

BIO4AFRICA

Novel application of green biorefinery whey

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B4A Final Conference/BLP 2025,
28-30 January, Montpellier

BLP 2025

BIOENERGY FOR LOCAL PRODUCTION
CONFÉRENCE INTERNATIONALE

Du 28 au 30 janvier 2025

www.Bio4Africa.eu



This project has received funding from
the European Union's Horizon 2020 research
and innovation programme under
grant agreement No. 101000762

Green biorefinery



Alfalfa

Collection of raw biomass



Pakchong



Plant residues processing



Presscake



Green juice



Protein concentrate



Whey (brown juice)

Assessment of the high-value bioactive ingredients in the biorefinery whey



Whey (brown juice)

Residual stream derived from the green biorefinery process

High-value ingredients



Cosmetics/cosmeceuticals
Pharmaceuticals

Assessment of the high-value bioactive ingredients in the biorefinery whey



Residual green biorefinery whey samples were generated in **Uganda** in **January** and **May 2024**.

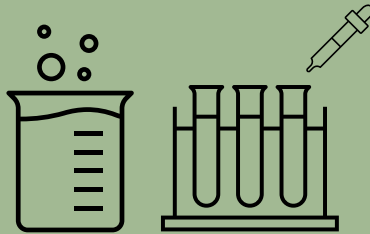
The biomass used for the biorefinery process is derived from:



Alfalfa (*Medicago sativa*)



Pakchong (*Pennisetum purpureum*)

