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Comparative Histological Study on Anti-Ageing Effect of Hyaluronic Acid Versus Nanofat Injection on the Epidermis of Thin Skin of Female Albino Rat

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ABSTRACT

Introduction: Intrinsic skin ageing is an inevitable and physiological process that is characterized by excess wrinkles. Hyaluronic acid (HA) filler is widely used as a dermal filler while nanofat represents a new technique for skin rejuvenation. **Aim of the work:** This work was carried out to compare between the effect of HA versus nanofat subcutaneous (S.C) injection on the epidermis of aged thin skin of female albino rats. **Materials and Methods:** 40 female albino rats were divided into four equal groups. Group I was the control adult one. Group II aged rats were subdivided equally into; subgroup IIa for preparation of the nanofat and subgroup IIb that received a single SC injection of 0.1ml of normal saline at the right flank region. Group III aged rats received a single SC injection of 0.1ml of cross-linked HA at the right flank region. Group IV aged rats received a single SC injection of 0.1ml of nanofat at the same site as group II&III. Skin specimens were examined by LM after their staining with H&E. Morphometric study and statistical analysis were performed to measure the mean total epidermal thickness. **Results:** LM examination of thin skin of group III and IV showed structural rejuvenation of their aged epidermis. Morphometric analysis displayed a significant increase in the mean total thickness of the epidermis in thin skin of both group III&IV compared to group II. **Conclusion**: From the present study, it could be concluded that both hyaluronic acid and nanofat subcutaneous injection have anti-ageing effect on the epidermis of thin skin of female albino rat.

Keywords: Histology, skin, epidermis, aged rats, hyaluronic acid, and nanofat

1. Introduction

Skin ageing is a dynamic process including two types; the first one is the intrinsic ageing, which occurs in a similar way to all internal organs. The second one is the extrinsic ageing which occurs as a consequence of external factors, such as ultraviolet (UV) radiation, poor nutrition, smoking and air pollution. The aged skin looks thinner, paler and translucent, in association with pigmented spots, wrinkles and volume loss. Ageing damages the elasticity of the skin and leads to fragmentation and fragility of the collagen bundles. Ageing creates morphological, structural and functional deterioration of the skin, which impacts not only on cosmetic health but also on psychological and physical health (He *et al.,* 2023; Liang *et al.,* 2023).

Senescence of cells is a complex process as a result of permanent cell cycle arrest, which leads to decline of their physiological functionality and subsequent acceleration of the ageing process of skin. They are distinguished by their failure to proliferate, resistance to apoptosis, and release of substances that encourage tissue degeneration and inflammation. Senescent cells have been demonstrated to accumulate with ageing and may play a role in multiple diseases and changes to the skin (Amor *et al.,* 2020).

Nowadays, more attention is being paid to improve skin conditions, and the demand for injectable dermal fillers has been increased. There are many types of ideal dermal fillers currently used in routine clinical practice (Guo *et al.,* 2023).

Hyaluronic acid is a non-sulphated-7-glycosaminoglycan, which is present in the dermis, articular cartilage, synovial fluid and vitreous humor. It can be naturally synthesized by fibroblasts

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and broken down by several elements, including hyaluronidase enzymes, acid-base, ultrasonic waves, heat, free radicals, and UV radiation, which hasten the degradation of HA. To boost HA stability, numerous technologies have been created such as crosslinking and stabilizers which is a natural component of the extracellular matrix. The injectable cross-linked hyaluronic acid is a gold standard and the commonest type used nowadays as a temporary dermal filler for its safety and efficacy in reducing wrinkles and restoring volume loss because its effect appears immediately (Haddad *et al*., 2022; Yasin *et al.,* 2022; Allen and Dodou, 2024)**.**

Nanofat is an ultra-purified adipose tissue-derived product without mature adipocytes. It can be obtained by emulsification and filtration of the lipo-aspirates. It is used for many indications, mainly for reshaping and filling contour defects. Nowadays, nanofat has been used for its anti-ageing effect and as a long-lasting soft tissue filler because it is easily accessible and can improve skin quality. It treats delicate areas such as fine wrinkles around the lips and dark circles around eyes. Furthermore, nanofat can fill the nasal grooves, cheek and lips (Bhooshan *et al.,* 2018; Ding *et al.,* 2022).

