The skin consists of three main layers, from the exterior to interior, the epidermis, the dermis and the hypodermis. The epidermis, in turn, is divided in 4 parts, from the inside to the outside, the stratum basale, stratum spinosum, stratum granulosum and stratum corneum which is in contact with the outside[1, 2].

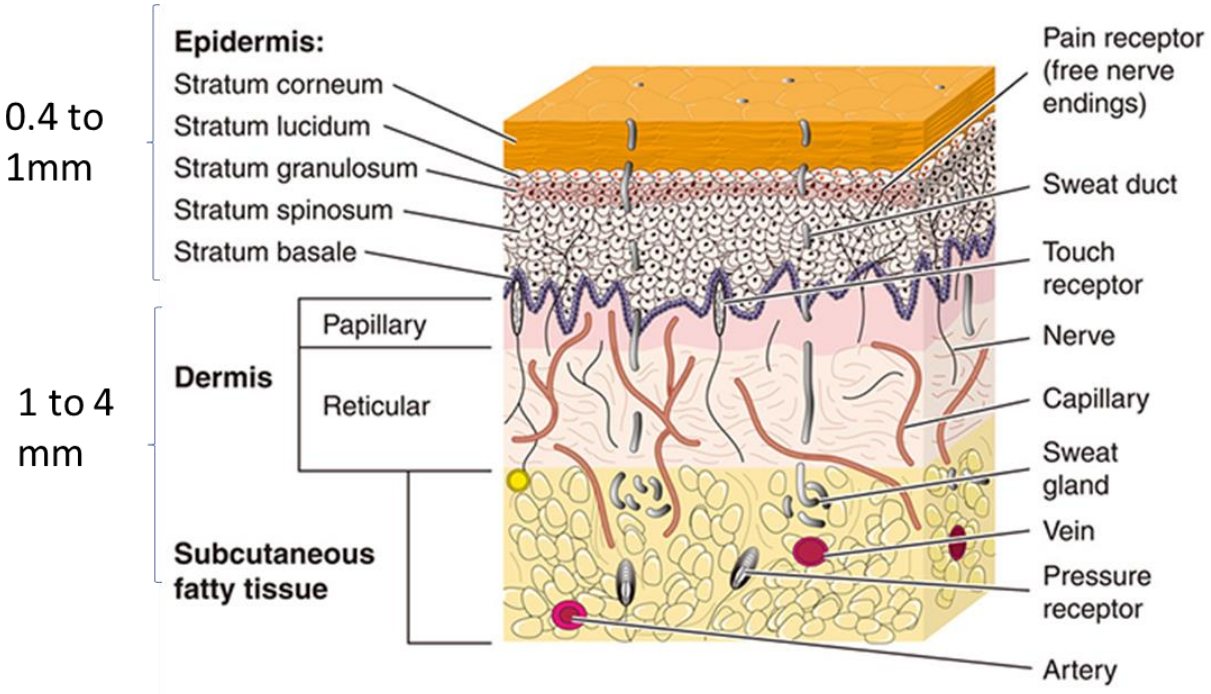

Figure 1: Human skin anatomy

Stratum corneum (SC) is the most contributing to the barrier function of the skin which functions as barrier for big molecules to pass through [2, 3] .

Model skin substrate

The skin of a piglet was used as model substrate for this study because of the many physiological and anatomical similarities between the pig skin and human skin, specifically its permeability behaviour[1]. Skins of both species has a relatively thick epidermis which is clearly separated from all the sub-layers. The dermis of both species contains a lot of elastic tissue with similar collagen fibrils thickness[4, 5].

Sample substrate preparation

In brief, stillborn piglet skin samples (of different sizes) were sourced and collected from a Dunedin (Otago) pig farm. The samples were immediately processed, reduced to the necessary size, and preserved following a thorough tissue preservation protocol. They were stored in the surgery department by the animal surgery team (HTRU facilities manager). The piglets skin samples were dissected into a desirable dimension in order to fit the Optical &RKHUHQW7RPRJUDSK\2&7 H[SHULPHQWDOXHPHQWVWHLLWLQVPRSWLFDO probes). On average, the dimension of the piglet skin samples was calculated at 10 mm x 10 mm with a thickness of 2-4 mm for the OCT assessment.