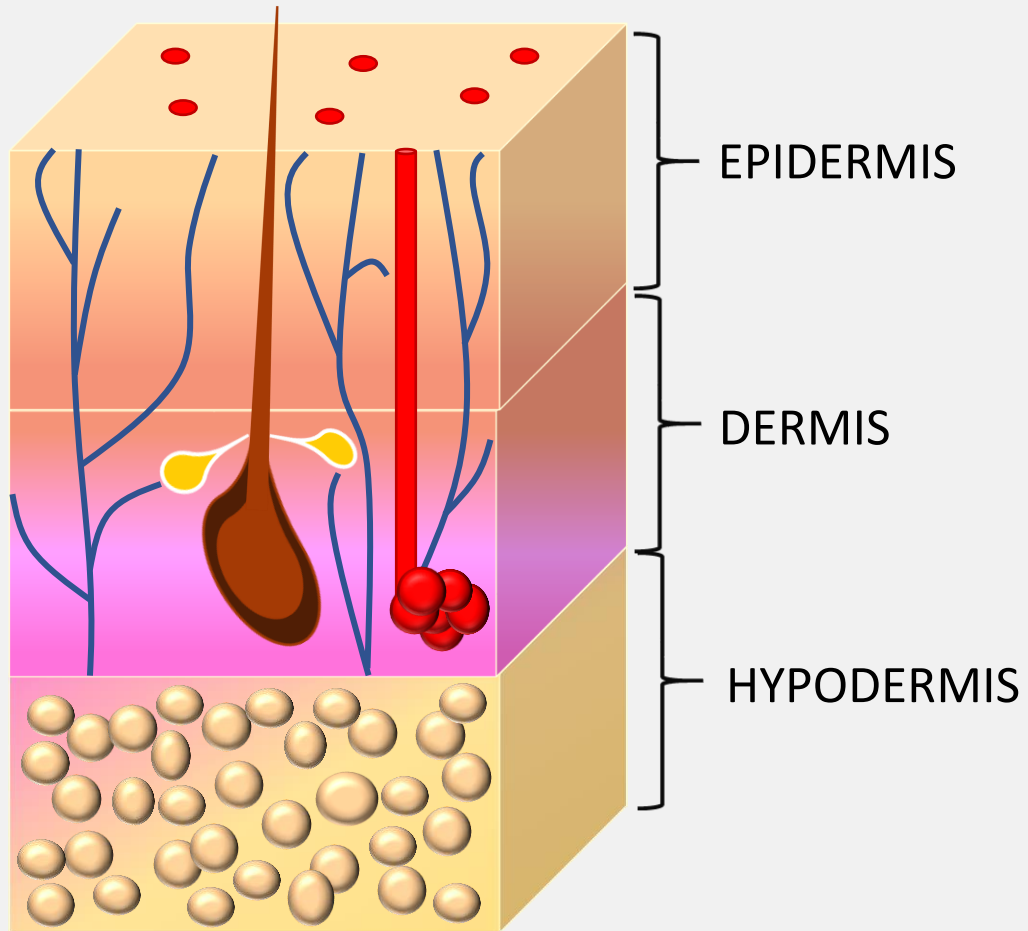


Biochemical assays

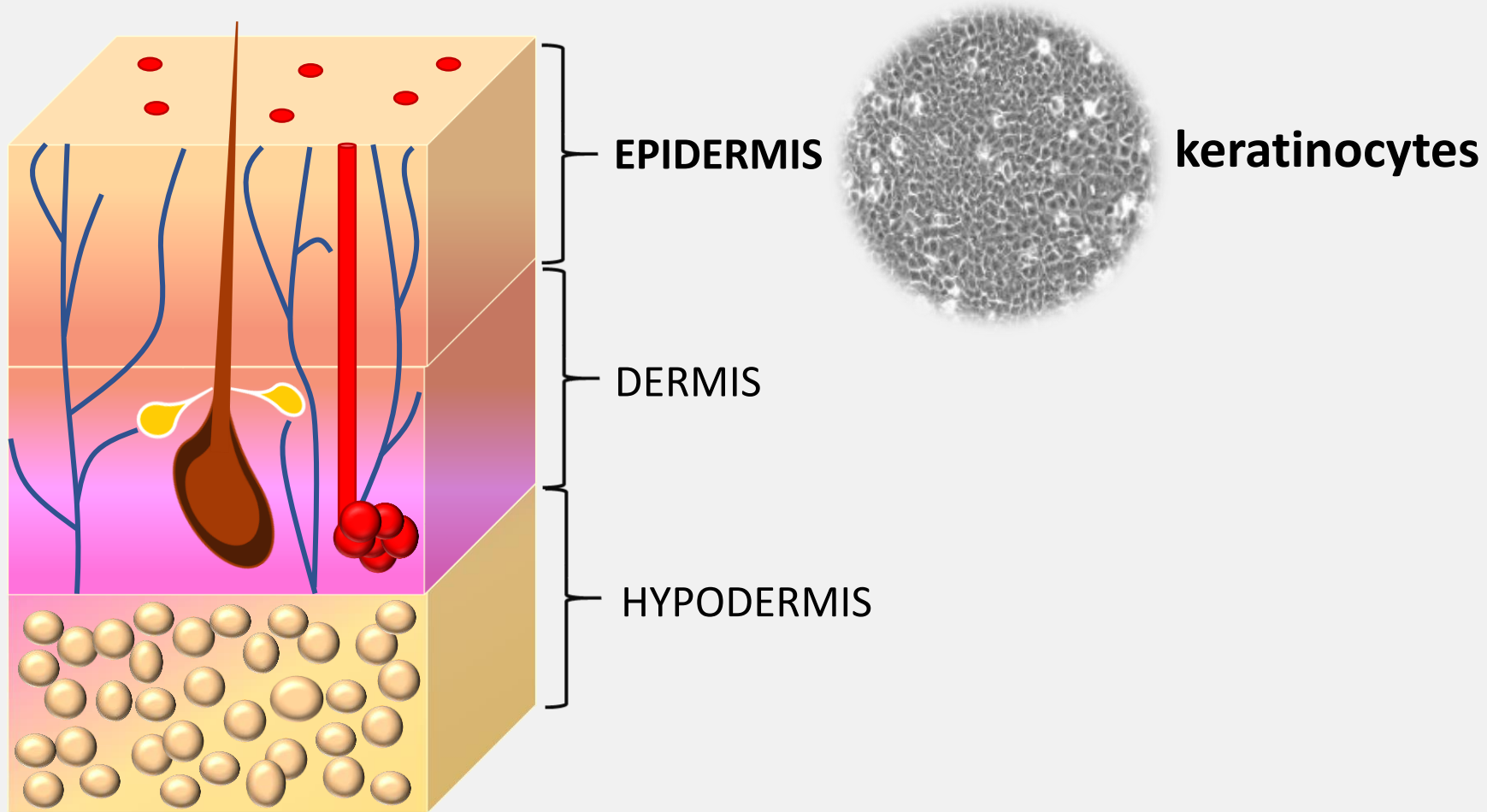


Cellular assays
Skin cells

