공학석사학위논문

Mechanical Enhancement of 3D Collagen Matrix by α-Synuclein On-Site Fibrillation

알파-시뉴클레인을 이용한 3D 콜라겐 매트릭스의 물리적 성질 강화

2014년 08월

서울대학교 대학원

화학생물공학부 전공

백운규

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이 논문을 공학석사 학위논문으로 제출함

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Abstract

Mechanical Enhancement of 3D Collagen Matrix by α-Synuclein On-Site Fibrillation

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Injectable dermal fillers are becoming increasingly popular for improving skin contour defects related to aging (wrinkles and lines), depressed acne scars, and other traumatic or congenital conditions. In this paper, we have evaluated self-assembling amyloidogenic protein, α-synuclein, as an alternative material for

dermal filler. α-synuclein successfully penetrated into 3D collagen matrix and formed nano-scaled α-fibril evenly throughout the whole matrix by matrix-localized fibrillation. We speculate that matrix-localized fibrillation of α-synuclein was due to the accelerated fibrillation within the matrix which caused continual inward flow of α-synuclein monomer and oligomer. In addition, formed α-fibrils were found to present structural support to collagen matrix by forming intertwined fiber-network: roping around and connecting neighboring fibers. Futhermore, self-assembled α-fibrils enhanced mechanical properties of collagen matrix, including elasticity and strength, and provided protection against protease degradation.

Keywords : α-synuclein, Amyloid, Dermal Filler, Self-Assembly, Collagen Matrix.

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1. Introduction

1.1 α-synucle and Parkinson's Disease

α-synuclein is a protein, with 140 amino acid residues, that is naturally expressed in various parts of human tissues including brain, heart, muscle, and etc,.. [1, 2]. α-synuclein and its self-assembling property have attracted much of interest from various scientific societies as a major constituent of the Lewy body formation and have implicated in the pathogenesis of Parkinson's disease(PD) and other neurodegenerative disorders [3, 4].

1.2 Self-Assembly of α-Synuclein

Upon incubation at elevated temperatures with agitation, self-assembly of α-synuclein results in the protein-based suprastructure formation of divers morphology including nano-scaled fiber with high β-structure content [5, 6]. These suprastructures and unique self-assembling property of amyloidogenic proteins, such as α-synuclein, are also suggested to be engineered in vitro to provide biologically compatible materials [7].

1.3 Dermal Fillers

Facial wrinkles can originate from the biological aging of the skin and are associated with various environmental factors such as photoaging, hyperdynamic facial expression, and etc [8]. Many different polymer-based and protein-based materials, including hyaluronic acid, bovine collagen, are currently being used as cosmetic surgical implants for improving aging-related skin defects [9, 10]. These dermal fillers offer patients who seek treatments

with minimally invasive therapy to alleviate facial wrinkles to reduce the signs of aging [11].

1.4 α-Synuclein as Dermal Filler

However, the treatment methods of currently commercialized dermal fillers are injected as a bulky prostheses, after produced and materialized in-vitro, to merely stuff the diminished skin collagen matrix rather than creating structural support and enhancing the mechanical properties of existing fiber matrix. In this report, we present data to propose α-synuclein, and its amyloidogenic self-assembly process, as an alternative material and method for improving skin contour defects by forming nano-scaled fibrillar network that grants extra structural support to weakening skin collagen matrix.

2. Materials and methods

2.1 Preparation of α-synuclein

Recombinant α-synuclein was prepared according to the procedures previously described [12]. Briefly, a-synuclein cloned in pRK172 was overexpressed in E. coli BL21(DE3) and completely purified via heat treatment of the cell lysate, DEAESephacel anion-exchange, Sephacryl S-200 size-exclusion, and SSepharose cation-exchange chromatography steps. Purified asynuclein was stored in aliquots at 280uC following dialysis against 6 liters of 20 mM Mes at pH 6.5.

2.2 Transmission electron microscope (TEM)

An aliquot containing amyloid fibrils of a-synuclein was adsorbed onto a carbon-coated 200-mesh copper grid (Ted Pella Inc., USA). Following negative staining of air-dried sample with 2% uranyl acetate (Electron Microscopy Sciences, USA) for 20 s, the amyloid fibrils were visualized with TEM (JEM 1010, Jeol, Japan).

2.3 Field-emission scanning electron microscope (FE-SEM)

For the SEM analysis, collagen gel samples were prepared by previously freezing at -80 °C, then according to previous freeze-drying method [13]. Freeze-dried collagen gels were sputter-coated with gold/palladium (approximately 5 nm of

thickness) using Emscope SC 400 (Electron Microscopy Sciences, USA). The interior and surface structures of collagen gels were examined with FE-SEM (SUPRA 55VP, Carl Zeiss, Germany) at 2.0 kV.

2.4Thioflavin-T binding fluorescence

Aliquots (20 ul) of samples were combined with 2.0 mM thioflavin-T in 50 mM glycine, pH 8.5, to a final volume of 200 ul. Amyloid formation was evaluated with thioflavin-T binding fluorescence at 482 nm with an excitation at 450 nm. The fluorescence intensity was measured with a luminescence spectrometer (LS-55, Perkin-Elmer).

2.5 Confocal Laser Scanning Microscope (CLSM II)

Collagen gel samples were subjected with excess amount of 2.0 mM thioflavin-T dye prior to analysis with . Presence of Amyloid fibrils was evaluated with thioflavin-T binding fluorescence at 483 nm with an excitation at 450 nm. The confocal images were taken with confocal laser scanning microscope (Carl Zeiss LSM710).

2.6 Advanced Rheometric Expansion System (ARES)

Collagen gels were prepare in circular coin-shape using cylindrical mold, 28 mm in diameter. mechanical strength and elasticity of prepared gels were measured by advanced rheometric expansion system (Rheometric scientific, UK) using two different

modes: steady sweep and strain sweep. The samples were places in between two disks of 25 mm in diameter with gap of 1.6 mm. The analysis were performed at room temperature.

