

## STRATEGIC SCIENCE & TECHNOLOGIES, LLC

# THE TECHNOLOGY

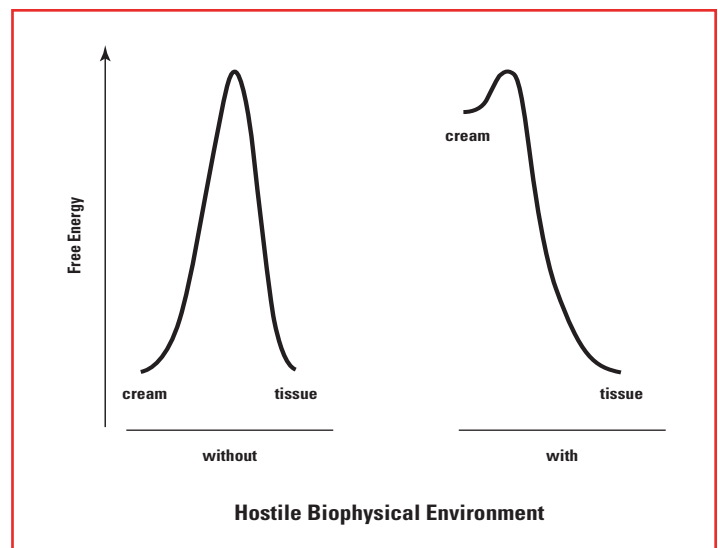
Strategic Science & Technologies (SST) brings a transformational technology to the field of localized transdermal drug delivery. Target therapeutic opportunities include delivery of new or current systemic therapeutics that treat localized physiological derangements as well as candidate drugs which failed due to systemic toxicity. Two completed formulations, transdermal L-Arginine for treatment of poor peripheral circulation in patients with diabetes, and transdermal ibuprofen for muscle and joint pain and inflammation will be the first marketed products.

The intellectual property and underlying science and technology facilitating the products of SST is effective, unique and transformational for the field of transdermal treatment of localized physiological derangements and their resulting medical conditions.

The many inherent advantages of transdermal delivery of pharmaceutical agents (PA) have long been recognized. They include reduction or elimination of many of the side effects, substantial reduction in total body dose, and a potential for higher localized dose. The PA is delivered where it is needed and other tissue in the body is not exposed to the PA.

Despite the general recognition of the advantages of transdermal delivery, only a limited number of agents have been successfully developed in the transdermal format. The SST technology greatly expands to range and scope of classes and types of PAs that can be successfully delivered transdermally by overcoming two major obstacles.

The outer layer of the skin, the *stratum corneum*, though only a few microns in thickness, constitutes a highly effective barrier to skin penetration. SST technology embodies a kenotic ability of the vehicle of the PA to cross this barrier. (Kenosis or kenotic is a Greek word meaning or describing an act of self-emptying.) The SST technology employs two complementary features addressing the two major barriers to transfer of the PA from the vehicle into the tissue through the *stratum corneum*. First, SST technology raises the free energy of the PA in the vehicle with respect to the free energy of the PA in tissue, creating a positive chemical potential thus driving the PA from the vehicle into the tissue.



Second, a major feature of the *stratum corneum* barrier is the ability of the membranes of its cellular constituents to form hydrogen bonds with the PA, preventing the PA from traversing the *stratum corneum*.



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vehicle, the chemical potential of L-Arginine (see above) was raised. In addition, this same high ionic strength vehicle prevents formation of hydrogen bonds between L-Arginine or another PA and the membranes of the cells of the *stratum corneum*.

The resulting transdermal L-Arginine system was first tested and demonstrated to be effective on people with cold hands. Cold hands or feet are the result of an insufficient supply of warm blood reaching the extremity. Warm blood from the core of the body is, among other things, the heating system of the body. Many tests were conducted. The warming effect of the transdermal L-Arginine preparation on cold hands was achieved by using the first product created by SST, Warm Cream. The table below summarizes the results of tests done by a

commercial testing lab. They selected a group of subjects with index finger temperatures between 22 and 26 °C. After equilibrating in the test room for a half an hour, index finger temperature was measured by an infrared thermometer. Ten minutes later the subjects rubbed the cream into their hands for five minutes and the index finger temperature was recorded every ten minutes for an hour. As can be seen in the figure, a temperature increase of approximately 10 °C was recorded at the end of the hour.