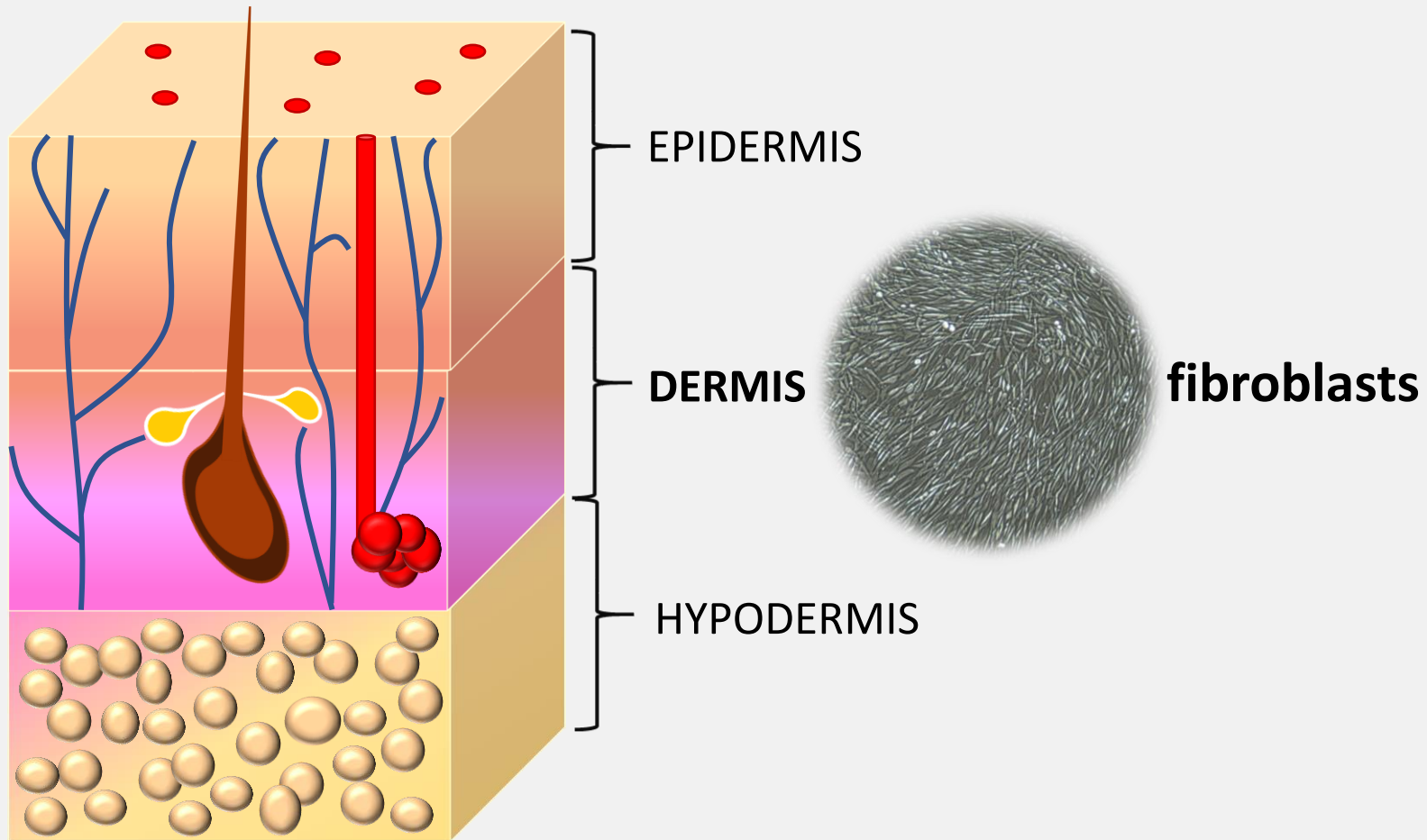
Human skin structure



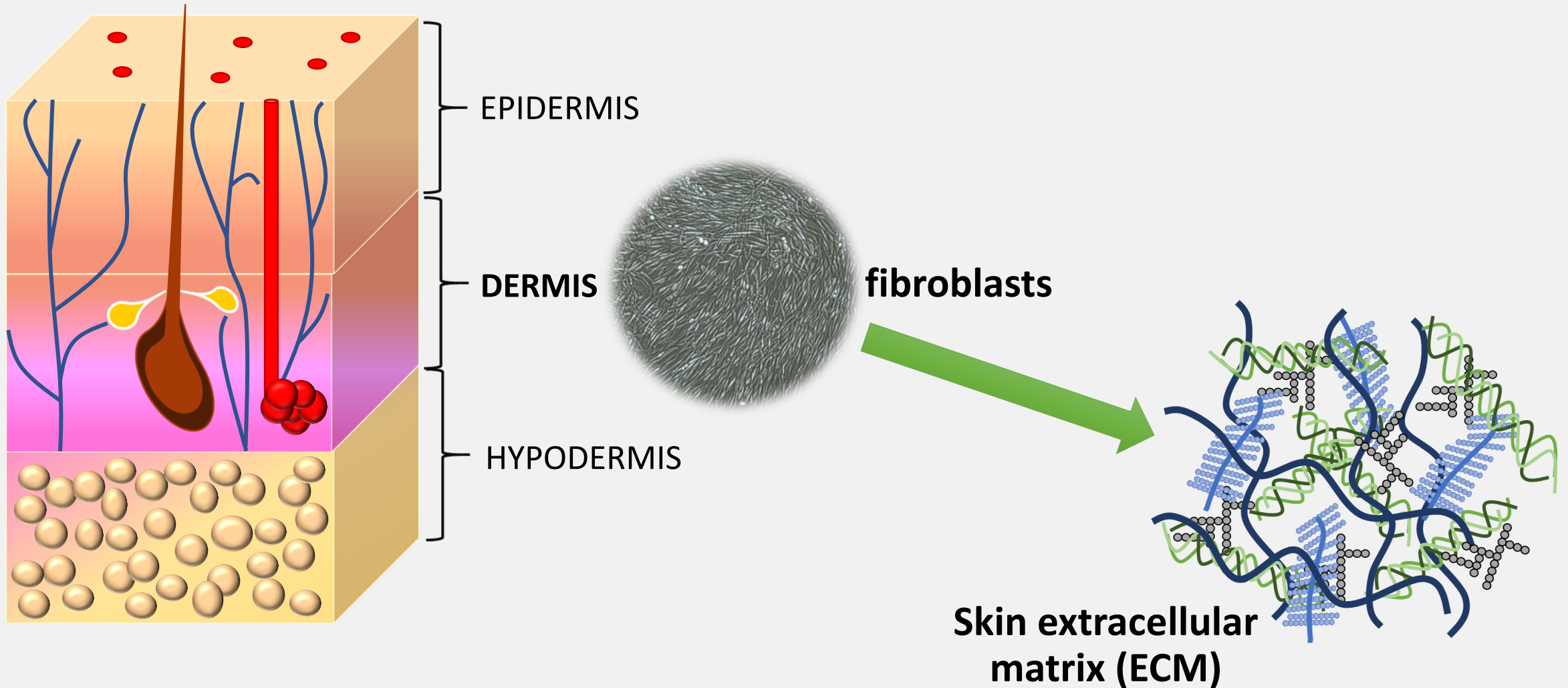
Human skin structure



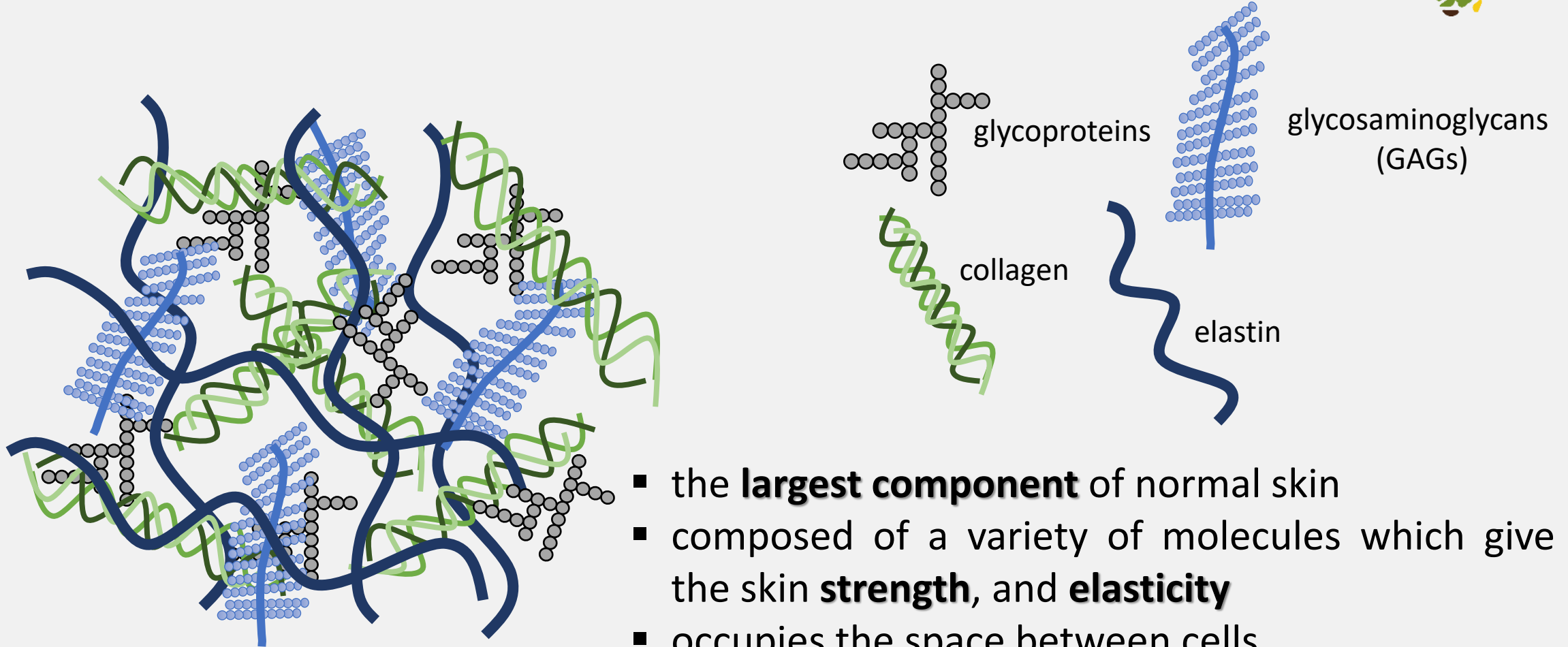
