Characterization on Properties of Modification Gelatin Films with Carboxymethylcellulose

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Abstract

Effects of carboxymethylcellulose (CMC), a derivative of cellulose, blended with biopolymer gelatin films has been studied. The films were fabricated by blending CMC with gelatin solution in various ratio and casted on glass cover slips. Thermal and chemical crosslinking techniques were used to induce conjugation of free amide and carboxyl groups in protein structures of the films. Physical and mechanical properties of different gelatin/CMC films were characterized by Atomic Force Microscope (AFM) which scans on film surfaces and evaluates their elasticity. The physical structures of the films from AFM analysis indicated that increasing of CMC ratio effected in more aggregated of the protein structures of all the films. The analysis mechanical properties demonstrated that increasing of CMC ratio in gelatin/CMC films resulted gradually increasing in modulus of elasticity compared to pure gelatin films. The physical and chemical crosslinking EDC/NHS in 50 mM MES buffer in 40% ethanol improved in mechanical strength of all the gelatin/CMC films by increasing in modulus of elasticity with an average at 62.71 ± 1.69 kPa and 63.24 ± 0.92 kPa, respectively compared to pure gelatin film. These results suggested that using CMC as an additive and crosslinking techniques including thermal treatment and EDC/NHS as a crosslinking agent strengthened in protein structures which enhanced in mechanical properties of gelatin. The additive of CMC had tendency to display some interesting properties for applying in biomedical applications.

Keywords*:* gelatin, carboxymethylcellulose, film, Atomic Force Microscope, Modulus of Elasticity

1. Introduction

Gelatin, a denatured structure of collagen, has been used for medical application as a biomaterial and as an additive or gel capsules in drugs[1]. However, gelatin has a very hydrophilic nature and relatively poor mechanical properties which limit their potential applications [2]. Several methods that have been widely used to improve gelatin properties are blending gelatin with other proteins or polysaccharides such as alginate [3], chitosan [4] and hyaluronic acid [5]. The other one is using crosslinking reagent such as glutaraldehyde (GA) that is the most widely used

in crosslinking biomaterials. However, there are some reported that GA has been shown to release toxic upon hydrolyzation of the material and exhibit reduced cellular ingrowth in vitro and in vivo [6,7]. To overcome this problems, we took an interest in using 1-ethyl-3-3-dimethylaminopropyl carbodiimide (EDC) and N-hydroxysuccinimide (NHS) as a crosslinking agents because they has been shown to be more biocompatible for biological applications than GA [8]. EDC/NHS does not remain in the chemical bond after the reaction was completed because they released as a substituted urea molecule instead [9].

An alternative polymer for blending with gelatin to improve its properties, we took an interest in using carboxymethylcellulose (CMC), a derivative of cellulose by reacted with sodium hydroxide and chloroacetic acid. Due to its good viscosity building, high shear stability, biocompatibility, easily available and very cheap compared to other polysaccharides, It has widely used in many fields [10,11]. In medical applications, CMC was used as a hydrogel for wound dressing [12], a scaffold for various tissue engineering applications [13] and an injectable material for bone augmentation [14]. The current study focused on the films made from gelatin modified with CMC in various ratios. Physical and mechanical properties of the modified films were evaluated in comparison to the conventional gelatin film.

2. Experimental

2.1 Materials

Gelatin was purchased from BIO BASIC INC, Canada. From certificate of analysis and specifications, gelatin was Type A, a reagent grade, derived from porkskin with bloom number of 240-270 and pH at 25°C was 4.5-5.5. Its viscosity was 3.5-4.5 cps and moisture less than 12.0%. Carboxymethylcellulose sodium salt (CMC) was purchased from Sigma-Aldrich, St. Louis, MO, USA. It was medium viscosity of 400-800 cps in a 2% aqueous solution at 25°C. Deionized water was used for preparing gelatin and CMC solutions.

Chemical crosslinking, 1-ethyl-3-3-dimethylaminopropyl carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (1-Hydroxy-2,5-pyrrolidinedione) (NHS), a reagent grade, and 2-[n-morpholino]ethanesulfonic acid (MES), Free acid were purchased from BIO BASIC INC, Canada.

2.2 Preparation of gelatin/CMC films

Powders of Type A gelatin were swollen in deionized water at room temperature for 0.5 h before dissolved at 50°C under agitation for 1 h to obtain 0.8 wt% (w/w) solution. Then, CMC was dissolved in deionized water at 50°C for 1 h to form a 0.8 wt% (w/w) solution. The gelatin solution was mixed with the CMC solution at the blending composition of gelatin/CMC as be shown in table 1.