Experimental analysis

In this study, OCT was implemented to visualize if and how the nanomatrices of actiVlayr containing a bioactive, would deliver it into the skin sample.

- The test sample measurement spots and the real-time measurement period, or rate, was established. The recommended time frame of an hour (60 minutes) to scan and obtain all the images and measurements was followed.
- The skin sample was placed/mounted onto the OCT platform precisely under the OCT probe avoiding any additional compression of the sample
- The OCT probe was precisely placed over the 'spot area' where a nanofibrous matrix) was placed and sited.
- For each sample analysis, the OCT dynamic range was adjusted to avoid random noise and guarantee optimal OCT images with reasonable depth and good contrast.
- Prior to the acquisition of the OCT data, images and background noise was removed.
- All OCT scanned images/data were captured and accumulated via Fiji.apps software. Fiji.apps software was used to analyse OCT data, and the images were reported as 2D or 3D image files, while the OCT signals' strength in relation to the skin depth were reported in graphs elaborated using the software MATLAB.

When the sample was placed on the skin surface the natural diffusion behaviour was observed with OCT. The diffusion of bioactives including the nanofibre matrix provides the changes of scattering properties of the skin and thus detected by OCT i.e., the permeability rate of bioactives into the skin will be measured by monitoring changes in the OCT signal slope and by using a special software the double-correlation analysis of 2D/3D OCT images will reorganized for validation of the skin penetration outcomes. In turn the increase of bioactive (s) concentration will result in a progressive change of scattering properties of skin. The permeability changes of bio actives can thus be examined up to 2mm in depth of skin which covers subcutaneous region such as Stratum Corneum (SC), epidermis, dermal-epidermal junction (DEJ) and reticular dermis.

ActivLayr composition and working principle

actiVlayr nanofibre matrix is composed of marine collagen (a combination of de-natured whole chain and hydrolysed collagen molecular chains), Hyaluronic acid (HA) and Vinanza.

Collagen has been used in cosmetics for its skin toning and moisturizing properties. Marine collagen is generally considered superior to bovine and procaine collagen due to its high hydroxy proline and lysine content which helps to combat antiaging by synthesizing longer triple helix alpha chains to build collagen fibrils and great moisture absorption properties.

Collagen has been widely known and used in the development of cosmetic formulations as a moisturizer and natural humectant [6, 7] component with a high substantivity to the skin [7]. Usually, proteins of high molecular weight (HMW), such as collagen, cannot be absorbed or pass through by the stratum corneum of the skin; they remain on the surface instead, working as water-uptake through hydration (keeping the skin moisturized)[6, 8] and as protectors against microbial infiltration in cases of wounded tissue. But the

moisturizing effect wont last longer as it is only superficially absorbed by the SC of the epidermal layer.

But by delivering the whole chain collagen molecule in a form of nanofibre matrix, even HMW collagen protein can break up in to small dissolvable pieces to enter stratum corneum. This base idea is used to construct actiVLayr which is composed of HMW denatured collagen, low molecular weight (LMW) hydrolysed collagen, bioactives such as HA and Vinanza.

A de-natured whole chain collagen is a long chain collagen molecule with intact α-chains, β- and γ-components accounting for high molecular weight. With a high hydroxyproline content denatured form (no triple helix) prove to have a good moisturizing effect through water absorption, preventing skin dehydration without signs of irritancy to the skin, as demonstrated by Alves and Silva etal [6].

On the other hand, the hydrolysed collagen with LMW can readily absorbed by the stratum corneum of the skin to penetrate faster and deeper. The bioactives that are chemically bonded with hydrolysed collagen will therefore penetrate deeper into the skin. Research published in 2015 in the *Open Nutraceuticals Journal* states how numerous clinical trials have now been performed showing the efficacy and benefits of collagen peptides on skin properties, including hydration, elasticity and reduction of wrinkles. Researchers conclude that hydrolysed collagen is a smart weapon in the everyday fight against the undesirable yet visible signs of aging [9].

Therefore, a collagen matrix containing a denature and hydrolysed collagen mix can have profound effect of skin by delivering the bioactives deeper while integrating into skin's own collagen framework to reduce skin wrinkles.

