



## LETTER TO THE EDITOR

## Pulsed current iontophoresis of hyaluronic acid in living rat skin

### KEYWORDS

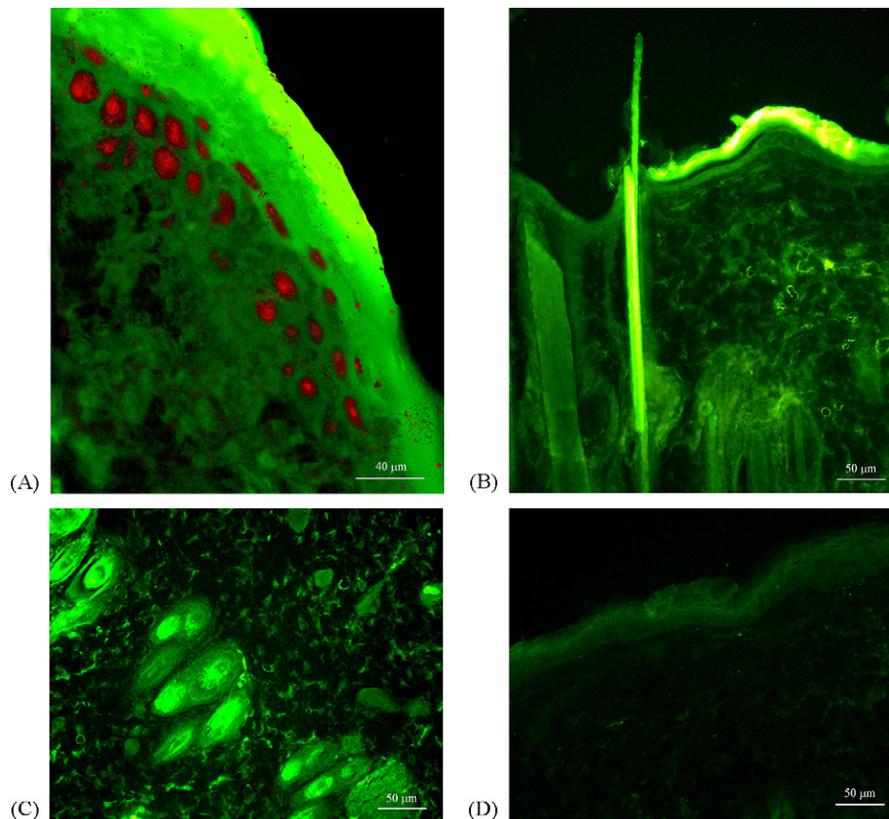
Hyaluronic acid;  
 Rat skin;  
 Transdermal delivery;  
 Iontophoresis

Hyaluronic acid (HA) is a natural glycosaminoglycan (GAG) used in treatment and prevention of different skin diseases and as a filler agent for improving wrinkles, scars, or increasing volumes [1]. Because of chemical structure, HA has to be injected repeatedly for many months [2], in tens or hundreds of sites [3], by experienced operators [4]. This feature contrasts with the needs of today's busy and demanding world that ask for painless "lunchtime procedures" [4,5]. In this study we evaluated the efficacy of a powered pulsed current iontophoresis drug delivery system (Transderm<sup>®</sup> Ionto System, Mattioli Engineering, McLean, VA, USA) approved for clinical use in humans by the US Food and Drug Administration (FDA, approval no. K042590, 10.14.2004), in delivering HA through living rat skin, following the procedure described in Ref. [6].

We applied 0.5 ml of fluorescent HA (mean molecular weight,  $M_w$  800 kDa; Molecular Probes, Carlsbad, CA, USA), at a final concentration of 2 mg/ml, or alternatively, 0.5 ml of stable, uniformly labeled radioactive [<sup>3</sup>H]-HA (American Radiolabeled Chemicals, St. Louis, MO, USA) (250 mCi/g, 1 mCi/ml, mean  $M_w$ , 800 kDa). [<sup>3</sup>H]-radioactivity is expressed as counts per minute (cpm). The applied volume (0.5 ml) was close to that routinely injected in patients (0.7 ml) [2]. Statistical analysis was performed using Student's *t*-test. Data are presented as means  $\pm$  S.D. ( $n = 3$ ), and were considered significantly different when  $p < 0.05$ .

First, we checked for modifications of skin structure following the electrically assisted procedure [6]. Although the device used in this study is

approved by the FDA for use in humans, alterations of skin structure are of utmost importance in plastic surgery and cosmetic dermatology. Images obtained by light microscopy after haematoxylin–eosin staining showed that epidermis, dermis and appendages, such as hair follicles and sebaceous glands, were not altered in shape, consistency, or staining. There was no morphological sign of tissue injury (i.e. abnormalities in cell shape or staining) in treated samples. These results were consistent with observation of treated areas; only pale redness of the skin was noticed (not shown). Measuring electric impedance of skin is a simple and safe method to determine skin barrier integrity [7]. Determination of skin impedance with an oscilloscope (Tektronik, Milan, Italy) provided results consistent with microscopic observation; impedance after treatment ( $805 \pm 48 \Omega$ ) was indicative of skin barrier integrity, being quite similar to that reported for intact mammal skin [7]. Pulsed current iontophoresis allowed the transdermal transport of fluorescent HA. The distribution of fluorescent (green) HA after treatment is shown in Fig. 1A, where cells were counterstained with propidium iodide (red). Fluorescence was more intense in the *Stratum corneum* (SC), where fluorescent HA was applied, and gradually less intense, but still bright, in the epidermis. Fluorescence in the epidermis was distributed along inter-cellular pathways so to clearly evidence both the outline of the corneocytes forming the SC and the outline of the keratinocytes in the underlying layers. Fluorescence was also observed in the dermis (Fig. 1B and C), thus demonstrating penetration and diffusion of fluorescent HA through the dermo-epidermal junction. Fluorescence was also detected in association to hair follicles and their sheaths as if these structures represented a sort of channel from the outside to the deepest skin layers (Fig. 1B and C). In control area (i.e. where fluorescent HA was applied onto the skin, but where no electrical treatment was performed), only a weak background fluorescence was detectable in the epidermis and dermis (Fig. 1D). Analysis of quantitative distribution of fluorescence, using a customized image analysis software