Human skin structure



Human skin structure



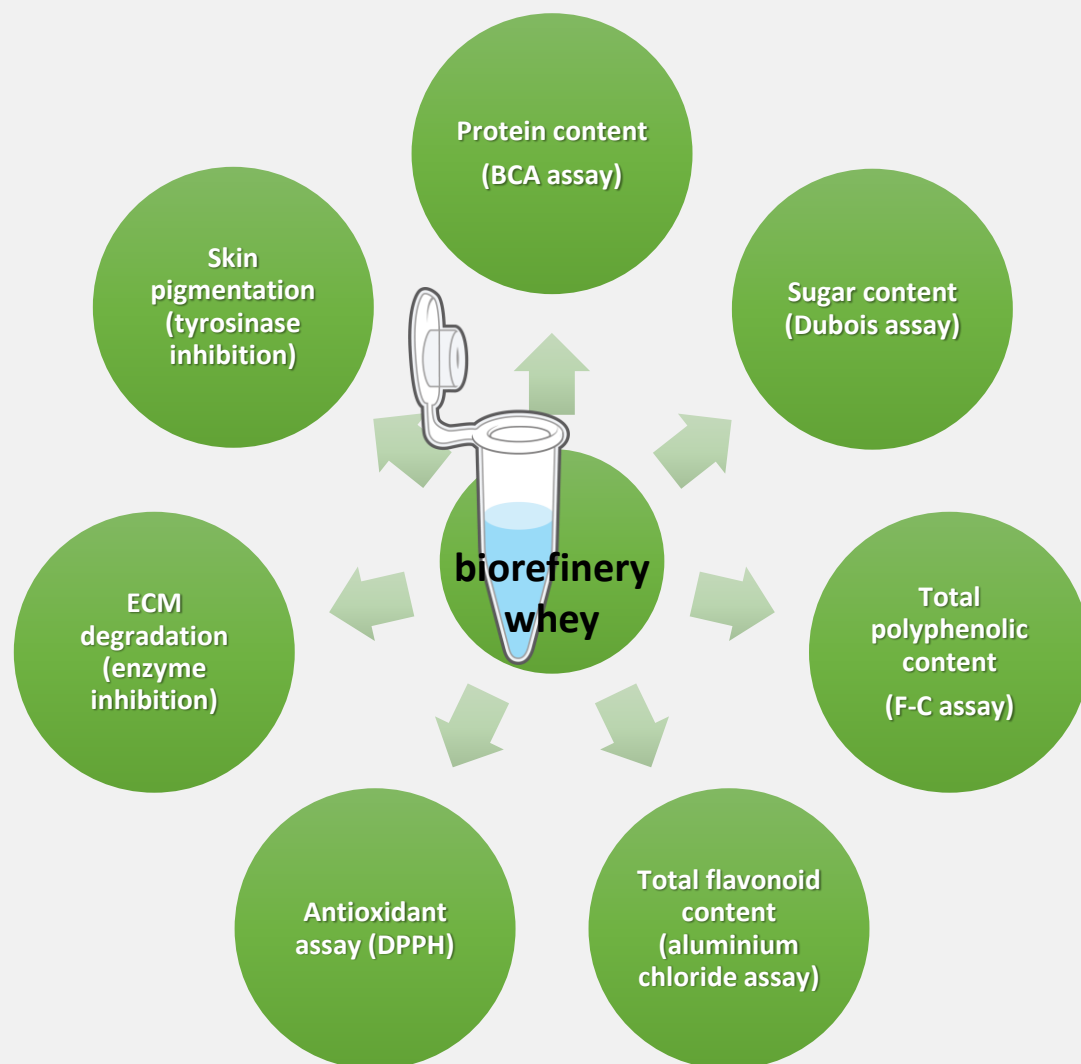
Skin extracellular matrix (ECM)