Three different placebo creams were also tested. In the table below the results are shown for a placebo which contains all components, including L-Arginine, but which omits the ionic components that constitute the delivery system. With this placebo, no temperature increase is seen.

**WARM CREAM TEST – SUBJECTS WITH COLD HANDS**

	time	10 min pre	0 min	10 min	20 min	30 min	40 min	50 min	60 min	90 min	120 min
n (number of subjects)		24	24	24	24	24	24	24	24	24	24
mean temperature		24.6	24.4	26.5	29.7	32.3	34.4	34.7	33.9	34.5	34.2
standard deviation		1.4	1.9	2.5	3.4	3.5	1.7	1.4	1.4	1.5	1.3
significance (p value) vs control (0 min) [lt = less than]				0.002	lt 0.001	lt 0.001	lt 0.001	lt 0.001	lt 0.001	lt 0.001	lt 0.001

**NOTE:** p values less than 0.1 indicate significance; less than 0.05 indicate great significance; less than 0.001 indicate extremely high significance  
p values greater than 0.1 indicate no difference between control and test group

**WARM CREAM TEST – SUBJECTS WITH COLD HANDS – PLACEBO C**

	time	10 min pre	0 min	10 min	20 min	30 min	40 min	50 min	60 min	90 min	120 min
n (number of subjects)		6	6	6	6	6	6	6	6	6	6
mean temperature		22.5	22.6	23.1	23.1	23.1	23.5	23.1	23.8	23.8	23
standard deviation		1.5	1.4	1.4	1.3	0.8	1.2	1	0.5	1	0.9
significance (p value) vs control (0 min) [ns = not significant]				ns	ns	ns	ns	ns	ns	ns	ns

**Placebo C – Exactly like active prep but without sodium chloride, magnesium chloride and choline chloride.**

Two other placebo creams were also tested (but not shown). In one the L-Arginine, but not the ionic components was omitted. In the second, the optical isomer, D-Arginine, was substituted for L-Arginine. The enzyme eNOS is only able to convert the natural L isomer to nitric oxide. Neither of these creams resulted in a temperature increase.

### **A Pilot Study of Transdermal L-Arginine Cream in Patients with Diabetes Who Have Impaired Circulation in Their Feet**

The transdermal L-Arginine cream was tested in a pilot study for its ability to improve temperature and flow in the feet of patients with diabetes with symptoms of impaired circulation in their feet. This study was published in the January 2004 issue of the journal *Diabetes Care* and is described below.

Poor blood circulation in the legs and feet is a major source of complications in patients with diabetes. These complications begin as coldness and progress first to pain (neuropathy) and then to open ulcers. Ulcers can progress to a point where the only treatment available is amputation. In the US approximately 90,000 amputations are performed each year on the toes, feet or limbs of patients with diabetes. These are directly caused by impaired blood flow.

The eighteen million people with diabetes in the US either have compromised peripheral circulation or are at high risk of developing it. In order to test the hypothesis that our transdermal L-Arginine cream would be beneficial to people with diabetes we conducted a pilot study in a patient population with type II (non-insulin dependent) diabetes who had some degree of neuropathy, but had not yet developed ulcers.

As background to this study, it has been shown that in diabetes the functionality of the endothelial nitric oxide (NO)/nitric oxide synthase (eNOS) system is impaired<sup>1,2,3</sup>. NO is generated in the endothelium through the oxidation of the amino acid, L-Arginine by the enzyme eNOS. NO causes vascular smooth muscle to relax resulting in increased blood flow. In addition to being a substrate of eNOS, L-Arginine facilitates the dimerization of two identical subunits forming a homodimer. The enzyme is only active in the dimeric form. Under proper conditions, dimerization occurs rapidly, on a timescale of minutes. Once formed the dimer is stable<sup>4</sup>.

