Photokinetic Transdermal Delivery of a Lidocaine-Prilocaine Eutectic Mixture

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ABSTRACT SUMMARY:

A novel facilitated transdermal delivery system using pulsed incoherent light directed onto a commercial 2.5% lidocaine-prilocaine eutectic preparation (EMLA®) applied to human skin in a modified Franz cell setup is described. Results demonstrate a significant increase in skin permeation and deposition using the facilitated light based (photokinetic) system vs. passive delivery.

INTRODUCTION:

The transdermal route offers several advantages over other routes for the delivery of drugs with systemic or local activity. However, the inherent barrier function of intact *stratum corneum* (SC) of the skin prevents or slows the permeation of most topically applied chemicals and drugs. Physical and chemical methods that alter the SC have been implemented in order to increase absorption of drugs into and through the skin[1]. A non-destructive, painless method of enhanced transdermal transport of a wide range of drugs is wanting.

The effectiveness of topically applied drugs for skin anesthesia is dependent on the drug concentration in the tissue. Drug concentration within the upper layers of the skin in passive transdermal delivery of anesthetics relies on a concentration gradient across the semi permeable dermal epithelium and the subsequent diffusion of the drug away from the upper skin levels into the dermis and capillary bed. Consequently, low permeation flux rates through the SC result in low drug concentrations at the site of action within the upper skin layers as the drug diffuses away into the deeper tissues. This explains the relative lack of anesthesia depth that can be achieved within the skin by passive transdermal methods[2]. It is therefore suggestive that if higher flux rates across the SC of topically applied anesthetics can be achieved without also increasing the permeation into the deeper tissues, then the concentration of the drug at the site of action can be increased and this may result in a greater depth of anesthesia as well as a faster onset of action can be realized.

It is widely recognized that stimulation of the optical sites of organic molecules may induce conformational changes which produce a physical change of the shape. When this stimulation is stopped the molecule would then return to a resting state and original physical shape. Low optical power and slow cyclical stimulation of the molecular optical sites would induce reversible conformational changes which may cause the molecule to flex and vibrate resulting in gross physical movement[3].

We hypothesized that if a drug molecule in a pharmacologically acceptable formulation is placed on the surface of the skin and cyclically illuminated with a selected wavelength of light at a selected pulse rate, the resulting cyclic physical shape change of the molecule may cause gross movement and migration of the molecule across the skin membrane. We further hypothesized that narrow wavelength incoherent (non-laser) visible light from a light emitting diode (LED) source could be used for optical stimulation and that light with these characteristics would not denature the drug molecule or harm the skin itself. We refer to this method of transdermal drug delivery by pulsed incoherent light as “photokinetic transdermal drug delivery” (PTDD)[4].

EXPERIMENTAL METHODS:

Traditional “up and down” Franz skin perfusion cells were adapted to receive LEDs on top of donor compartment. Control cells were identical absent the LEDs. Several light wavelengths were tested first to determine the optimum wavelength of light for lidocaine and prilocaine enhanced photokinetic delivery over 24 hours. Secondly, once a suitable wavelength of light was found to be effective for facilitated delivery, the achieved drug concentration within the skin was evaluated at 15, 30 and 60 minutes of photokinetic exposure and compared to passive controls.

PTDD modified Franz cell testing device was configured so that it accommodated the placement of LEDs with a peak light emitting wavelengths as follows: 390nm, 405nm 436nm, and 450nm (±10-30nm at the 50% radiance output @ 1.3-25 mW).

The LEDs were driven by a square wave pulse generator providing electrical energy set at 24 cycles per second with a 50% duty cycle (ON 50%, OFF 50% of the time). The pulse generator driving current was at or slightly below the manufacturers specified drive current level to avoid exogenous heat generation.

Dermatomed split thickness skin approximately 0.6mm thick from cadaver donors was used throughout the study. Harvested skin was refrigerated at 2ºC in RPMI medium with antibiotics and held until use. Study skin was used within 3 days after procurement. Body area origin of individual skin sections used is essentially random from any area that may normally be harvested from

the trunk and legs circumferentially during the skin donation procedure.

