forum, London, England, Feb 23, 2004.
6. Shergold, O.A. The Mechanics of Needle-Free Injection. Ph.D. thesis, Cambridge University, Cambridge, England, 2004.
7. Hingson, R.A.; Davis, H.S.; Rosen, M. The historical development of jet injection and envisioned uses in mass immunization and mass therapy based upon two decades' experience. Mil. Med. 1963, 128, 516–524.
8. Hingson, R.A.; Davis, H.S.; Rosen, M. Clinical experience with one and a half million jet injections in parenteral therapy and in preventive medicine. Mil. Med. 1963, 128, 525–528.
9. Canter, J.; Mackey, K.; Good, L.S.; Roberto, R.R.; Chin, J.; Bond, W.W.; Alter, M.J.; Horan, J.M. An outbreak of hepatitis B associated with jet injections in a weight reduction clinic. Arch. Intern. Med. 1990, 150, 1923–1927.
10. King, T. Needle-free injection: protein delivery via pre-filled needle-free liquid injection. Drug Delivery Technol. 2003, 3 (7), 52–57.
11. Baker, A.B.; Sanders, J.E. Fluid mechanics of a springloaded jet injector. IEEE Trans. Biomech. Eng. 1999, 46, 235–242.
12. Schramm, J.; Mitragotri, S. Transdermal drug delivery by jet injectors: energetics of jet formation and penetration. Pharm. Res. 2002, 19 (11), 1673–1679.
13. Taylor, R.; Home, P.D.; Alberti, K.G.M.M. Plasma free insulin profiles after administration of insulin by jet and conventional syringe injection. Diabetes Care 1981, 4, 377–379.
14. Weller, C.; Linder, M. Jet injection of insulin vs. the syringe- and-needle method. J. Am. Med. Assoc. 1966, 195, 156–159.
15. Worth, R.; Anderson, J.; Taylor, R.; Alberti, K.G.M.M. Jet injection of insulin. A comparison with conventional injection by syringe and needle. Br. Med. J. 1980, 281, 713–714.
16. Sarphie, D.; Johnson, B.; Cormier, M.; Burkoth, T.L.; Bellhouse, B.J. Bioavailability following transdermal powdered delivery (TBD) of radiolabeled insulin to hairless guinea pigs. J. Controlled Release 1997, 47, 61–69.
17. King, S.; Bareille, P.; Stanhope, R. Re: growth hormone treatment without a needle [letter]. J. Pediatr. Endocrinol. Metab. 1998, 11 (1), 87.
18. Verhagen, A.; Ebels, J.T.; Dogterom, A.A.; Jonkman, J.H. Pharmacokinetics and pharmacodynamics of a single dose of recombinant human growth hormone after subcutaneous administration by jet-injection: comparison with conventional needle-injection. Eur. J. Clin. Pharmacol. 1995, 49 (1–2), 69–72.