2.7 Tensile Test

Collagen gels were prepare as long quadrilateral shape in PDMS mold of 17 mm by 85 mm with excess height. 2 ml of collagen solution was evenly spread in the mold to form solidified gel. Formed gels were subjected to freeze-drying according to previous method [13]. Resulting quadrilateral fiber strips were precisely cut into equal dimension of 14 mm by 85 mm for analysis of mechanical tensile strength with Universal Testing Machine (UTM), 20 N load cell, testing speed of 5 mm/min, at 23±2 ℃.

3. Results and Discussion

3.1 Preparation and Structural Analysis of Fibrillar Composite.

3.1.1 Formation of α-fibrils in 3D Collagen Matrix.

In order to evaluate the α-synuclein as an alternative material for dermal filler and its unique amyloidogenic self-assembling property as a new method for replenishing diminished collagen ECM in skin, we first analyzed if the α-synuclein can penetrate into the 3D collagen matrix and successfully form a collagen matrix α-fibril composite through amyloidogenic self-assembly. 0.5 ml of 1 mg/ml collagen matrix were prepared using, collagen type I, according to the generalized protocol for making of 3D collagen matrix [14]. After subjecting formed matrix to thorough washing process, control group was incubated with 1 ml of 20 mM Mes at pH 6.5, while experimental group was incubated with 1 ml of 1mg/ml α-synuclein oligomer for 36 hrs. The prepared gels were treated with the amyloid-specific dye, thioflavin-T, for confocal laser scanning microscopy analysis, in order to detect the presence of α-fibrils within 3D matrix Figure 1A and 1B shows the merged image of optical and fluorescence view of the samples. Optical and fluorescence images were taken to view the collagen matrix, and α-fibers, respectively. Opaque areas represent collagen gel while green fluorescence represent α-fibers. As shown in Figure 1, the fluorescence of α-fibers were detected in experimental group (Figure 1B) indicating that α-fibrils were succesfully formed within the collagen matrix.

To further analyze the formation of α-fibrils in 3D collagen gel, the cross-section images along the depth of the 3D gel were taken (Figure 2A) to create a 3D image (Figure 2B). α-fibrils were formed through out the all depth of the 3D gel, and the high density of the formed α-fibrils throughout the whole matrix can be indirectly concluded from the high fluorescence intensity in the 3D image. From the results, we can conclude that α-synuclein successfully penetrated into all depth of the 3D gel and underwent fibrillation through self-assembly.

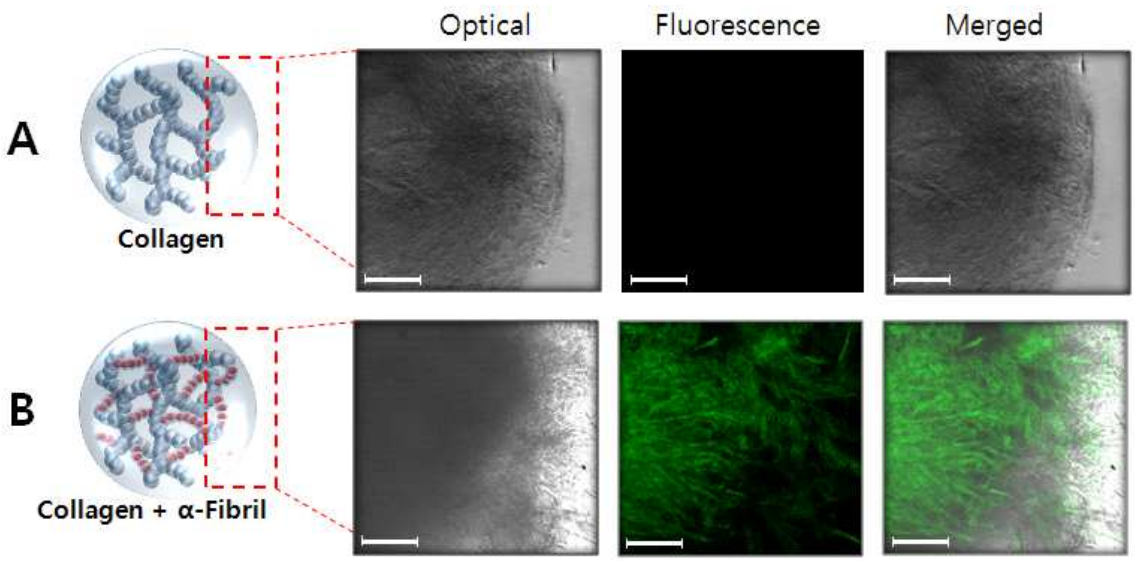

Figure 1. Confocal Laser Scanning Microscopy Images of Collagen and Composite Gels. Scale bar represents 100 μm. Optical, fluorescence, and merged images taken from the edges of collagen gel and α-fibril embedded collagen gel are shown in penal A and B, respectively.