2. Materials and Methods

This work was carried out on forty female albino rats, including 10 adults and 30 aged rats. They were divided equally into four main groups:

1. Group I (Control-adult group)

This group included ten adult female albino rats (6-8 months) that was kept without any treatment for the study of the normal histological structure of the adult skin.

2. Group II (Control-aged group)

This group included ten aged female albino rats (12 months) and was used for studying the histological structure of the aged skin and was randomly subdivided into two equal subgroups:

• Subgroup IIa

It was kept without treatment. The fatty tissue used in the preparation of nanofat was harvested from perirenal and inguinal fat.

• Subgroup IIb

It received a single subcutaneous injection of 0.1ml of normal saline at the right flank region.

3. Group III (Hyaluronic acid-treated group)

It included ten aged rats that received a single subcutaneous injection of 0.1ml of cross-linked hyaluronic acid in the right flank region, then they were sacrificed and dissected after 30 days (Aziz *et al.,* 2019).

4. Group IV (Nanofat-treated group)

It included ten aged rats that received a single subcutaneous injection of 0.1ml of nanofat in the right flank region, then they were sacrificed and dissected after 30 days (Weinzierl *et al.,* 2022).

Stereoscope was used to show the whole thickness of the injected area after subcutaneous injection with fillers. All the slides were examined and photographed by Zeiss camera attached to stereoscope (Jena, Germany) and Olympus light microscope (Tokyo, Japan) coupled to (E-420, 10 megapixels) Olympus digital camera at Histology Department, Faculty of Medicine, Tanta University.

Preparation of nanofat

The macrofat was obtained from areas rich in adipose tissue, like the inguinal region and perirenal fat. The macrofat was harvested from subgroup IIa then minced by a tissue cutter to produce fat fragments of an identical volume to produce microfat (Weinzierl *et al.,* 2022). Mechanical emulsification was then done between two 10-cc syringes using 3-way adapter to produce the nanofat by a standardized manner according to Tonnard *et al.* (2013).

Morphometric study

The software "Image J, (1.48v)" was used for image analysis obtained from the National Institute of Health, Bethesda, Maryland, USA for morphometric study of the specimens. Ten different, non-overlapping, randomly selected fields from each slide from each rat of each group were microscopically examined. The mean total thickness of the epidermis in H&E stained sections was quantified (at a magnification power of 400x).

3. Results

A-Stereoscope Results

Stereoscopic examination of haematoxylin and eosin-stained sections showed the histological structure of the rat skin formed of epidermis and dermis. The subcutaneous tissue ~~that~~ consists of an outer layer of adipose tissue, a middle layer of thin sheet of striated muscle called panniculus carnosus, and an inner layer of loose connective tissue as shown in (figure i and ii) which represent group I, (the control-adult group) and group II (the control-aged group) respectively. In group III, there was an expansion of the subcutaneous tissue (site of injection) of the aged skin by a large homogenous basophilic pool of hyaluronic acid (dermal filler) (figure iii). There was also an expansion of the subcutaneous tissue of the aged skin of group IV by the faintly stained nanofat as shown in (figure iv). Stereoscope revealed the whole thickness of the injected areas with the filling materials (Fig.1).

