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Final Report

H. Zingboim

Evaluation of Hyaluronic acid (HA) skin penetration in several cream formulations using ex vivo Human skin

AUTHOR:	**Ella Kenigswald Lax and Dr. Rinat Borenshtain Koreh**			
APPROVED BY:	**Fanny Ben-Ezra**	**Date -**	18-10-19	**Signature - RBK**
Run details:	**Name - Fanny Ben-Ezra**	**Date -**	15-10-19	**Signature -**

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דע-תא ביוטק בע"מ
רח' ברגמן 2ב, קומה 3 פרק ת.מ.ר , רחובות. טל. 08-9100575
כתובת לדואר: דע-תא ביוטק בע"מ. ת.ד. 4194 רחובות 74140
rinat@databiotech.co.il **www.databiotech.co.il**

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Protocol	
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Sponsor:	**Hava Zingboim**
Sponsor Representative:	**Moti Zingboim and Prof. Lubart**
E. Mail address:	**motizingboim@gmail.com**

Facility:	**Da-Ta Biotech**
Address:	**2 Bergman st. Science Park, Rehovot, Israel.**
Telephone number:	**08-9100575**
E. Mail address:	**rinat@databiotech.co.il**
Project Manager:	**Fanny Ben Ezra, M.Sc.**
Principal Investigator:	**Fanny Ben Ezra**

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CONTENTS

1. Goal

This Report is submitted further to a request of Professor Rachel Lubart from H. Zingboim to assess the penetration of a compound into ex vivo Human skin samples using IHC method.

2. Background

This document is submitted on the basis that it remains the property of Da-Ta Biotech Ltd. and its contents are confidential to H. Zingboim.

3. Protocol outline

I. Sample preparation and treatment:

Fresh Full thickness Human skin pieces (2cmx2cm, Sample obtained under Helsinki approval # 0443-19-TLV) were used for the experiment. The pieces were laid on a PBS soaked diaper (See Appendix). The amount of 0.2gr of each sample was spread on skin pieces for 2 minutes and then rubbed into the piece gently for 30 seconds. A thin layer was left for 5 hours then the sample left overs were wiped off the skin pieces using a paper and inserted into formalin for 1 week.

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Table 1- Samples List (Each sample was performed in duplicate)

	Sample	Skin Treatment*
HA Samples	31	m. פורמולציה בוגר
	32	l. פורמולציה בוגר
control	10a	פורמולציה - בוגר
	10b	פורמולציה - בוגר "No 1st Antibody" control – skin PBS at 1st antibody
	10c	פורמולציה – בוגר (not pictured) "No 2nd Antibody" control – skin PBS at 2nd antibody
	11	Non treated control - skin

*treatments were provided ready to use with numbering by Prof. Lubart from H.Zingboim

II. Slides preparation and staining:

Samples were transferred to 70% Ethanol (after 1 week in Formalin). Paraffin blocks were prepared. Slides were cut, Deparaffinized using xylene and stained using anti-Hyaluronic acid primary antibody (Abcam Cat# ab53842) followed by polyclonal Secondary Antibody (Abcam Cat# ab150181).

Negative control slides ("No 1st antibody" or "No 2nd Antibody" control) were incubated with 1X PBS instead.

4. Results:

The Leica SP8 Confocal Microscope was used for data collection with magnification of x20

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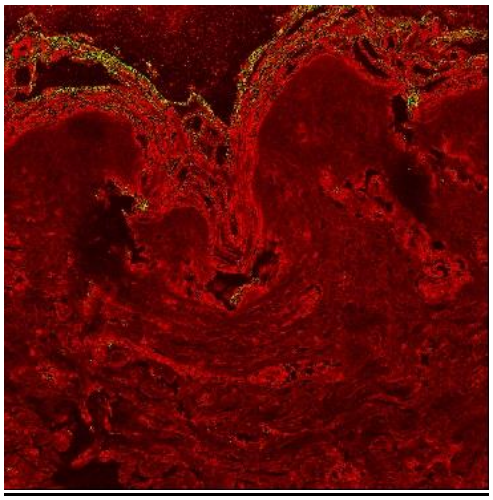
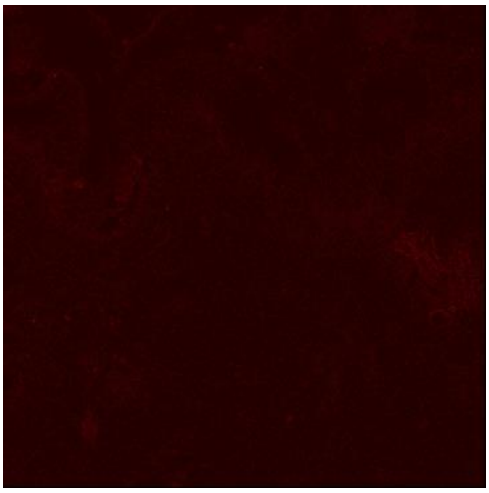
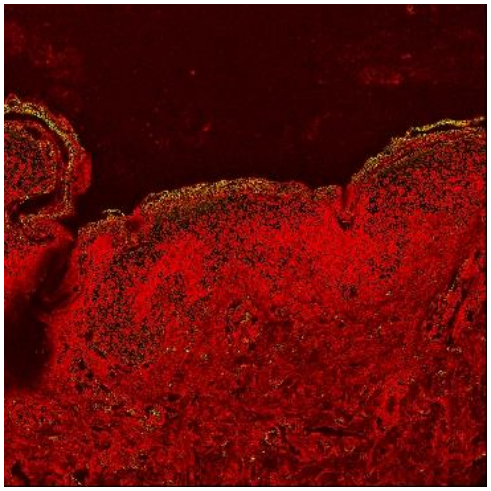


