Short Communication

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Determining the Collagen Content and Concentration of Nanofat and Microfat

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ABSTRACT

This study investigates the collagen content and concentration in Microfat and Nanofat using SDS-PAGE and Sirius Red Dye (SRD) assay analysis. Microfat consists of small clumps of fat derived from lipoaspirate, while Nanofat is a broken-down lipoaspirate with no adipose cells but rich in stromal vascular fractions. The hypothesis posits that Nanofat, devoid of adipocytes, would contain higher collagen concentrations. However, Microfat had an average absorbance level of 0.09921, while Nanofat was 0.56112. These results indicated that Microfat contains more collagen than Nanofat, disproving the hypothesis. These findings suggest that Nanofat's anti-aging properties may be due to growth factors rather than collagen concentration. This study will help to spur more knowledge of the beneficial effects of NF for burn survivors and severely scarred patients.

Introduction

Collagen, a primary structural protein in skin, muscles, bones, and connective tissues, is essential for providing strength and support. Comprising one-third of the human body's total protein and three-quarters of the skin's dry weight, collagen is rigid and resistant to stretching due to its unique triple helix structure and amino acid composition (Wu et al., 2022). This protein is widely used in medical applications, including tissue repair and augmentation (Ramshaw, et al. [1]). Microfat and Nanofat, derived from autologous adipose tissue, are popular in cosmetic and reconstructive procedures such as treating scars or eliminating aspects of facial volume loss that occur with age. Microfat is classic lipoaspirate harvested with a multiport small-hole cannula which retains adipocytes within an extracellular matrix, while Nanofat, devoid of adipocytes, contains stromal vascular fractions rich in stem cells and growth factors (Alkhouli, et al. [2]). These cells allow nanofat to serve as a regenerative modality and stimulate collagen production (Alessi, et al. [3]). Previous studies suggest these fats promote collagen and extracellular matrix regeneration, but detailed collagen content and concentration analyses are lacking.

Moreover, nanofat, essentially microfat without adipocytes, contains exosomes and growth factors, including collagen-stimulating factors. SRD-polyacrylamide gel electrophoresis (SDS-PAGE) and Sirius Red Dye (SRD) assays are standard techniques for analyzing protein concentration and molecular weight. SDS-PAGE separates proteins based on their molecular weight, while SRD specifically binds to collagen, allowing quantification through UV absorbance measurements. SDS, also known as lauryl sulfate, is an anionic detergent and negative charges on SDS destroy most of the complex structure of proteins, and are strongly attracted toward an anode in an electric field (Caprette [4]). SDS PAGE yields this information based on their differential rates of migration through a sieving matrix under the influence of an applied electrical field (How SDS-PAGE Works [5]). This

study aims to compare the collagen content in Microfat and Nanofat, using OviGenex Ovine collagen as a control—a native collagen that has not been degraded. OviGenex collagen is a soluble ovine (sheep) collagen containing 93-97% Type I and 3-7% Type III collagen. The hypothesis is that Nanofat will show a higher collagen concentration due to the absence of adipocytes.

Methods

For the SDS-PAGE assay, microfat, nanofat, and CollOvine collagen samples were prepared in microfuge tubes with loading dye. The samples were heated to denature proteins and loaded onto NuPAGE 4-12% bis-tris gels. After filling the electrophoresis apparatus with MES running buffer, gels were placed in the apparatus and samples were pipetted into wells. The gels ran at 155V and 225mA under constant voltage. After electrophoresis, gels were stained with Coomassie Brilliant Blue G-250 and de-stained to visualize protein bands. The gels were scanned for analysis. For the Sirius Red Dye assay, collagen standards and microfat and nanofat test samples were prepared and reacted with 0.5 mmol/L Sirius Red Dye solution. The samples were centrifuged to separate the collagen-dye precipitate. The supernatant was carefully removed, and the precipitate was dissolved in acetic acid. The solutions were transferred to a 96-well plate, and absorbance at 540 nm was measured using the Varioskan LUX plate reader. The data was exported to Excel for analysis.

Results

For the SDS-PAGE assay, Microfat samples showed more distinct and concentrated collagen bands compared to Nanofat or the CollOvine collagen sample, indicating higher collagen concentration (Figures 1 & 2). In the SRD assay, the UV absorbance measurements 540 nm showed that Microfat had a lower absorbance (0.09921), indicating higher collagen content compared to Nanofat (0.56112). The SRD assay results demonstrated Nanofat absorbing 566% more UV absorbance than Microfat and Microfat containing 493% more collagen (mg/mL) than Nanofat. The R-squared value of the Sirius Red Dye assay comparing Microfat, Nanofat, and CollOvine standard came out to be 0.9952, demonstrating that this assay's results are extremely accurate and reliable (Figure 3, Tables 1-3). Therefore, through experimentation I found that Microfat has a higher content and concentration of collagen than Nanofat.