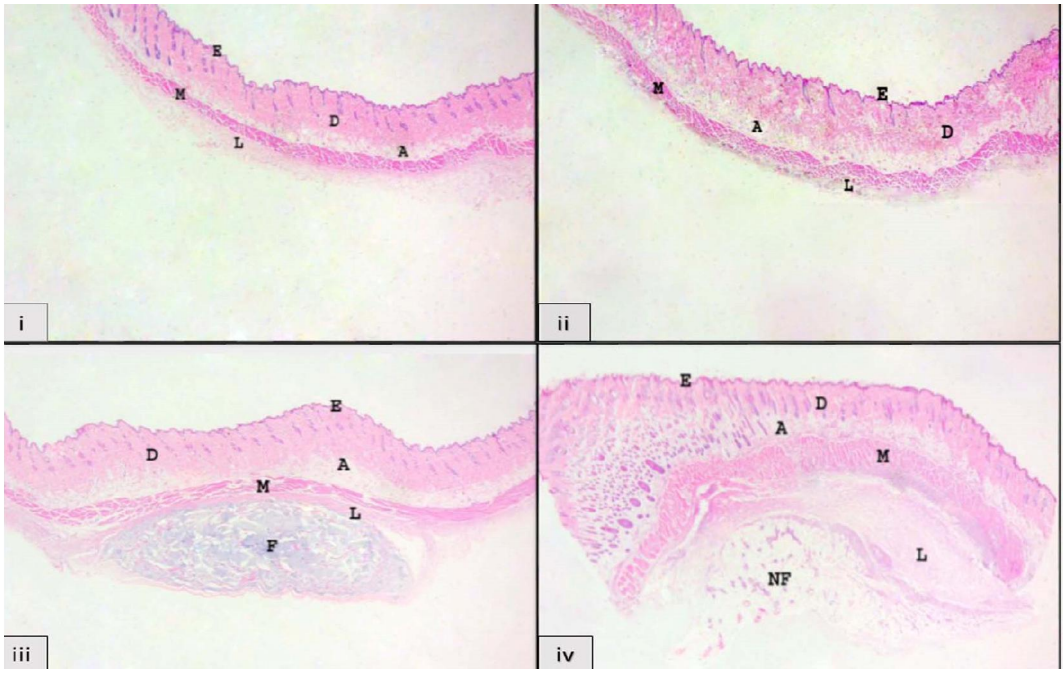

Fig. 1: A photomicrograph of rat thin skin sections of the four groups, showing the normal histological structure of the skin formed of the epidermis **(E),** dermis **(D)** and subcutaneous tissue that consists of an outer layer of adipose tissue **(A),** a middle layer of thin sheet of striated muscle **(M)** and an inner layer of loose connective tissue **(L)** (Figure i; control adult), (Figure ii; control aged). Notice the expansion of the subcutaneous tissue of group III injected by hyaluronic acid (dermal filler) **(F)** (Figure iii), as well as an apparent increase in the thickness of three layers of subcutaneous tissue of group IV, injected by nanofat **(NF)** specifically the adipose tissue layer that displays many hair follicles (Figure iv). **(Stereoscope).**

3.1. Results of light microscope

• Hematoxylin and eosin-stained sections

Group I (control-adult group)

Light microscopic examination of the thin skin sections obtained from the rats of group I was similar to the normal histological structure of the epidermis with prominence of epidermal-dermal junction. The epidermis consisted of stratified squamous keratinized epithelium containing keratinocytes arranged in four layers; stratum basale, spinosum, granulosum and corneum (Fig. 2).

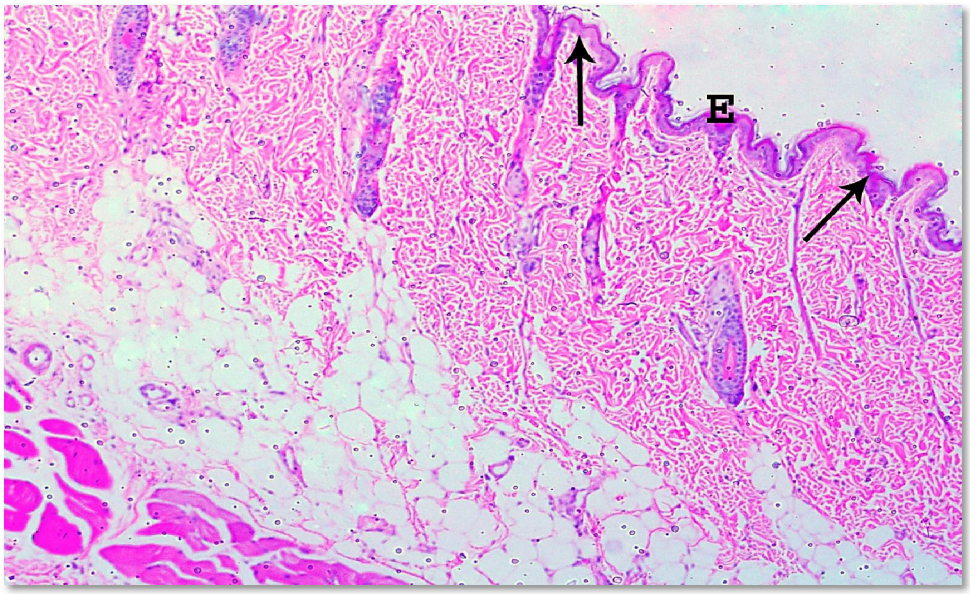

Fig. 2: A photomicrograph of a section in thin skin of group I, showing the epidermis (E). Notice the prominent epidermal dermal junction (thin arrows). (group I H&E, x100).