Subjects with diabetes have abnormally low levels of L-Arginine<sup>5</sup> and elevated levels of the eNOS inhibitor, asymmetric dimethylarginine (ADME)<sup>6</sup> in their plasma. Though the value of increasing L-Arginine levels in cases of impaired circulation is now recognized, practical schemes for therapeutic use of L-Arginine have been elusive. In this pilot study we sought to determine whether supplying L-Arginine transdermally would improve vascular function of the feet in patients with diabetes as indicated by flow and temperature.

The study was designed as a double-blind vehicle-controlled two-period crossover protocol, with washout periods of one week. Sixteen subjects were enrolled and thirteen completed the study (age 56 +/- 8 yrs.). After analyzing the data it was clear that the effect of L-Arginine persisted throughout the washout periods. Because of this, except for the initial exposure of L-Arginine virgin feet, the analysis was altered to determine the effect from cumulative exposure to L-Arginine throughout the protocol. Flow was measured at the metatarsal and Achilles area using a Doppler flow meter<sup>7</sup> and temperature was measured at the metatarsal and big toe areas using an infrared thermometer. The active cream

was a water-based moisturizing vehicle containing 12.5% L-Arginine hydrochloride in a hostile biophysical environment comprised of high concentrations of choline chloride, sodium chloride and magnesium chloride. The vehicle control was identical except that the L-Arginine was omitted.

At the first visit, after baseline measurements were made each subject rubbed active cream (4 mg. L-Arginine/cm<sup>2</sup>) into one foot and vehicle into the other. After thirty minutes measurements were made again. A one-week wash out period followed. Patients returned after the wash out period and flow and temperature measurements were made. They were then randomly given either active or placebo cream and told to rub it into their feet in the morning and evening every day for two weeks. At the end of two weeks subjects returned and again measurements were made. A second one week wash out period followed that third visit. At the end of that period subjects returned and measurements were made. They were given the cross over product and told again to rub it into their feet morning and evening for two weeks. The subjects returned for final flow and temperature measurements at the end of that period.

At the first visit flow was increased at the Achilles in the foot with active cream from 8.1 +/- 3.3 Absolute Units (AU) to 11.5 +/- 5.5 (p=0.05) thirty minutes after application. In the foot that received placebo cream flow failed to increase (8.1 +/-1.4 vs 8.3 +/- 2.2). Further, at the last visit the temperature at the metatarsal area had risen from the initial value of 82.0° F. +/- 2.3 to 86.9 +/-2.4 (p<0.0001) and the temperature of the big toe had risen from the initial visit value of 74.4 +/- 4.2 to 82.4 +/-4.8 (p< 0.0001). And at the last visit the flow at the metatarsal area had risen from 8.7 +/-4.3 to 11.6 +/- 5.5 (p<0.0001) and flow

at the Achilles area had risen from 8.4 +/- 2.5 to 11.4 +/- 5.5 (p=0.02). While the failure of the L-Arginine effect to wash out removed the opportunity for placebo control, the improvement in temperature and flow were substantial and highly statistically significant. Though puzzling, one explanation of the persistence of the L-Arginine effect is that the local tissue concentration of L-Arginine becomes high enough to cause inactive monomers of eNOS to form active dimers.

From these data it is clear that, in the patients we studied with diabetes, treatment of their feet with a transdermal preparation of L-Arginine improved both flow and temperature and that this effect was surprisingly long lasting. Such improvement of compromised local blood flow should be beneficial and could reduce the complications of the disease.