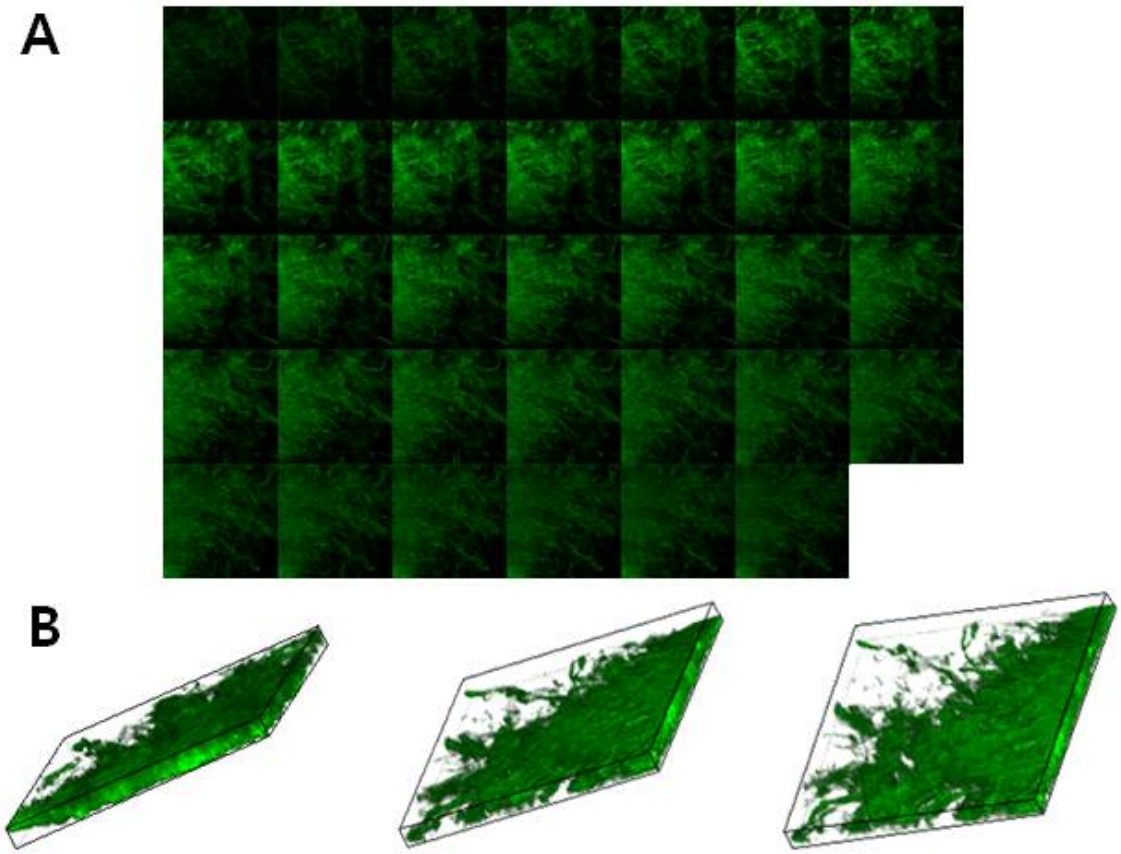

Figure 2. Evenly Dispersed Formation of α-Fibril in Collagen Matrix. a, Cutaway images of 3D collagen gel cross sections along the overall depth. **b**, Constructed 3D images of α-Fibril positioning within collagen matrix.

3.1.2 Nano-scaled Analysis of Fibrillar Composite Structure.

With confirmed penetration and fibrillation of α-synuclein within 3D matrix, the structural analysis of the composite was proceeded by field-emission scanning electron microscopy. Redundantly, as shown in SEM images in Figure 3B, C, D, α-fibrils were succesfully formed within the collagen matrix. According to the SEM images obtained, collagen fibers were in the range of 100~200 nm in thickness (Figure 3A). α-fibrils were in the range of 20-30 nm in thickness (Figure 3B, C, D). Considering the size of the gold particles (5 nm) spreaded on to the sample for SEM analysis, the size of the observed α-synuclein filaments and collagen fiber correspond with reported data [15-17].

Furthermore, α-fibrils were actively intertwined with collagen fibers: roping around and connecting neighboring collagen fibers (Figure 3C). As a result, α-fibrils were actively filling up the empty pores within the collagen matrix, increasing the overall density of the matrix (Figure 3D). By formation of intertwined fiber-network within 3D matrix, α-fibrils were found to present structural support to the pre-exisiting collagen fibers.

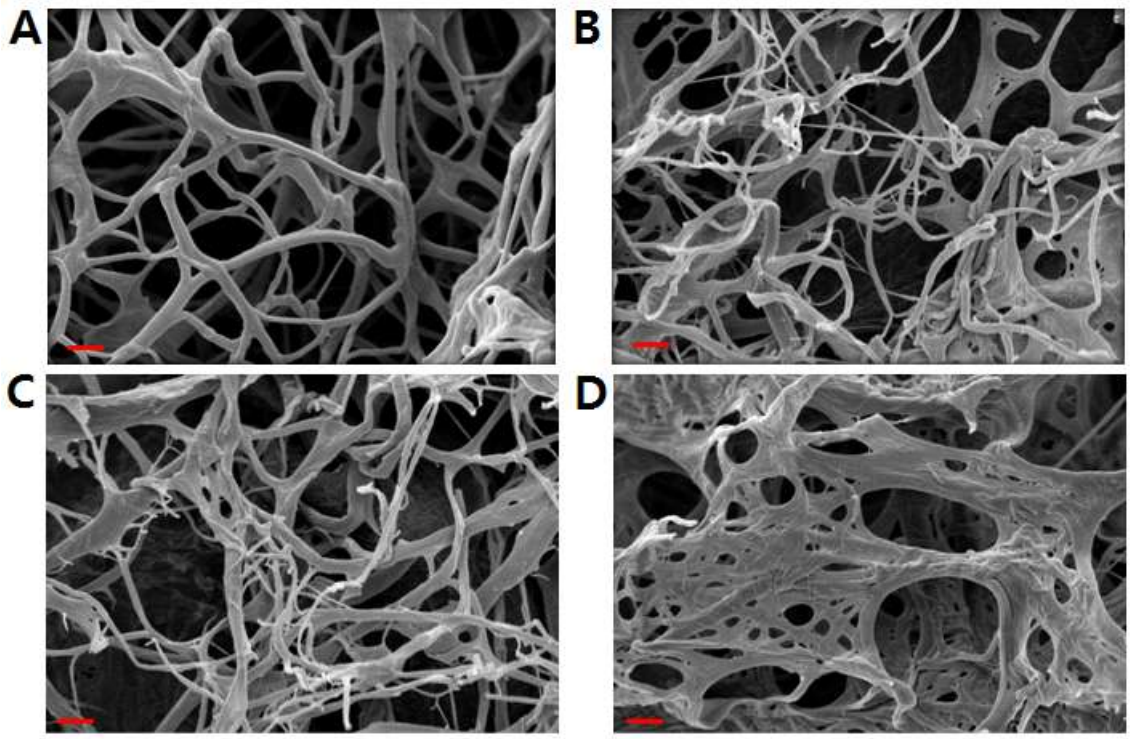

Figure 3. SEM Images. Nano-scaled structural analysis of collagen gel matrix **(a)**, and α-synuclein-treated collagen matrix **(b, c, d)**. All scale bar representing 400 nm. α-fibrils formed intertwined fibrillar network around collagen fibers, creating structural support to the 3D matrix **(b, c, d)**.