Group II (control-aged group)

Results of both subgroups IIa and IIb were the same, the light microscopic examination of the thin skin sections showed an apparent reduction of the epidermal thickness with focal areas of discontinuation of the keratin layer (Fig. 3). Epidermal-dermal junction appeared flattened with effacement of the rete ridges.

Group III (HA-injected group)

Examination of the thin skin of the aged rats that were injected with hyaluronic acid (dermal filler) in the subcutaneous tissue and sacrificed after 30 days, revealed that the epidermis was nearly similar to the picture seen in the control adult rats. The epidermis appeared to be formed of the four usual layers, stratum basale, stratum spinosum, stratum granulosum and stratum corneum with a continuously arranged keratin layer. A prominent epidermal-dermal junction was detected (Fig. 4).

Group IV (nanofat-injected group)

Examination of the aged skin after subcutaneous injection of nanofat revealed that the epidermis was nearly similar to the picture seen in the control adult rats with increase in the epidermal thickness. Epidermal-dermal junction became prominent with appearance of epidermal ridges in some areas (Fig.5).

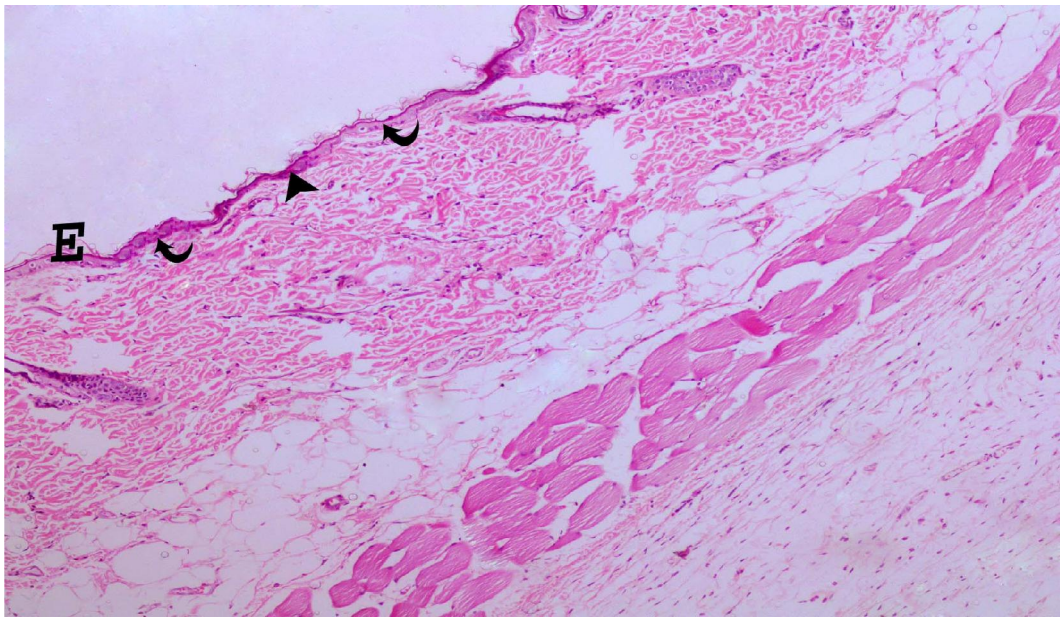

Fig.3: A photomicrograph of a section in thin skin of group II showing the epidermis (E) with an apparent reduction in its thickness. Notice flat epidermal-dermal junction (curved arrows) and effacement of rete ridges (arrow heads). (group II: H&E, x100).