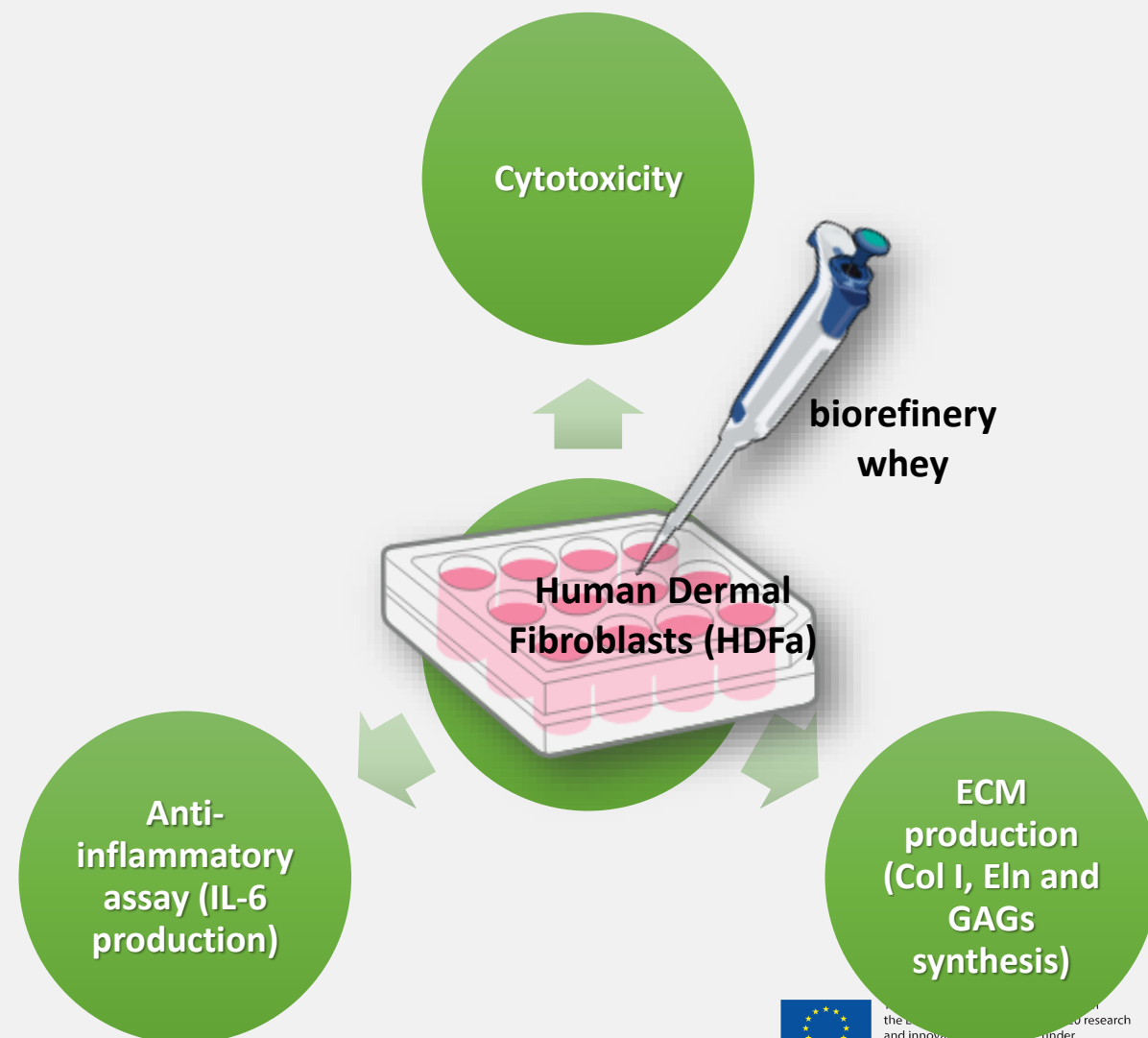
- the **largest component** of normal skin
- composed of a variety of molecules which give the skin **strength**, and **elasticity**
- occupies the space between cells
- serves as a sort of intranet which connects all the components of the skin