Table. 1 Blending composition of gelatin/CMC films

Blending composition of gelatin/CMC	Type of non-crosslinked Gelatin/CMC films
100/0	nG100
90/10	nGC91
80/20	nGC82
70/30	nGC73
60/40	nGC64

The mixed suspensions were stirred at 50°C for 1 h, then degassed. The blended gelatin/CMC solutions were casted on glass cover lips with diameter of 1 cm. before dry at 37°C for 2 h.

2.3 Crosslinking treatments

All the films were dehydrated at 140°C for 48 h prior to crosslink with EDC/NHS. The films were immersed in a solution of EDC/NHS (14/5.5 mM) in 50 mM MES buffer in 40% ethanol for 2 h at room temperature. Subsequently, the films were washed with deionized water (30 min × four times). The type of crosslinked gelatin/CMC films were shown in table 2.

Table. 2 Type of crosslinked gelatin/CMC films

Blending composition of gelatin/CMC	Thermal crosslinked gelatin/CMC films	EDC/NHS-crosslinked gelatin/CMC films
100/0	G100T	G100EN
90/10	GC91T	GC91EN
80/20	GC82T	GC82EN
70/30	GC73T	GC73EN
60/40	GC64T	GC64EN

2.4 Observation on structures of the films

The surface structures of the gelatin/CMC films were observed via AFM instrument (XE-70, Park Systems Corp., Suwon, Korea). AFM was controlled by a piezo translator, a maximum xy scan range of 50 μm and a z range of 12 μm., and used XE Data Acquisition Program.

2.5 Evaluation on mechanical properties of the films

The modulus of elasticity performed by AFM. We used a 130-μm-long Si_3N_4 cantilever (Park Systems Corp., Suwon, Korea) with a spring constant of 0.6 N/m. The force curve analysis module performed analysis of single force curve pairs to describe how adhesion and elasticity properties were distributed over the surface. We used Sneddon cone-on-flat model which a rigid cone is punched into a soft flat surface. The Modulus of Elasticity of the films calculated from relation between indentation (δ) and loading force (F) as shown in equation 1 [15,16].

$$F = \frac{2}{\pi}\frac{E}{1-\nu^2}\tan(\alpha)(\delta)^2 \qquad (1)$$

E = Young's modulus (modulus of elasticity)

ν = Poisson's ratio (0.35) [17]

α = Tip half cone opening angle (15°) [18]

δ = Indentation

F determined from multiplication of cantilever deflection, $d(z)$ and spring constant, k as shown in equation 2. $d(z)$ is equal to the movement of z axis of piezo that is decreased due to δ on the surface of the film as shown in equation 3 [19].

$$F = kd(z) \qquad (2)$$

$$d(z) = z - \delta \qquad (3)$$

3. Results and discussion

3.1 Morphology of the films

Figures 1 and 2 showed representative AFM images of the film surfaces at different mixing ratios of gelatin to CMC before and after thermal crosslinking, respectively. The morphology of the gelatin/CMC films seemed to

be mainly dependent upon the mixing ratios of gelatin and CMC solutions. Adding of CMC to the films increased in molecular aggregates between gelatin and CMC as shown striking features in Fig. 1b – 1e and Fig. 2b – 2e both before and after crosslinked by thermal treatment.