3.1.3 Effect of α-synuclein concentration on formation of α-fibrils within matrix.

To make an assurance that the nano-sized filaments in SEM images and the thioflavin-T fluorescence in confocal laser scanning microscope images are indeed the result of the formation of α-fibrils, the effect of varying concentrations of α-synuclein in the formation of composite gel was analyzed. In this experiment, equal amount of α-synuclein solutions with varying concentrations were used to induce self-assembly within the collagen matrix. Three different concentrations (0.35, 0.65, 1 mg/ml) of α-synuclein oligomers were used as experimental groups while Mes buffer was used as a control group. The resulting gels were treated with equal amount of thioflavin-T dye for an emission scan test by fluorescence spectrometry. Since α-fibrils are know to have an excitation wavelength of 450 nm and an emission wavelength of 482 nm, excitation was fixed at 450 nm and the emissions were scanned along the range of 400 nm to 550 nm [18, 19].

As shown in Figure 4, the peaks of all the experimental groups were reached around at the wavelength of 482 nm, the same emission wavelength of α-fibrils at 450 nm excitation. Moreover, the intensities of the fluorescence increased according to the increased concentration of α-synuclein oligomer used in the preparation of the gel. These results show that the thioflavin-T fluorescence detection in collagen gel is dependant on α-synuclein and therefore, the results of SEM and CLSM II were indeed due to the presence of α-fibrils formed within collagen matrix.

Furthermore, this ability to control the amount of α-fibrils formation by adjusting the concentration of α-synuclein suggests

that the amount of structural filler formed in skin ECM can be controlled according to the patients' skin condition in future applications.

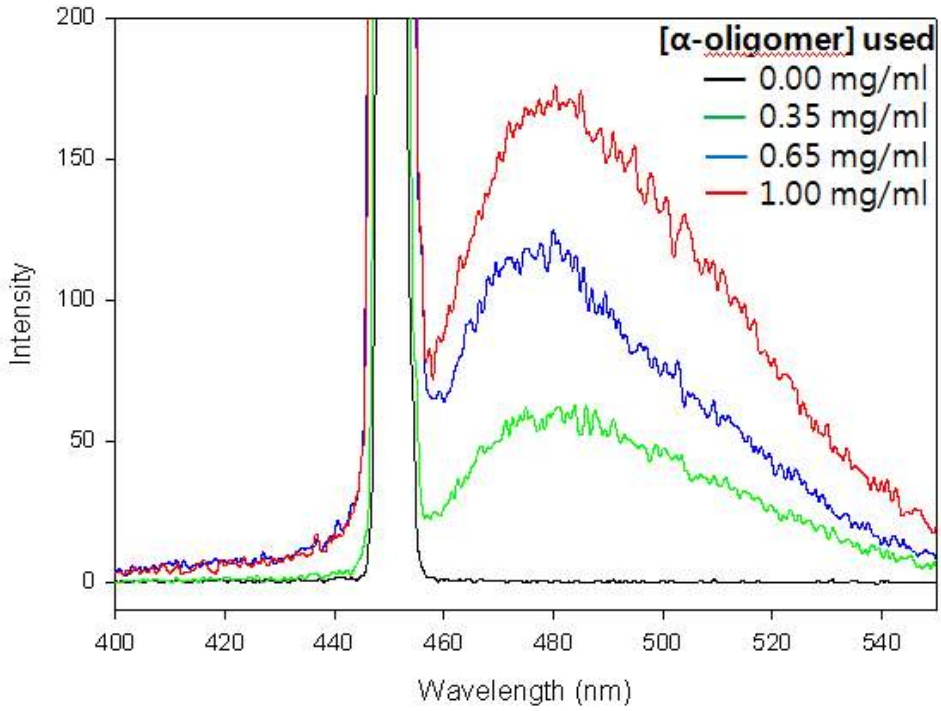

Figure 4. Emission Fluorescence Spectra Scan by Fluorescence Spectrometer. Four different concentrations (0, 0.35, 0.65, 1 mg/ml) of α-synuclein oligomers were treated to collagen matrixes. The amount of α-fibril formed in resulting matrixes were analyzed by thioflavin-T binding fluorescence spectra at between 400 nm and 550 nm with an excitation at 450 nm.

3.2 Characterization

3.2.1 3D Collagen Matrix Protection Against Protease-Degradation by α-synuclein.

Currently commercialized dermal fillers are being injected as bulky prostheses after synthesized and materialized in-vitro. These bulky prostheses are commonly subjected to biological degradation by various proteases produced by body's natural defence mechanism [20, 21, 22]. Also, many reports suggest age-related increase in skin proteases expression as one of the major causes of the regression of skin ECM matrix [23, 24]. Unlike many other protein materials α-synuclein is known to have high resistance against various protease-degradation in general [25, 26]. To investigate if the protease-resistance of α-fibrils may protect the composite gel from the protease-degradation, we proceeded following experiment.