In addition, HA which is also part of the matrix is a naturally occurring polysaccharide frequently used as a functional ingredient and as a dermal filler in topical and subcutaneous anti-aging treatments. As it is one of the most hydrophilic molecules in nature when administered subcutaneously, HA builds a network within wrinkles and rhytids that draws in water to form gel-like structures and increases the elasticity of the skin, giving it a plumper, fuller appearance [10].

Similarly, Vinanza® is a blend of potent antioxidants including catechins, vitamin C, gallic acid, flavonoids, and proanthocyanins that helps with skin brightening and improves skin appearance.

Unfortunately, in its native state, HA has certain characteristics that limit its performance—i.e., it exhibits unusually rapid degradation and systemic removal. Also can displays mechanical and structural weaknesses that limit its durability and performance[10].

Despite this, HA-based treatments are still the preferred choice for many in vivo applications such as dermal fillers since it is a natural component of the body (bio-compatible) and has proven effect on anti-aging and skin hydration [10].

On the other hand, Vinanza composed natural polyphenols is naturally prone to oxidation which shorten its shelf life.

These limitations can be addressed by modifying the MW and rheological characteristics by entangling the molecular chain of HA [10] and by stabilizing the polyphenols in case of Vinanza. Both are achieved by Revolution Fibres Proprietary Sonic electrospinning technology.

With sonic electrospinning a unique nanofibre matrix has been developed that stretches the HA polymer chain to form inter penetrated network with collagen polymer chain to increase its stability. The collagen nanofibre also encapsulates Vinanza molecule within the chain during the process protecting its potency and its stability against photo oxidation.

The capability to encapsulate HA and Vinanza in actiVlayr is therefore an important strategy for enhancing and improving its value and performance.

RESULTS AND DISCUSSION

The scanned OCT data/results for actiVlayr showed both strong OCT signal, ~0.41 a.u., and a good depth, ~2.5 mm. Consequently, it appears to be a technology that assures a deeper penetration of the bioactive to the dermal layer of the skin.

In detail, the following graph shows the degree of penetration of the bioactive in each nanofibrous matrix. In order to obtain, an optimal visualisation and a consequential better understanding of the penetration behaviour, each skin sample was arbitrarily subdivided into 256 smaller volumes during the OCT analysis and each section was photographed and analysed separately.

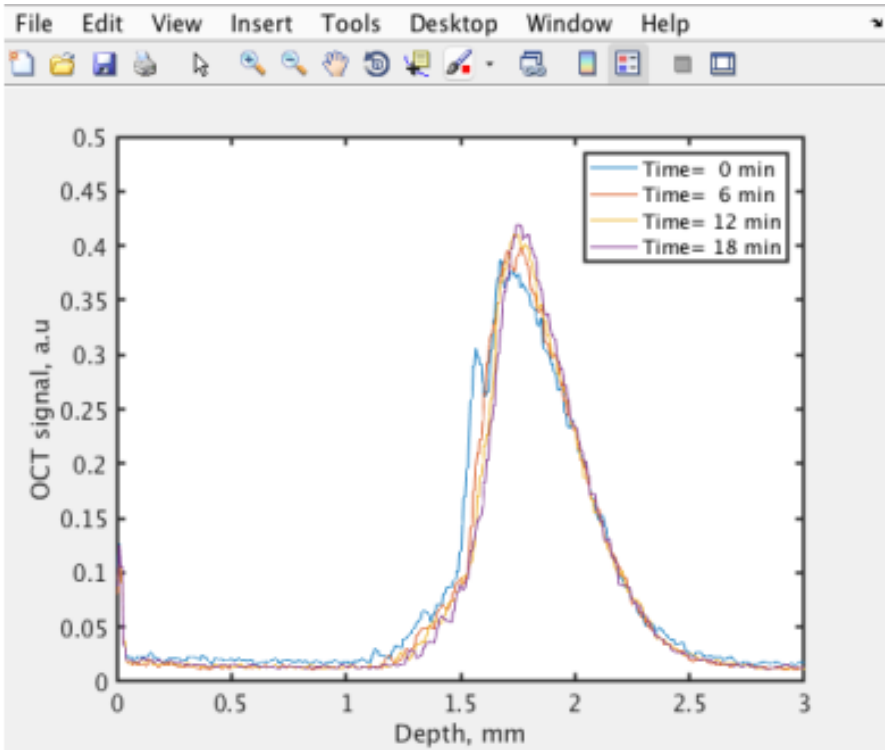

Figure 2: actiVlayr penetration 0-18min

Within 0 min actiVLayr exhibits to penetrate deep, crosses stratum corneum and reaches 1.5 mm which is dermal- epidermal junction layer of the skin and moves further deep up to 1.75 mm in 18 min.