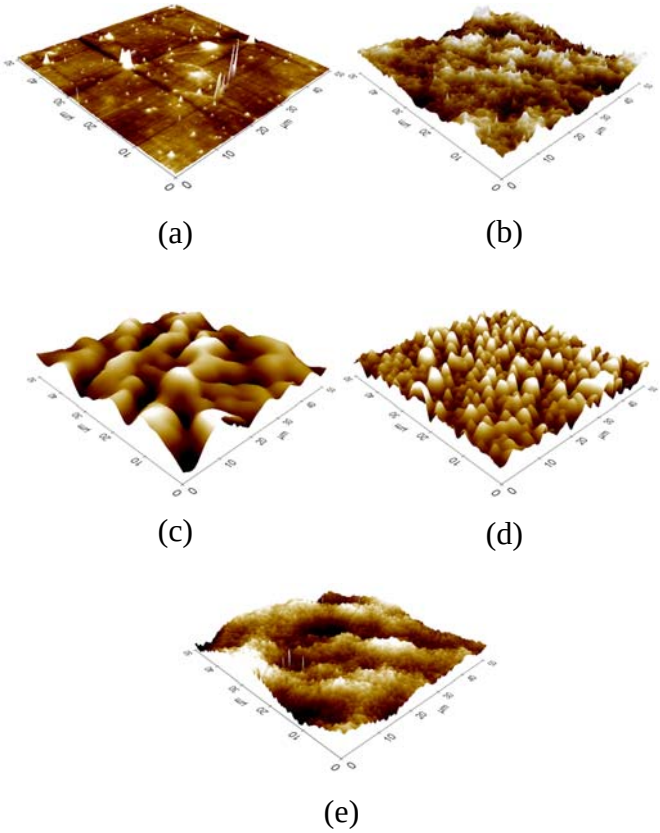

Fig. 1 AFM image (50 x 50 μm^2) of non-crosslinked gelatin/CMC films (a) nG100 (b) nGC91 (c) nGC82 (d) nGC73 (e) nGC64

The most molecular aggregates on non-crosslinked film was nGC73 (Fig. 1d) and the thermal crosslinked film was found in GC73T (Fig. 2d). The morphologies of the films both before and after using thermal crosslinking were no significant different indicating that thermal crosslinking technique did not effect on surface structure of the gelatin/CMC films. Both pure gelatin films before and after crosslinking (Fig. 1a, 2a and 3a) displayed fairly flat and nearly homogeneous layers. Corresponding AFM images, there were no significant different between thermal crosslinked and non-crosslinked gelatin/CMC films.

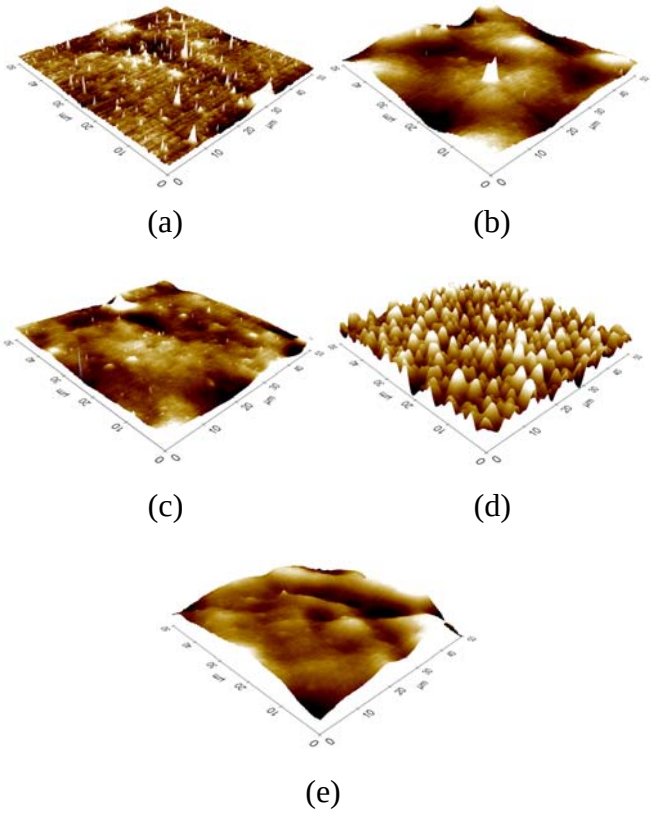

Fig. 2 AFM image (50 x 50 μm^2) of thermal crosslinked gelatin/CMC films (a) G100T (b) GC91T (c) GC82T (d) GC73T (e) GC64T

After using chemical crosslinking (EDC/NHS) on gelatin/CMC films. The surface structures of the EDC/NHS crosslinked films were shown in Fig. 3. All of the crosslinked films seemed to be more homogeneous and flat when compared to the gelatin/CMC films both non-crosslinked films and thermal crosslinked films

which displayed more molecular aggregates on the films as shown in Fig. 1 and 2, respectively.