Cylindrical molds of equal size were used to create collagen matrixes of equal size and shape and the experimental group was incubated with α-synuclein oligomer to form fibrillar composite. Then, equal amounts of protease K (200 ug) were treated to both samples and were incubated at 37 ℃ for 40 hrs. After 40 hrs of incubation with protease K, the resulting samples are shown as in Figure 5. As shown in pictures the structure of collagen control was collapsed while collagen matrix with protease-resistance α-synuclein are found to retain its overall structure and shape. Also, due to extensive degradation, matrix volume was greatly diminished in control group while no significant volume decrease was detected in experimental group.

This data suggest the ability of α-synuclein fibril to protect

the 3D matrix from protease degradation and gives a unique advantage to α-synuclein in future application as dermal filler.

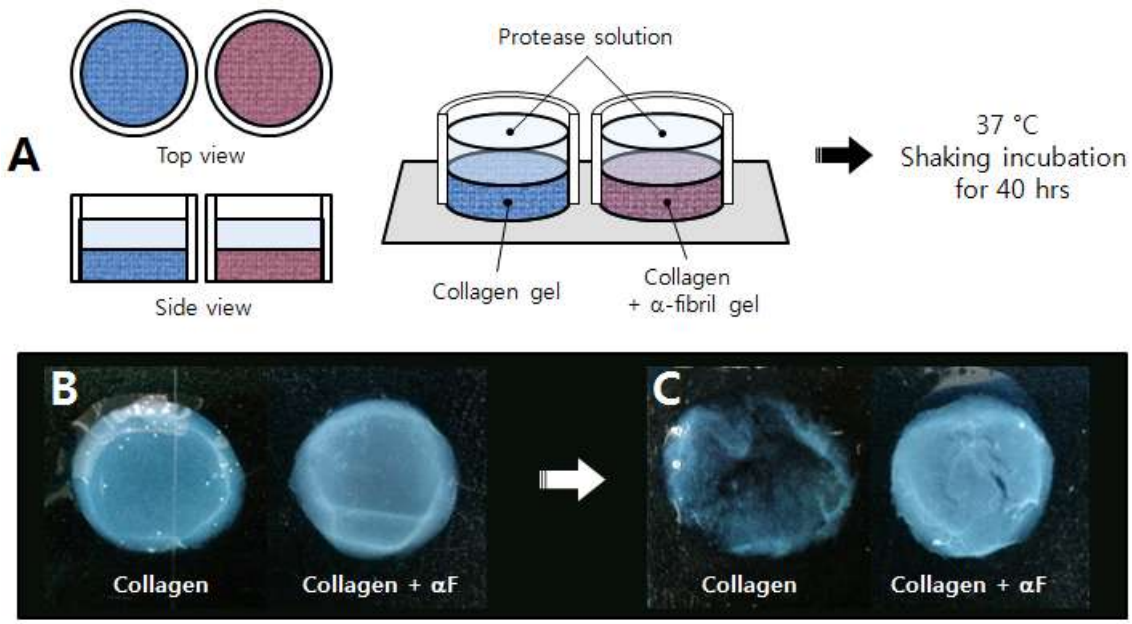

Figure 5. Matrix Protection Against Protease-Degradation by *α*-synuclein. a, Schematic of experimental design. **b**, Images of coin-shaped collagen gel matrixes before the protease K treatment. **c,** Images of resulting collagen gel matrixes after the 40 hrs. of protease K treatment.

3.2.2 Matrix-Localized-Fibrillation of α-synuclein.

During the experiments inducing α-synuclein fibrillation in 3D collagen matrix, interesting phenomena of matrix-localized-fibrillation of α-synuclein was observed. It was speculated that α-synuclein underwent fibrillation process exclusively in the 3D matrix of collagen. To confirm such property of α-synuclein, the following experiment was conducted.

Experimental and control group were prepare according to general method described previously in this paper. During the incubation (experimental group with α-synuclein and control group with Mes buffer), 20 μl of samples were taken from the solutions of both groups at various different incubation time to detect the presence of α-fibril by thioflavin-T binding fluorescence analysis. As result suggests, the formation of α-fibrils was not detected in the solutions of both experimental and control group through the toatal incubation time of 168 hrs (Figure 6A). Although there was no formation of α-fibrils in solution, α-monomer concentration in the solution of experimental group decreased along the incubation time (data not shown). The decrease of monomer concentration of α-synuclein conveys the formation of α-fibrils somewhere in the sample. Since no fibril formation occurred in the solution, we speculated the fibril formation in 3D collagen matrix.

After 168 hrs. of incubation, the resulting gels were isolated and broken down for the analysis of α-fibril presence. The resulting data, Figure 6B, shows the detection of α-fibrils in the 3D collagen matrix of experimental group and a mere noise level detected in control group due to the broken down residues of collagen matrix which can interfere with light wave of fluorescence spectrometer. Gathering the informations taken from Figure 6A and

6B, α-synuclein underwent fibrillation process exclusively in the 3D collagen matrix, which resulted complete localization of α-fibrils within the matrix. This result shows the possibility of collagen ECM targeting α-synuclein dermal filler.