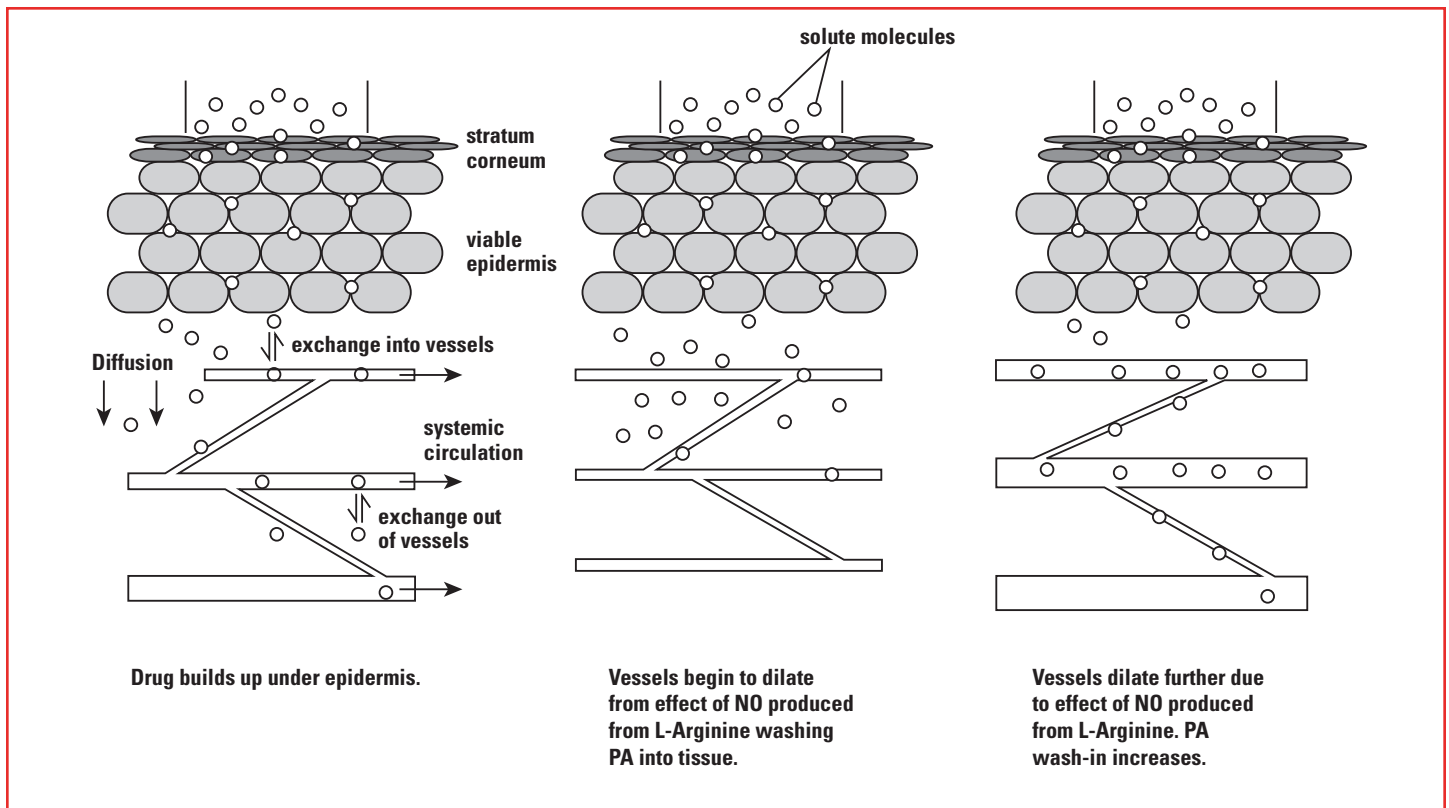
### Flow-Assisted TransDermal Drug Delivery

One of the classical limitations of transdermal drug delivery is imposed by Fick's first law of diffusion.

$$J_j = -D_j \left( \frac{\partial C_j}{\partial x} \right)$$

Simply put, the amount of PA transferred across the skin J, is governed by a constant D and is directly proportional to the concentration gradient C, and inversely proportional to the thickness of the barrier x posed by the *stratum corneum*. If the gradient C collapses (becomes 0), drug delivery comes to a halt. The gradient becomes 0 when the amount inside the skin and outside the skin becomes equal.

In order to prevent the collapse of the gradient and maintain the flux of PA across the stratum corneum, SST's flow assisted transdermal delivery system incorporates the transdermal L-Arginine technology described above.



Blood flow is enhanced in the target tissue by providing L-Arginine for the production of nitric oxide. The enhanced blood flow prevents the gradient from collapsing by not allowing build up of PA under the skin, but rather washing it deeper into the tissue.

In principle this is a generally applicable technique for a wide range of PAs. It has been put to practice in the first instance with ibuprofen as described below. It is illustrated above in diagrams showing how increased blood flow removes the PA from the viable epidermis, preventing collapse of the gradient.