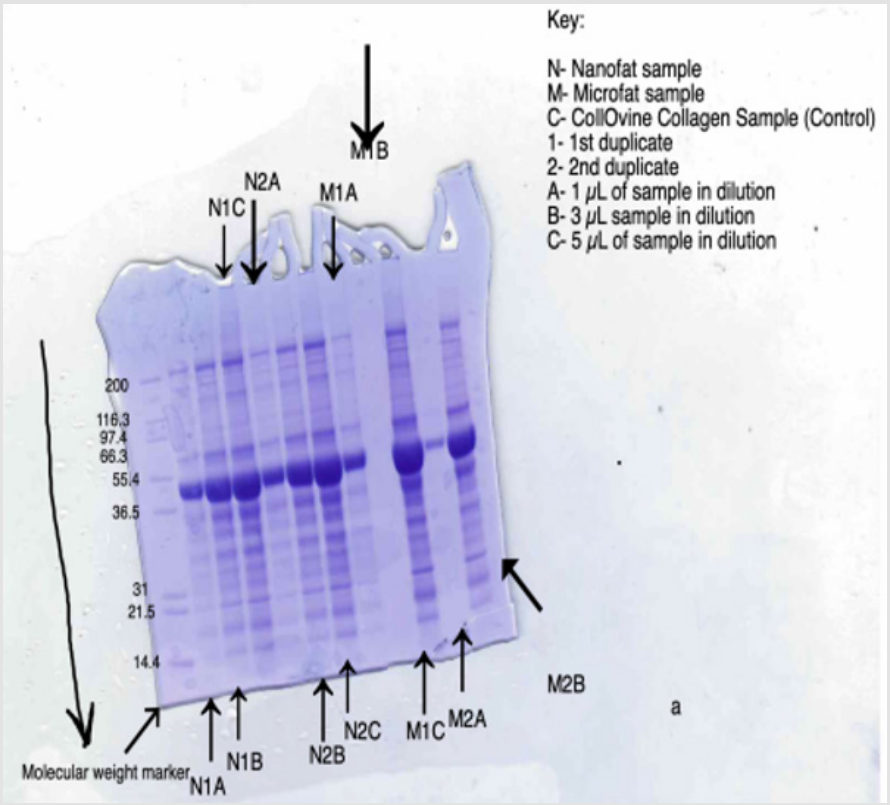

Figure 1: SDS-PAGE Gel Results. Wells 1-12.

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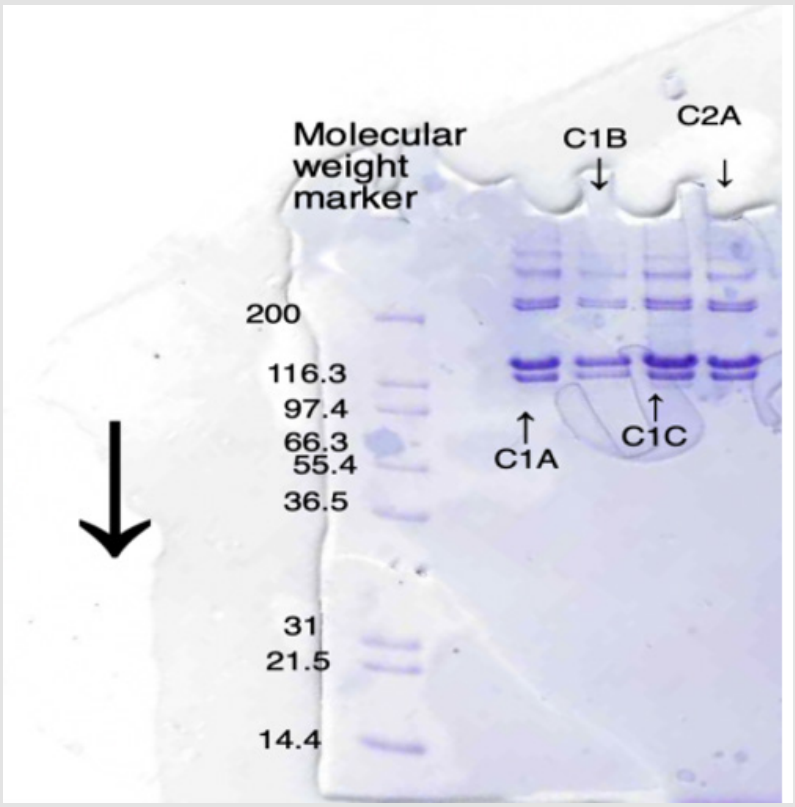

Figure 2: SDS-PAGE Gel Results. Collagen Samples.