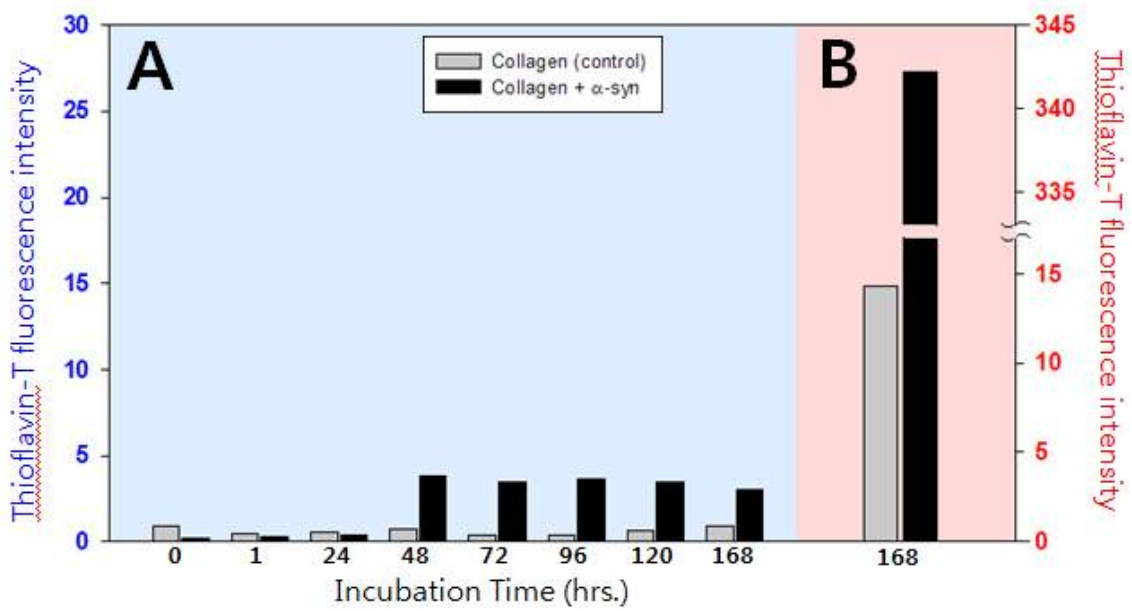

Figure 6. Matrix-Localized-Fibrillation of α-synuclein. Thioflavin-T binding fluorescence spectra for the detection of α-fibril formation in surrounding bulk solution **(a)**, and in gel matrix **(b)**. The presence α-syunclein was detected, with fluorescence intensity of 342.177, only from the inside of gel matrix of experimental group. Fluorescence tensity of 14.282 detected from gel matrix of control group can be understood as a noise level due to the optical interference caused by broken down gel residues.

3.2.3 Matrix-Assisted Acceleration of α-Synuclein Fibril Formation.

To further analyze the collagen-matrix-localized fibrillation of α-synuclein, we set to find the underlying mechanism of such phenomena. As a result we found that 3D collagen matrix assists in the acceleration of α-synuclein fibrillation.

The experiment was proceeded as follows. 1ml of 1.5 mg/ml α-synuclein monomer was incubated at 37 ° C at 200 rpm shaking condition (generalized condition for α-synuclein fibrillation). 0.5 ml of Mes buffer and 0.5 ml of gelated collagen matrix was added to control and experimental group, respectively, to equalized the final concentration of the α-synuclein in both groups. At different time points along the incubation, thioflavin-T fluorescence intensity was analyzed to detect the formation of α-fibrils in both samples: collagen matrix in experimental group was broken down for this analysis since α-fibrils form only in the collagen matrix. After breaking down the collagen matrix the sample was mixed vigorously to well disperse the concentrated α-fibrils from the 3D matrix. Multiple samples of experimental groups were prepare to analyze the α-fibrils formation at multiple time points along the course of incubation.

On Figure 7, red curve and blue curve (control) represent the thioflavin-T fluorescence intensity graph of α-synuclein fibrillation with and without the presence of collagen matrix, respectively, along the time course of incubation. As it can be concluded from Figure 7, the α-synuclein was found to have an accelerated fibrillation process in the presence of collagen matrix. This result can be promising to quick or fast-acting cosmetic result

of α-synuclein when use in collagen ECM matrix as a dermal filler.

To add, with the finding of matrix-assisted acceleration of α-synuclein fibril formation, the found phenomena of collagen-matrix-localized fibrillation of α-synuclein can be explained. When collagen matrix is introduced to the solution of α-synuclein, α-synuclein will penetrate into the 3D matrix due to the concentration gradient. Then, collagen matrix, by an unknown interaction, accelerates fibrillation process of α-synuclein monomer in the matrix into α-fibers, which results in decrease of α-synuclein monomer in the gel matrix creating continual inward flow of monomer into the matrix and accumulation of produced α-fibrils only within the matrix.

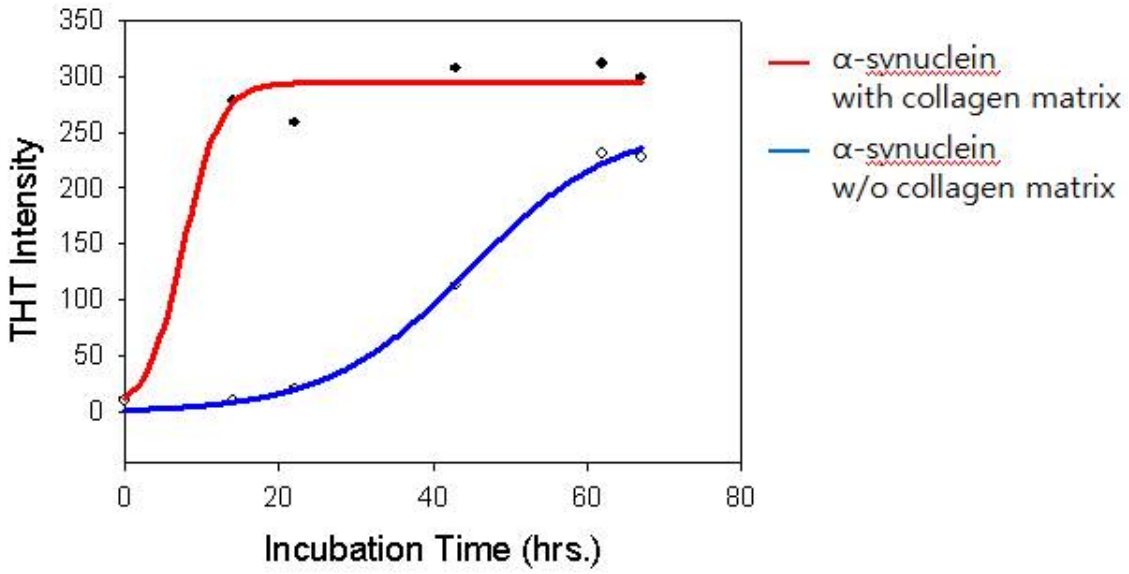

Figure 7. Accelerated α-Synuclein Fibrillation within Collagen Matrix. Formation of α-fibrils were detected, along the time course of incubation, within the collagen matrix (red curve) and without the presence of collagen matrix (blue curve). Time required to complete fibrillation was reduced by 53 hrs. by the presence of collagen matrix.