Experimental design

Non-cellular assays



Cellular assays



Proximate composition of the green biorefinery whey

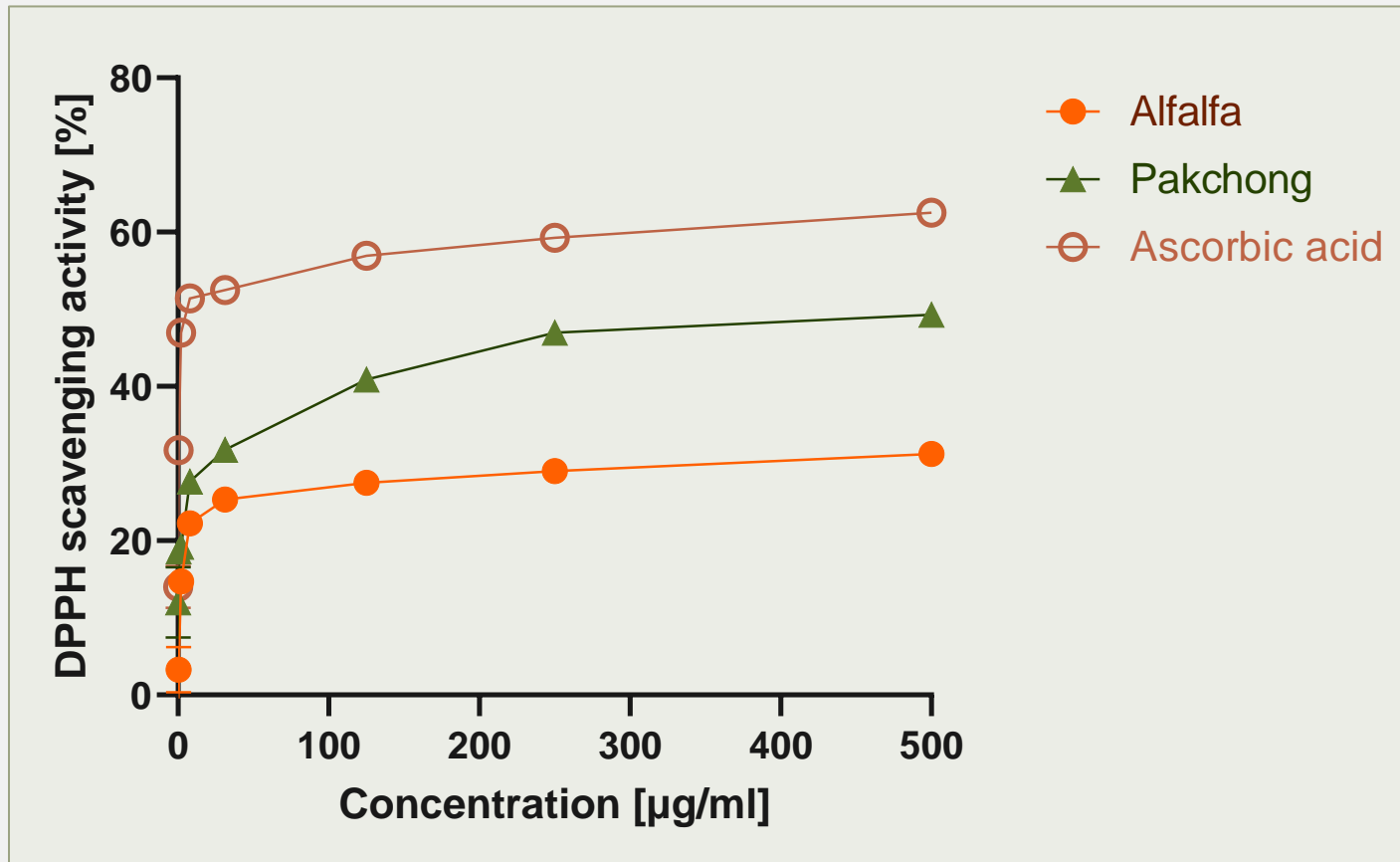


Sample	Protein [mg/ml]	SEM	Sugar [µg/ml]	SEM	TPC [mg GAE/g]	SEM	TFC [mg QE/g]	SEM
Alfalfa	0.66	0.05	17.36	1.61	20.1	0.64	13.06	0.77
Pakchong	0.4	0.06	31.04	1.99	33.26	1.1	18.97	2.19

Phenolic compounds are well-known for their **antioxidant properties**, and a higher TPC typically correlates with greater potential health benefits.

Antioxidant potential of biorefinery whey

DPPH radical scavenging potential of whey samples and positive control (ascorbic acid).



Values correspond to mean \pm SEM of three independent experiments.

Skin health-related anti-enzymatic activity

Effect of biorefinery whey on tyrosinase, elastase and collagenase activity.