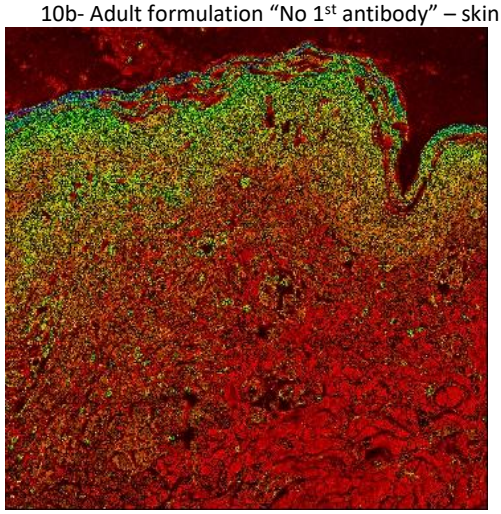
10a- Adult formulation



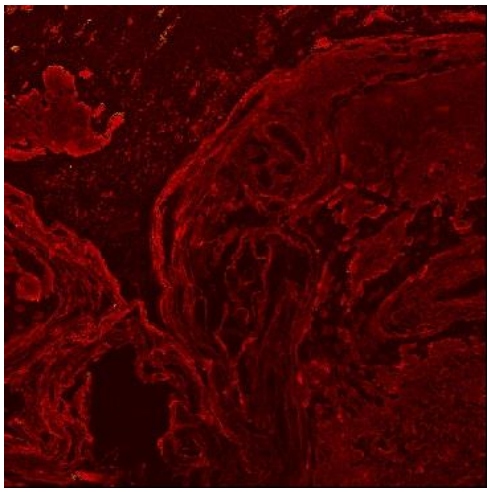
10b- Adult formulation "No 1st antibody" – skin



11- Non treatment control- skin



31- Adults formulation m.



32- Adult formulation l.

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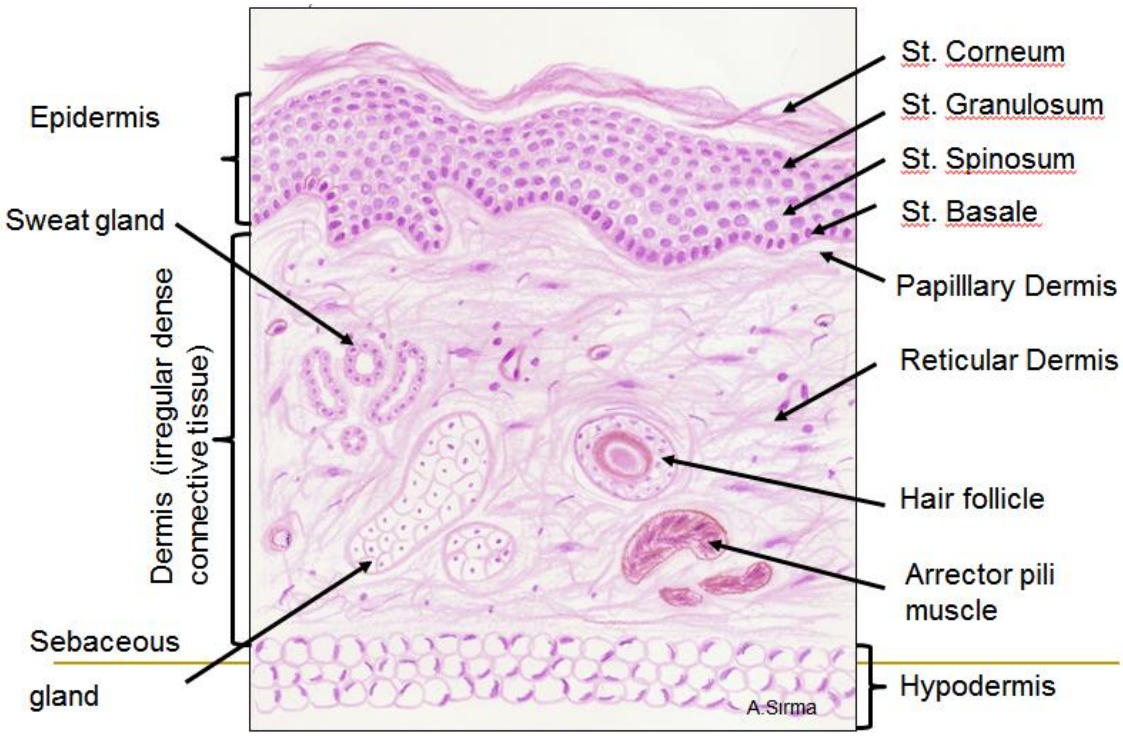
5. **Conclusions:**