EMLA® (AstraZeneca), a commercial eutectic mixture of 2.5% lidocaine, 2.5% prilocaine was used as the test drug formulation. 0.75 grams of the drug formulation (1.875 mg each lidocaine-prilocaine) was placed in the Franz donor cells. HPLC grade water served as the Franz recipient cell fluid.

For the light wavelength 24 hour evaluation, the Franz recipient cell water was analyzed by HPLC method. For the timed skin concentration study, at the exposure time points of 15, 30 and 60 minutes, the drug formulation was washed from the skin several times with HPLC grade water. The skin area exposed to the drug was cut away from the surrounding non-exposed area under the cell flanges. The skin pieces were then freeze-fractured in a cryogenic tissue pulverizer and weighed. Freeze fractured skin was placed in a Tris extraction buffer. The samples were further ground using a wet method and centrifuged to separate the solids from the buffer. The supernatant was submitted for HPLC analysis.

Representative skin samples (control skin no exposure, 60 min. drug exposure, and 60 min w/ 450nm light and drug exposure) from the timed studies were taken and placed in formalin and stained with hematoxylin and eosin. Slides were read by two pathologists blinded as to sample group origin.

RESULTS AND DISCUSSION:

The 24 hour light wavelength determination of the four wavelengths of light tested showed a significant ($p<0.001$) increased permeation was achieved with 450nm light versus controls as summarized in Fig. 1.

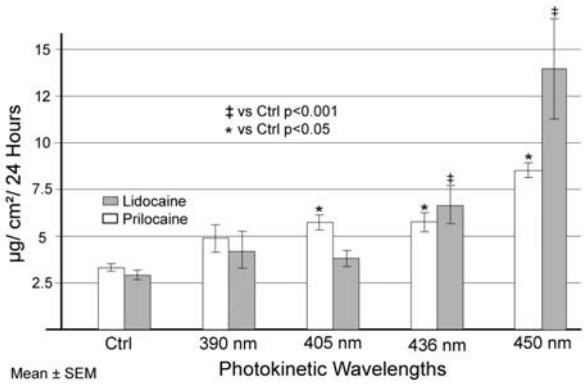


Fig. 1: Photokinetic wavelengths tested. Micrograms lidocaine and prilocaine recovered from Franz recipient cell fluid per cm^2 /24hours.

In the 3 time points skin deposition test, significant ($p<0.001$) levels of lidocaine compared to controls was achieved as shown in Fig. 2. Significant ($p<0.05$) increased levels of prilocaine compared to controls was achieved at 30 and 60 minutes.

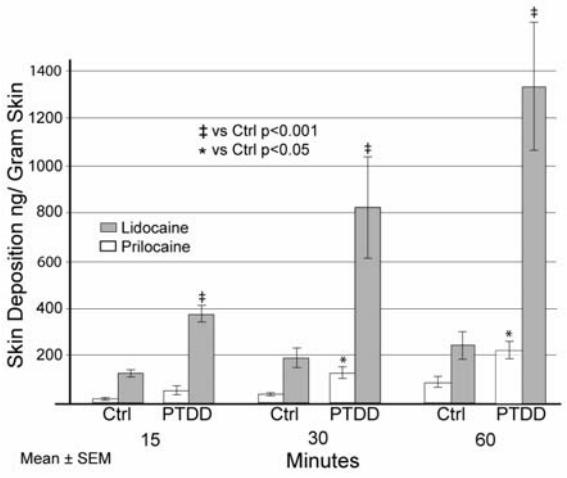


Fig. 2: Nanograms lidocaine and prilocaine per cm^2 per gram skin at each time point using 450nm LED.

Histological examination of the non-exposed control skin, 60 minute control drug exposure and the 60 minute PTDD exposure revealed no differences between the sample groups. Microscopically the *stratum corneum*, epidermis and dermis in all three sample groups were intact. The SC in all samples showed no structural changes and was firmly adhered to the epidermis.

CONCLUSION:

This *in vitro* model demonstrates that pulsed visible incoherent light directed onto a mixture of lidocaine and prilocaine applied to human skin can be used to facilitate transdermal delivery. The transdermal flux rate of lidocaine using PTDD was increased >4 times over passive control in a human skin Franz cell model over 24 hours. Thirty minutes exposure with the PTDD system resulted in an increase in deposition of lidocaine into split thickness human skin by a factor of 4 compared to passive permeation controls. The PTDD system did not damage or alter the skin exposed to light energy at the wavelengths and intensity used herein.