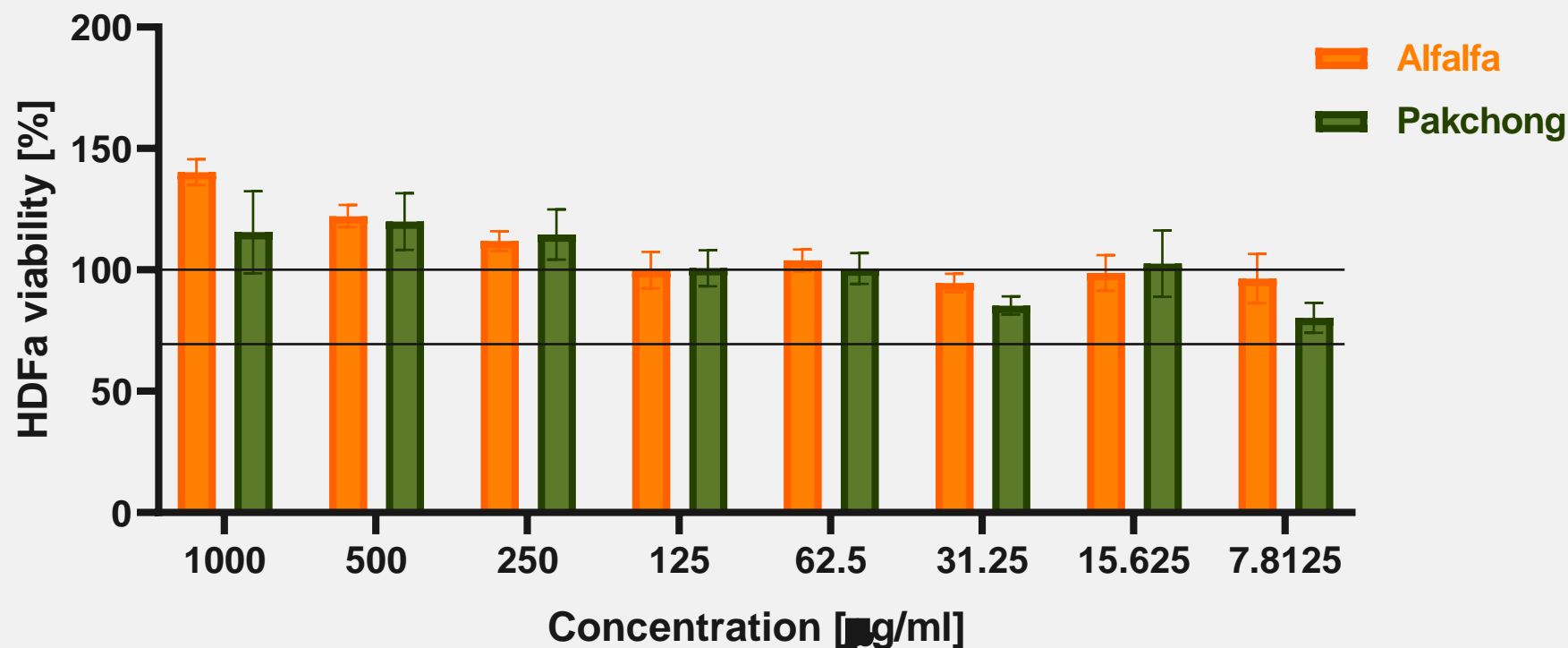
		Tyrosinase inhibition [%]	SEM	Elastase inhibition [%]	SEM	Collagenase inhibition [%]	SEM
positive control		91.46****	0.41	26.86****	3.90	75.6****	1.48
Alfalfa [mg/ml]	10	7.40	2.14	2.21	1.44	35.27****	0.97
	1	17.90**	4.97	5.42	1.07	25.402****	1.06
	0.1	26.15****	4.33	5.10	1.50	22.08***	4.47
	0.01	22.94****	3.13	5.27	1.29	13.75***	0.73
Pakchong [mg/ml]	10	6.62	2.78	25.90****	1.15	79.44****	2.37
	1	19.53**	2.72	4.60	2.03	70.38****	1.88
	0.1	19.19**	2.00	6.74	1.87	47.07****	3.92
	0.01	13.42	5.74	5.07	2.60	21.32****	0.81

Results are presented as a percentage of the enzyme activity. 10 mg/ml kojic acid, 1 mM MeOSuc-Ala-Ala-Pro-Val-CMK and 0.5 mM phenantroline were used as positive controls (tyrosinase, elastase and collagenase inhibitor, respectively). Bars correspond to mean \pm SEM of three independent experiments.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ vs. negative control (enzyme activity; 100%).

Cytotoxicity assessment

Effect of the brown juice on the human dermal fibroblasts (HDFa) viability after 24 h incubation.



The data are presented as the mean \pm SEM from three independent experiments. Statistical significance in comparison to the negative control (100%) was assessed using one-way ANOVA followed by Dunnett's multiple comparison test; * $p < 0.05$; ** $p < 0.01$. The black line indicates the cytotoxicity limit according to ISO 10993-5 (70%).