It has been established through experiments that the size of the molecule and its lipophilicity are major determinants of the penetration processes through the stratum corneum. This means that the permeability of a molecule is directly related to its lipophilicity and inversely proportional to molecular size[1, 3]. actiVlayr being a water-soluble nanofibre material breaks down into nano sized fragments that enters through pores of the stratum corneum (within 5 sec) to pass through even the strong barrier layers of epidermis within 6 min.

During the diffusion, the hygroscopic epidermis layer naturally absorbs actiVlayr (thanks to its hydrophilicity) to fill up the fine lines and wrinkles and gets hydrated.

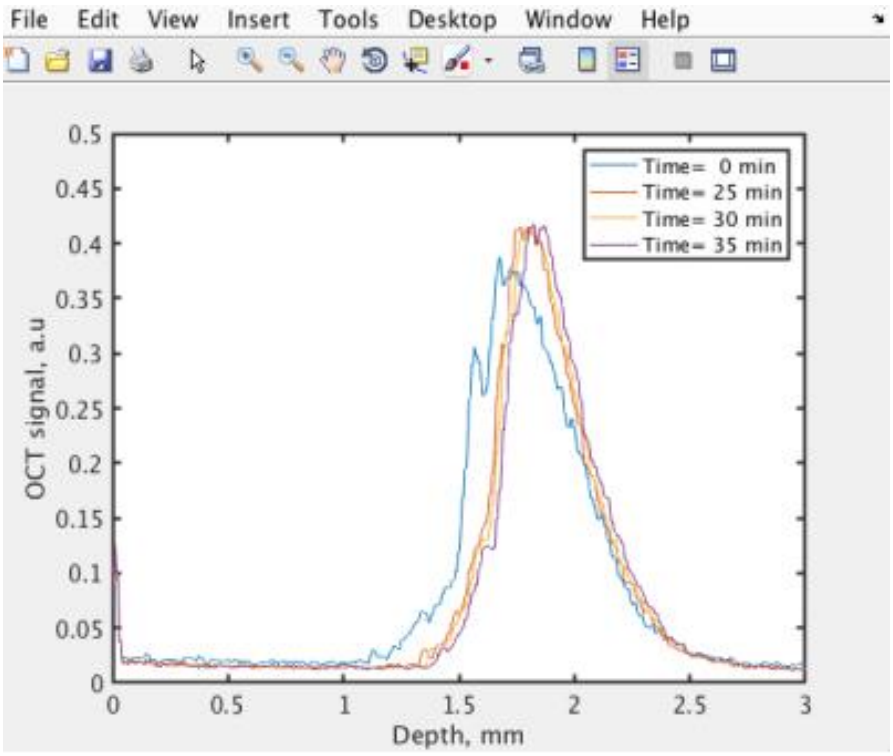

Figure 3: actiVlayr penetration 0-35 min

Within the next 35 min actiVLayr crosses 2mm which is dermal- epidermal junction (DJS) and reaches dermal layer.

This zone between the dermis and epidermis is the cutaneous basement membrane, or the dermal-epidermal junction (DEJ), leads to a dynamic interface and adhesion between both layers. It consists of interconnected proteins in a complex network. The DEJ helps for remodelling, healing of wounds, and development of the skin. The DEJ restricts the passage of molecules, based on charge and size, between the epidermis and dermis[1]. And of course, due to the advantage of the size of nanofibre the actiVLayr easily permeates the DEJ layer and spread across the network. This would help in repairing deep scars and deep wrinkles.