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The Galveston Shriners Skin Bank graciously provided tissue for these studies.

Photokinetic Transdermal Delivery of Cyanocobalamin

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ABSTRACT SUMMARY:

A novel transdermal delivery system using pulsed incoherent light directed onto 1% Cyanocobalamin (1380 Daltons) in a suitable topical formulation applied to human skin in modified Franz cell setup is described. Results show a significant ($p<0.001$) increase in skin permeation of the vitamin with photokinetic vs. passive delivery.

INTRODUCTION:

The molecular weight of 1380 Daltons (Da) and hydrophylic properties of Cyanocobalamin (vitamin B-12) limit passive permeation through intact *stratum corneum* (SC) reducing the applicability of water soluble vitamin therapies for systemic delivery via the transdermal route. Injectable forms of B-12 are the only approved therapy for B-12 deficiency in patients with reduced gut absorption condition.

Physical and chemical methods that alter the SC have been implemented in order to increase absorption of hydrophilic compounds >500 Da into and through the skin[1] however, these methods may be too irritating or destructive to be clinically acceptable. A non-destructive, painless method of enhanced transdermal transport of hydrophilic compounds >500 Da is wanting.

It is widely recognized that stimulation of the optical sites of organic molecules result in conformational changes of the molecule which may induce a physical change in the shape of the molecule. When this stimulation is stopped the molecule would then return to a resting state and original physical shape. Low optical power and slow cyclical stimulation of the molecular optical sites would result in reversible conformational changes which may cause the molecule to flex and vibrate resulting in gross physical movement[3].

We hypothesized that if cyanocobalamin in a pharmacologically acceptable formulation is placed on the surface of the skin and cyclically illuminated with a wavelength of light near it's absorption band of 550nm at a selected pulse rate, the resulting cyclic physical shape change of the molecule may facilitate migration of the molecule across the skin membrane. We further hypothesized that narrow wavelength incoherent (non-laser) visible light from a light emitting diode (LED) source could be used for optical stimulation and that light would not denature the drug molecule or harm the skin itself. We refer to this method of transdermal drug delivery by pulsed incoherent light as "photokinetic transdermal drug delivery" (PTDD)[4].

EXPERIMENTAL METHODS:

Traditional "up and down" Franz skin perfusion cells were adapted to receive LEDs on the donor compartment. Control cell setup was identical without LEDs. Three light wavelengths were tested to determine the optimal conditions for enhanced photokinetic delivery of Cyanocobalamin in a formulation over 24 hours. The effect of light pulse rate was also determined.

PTDD modified Franz cell testing device was configured so that it accommodated the placement of LEDs with a peak light emitting wavelengths @ 80-120 mW as follows: 528nm, 565nm and 570nm, (±25-30nm at the 50% radiance output).

The LEDs were driven by a square wave pulse generator providing electrical energy set at 8, 24, 60 or 100 cycles per second (CPS) with a 50% duty cycle (ON 50%, OFF 50% of the time). The pulse generator driving current was at or slightly below the manufacturers specified drive current level to avoid exogenous heat generation within the cell, which could not be detected.

Dermatomed split thickness skin approximately 0.6mm thick from cadaver donors was used as the Franz cell membrane throughout the study. Harvested skin was refrigerated at 4ºC in RPMI 1640 medium with antibiotics and held until use. Study skin was used within 3 days after procurement. Body area of origin of individual skin sections used was essentially random from any area that may normally be harvested from the trunk and legs circumferentially during the skin procurement procedure.

Skin sections are inspected under magnification for tears or holes prior to placement between the Franz cell chambers. Skin was placed with the epidermal side facing the donor chamber. Cells were held in a heat block/stir base arrangement at 35.5ºC. A magnetic stir bar constantly stirred the recipient cells.

A topical drug carrier formulation was developed comprising 30% propylene glycol, 5% Ethyl Lactate, 0.1% Azone, 0.75% hyaluronic acid as a gelling

agent with 0.1% neolone 950 as a preservative with the balance being HPLC grade water.