3.3 Mechanical Properties.

3.3.1 Advanced Rheometric Expansion System (ARES)

To analize the mechanical enhancement of 3D collagen gel matrix by formation of α-fibril, tests by ARES was performed and the resulting mechanical differences between collagen gel and α-fibril embedded collagen gel was analyzed. Two different modes of ARES were performed: steady sweep mode and strain sweep mode.

Steady sweep mode, results shown in Figure 8A, detects the degree of stress experienced by gel sample when varying magnitudes of steady shear deformation is applied. In Figure 8A, x-axis represents the increasing rate of spinning motor disk, which can translated to increasing magnitudes of shear deformation applied to the sample. The mechanical strength of the sample can indirectly compared by the initiation point of stress and the degree of experienced stress. Sample with superior mechanical strength is known to have early initiation point of stress, and greater degree of experienced stress [28].

As shown in Figure 8A, α-fibril embedded collagen gel (represented by red curve) shows earlier initiation point of stress, as well as, greater degree of experienced stress than blue curve representing control group. Through indirect measurement of mechanical strength by ARES steady sweep mode, α-synuclein fibrillation within collagen gel matrix was found to increase the strength of the resulting matrix.

Strain sweep mode test of ARES, result shown in Figure 8B, detects the elasticity of the sample when shear strain is applied. It measures for the tendency of the sample, at various degree of

deformation, to restore to its original structure [29]. The resulting graph is given in the value of G', elastic modulus. As show in Figure 8B, α-fibril embedded collagen gel (represented by red curve) shows higher elastic modulus values than blue curve representing control group. Through indirect measurement of mechanical elasticity by ARES strain sweep mode, α-synuclein fibrillation within collagen gel matrix was found to increase the elasticity of the resulting matrix.

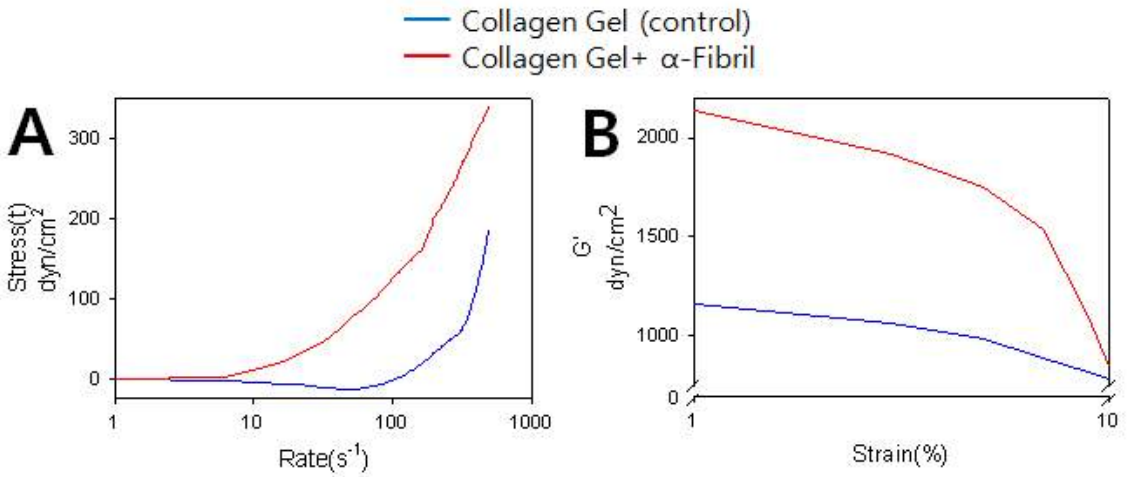

Figure 8. Enhancement of Strength and Elasticity Properties of Collagen Matrix by *α*-Synuclein Treatment. a, Increase in mechanical property of *α*-synuclein-treated collagen gel analyzed by ARES steady sweep mode test. **b,** Increase in elasticity of *α*-synuclein-treated collagen gel analyzed by ARES strain sweep mode test.

3.3.2 Tensile Test

Due to the specifications of ARES and its limitations in comparative mechanical analysis, the degree of mechanical enhancement of collagen gel by α-fibril was analyzed indirectly. However, for analysis of direct measurement of mechanical enhancement of 3D collagen gel matrix by α-synuclein treatment, the tensile test was performed and the resulting mechanical differences between collagen gel and α-fibril-treated collagen gel was compared.

Collagen gels were prepare as long quadrilateral shape in PDMS mold of 17 mm by 85 mm with excess height. 2 ml of collagen solution was evenly spread in the PDMS mold to form solidified gel. Formed gel of the control group was incubated with 2 ml of Mes buffer, pH 6.5, while that of the experimental group was incubated with 2 ml of 1 mg/ml α-synuclein oligomer. After the incubation, resulting gels of both group was subjected to freeze-drying according to previous method [13]. Resulting quadrilateral fiber strips were precisely cut into equal dimension of 14 mm by 85 mm for analysis of mechanical tensile strength with Universal Testing Machine (UTM), 20 N load cell, testing speed of 5 mm/min, at 23±2 ℃.

As shown by the result of the tensile test, stress vs. strain curve, in Figure 9, the tensile strengths of collagen gel and α-synuclein-treated collagen gel were measured to be 0.283 N/mm^2 and 0.463 N/mm^2 , respectively. It is a 163% increase in strength of collagen matrix by the formation of structural supporting α-fibril, when α-synuclein was treated as matrix filler.