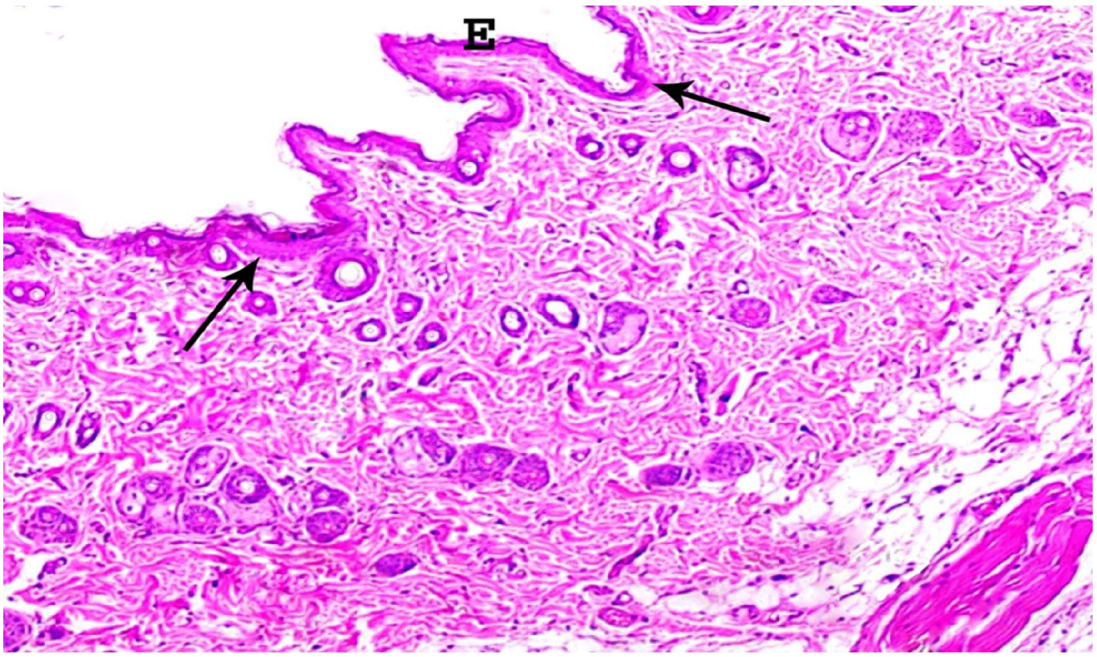

Fig.4: A photomicrograph of a section in thin skin of group III showing that the epidermis (E) is formed of the known layers with a continuous well-arranged keratin layer. Well demarcated undulated epidermal-dermal junction (thin arrows). (group III: H&E, x100).