Cyanocobalamin was dissolved into the topical carrier formulation providing a final concentration of 1% weight/volume. 1.5 grams of the cyanocobalamin mixture/topical formulation was placed in the Franz donor cells. HPLC grade water served as the Franz recipient cell fluid. Recipient cell fluid was removed at 24 hours and held for HPLC analysis.

Representative skin samples (control skin no exposure, 24 hour passive drug exposure, and 24 hour light and drug exposure) from the studies were taken and placed in formalin and stained with hematoxylin and eosin. Slides were read by two pathologists blinded as to sample group origin.

HPLC analysis was performed on the recipient fluid according to a method developed in our lab.

Statistical analysis was performed using two way analysis of variance (ANOVA) with Bonferroni's correction. Significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION:

Recipient cell fluid HPLC results from the passive permeation control cells (n=11) and the three PTDD light groups at 100 CPS (n=4 528nm and 570nm, n=7 565nm) expressed in micrograms after 24 hours are summarized in Fig.1.

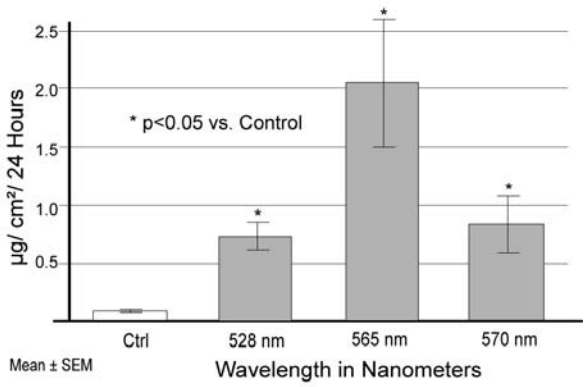


Fig 1: Micrograms cyanocobalamin recovered from Franz recipient cell fluid per cm^2 / 24 hours. Passive control vs. Photokinetic wavelengths tested for cyanocobalamin (1380 Da) at 3 PTDD wavelengths @ 100CPS.

Relatively poor permeation was noted in the passive control group as would be expected with a 1380 Da hydrophilic compound. The 3 light groups showed significant ($p<0.05$) increased permeation. The 565nm group showed a >20 times permeation over controls.

Histological examination of the non-exposed control skin, 24 hour control passive exposure and the three 24 hour PTDD exposure revealed no differences between the sample groups. The *stratum corneum*, epidermis and dermis in all four sample groups were grossly intact. The SC in all samples showed no structural changes and was firmly adhered to the epidermis.

Light pulse rate results demonstrated lower permeation as the pulse rate was diminished below 100CPS (Fig. 2).

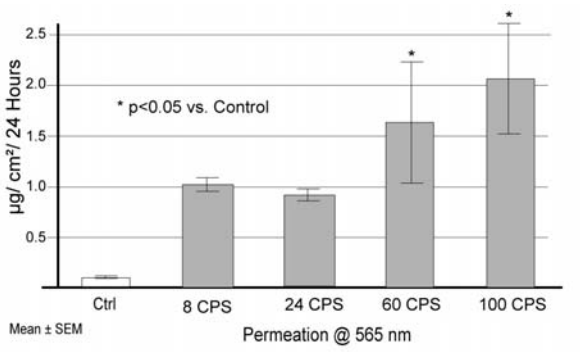


Fig. 2: Permeation as affected by PTDD light pulse rate.

CONCLUSION:

This *in vitro* model demonstrates that pulsed visible incoherent light at selected wavelengths and light pulse rates directed onto a mixture of cyanocobalamin in an aqueous solvent carrier applied to human skin can be used to facilitate transdermal delivery. Transdermal flux rates of a 1380 Da hydrophilic compound using the PTDD system significantly ($p<0.05$) increased over passive controls in a human skin Franz cell model over 24 hours. Flux rates were wavelength and pulse rate dependent.

The PTDD system did not damage or alter the skin exposed to light energy at the wavelengths and intensity used herein.