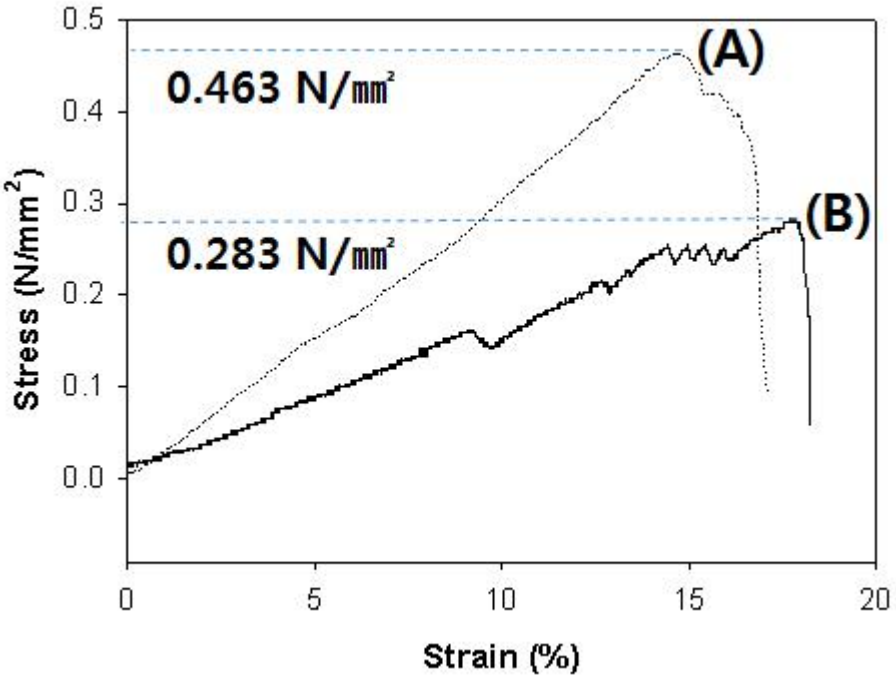

Figure 9. Direct Comparison of Strength Enhancement of α-Synuclein as Matrix Filler. Resulting stress vs. strain curve acquired from tensile test of collagen matrix **(a)**, and α-synuclein-treated collagen matrix **(b)**. The tensile strength were measured to be 0.283 N/mm² in control group and 0.463 N/mm² in experimental group, showing 163% increase in strength of collagen matrix by the formation of α-fibrils.

4. Conclusion

To address the problems of currently commercialized dermal fillers, mere stuffing of the diminished skin collagen matrix with bulky prostheses rather than enhancing the mechanical properties or providing extra structural support of existing fiber matrix, we evaluated α-synuclein, and its amyloidogenic self-assembly process as an alternative material and method for improving skin contour defects by forming nano-scaled fibrillar network that grants extra structural support to weakening skin collagen matrix.

First of all, α-synuclein was found to be well penetrated and successfully form α-fibrils evenly throughout the whole matrix by its amyloidogenic self-assembly. In addition, formed α-fibrils were found to present extra structural support to collagen matrix by forming intertwined fiber-network: roping around and connecting neighboring fibers and effectively increasing the density of the matrix.

Further, the amount of α-fibrils form within the collagen matrix could be controlled by adjusting the concentration of applied α-synuclein oligomer as evidenced by the results of emission fluorescence spectra scanning: proportional increase in fluorescence intensity with increased oligomer concentration. Also, α-synuclein treatment was proven to be effective in protecting the collagen gel matrix from protease degradation, enabling the gel to retain much of its original structure even after 40 hrs. of degradative incubation with excess amount of protease.

Moreover, the self-assembly process of α-synuclein was being accelerated and exclusively localized within collagen matrix, which can lead to collagen ECM matrix targeted dermal filler in

future application.

Most importantly, as proven with various mechanical testings by ARES and tensile test, matrix-embedded α-fibrils were effectively enhancing the mechanical properties of collagen matrix including elasticity and strength.

Although, in order to be used in clinical application, many further tests and examination, such as skin-permeation test, toxicity test, cell-compatibility test and etc, are required, many data (some unique to α-synuclein) we presented in this paper shows promising possibility of α-synuclein as an alternative material for the dermal filler that enhances the mechanical properties and provides extra structural support to endogenous collagen ECM fiber matrix.

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국문 초록

주름과 같은 노화와 관련된 피부 결점들을 개선시켜주는 피부 필러는 요즘 매우 빠른 속도로 성장하고 있는 분야이다. 하지만 현재 사용되고 있는 피부 필러들에게도 여러 가지 문제점과 개선 되야 할 점들이 있다. 우리는 이 논문에서 가기조립 특성을 지닌 알파-시뉴클레인 단백질의 새로운 피부 필러 재료로써의 가능성을 확인해보았다. 실험 결과, 알파-시뉴클레인은 3D 콜라겐 매트릭스 안으로 특이적으로 침투하였고 콜라겐 매트릭스와의 상호작용을 통하여 빠른 속도로 수 나노 크기의 단백질 섬유를 매트릭스 전체에 걸쳐 골고루 형성하는 것을 관찰하였다. 또한, 매트릭스 안에 형성된 알파-시뉴클레인 섬유를 확인해본 결과 콜라겐 섬유를 나무덩굴처럼 감싸거나 주위의 여러 콜라겐 섬유들을 서로 연결시킴으로써 콜라겐 매트릭스를 구조적으로 보강해주고 있는 것을 확인하였다. 더 나아가, 알파-시뉴클레인에 의한 구조적 보강이 콜라겐 매트릭스의 강도와 탄성력을 증진 시키는 것을 확인하였고 단백질분해효소로부터 콜라겐 매트릭스를 보호하는 것을 확인하였다.

주요단어: 알파-시뉴클레인, 가기조립현상, 아밀로이드, 피부 필러, 콜라겐 매트릭스

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