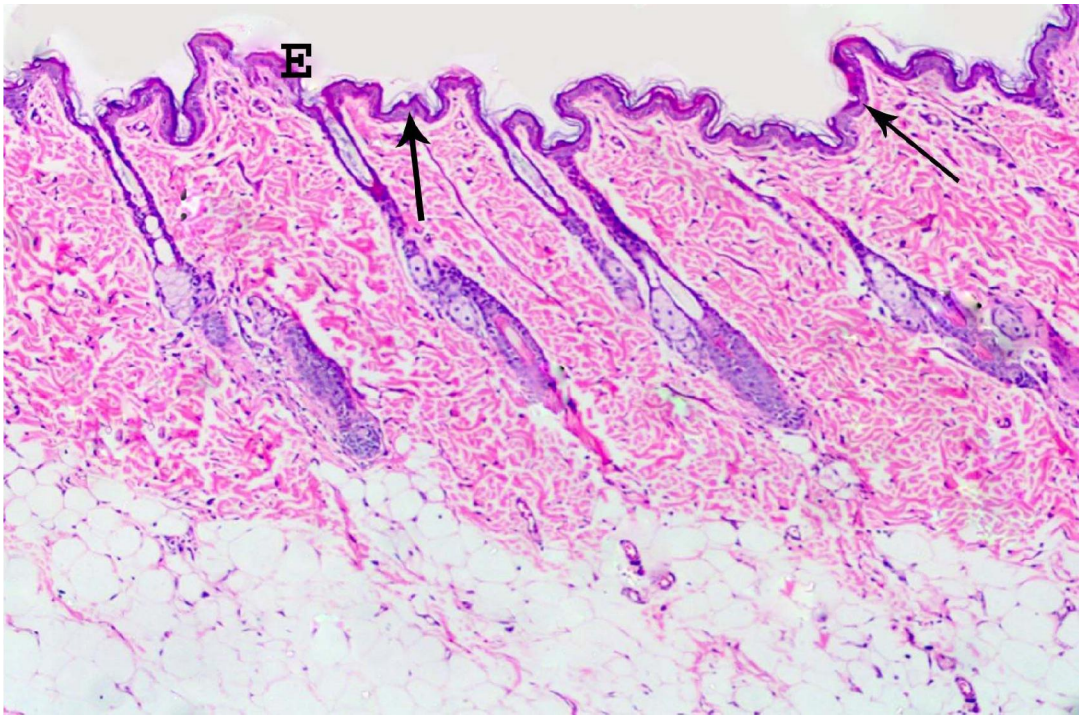

Fig. 5: A photomicrograph of a section in thin skin of group IV showing nearly normal epidermis (E) with prominent of rete ridges. (group IV: H & E, x100).

C- Morphometric Result.

- **The mean total thickness of the epidermis in H and E-stained sections:**

A significant reduction of the mean total epidermal thickness in the aged rats of group II (control aged group) compared to the control adult rats of group I ($p < 0.001$) was observed. As regards group III and group IV, there was a significant increase in the mean total epidermal thickness ($p<0.001$) compared to the aged rats of group II. A non-significant change was detected in the mean total epidermal thickness of rats of groups III (HA injected group) and group IV (nanofat injected group) as compared to group I ($P>0.001$). (Table 1 and Fig 6).

Table 1: Mean total thickness of the epidermis in the different studied groups.

Groups	**Mean total epidermal thickness (um)**						**ANOVA**	
	Range			**Mean**	**±**	**SD**	**F**	**P-value**
Group I	49.002	-	133.119	86.202	±	31.822	10.891	<0.001*
Group II	13.546	-	41.697	28.385	±	10.281		
Group III	41.338	-	101.91	66.228	±	22.419		
Group IV	20.92	-	97.552	60.011	±	22.146		

TUKEY'S Test					
I&II	**I&III**	**I&IV**	**II&III**	**II&IV**	**III&IV**
<0.001*	0.228	0.069	0.004*	0.020*	0.930

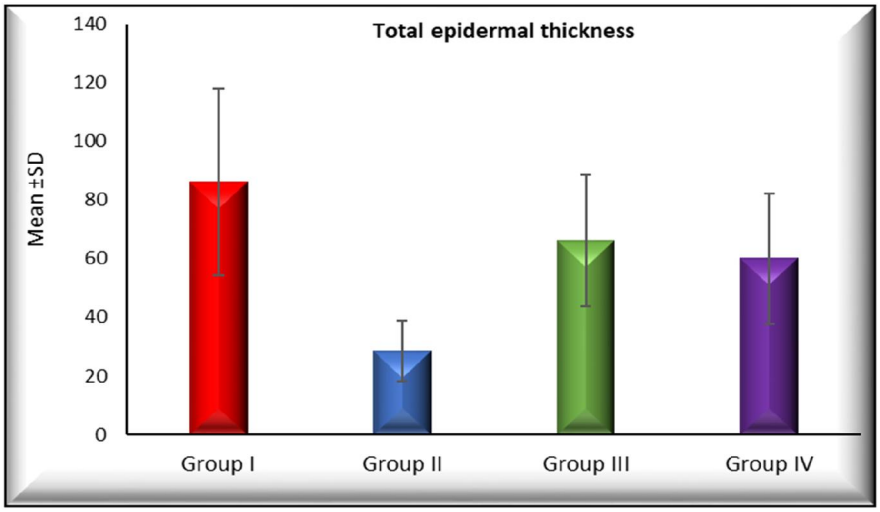

Fig. 6: Comparison between the studied groups regarding mean ± SD of total thickness of the epidermis.

4. Discussion

Dermal fillers are commonly used nowadays to create volume or reverse any soft tissue loss. HA and nanofat are commonly used filler materials. Subcutaneous injection of HA is widely used in medical cosmetology to locally improve the signs and symptoms of skin ageing. On the other hand, the biocompatibility, biodegradability, and high-water absorption of HA make it the common material for dermal fillers (Hernandez *et al.,* 2023).