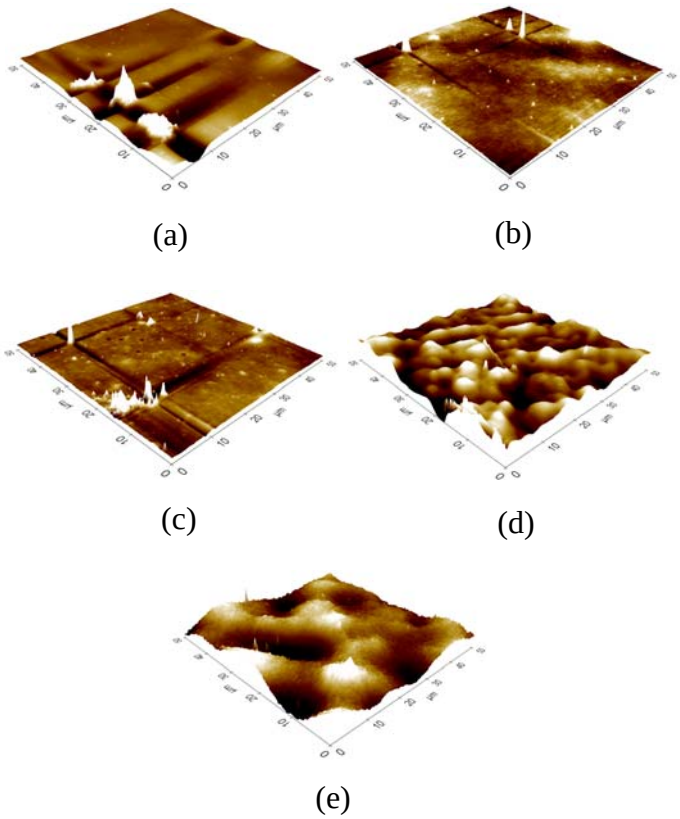

Fig. 3 AFM image (50 x 50 μm^2) of EDC/NHS crosslinked gelatin/CMC films (a) G100EN (b) GC91EN (c) GC82EN (d) GC73EN (e) GC64EN

3.2 Mechanical properties

The mechanical properties of the modified gelatin films as shown in Fig. 4, Fig. 5 and Fig. 6 were evaluated with the AFM force curve analysis mode as mentioned above at various points on the surface of the films. The relationships between the cantilever deflection and the indentation of the films were measured. The observed modulus of elasticity of different gelatin/CMC films were not constant which varied from 48.75 ± 5.48 kPa to 65.64 ± 2.68 kPa and 56.57 ± 6.77 kPa to 67.88 ± 2.27 kPa for non-crosslinked (Fig. 4) and thermal crosslinked gelatin/CMC films (Fig. 5), respectively.

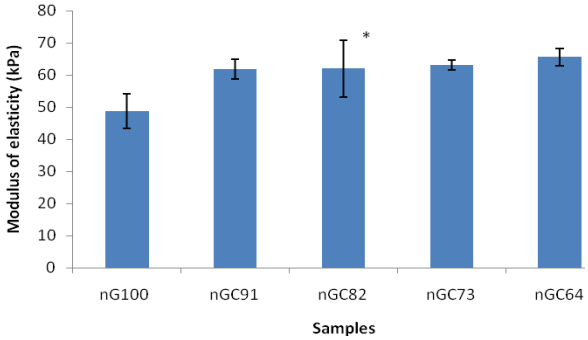

Fig. 4 Modulus of elasticity of non-crosslinked gelatin/CMC films

(significant different p<0.05 relative to nG100)*

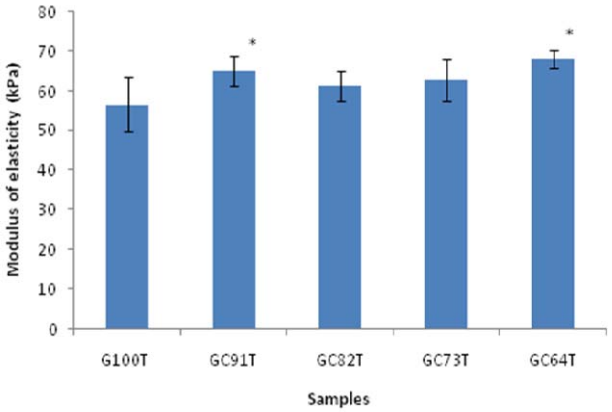

Fig. 5 Modulus of elasticity of thermal crosslinked gelatin/CMC films

(significant different p<0.05 relative to G100T)*

Adding CMC effected of the film stiffness which the modulus of elasticity increased when increased of CMC ratios in the films. It was clearly found in GC91T and GC64T thermal crosslinked films (Fig. 5) which their modulus of elasticity (64.97 ± 3.83 kPa and 67.88 ± 2.27 kPa, respectively) increased with significantly difference compared to pure gelatin film G100T which its modulus of elasticity was 56.57 ± 6.77 kPa. Although there was slightly different between modulus of elasticity of non-

crosslinked films and thermal-crosslinked films, but this results indicated that the modulus of elasticity increased gradually according to increase the CMC ratios.