19. de la Motte, S.; Klinger, J.; Kefer, G.; King, T.; Harrison, F. Pharmacokinetics of human growth hormone administered subcutaneously with two different injection systems. *Arzneimittelforschung* 2001, 51 (7), 613–617.
20. Suzuki, T.; Takahashi, I.; Takada, G. Daily subcutaneous erythropoietin by jet injection in pediatric dialysis patients. *Nephron* 1995, 69, 347.
21. Cooper, J.A.; Bromley, L.M.; Baranowski, A.P.; Barker, S.G.E. Evaluation of a needle-free injection system for local anaesthesia prior to venous cannulation. *Anaesthesia* 2000, 55, 247–250.
22. Zsigmond, E.K.; Darby, P.; Koenig, H.M.; Goll, E.F. Painless intravenous catheterization by intradermal jet injection of lidocaine: a randomized trial. *J. Clin. Anesth.* 1999, 11, 87–94.
23. Peter, D.J.; Scott, J.P.; Watkins, H.C.; Frasure, H.E. Subcutaneous lidocaine delivered by jet-injector for pain control before IV catheterization in the ED: the patients' perception and preference. *Am. J. Emerg. Med.* 2002, 20 (6), 562–566.
24. Varley, P.G.; Uddin, S.; Hlodan, R.; Edwards, S.; King, T. Monoclonal antibody injection without a needle. *Br. J. Pharmacol.* 2000, 131 (Proceedings Suppl.), 218.
25. Jackson, J.; Dworkin, R.; Tsai, T.; McMullen, R.; Kuchmak, N. Comparison of antibody response and patient tolerance of yellow fever vaccine administered by the Biojector_ needle-free injection system versus conventional needle/syringe injection. *International Society of Travel Medicine Conference, Paris, 1993.*
26. Whittle, H.C.; Lamb, W.H.; Ryder, R.W. Trial of intradermal hepatitis B vaccines in Gambian children. *Ann. Trop. Paediatr.* 1987, 7, 6–9.
27. Sarno, M.J.; Blase, E.; Galindo, N.; Ramirez, R.; Schirmer, C.L.; Trujillo-Juarez, D.F. Clinical immunogenicity of measles, mumps and rubella vaccine delivered by the Injex jet injector: comparison with standard syringe injection. *Pediatr. Infect. Dis. J.* 2000, 19, 839–842.
28. Wilson, H.D. Experience of BCG vaccination by jet injection in an outbreak of primary tuberculosis. *Lancet* 1973, 1, 927–928.
29. Payler, D.K.; Skirrow, M.B. Intradermal influenza vaccination. *Br. Med. J.* 1974, 2, 727.
30. Williams, J.; Fox-Leyva, L.; Christensen, C.; Fisher, D.; Schlicting, E.; Snowball, M.; Negus, S.; Mayers, J.; Koller, R.; Stout, R. Hepatitis A vaccine administration: comparison between jet-injector and needle injection. *Vaccine* 2000, 18, 1939–1943.
31. Brodell, R.T.; Bredle, D.L. The treatment of palmar and plantar warts using natural alpha interferon and a needleless injector. *Dermatol. Surg.* 1995, 21, 213–218.
32. Nathan, C.F. Local and systemic effects of intradermal recombinant interferon-gamma in patients with lepromatous leprosy. *N. Eng. J. Med.* 1986, 315, 6–15.
33. Baer, C.L.; Bennett, W.M.; Folwick, D.A.; Erickson, R.S. Effectiveness of a jet injection system in administering morphine and heparin to healthy adults. *Am. J. Crit. Care* 1996, 5, 42–48.
34. Berry, R.B. A comparison of spring and CO₂-powered needleless injectors in the treatment of keloids with triamcinolone. *Br. J. Plast. Surg.* 1981, 34 (4), 458–461.
35. Hartikka, J.; Bozoukova, V.; Ferrari, M.; Sukhu, L.; Enas, J.; Sawdey, M.; Wloch, M.K.; Tonsky, K.; Norman, J.; Manthorpe, M.; Wheeler, C.J. Vaxfectin enhances the humoral immune response to plasmid DNA-encoded antigens. *Vaccine* 2001, 19 (15–16), 1911–1923.
36. Cui, Z.; Baizer, L.; Mumper, R.J. Intradermal immunization with novel plasmid DNA-coated nanoparticles via a needle-free injection device. *J. Biotechnol.* 2003, 102, 105–115.
37. Hollingsworth, S.J.; Hoque, K.; Linnard, D.; Corry, D.G.; Barker, S.G.E. Delivery of low molecular weight heparin for prophylaxis against deep vein thrombosis using a novel, needle-less injection device (J-Tip_). *Ann. R. Coll. Surg. Engl.* 2000, 82, 428–431.
38. Obery, J.; Mannaerts, B.; Huisman, J.; Timmer, C. Local tolerance, pharmacokinetics, and dynamics of ganirelix (Orgalutran) administration by Medi-Jector compared to conventional needle injections. *Hum. Reprod.* 2000, 15 (2), 245–249.

39. Gonelli, R. The Different Regulatory Strategies for Commercialization of Needle Free Delivery Technologies. BIO2004, San Francisco, June 2004.

40. King, T. Pre-Filled Needle-Free Injectors: A Commercial Reality. BIO2004, San Francisco, 2004.

41. King, T. A review of needlefree injection technologies. In World Pharma Web [article 4]; Pharma Ventures, Ltd., 2001; 1–5.