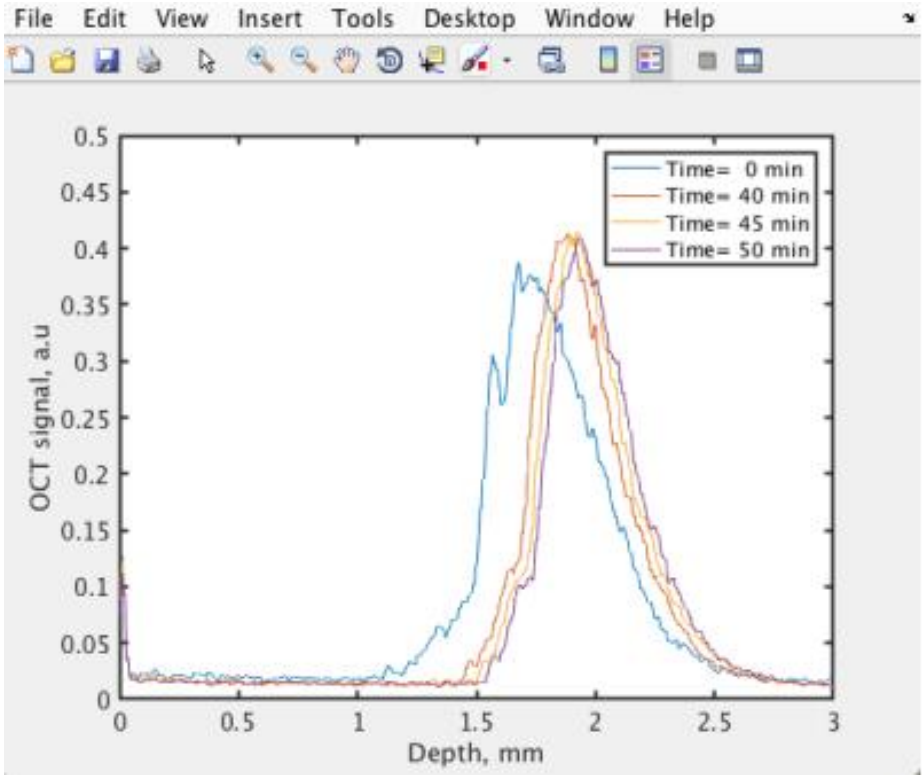

Figure 3: actiVlayr penetration behaviour 0-50 minutes

In 50 min actiVLayr reaches 2.5 mm which is dermal layer of the skin. In this layer is where the fibroblast is present which synthesis the collagen.
actiVlayr consists of collagen can very well disperse at this layer and form an integrated framework with the layer while Vitamin C from Vinanza and HA helps in hydroxylation of collagen molecules building more collagen fibrils.

For every nanofibrous matrix, the following images, Fig 4 and 5. meet the penetration level of the initial volume 1 and the final volume 256 at 0 minutes and 50 minutes, showcasing how
the bioactive is widespread in the whole sample and not located only in one section. This observation is a very interesting to note. Through electrospinning the bioactives has been homogeneously distributed throughout the samples ensures optimal and even dose every single time of dermal application.

Furthermore, an effort was applied to differentiate and distinguishing the widespread penetration of bioactives into the skin substrate. The images have been colour coded though an appropriate MATLAB code using both a copper colour and 'hsv' which guarantees a clearer visualisation of the absorbed bioactive (Figure 4c, d and 5c, d). Further work is underway to exactly identify and differentiate the individual components.