**Fig. 1** Fluorescent HA distribution in rat skin. After pulsed current iontophoresis, green fluorescent HA was detectable in the *Stratum corneum* and in all the underlying epidermis layers. Panel A shows the outline of corneocytes and of keratinocytes counterstained with red propidium iodide. Cells' nucleus and nucleolus were strongly stained in red as well as the cytoplasm, although to a lesser extent. Fluorescence was also detected in association to hair follicles and in the dermis where it was evenly distributed (B and C). Panel B refers to a transverse section of skin, whereas panel C refers to a parallel section. Untreated skin (panel D) showed a weak green fluorescent background both in the epidermis and in the dermis. Confocal laser scanning microscopy. Total magnification; 700 $\times$  (A), and 400 $\times$  (B–D).

program, showed that after pulsed current iontophoresis, fluorescence, measured on images of full thickness skin comprising epidermis, dermis, and hair follicles, was  $61.482 \pm 5.345$  pixels; fluorescence measured on images of dermis was about 25% of total fluorescence ( $20.384 \pm 1.761$  pixels). Background fluorescence measured on images of control skin was  $1.377 \pm 56$  pixels. Mean value of measured area was  $55.4 \pm 6$  cm<sup>2</sup>. Finally, we measured transdermal transport of [<sup>3</sup>H]-HA (Table 1). The table shows that there was minimal amount of [<sup>3</sup>H]-radioactivity recovered in control biopsies. After pulsed current iontophoresis, a significant

amount of [<sup>3</sup>H]-radioactivity was recovered within the biopsies. This result reflected electrically assisted transdermal passage of [<sup>3</sup>H]-HA, and it was statistically significant *versus* control ( $p < 0.05$ ). About 10% of applied [<sup>3</sup>H]-HA was recovered in the biopsies of treated areas. Analysis of [<sup>3</sup>H]-HA distribution within the biopsies showed that about 25% of [<sup>3</sup>H]-HA was recovered in the dermis; the rest was recovered in the epidermis. This distribution paralleled that observed with fluorescent HA. It is worth noting that the mean  $M_w$  of HA (800 kDa) is significantly greater than that of most molecules that are delivered by iontophoresis [8]. However, GAGs behave quite differently than other macromolecules because of their chemical structure. They are long, charged molecules endowed with high flexibility that allows them to slip through molecular weight sieves where proteins and peptides remain entrapped. Because of this, polyacrylamide gel electrophoresis (PAGE) is a method to separate GAGs from proteins; proteins and peptides remain in the gel, whereas intact GAGs, driven by

**Table 1** Transdermal delivery of [<sup>3</sup>H]-HA

cpm	Control	Treated (pulsed iontophoresis)
Applied	$235.721 \pm 1.322$	$234.813 \pm 1.415$
Recovered	$825 \pm 69$	$22.918 \pm 772$
Epidermis	–	$16.964 \pm 713$
Dermis	–	$5.654 \pm 191$

current, escape the gel and are recovered in the buffer [9]. This procedure does not cause breakdown of GAGs or loss of their biological activities; furthermore, GAGs are able to escape the gel independently of their  $M_w$  [9]. Thus, we hypothesize that pulsed current iontophoresis might allow transdermal passage of HA in a manner similar to that observed with GAGs in PAGE. Commercial preparations of HA such as those used in this study are mixtures containing different molecular species differing in size and  $M_w$ . Although we cannot rule out the possibility that molecular species with lower  $M_w$  were preferentially transported into the skin, analysis by cellulose acetate electrophoresis of HA extracted from skin biopsies after iontophoresis, showed that recovered HA had a pattern of electrophoretic migration superimposable to that of applied HA (not shown). The data presented in Table 1 demonstrate that the amount of HA that reached into the dermis following iontophoresis was significant, although not impressive. However, it should be considered that being the treatment fast and painless, and avoiding all the problems associated with syringes and needles, it could be proposed as a safe alternative to injection.

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Stefania Pacini\*  
Tiziana Punzi  
Massimo Gulisano  
Department of Anatomy,  
Histology and Forensic Medicine,  
University of Firenze,  
Viale Morgagni 85, 50134 Firenze,  
Italy

Marco Ruggiero  
Department of Experimental  
Pathology and Oncology,  
University of Firenze,  
Italy

\*Corresponding author. Tel.: +39 055 410084;  
fax: +39 055 4379500  
E-mail address: [stefania.pacini@unifi.it](mailto:stefania.pacini@unifi.it)  
(S. Pacini)

12 May 2006

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