### TransDermal Ibuprofen: LOCALIZED TREATMENT OF PAIN AND INFLAMATION

Ibuprofen has a history of 26 years of use, first by prescription and then by OTC and prescription. Since its introduction it has become obvious that it can cause serious side effects including gastrointestinal (GI) bleeding and renal insufficiency or failure. In 1986 when generic ibuprofen products were marketed, the FDA required a general safety warning: *If you experience any symptoms which are unusual or seem unrelated to the condition for which you took ibuprofen consult a doctor before taking any more of it.*

By 1998, data about serious side effects had accumulated and the FDA required the addition of further warnings.

In August of 2002 the agency published the current warnings required on all packaging which include specific warnings about GI bleeding and renal problems.

Despite the side effects and warnings, ibuprofen and other NSAIDs are widely used orally for relief of pain and inflammation of muscles and joints. GI bleeding in a subset of patients results in more than 100,000 hospitalizations per year at a substantial cost to the health care system and substantial risk to the patients. In addition to this complication, oral administration is less than ideal as the whole body is dosed when only a small portion of the body usually needs treatment. Transdermal delivery of ibuprofen avoids exposure to the susceptible GI system and focuses the therapy on the afflicted tissue. In addition to reducing the whole body dose, transdermal ibuprofen may achieve higher local tissue levels than oral ibuprofen.

The effective concentration of ibuprofen ( $EC_{50}$ ) is 6-10  $\mu\text{g/ml}$ . If a painful joint has a volume of 50 ml it would require 500  $\mu\text{g}$  which is the same as 0.5 mg of ibuprofen for successful treatment. If this were delivered transdermally the local dose (also the total body dose) would be 0.5 mg. To achieve this concentration of ibuprofen in the wrist by oral administration of ibuprofen 500 mg would need to be ingested by a 50 kg person assuming 100% adsorption. Thus treatment of the wrist in this example could be accomplished transdermally with a total body dose 1/1000<sup>th</sup> that of the oral dose.

SST has been successful in producing an effective flow assisted transdermal ibuprofen preparation as well as an effective naproxen preparation. These are ready to begin the 505 (b) (2) NDA route to FDA approval. ●

### Footnotes:

- <sup>1</sup> Cooke J.P., Dzau V: Nitric Oxide Synthase: Role in the Genesis of Vascular Disease. *Ann Rev Med* 28:489-501, 1997.
- <sup>2</sup> Calles-Escandon J, Cipolla M: Diabetes and Endothelial Dysfunction: A Clinical Prospective. *Endocrine Reviews* 22:36-52, 2001.
- <sup>3</sup> Kin KY, Ito A, Asagami T, Tsai PSS, Adimoolan S, Kimoto M, Hideaki T, Reaven GM, Cooke J.P.: Impaired Nitric Oxide Synthase Pathway in Diabetes Mellitus. Role of Asymmetric Dimethylarginine and Dimethylarginine Dimethyaminohydrolase. *Circulation* 106:987-992, 2002.
- <sup>4</sup> Panda K, Rosenfeld RF, Ghosh S, Meade AL, Getzoff ED, Stuehr DJ: Distinct Dimer Interaction and Regulation in Nitric Oxide Synthase Types I, II, and III. *J Biol Chem* 277:31020-31030, 2002.
- <sup>5</sup> Pieper GM, Siebeneich W, Dondlinger LA: Short-term oral administration of L-Arginine Reverses Defective Endothelium-Dependent Relaxation and cGMP Generation in Diabetes. *Eur J Pharm* 317:317-320, 1996.
- <sup>6</sup> Abbasi F, Asagami T, Cooke J.P., Lamendola C, McLaughlin T, Reaven GM, Stuehlinger M, Tsao PS: Plasma Concentrations of Asymmetric Dimethylarginine Are Increased in Patients with Type 2 Diabetes Mellitus. *Am J Cardiol* 88:1201-1203, 2001.
- <sup>7</sup> Vongsavan N, Matthews B. Some Aspects of the Use of Laser Doppler Flow Meters For Recording Tissue Blood Flow. *Exper Physiol* 78:1-14, 1993.