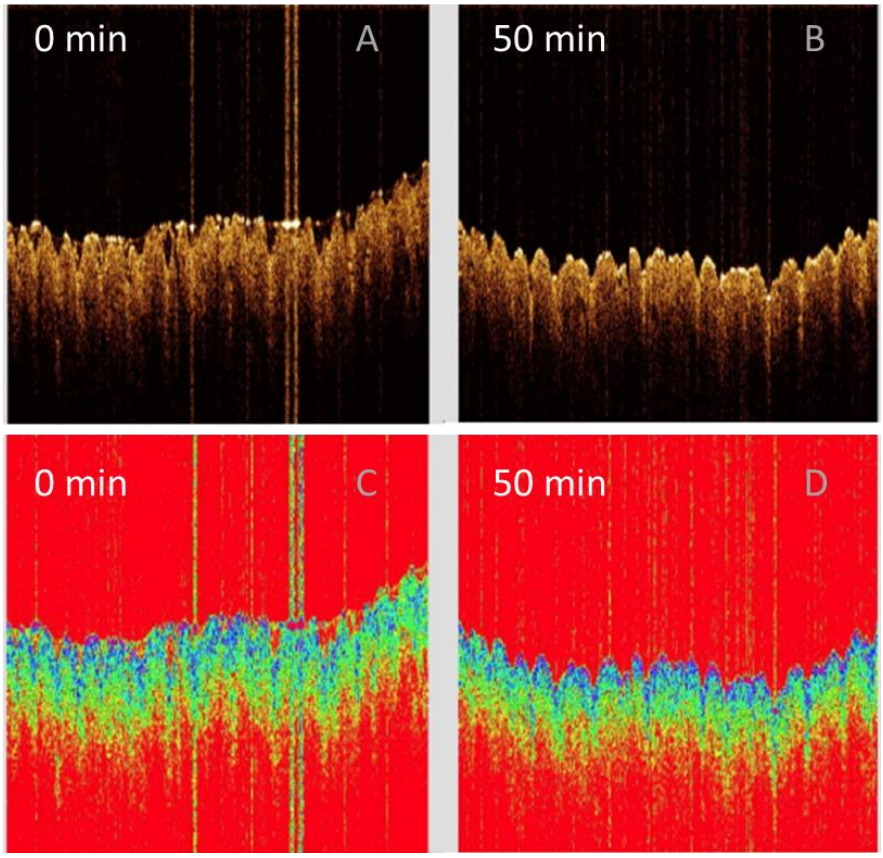

Figure 4: Volume 1 - difference between 0 and 50 minutes. A & B are non-coloured coded and C & d are colour coded.

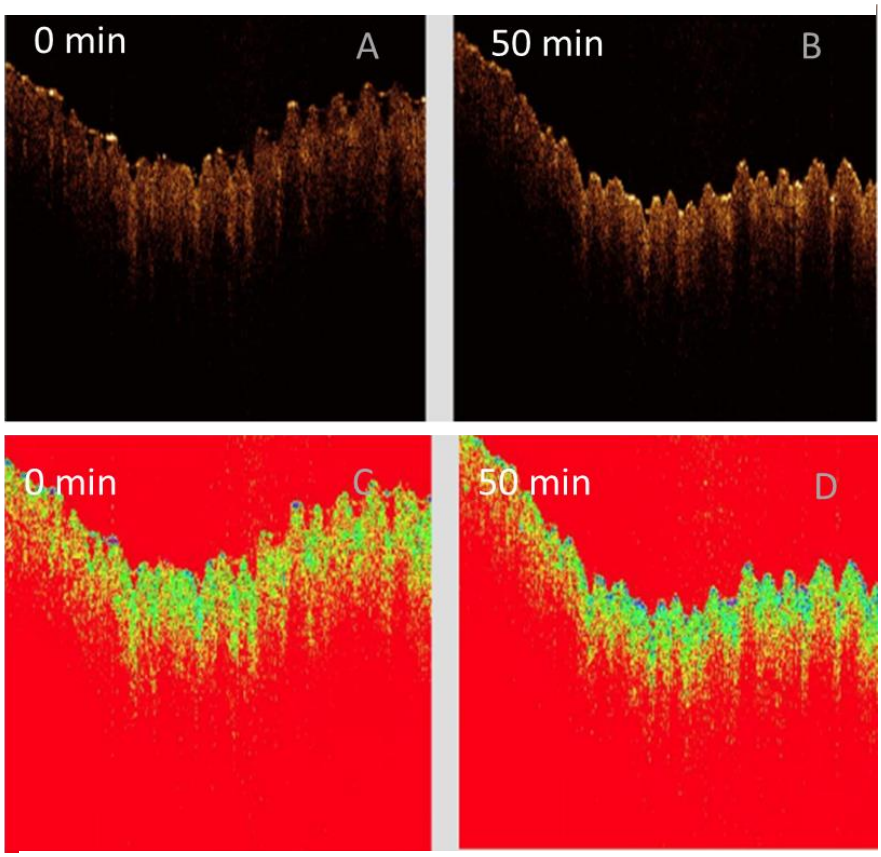

Figure 5: Volume 200 - difference between 0 and 50 minutes. A & B are non-coloured coded and C & d are colour coded.