All negative controls were without staining and served for background threshold setup. Sample 31 had high fluorescent detection across the epidermis and outer dermal layer. Sample 32 had no fluorescent detection.

Human skin histological scheme:

6.



https://www.google.com/search?q=skin+histology+layers&rlz=1C1SQJL_enIL838IL839&sxsrf=ACYBGNSObNBk85kaEhz7misXSAVShy5Q3Q:1574004428699&tbm=isch&source=iu&ictx=1&fir=Wvurzwt80g0pRM%253A%252Cp2lv3TLcBtT9vM%252C_&vet=1&usg=AI4_-kRwLYjWOWD_zUu6B2XbwWzZQonNCw&sa=X&ved=2ahUKEwi8veO2x_HlAhVEJ1AKHcMADnkQ9QEwA3oECAYQCg#imgrc=21quJutv4dp-yM:&vet=1

This picture is copied from the web link of "Human Biology Online Lab".

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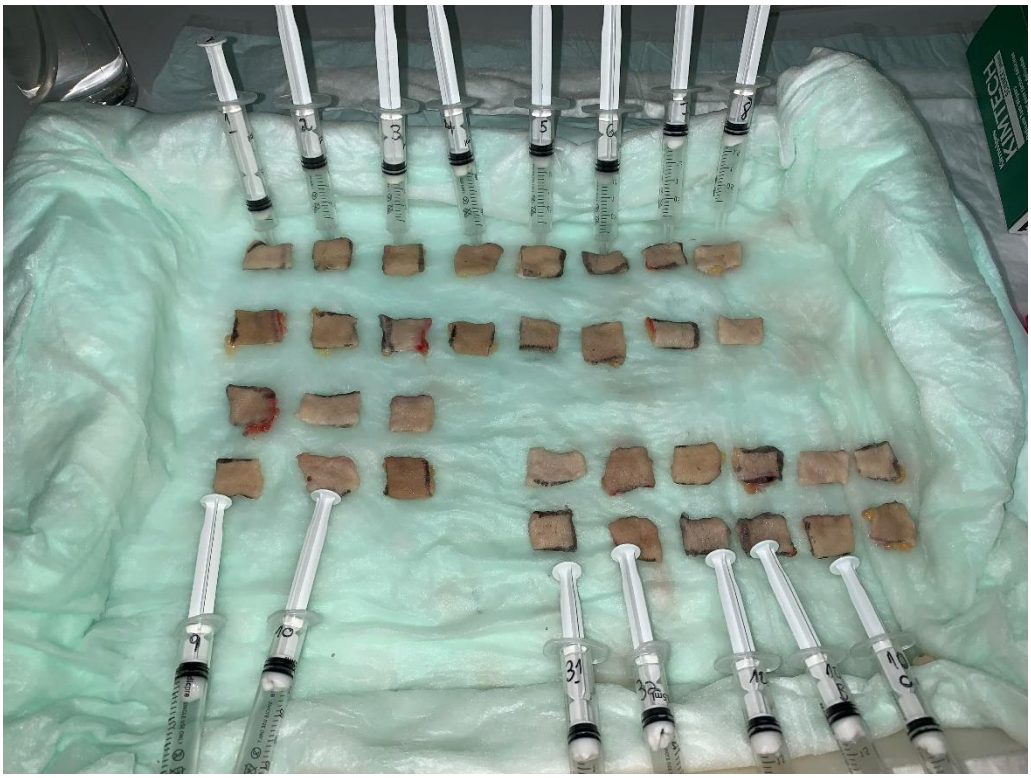
7. **Appendix 1:** Assay set up



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