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3. W. R. Brown *et al*. Nature Nanotech. 1: 25 (2006)
4. E. Kraft et al. European Patent Specification EP 1556061 B1, (2007)

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Photokinetic Transdermal Delivery of Peptides

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ABSTRACT SUMMARY:

A novel transdermal delivery system using pulsed incoherent light directed onto a mixture of 5 peptides (238-1046 Daltons) in a suitable topical formulation applied to human skin in modified Franz cell setup is described. Results show a significant ($p<0.001$) increase in skin permeation of peptides with photokinetic vs. passive delivery.

INTRODUCTION:

The hydrophilic properties of peptides limit passive permeation through intact *stratum corneum* (SC) reducing the applicability of peptide therapies for local skin targeted sites and systemic delivery. Topical peptide formulations are widely marketed for cosmetic applications despite the lack of skin permeation even while using high peptide concentrations.

Physical and chemical methods that alter the SC have been implemented in order to increase absorption of peptides into and through the skin[1] however, these methods may be too irritating or destructive to be clinically acceptable. A non-destructive, painless method of enhanced transdermal transport of peptides is wanting.

It is widely recognized that stimulation of the optical sites of organic molecules result in conformational changes of the molecule which may produce a physical change in the shape of the molecule. When this stimulation is stopped the molecule would then return to a resting state and original physical shape. Low optical power and slow cyclical stimulation of the molecular optical sites would result in reversible conformational changes which may cause the molecule to flex and vibrate resulting in gross physical movement[3].

We hypothesized that if peptides in a pharmacologically acceptable formulation are placed on the surface of the skin and cyclically illuminated with a selected wavelength of light at a selected pulse rate, the resulting cyclic physical shape change of the molecule may cause gross movement and migration of the molecule across the skin membrane. We further hypothesized that narrow wavelength incoherent (non-laser) visible light from a light emitting diode (LED) source could be used for optical stimulation and that light would not denature the drug molecule or harm the skin itself. We refer to this method of transdermal drug delivery by pulsed incoherent light as "photokinetic transdermal drug delivery" (PTDD)[4].

EXPERIMENTAL METHODS:

Traditional "up and down" Franz skin perfusion cells were adapted to receive LEDs on the donor compartment. Control cell setup was identical without the LEDs. Several light wavelengths were tested to determine optimum wavelength for enhanced photokinetic delivery of a five peptides mixture in a formulation over 24 hours.

PTDD modified Franz cell testing device was configured so that it accommodated the placement of LEDs with a peak light emitting wavelengths @ 1.3-25 mW as follows: 390nm, 405nm 436nm, and 450nm (±10-30nm at the 50% radiance output).

The LEDs were driven by a square wave pulse generator providing electrical energy set at 24 cycles per second with a 50% duty cycle (ON 50%, OFF 50% of the time). The pulse generator driving current was at or slightly below the manufacturers specified drive current level to avoid exogenous heat generation within the cell, which could not be detected.

Dermatomed split thickness skin approximately 0.6mm thick from cadaver donors was used as the Franz cell membrane throughout the study. Harvested skin was refrigerated at 4ºC in RPMI 1640 medium with antibiotics and held until use. Study skin was used within 3 days after procurement. Body area of origin of individual skin sections used was essentially random from any area that may normally be harvested from the trunk and legs circumferentially during the skin donation procedure.

Skin sections are inspected under magnification for tears or holes prior to placement between the Franz cell chambers. Skin was placed with the epidermal side facing the donor chamber. Cells were held in a heat block/stir base arrangement at 35.5ºC. A magnetic stir bar constantly stirred the recipient cells.

A topical drug carrier formulation was developed comprising 30% propylene glycol, 5% Ethyl Lactate, 0.1% Azone, 0.75% hyaluronic acid as a gelling agent with 0.1% neolone 950 as a preservative with the balance being HPLC grade water.

A mixture of five peptides at the stated final concentrations (weight/volume): Gly-Try 0.1% (GT), Gly-His-Lys 0.1% (GHK), Val-Try-Val 0.1% (VT), Leucine-Enkephalin 0.05% (LE), and Angiotensin-II 0.01% (ANG II), were dissolved into the topical

carrier formulation. 1.5 grams of the peptide mixture/topical formulation was placed in the Franz donor cells. HPLC grade water served as the Franz recipient cell fluid. Recipient cell fluid was removed at 24 hours and held for HPLC analysis.

HPLC analysis was performed on the recipient fluid according to a method developed in our lab.