In conclusion, based on the observations of the penetration behaviour of actiVlayr represented in the graphs, it appears that the actiVLayr guarantees a deeper up to 2.5 mm through epidermal, epidermal-dermal junction and dermal layer and more widespread penetration of the bioactive.

In cosmetics, molecular penetration is limited to epidermis. However, since deeper penetration is needed for ultimate skin enrichment mixture of emulsifiers are used to drive the penetration to maximize the benefits of the rest of the formulation[2, 3].

Usually such intense penetration is only achievable through micro-needle, ultra-sonic or light rays[3].

CONCLUSION

The ultimate goal of cosmetics is to use intelligent vehicle to deliver cosmetic actives deeper in to the skin yet not affect the skin integrity. Several enhancer vehicles have been synthesized to enhance penetration mechanism of actives such as Retinol, Vitamin C, HA etc. This includes chemical enhancers which are water or ester based or physical enhancing techniques like Phonophoresis or sonophoresis using ultrasound energy, iontophoresis technique that applies a small electric current to the skin or a microneedle delivery technique.
These techniques aim to alleviate immediate dryness and restore innate moisture contents along with other replenishers at the epidermis/dermis level of the skin for various other benefits.

Based on the OCT results, actiVLayr technology which is water based, 100% natural proved to outperform all the cream based cosmetic formulations with respect to penetration time, level and effect without uses of physical enhancement methods. To our knowledge and literature research no skincare cosmetic has claimed such deeper penetration of actives.

Therefore, based on the penetration profile actiVlayr could for various skin concerns including but not limited to

- Increasing bioactives absorption
- Fine lines
- Deeper wrinkle lines
- Anti-ageing
- Pigmentation marks
- Acne scars
- Light scars
- Deep scars

REFERENCES

1. ZAMAN, N., *INFLUENCE OF THE (ULTRA) STRUCTURE OF SKIN SPECIMENS USED FOR PHARMACOLOGICAL STUDIES ON THE TISSUE PERMEABILITY*, in *Department of Pharmaceutics, laboratory for medicinal chemistry*. 2014, Universiteit Gent: Belgium.
2. Mark R Prausnitz , P.M.E., Thomas J Franz , Matthias Schmuth , Jui-Chen Tsai ,Gopinathan K Menon , Walter M Holleran and Kenneth R Feingold, *Skin Barrier and Transdermal Drug Delivery*, in *Medical theraphy*. 2012. p. 2065-2073.
3. R.H.H., T.H.N., *Overcoming the Stratum Corneum: The Modulation of Skin Penetration.* Skin Pharmacol Appl Skin Physiol, 2006. **19**: p. 106-121.
4. Abd, E., et al., *Skin models for the testing of transdermal drugs.* Clinical Pharmacology : Advances and Applications, 2016. **8**: p. 163-176.
5. Dick, I.P. and R.C. Scott, *Pig Ear Skin as an In-vitro Model for Human Skin Permeability.* Journal of Pharmacy and Pharmacology, 1992. **44**(8): p. 640-645.
6. Alves, A., et al., *Cosmetic Potential of Marine Fish Skin Collagen.* Cosmetics, 2017. **4**(4): p. 39.
7. Peng, Y., et al., *Evaluation for collagen products for cosmetic application.* International Journal of Cosmetic Science, 2004. **26**(6): p. 313-313.
8. Swatschek, D., et al., *Marine sponge collagen: isolation, characterization and effects on the skin parameters surface-pH, moisture and sebum.* European Journal of Pharmaceutics and Biopharmaceutics, 2002. **53**(1): p. 107-113.
9. Sara Sibilla, M.G., Sarah Brewer, Anil Budh-Raja, Licia Genovese, *An Overview of the Beneficial Effects of Hydrolysed Collagen as a Nutraceutical on Skin Properties: Scientific Background and Clinical Studies.* The Open Nutraceuticals Journal, 2015. **8**: p. 29-42.
10. Sabagh, J.D.a.B., *Analytical Techniques for Hyaluronic Acid Formulation.* Cosm & Toil, 2015. **130**(7): p. 42-48.

Notes: Reccomdations on actiVlayr for skin concerns (in the conclusion section) is based on white paper of a microneedle technology called Dermaroller.
https://www.dermarollershop.com/blog/dermaroller-size-guide/