In the current study, examination of the epidermis in the H&E-stained sections of the aged group II revealed a significant reduction in thickness compared to group I; this was confirmed by morphometric and statistical analysis. Zorina *et al.* (2022) reported that the epidermis thins because the basal keratinocytes lose their proliferative potential in the ageing and migrate to the upper spinous layer. This leads to a decrease in both the quantity and renewal of the basal keratinocytes, which impairs epidermal morphogenesis, renewal, and thinning. Moreover, flattening of the epidermal-dermal junctions is thought to have significant functional consequences. It compromises the engagement of the downwardly projected epidermal rete ridges with the upwardly protruding dermal papillae of the dermis. This flattening also reduces the surface area over which nutrients and signalling molecules may flow from the dermis into the avascular epidermis (Russell-Goldman and Murphy, 2020).

Light microscopic examination of the epidermis of the HA-treated group, displayed normal structure nearly similar to that of the control adult group with restoration of the epidermal-dermal junction. Furthermore, there was a significant increase in its thickness compared to group II and a non-significant change compared to group I. These results came in accordance with Chen *et al.* (2023), who noticed that the low molecular weight HA complex caused an increase in the epidermal thickness as well as an enhancement of tightness between the basal keratinocytes and the basement membrane, resulting in reconstruction of the full thickness of skin. It also enhanced the expression of epidermal-dermal junction proteins such as laminin-332 and fibrillin-1.

In regards to the epidermal examination of the nanofat-injected group IV by light microscopes, it appeared to be nearly similar to the control adult group I with the appearance of epidermal ridges and prominent epidermal-dermal junction. There was a significant increase in the epidermal thickness compared to group II and a non-significant change compared to group I and III. This was in line with Menkes *et al.* (2020), who studied the effects of nanofat on the epidermis following a subcutaneous injection. The underlying mechanism is not fully clear, but the pro-angiogenic growth factors contained in the nanofat and the increased capillary density in the dermis caused by nanofat may attribute to this change. Rageh *et al.* (2021) attributed the significant increase in the epidermal thickness after intradermal injection of nanofat to the presence of ADSCs (adipose derived stem cells) within nanofat that could stimulate hyperplasia of the epithelium. The increased epidermal thickness came in agreement also with Elsherbeny *et al.* (2023), who studied the effect of topical nanofat grafts

on raw skin wounds. They found that nanofat improved the healing time and helped re-epithelialization, forming a para-keratinized, immature stratified epithelium with the main characteristic layers. They attributed their results to the huge proliferative activity of ADSCs and their ability to differentiate into mesoderm, ectoderm, and endoderm lineages. They can differentiate into keratinocytes as well as secrete soluble mediators with angiogenic and anti-inflammatory properties. The latter leads to better quality and a faster rate of new epithelium formation, which results in increased epidermal thickness after nanofat grafting.

5. Conclusion

Both HA and nanofat has a significant effect in improving the signs of skin ageing by increasing the thickness of the epidermis and restoring the epidermal-dermal junction. However, injecting hyaluronic acid is easier and faster than nanofat which is minimally invasive.

References

Allen, J. and K. Dodou, 2024. Current Knowledge and Regulatory Framework on the Use of Hyaluronic Acid for Aesthetic Injectable Skin Rejuvenation Treatments. Cosmetics, 11, 54.

Amor, C., J. Feucht, J. Leibold, Y.J. Ho, C. Zhu, D. Alonso-Curbelo, J. Mansilla-Soto, J.A. Boyer, X. Li, T. Giavridis, A. Kulick, S. Houlihan, E. Peerschke, S.L. Friedman, V. Ponomarev, A. Piersigilli, M. Sadelain, and S.W. Lowe, 2020. Senolytic CAR T Cells Reverse Senescence-Associated Pathologies. Nature, 583(7814): 127–132.

Aziz, M., E. Gamal El Din, S. Ahmed, and M. Ahmed Mekway, 2019. Histological Study on the Effect of Hyaluronic Acid Injection (Dermal Filler) on the Skin of the Adult and Aged Albino Rat. Egyptian Journal of Histology, 42(2):245-261.