Extracellular matrix (ECM) molecules production

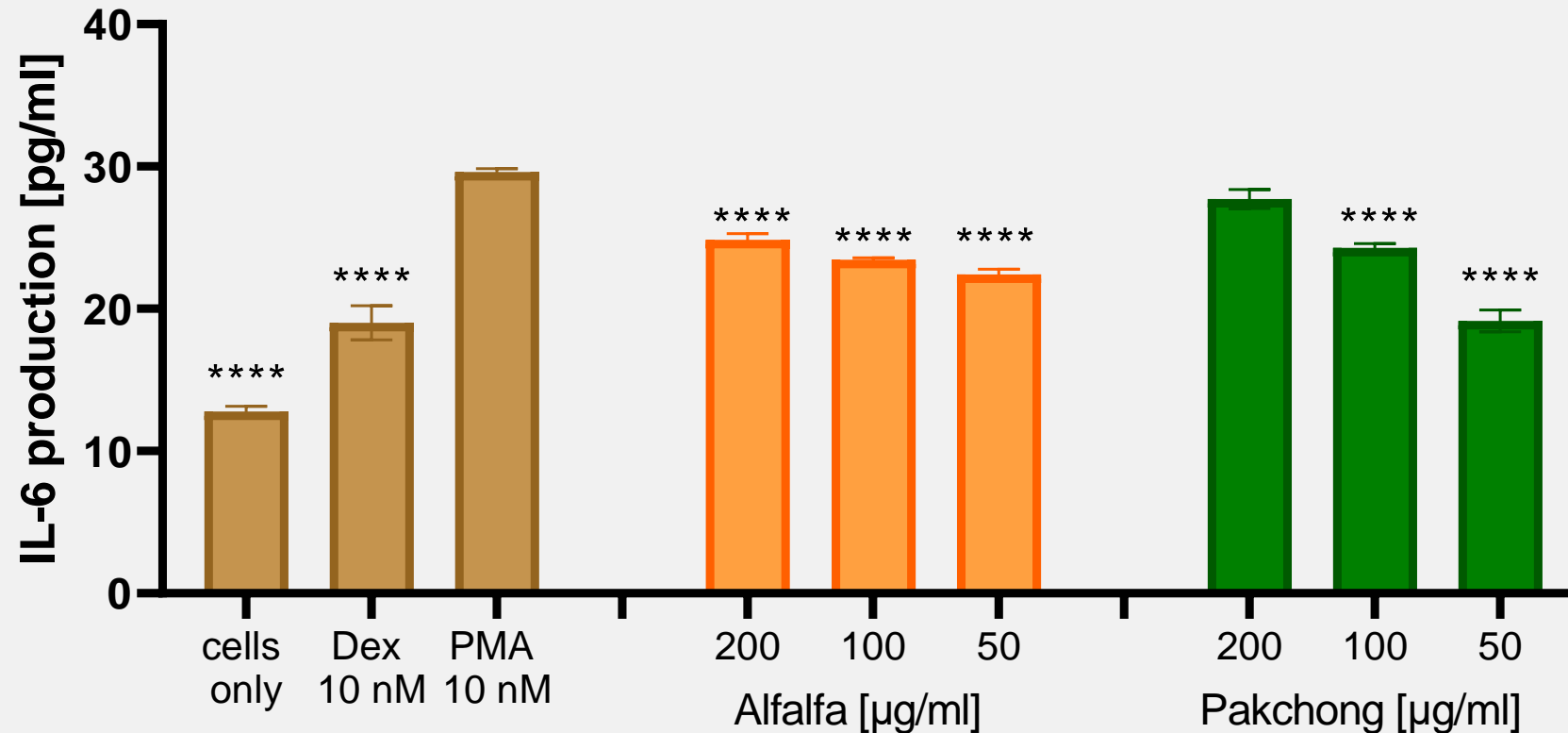
Effect of biorefinery whey on collagen I (Col I), elastin (Eln) and glycosaminoglycans (GAGs) production.

		Col I production [%]	SEM	Eln production [%]	SEM	GAGs production [%]	SEM
cells only		100.00	0.00	100.00	0.00	100.00	0.00
positive control		174.11****	3.58	123.04***	4.32	151.48***	4.82
Alfalfa [µg/ml]	200	92.49	3.73	110.19*	3.94	161.99****	20.28
	100	95.47	5.04	110.14*	2.92	156.27****	2.93
	50	97.46	2.59	106.63*	2.65	144.77**	6.60
Pakchong [µg/ml]	200	76.44	2.53	110.28*	1.67	136.20*	6.21
	100	84.46	2.90	108.90*	3.20	149.76***	4.07
	50	90.45	3.11	104.89	1.32	150.97***	7.73

Results are presented as a percentage of the untreated cells. Bars correspond to mean \pm SEM of three independent experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ vs. negative control (cells only; 100%).

Anti-inflammatory activity of biorefinery whey

Interleukin 6 production in PMA-activated skin cells



The data are presented as the mean \pm SEM. Statistical significance in comparison to the activated cells was assessed using one-way ANOVA followed by Dunnett's multiple comparison test; **** $p < 0.0001$.

Potential application of biorefinery whey

Assay \ Sample	Alfalfa	Pakchong	
Antioxidant assay	√	√√	antioxidant nutraceuticals; anti-ageing, anti-wrinkle cosmetic products
Anti-elastase assay	√	√	anti-ageing/anti-wrinkle cosmetic/cosmeceutical products
Anti-collagenase assay	√	√√	anti-ageing/anti-wrinkle cosmetic/cosmeceutical products
Anti-tyrosinase assay	√	√	cosmetic/cosmeceutical products (skin hyperpigmentation treatment)
Viability assay	√√	√	
Elastin production	√	√	anti-ageing/anti-wrinkle cosmetic/cosmeceutical products
Collagen production			cosmetic/cosmeceutical products (anti-ageing, anti-wrinkle, stretch marks treatment)
Glycosaminoglycans production	√√	√	cosmetic/cosmeceutical products (anti-ageing, anti-wrinkle, moisturisers)
Anti-inflammatory assay	√√	√	cosmetic/cosmeceutical products (treatment of skin conditions related to inflammation, e.g. atopic dermatitis)

Conclusions

Biorefinery whey (brown juice), the by-product of the green biorefinery was investigated for its potential use by the cosmetic/nutraceutical/pharmaceutical industry.

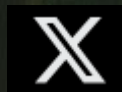
The obtained results show that:

- ✓ tested whey is **not cytotoxic** against used skin cell culture models, human dermal fibroblasts
- ✓ tested samples have **antioxidant** activity
- ✓ whey **increases the expression of extracellular matrix molecules**, such as **elastin** and **glycosaminoglycans**
- ✓ tested whey has the ability to **inhibit elastase, collagenase** and **tyrosinase** activity
- ✓ residual biorefinery whey has anti-inflammatory activity in skin cells

The obtained results indicate that whey offers promising potential as a cosmetic/cosmeceutical/pharmaceutical ingredient, because of its lack of toxicity, antioxidant, anti-hyperpigmentation, anti-wrinkle and anti-inflammatory properties.



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This project has recieved funding from
the European Union's Horizon 2020 research
and innovation programme under
grant agreement No. 101000762

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