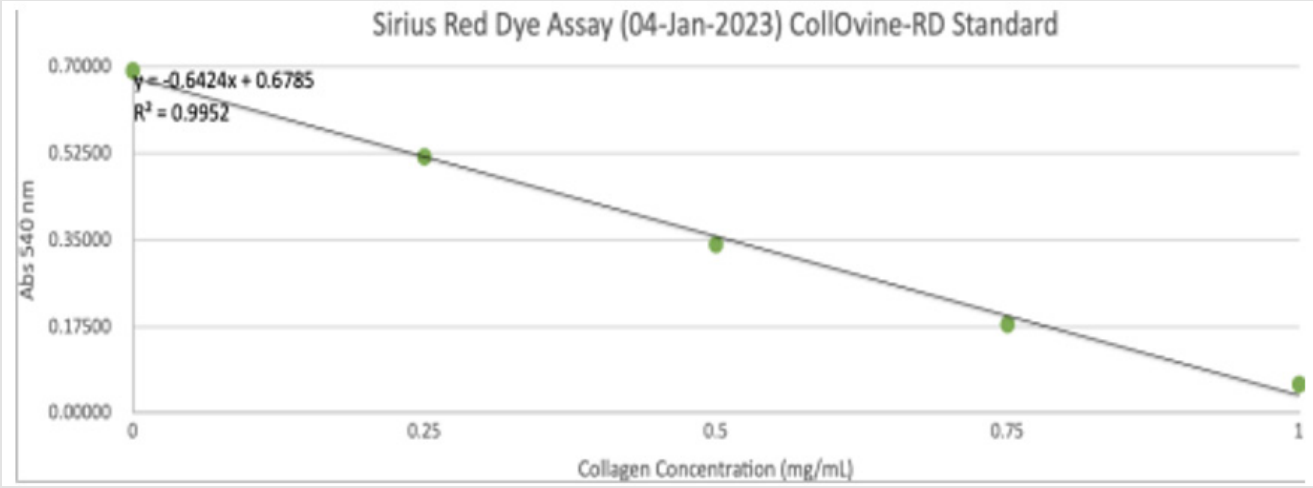

Figure 3: Sirius Red Dye Assay Standard Curve Graph.

Table 1: Standard Curve of Collagen Samples in Sirius Red Dye Assay.

	CollOvine-R Collagen Concentration (mg/mL) D CollOvine-RD D CollOvine-RD	Avg. CollOvine-RD
	1 0.06167 0.05276 0.05926	0.0579
	0.75 0.17472 0.17664 0.18556	0.17897
Triplicates used to average----	0.5 0.34196 0.34062 0.33862	0.3404
Used for Standard Curve	0.25 0.52690 0.52402 0.50188	0.5176
	0 0.69596 0.68701 0.69171	0.69156

Table 2: Sirius Red Dye Assay Results on Microfat.

	y Abs 540nm	y Abs 540nm	y Abs 540nm	Average Abs Dilution Factor 540nm (df)	x=(y-b)/m Collagen Dilution (mg/mL)	Collagen (mg/mL)
Microfat	0.13951	0.09149	0.06662	0.09921 10	0.9	9.02

Table 3: Sirius Red Dye Assay Results on Nanofat.

y	y	x=(y-b)/m	Collagen (mg/mL)
Abs 540nm	Abs 540nm	Average Abs Dilution Factor Collagen Dilution Abs 540nm (df)(mg/mL)	

Note: Nanofat 0.56006 0.56520 0.55809 0.56112 10 0.18 1.83.

Discussion

The hypothesis that Nanofat contains higher collagen concentration was disproven, as Microfat demonstrated higher collagen levels in both SDS-PAGE and SRD assays. The results of this study indicate that the fibrous proteins on the extracellular membrane of the adipocytes in Microfat contain more collagen than the stromal vascular fraction in Nanofat. Unlike Microfat, Nanofat lacks filling qualities as it contains only the stromal vascular fraction and adipose-derived stem cells, devoid of adipocytes. Increased synthesis of collagen in Nanofat is triggered by stem cells of the adipocytes becoming destroyed during the breakdown of the lipoaspirate (Fakih-Gomez, et al. 2020). The SDS-PAGE results demonstrated a similar distribution of proteins between Microfat and Nanofat. However, the absence of adipocytes in Nanofat significantly lowered its collagen content and concentration. These findings are crucial for understanding the mechanisms behind Nanofat's regenerative properties and could inform future applications in treating scars and skin damage [6-11].

Conclusion

This study concludes that Microfat contains higher collagen concentrations than Nanofat. The observed rejuvenating effects of Nanofat are likely due to growth factors rather than collagen content. These insights could enhance the use of Nanofat in medical treatments for burn survivors and patients with severe scarring, providing a deeper understanding of its regenerative mechanisms. Advancements in this field could lead to the development of a hybrid technology combining Microfat and Nanofat, offering both volumizing and skin-smoothing benefits. Future research should investigate the impact of increased collagen content and concentration on both Microfat and Nanofat. As new methods of Nanofat preparation are being explored, understanding the exact contents and processes involved is essential for analyzing these new techniques. This study aims to contribute to research on the beneficial effects of Microfat and Nanofat for burn survivors and patients with severe scarring.

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