Statistical analysis was performed using two way analysis of variance (ANOVA) with Bonferroni's correction. Significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION:

Recipient cell fluid HPLC results from the passive permeation control cells (n=11) and the four PTDD light groups (n=8) expressed in micrograms after 24 hours are summarized in Table 1.

	GT 238 Da	GHK 430 Da	VTV 380 Da	LE 557Da	ANG II 1046 Da
Ctrl n=11	0.097 ±0.030	0.139 ±0.018	0.387 ±0.029	0.199 ±0.017	1.005 ±0.113
390nm n=8	0.993 ±0.138	3.059 ±0.411	8.748 ±0.600	3.716 ±0.398	34.592 ±10.600
405nm n=8	1.997 ±0.188	2.721 ±0.210	7.011 ±0.574	5.787 ±0.937	21.379 ±5.968
436nm n=8	2.263 ±0.211	2.517 ±0.373	9.874 ±1.064	3.647 ±0.395	19.678 ±1.780
450nm n=8	2.113 ±0.522	4.727 ±0.997	10.058 ±1.453	3.983 ±0.424	18.041 ±3.013

Mean ± SEM

Table 1: Micrograms peptide recovered from Franz recipient cell fluid per cm^2 / 24 hours. Passive control vs. Photokinetic wavelengths tested for test group peptides with various molecular weights in Daltons (Da).

Relatively poor permeation was noted for each peptide in all passive control groups. The four light groups showed significant ($p<0.001$) increased permeation by a factor of 19 to 34 times was achieved for each of the peptides under specific PTDD conditions. Best PTDD mode results are summarized in Fig. 1.

All the peptides in the mixture were delivered and analyzed concurrently within the control and the four light groups. In the 390nm group, the permeation of the 238 Da GT peptide was much less than the permeation of the 1046 Da ANG II peptide under the same conditions. No one PTDD group demonstrated the highest flux rates for all of the peptides. Each peptide behaved differently within each light group. This suggests that each peptide may be affected differently with the selected light wavelength stimulation.

No one light group demonstrated higher flux rates for all the individual peptides, then it can be reasonably concluded that the possibility of skin poration, weather physically pre-existing or caused by the light exposure, is negated. High flux rates did not correlate with low molecular weight in the PTDD system as might be expected. LE (557 Da) and ANG II (1046 Da) exhibited relatively high permeation rates compared to smaller peptides.

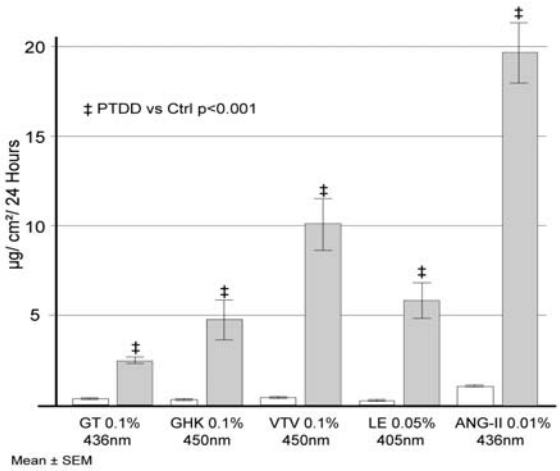


Fig. 1: Micrograms of individual peptides per cm^2 at 24 hours under various PTDD conditions compared to controls. Significant ($p<0.001$) increased flux rates, (19-34 times) over passive controls were achieved.

Relatively low donor concentrations (0.1% for GT, GHK, VTV, 0.05% for LE and 0.01% for ANG II) of the individual peptides demonstrates that the PTDD system can be useful to transport peptides even at low drug concentrations in a topical formulation.

CONCLUSION:

This *in vitro* model demonstrates that pulsed visible incoherent light at selected wavelengths directed onto a mixture of peptides in an aqueous solvent carrier applied to human skin can be used to facilitate transdermal delivery. Transdermal flux rates of peptides using the PTDD system significantly ($p<0.001$) increased flux rates 19-34 times over passive controls in a human skin Franz cell model over 24 hours.

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3. Brown, W.R., Feringa, B.L. Nature Nanotech. 1 (2006) 25-35
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