Bhooshan, L.S., M.G. Devi, R. Aniraj, P. Binod, and M. Lekshmi, 2018. Autologous Emulsified Fat Injection for Rejuvenation of Scars: A Prospective Observational Study. Indian Journal of Plastic Surgery: Official Publication of the Association of Plastic Surgeons of India, 51(1): 77–83.

Chen, F., X. Guo, and Y. Wu, 2023. Skin Anti-Ageing Effects of a Multiple Mechanisms Hyaluronan Complex. Skin Research and Technology: Official Journal of International Society for Bioengineering and the Skin (ISBS), International Society for Digital Imaging of Skin (ISDIS) and International Society for Skin Imaging (ISSI), 29(6):e13350.

Ding, P., E. Lu, G. Li, Y. Sun, W. Yang, and Z. Zhao, 2022. Research Progress on Preparation, Mechanism, and Clinical Application of Nanofat, Journal of Burn Care and Research, 43(5): 1140–1144.

Elsherbeny, K., A. Elshahat, and A. Gad, 2023. Effect of Autologous Nanofat Graft on the Healing of Donor Site of Split Thickness Graft. The Egyptian Journal of Plastic and Reconstructive Surgery, 47(2): 79-88, Issue 110-0044. Http://doi: 10.21608/ejprs.2023.180880.1236.

Guo, J., W. Fang, and F. Wang, 2023. Injectable fillers: current status, physicochemical properties, function mechanism, and perspectives. RSC advances, 13(34): 23841–23858.

Haddad, S., H. Galadari, A. Patil, M. Goldust, S. Al Salam, and S. Guida, 2022. Evaluation of the Biostimulatory Effects and the Level of Neocollagenesis of Dermal Fillers: A Review. International Journal of Dermatology, 61(10): 1284–1288.He, T., Fisher, G. J., Kim, A. J. and Quan, T. (2023). Age-Related Changes in Dermal Collagen Physical Properties in Human Skin. PloS one, 18(12): e0292791.

Hernandez, P.M., P. Cotrin, and F.P. Valarelli, 2023. Evaluation of the Attractiveness of Lips with Different Volumes after Filling with Hyaluronic Acid. Sci. Rep., 13, 4589.

Liang, Y., W. Su, and F. Wang, 2023. Skin Ageing: A Progressive, Multi-Factorial Condition Demanding an Integrated, Multilayer-Targeted Remedy. Clinical, cosmetic and investigational dermatology, 16: 1215–1229.

Menkes, S., M. Luca, G. Soldati, and L. Polla, 2020. Subcutaneous Injections of Nanofat Adipose-derived Stem Cell Grafting in Facial Rejuvenation. Plastic and reconstructive surgery. Global open, 8(1): e2550.

Rageh, M., M. El-Khalawany, and S. Ibrahim, 2021. Role of Nanofat Injection in Treating Post-Traumatic Scars. Al-Azhar International Medical Journal. 10.21608/aimj.2021.81579.1507.

Russell-Goldman, E. and G.F. Murphy, 2020. The Pathobiology of Skin Ageing: New Insights into an Old Dilemma. The American Journal of Pathology, 190(7): 1356–1369.

Tonnard, P., A. Verpaele, G. Peeters, M. Hamdi, M. Cornelissen, and H. Declercq, 2013. Nanofat Grafting: Basic Research and Clinical Applications. Plastic and Reconstructive Surgery, 132(4): 1017–1026.

Weinzierl, A., Y. Harder, D. Schmauss, M. Menger, M. Laschke, 2022. Boosting Tissue Vascularization: Nanofat as a Potential Source of Functional Microvessel Segments. Frontiers in Bioengineering and Biotechnology. 10. 820835.

Yasin, A., Y. Ren, J. Li, Y. Sheng, C. Cao, and K. Zhang, 2022. Advances in Hyaluronic Acid for Biomedical Applications. Frontiers in Bioengineering and Biotechnology. 10: 910290.

Zorina, A., V. Zorin, D. Kudlay, and P. Kopnin, 2022. Molecular Mechanisms of Changes in Homeostasis of the Dermal Extracellular Matrix: Both Involutional and Mediated by Ultraviolet Radiation. International Journal of Molecular Sciences, 23(12):6655.