The influence of EDC/NHS crosslinking on mechanical properties of the films was shown in Fig. 6. After treated by EDC/NHS, the modulus of elasticity of all the films were in range of 57.50 ± 1.80 kPa and 65.33 ± 3.17 kPa. Addition of CMC into the films gradually increased in modulus of elasticity of all the modified films. The significant difference can be detected on GC91EN and GC82EN films whose modulus of elasticity were 64.86 ± 1.08 kPa and 64.16 ± 2.22 kPa, respectively compared to pure gelatin film G100EN which its modulus of elasticity was 57.50 ± 1.80 kPa.

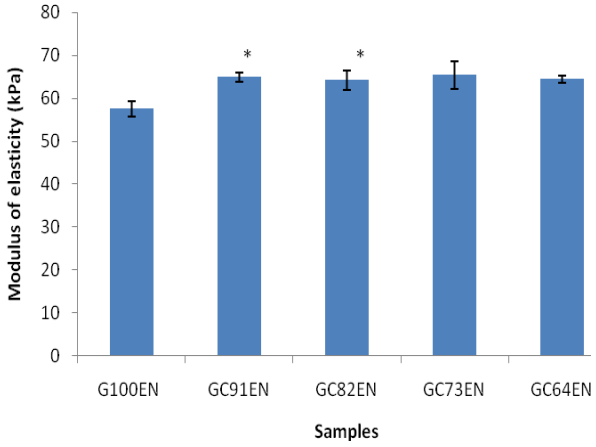

Fig. 6 Modulus of elasticity of EDC/NHS crosslinked gelatin/CMC films

(significant different p<0.05 relative to G100EN)*

4. Conclusions

Morphology and modulus of elasticity of the modified gelatin films with CMC were evaluated using the AFM. Adding of CMC to the films increased in molecular aggregates between gelatin and CMC. It was also gradually increased in modulus of elasticity of the films both non-crosslinked films and crosslinked films by using thermal treatment and EDC/NHS. The more molecular aggregates on the films can be implied that the root-mean square roughness (R_{rms}) measured on cross section profile along the corresponding line of the films will be higher than the other films. This results suggested that improving gelatin properties by blending with CMC and using crosslinking techniques including thermal and EDC/NHS treatment can be applied for biomedical applications.

5. Acknowledgement

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6. References

[1] Olsen, D., Yang, C., Bodo, M., Chang, R., Leigh, S., Baez, J., Carmichael, D., Peral, M., Hamalainen, E.R., Jarvinen, M. and Polarek, J. (2003). Recombinant collagen and gelatin for drug Delivery, *Advanced Drug Delivery Reviews*, Vol.55, pp. 1547– 1567.

[2] Chiou, B-S., Avena-Bustillos, R.J., Bechtel, P.J., Jafri, H., Narayan, R., Imam, S.H., Glenn, G.M. and Orts, W.J. (2008). Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties, *European Polymer Journal*, Vol.44, pp. 3748–3753.

[3] Choi, Y.S., Hong, S.R., Lee, Y.M., Song, K.W., Park, M.H. and Nam, Y.S. (1999). Study on gelatin-containing artificial skin: I. Preparation and characteristics of novel gelatin-alginate sponge, *Biomaterials*, Vol.20, pp. 409-417.

[4] Mao, J.S., Zhao, L.G., Yin, Y.J. and Yao, K.D. (2003). Structure and properties of bilayer

chitosan-gelatin scaffolds, *Biomaterials*, Vol.24, pp. 1067-1074.

[5] Mao, J.S., Liu, H.F., Yin, Y.J. and Yao, K.D. (2003). The properties of chitosan-gelatin membranes and scaffolds modified with hyaluronic acid by different methods, *Biomaterials*, Vol.24, pp. 1621-1629.

[6] Speer, D.P., Chvapil, M., Eskelson, C.D. and Ulreich, J. (1980). Biological effects of residual glutaraldehyde in glutaraldehyde-tanned collagen biomaterials, *Journal of Biomedial Materials Research*, Vol.14, pp. 753-764.

[7] Cooke, A., Oliver, R.F. and Edward, M. (1983). An in vitro cytotoxicity study of aldehyde-treated pig dermal collagen, *British Journal of Experimental Pathology*, Vol.64, pp. 172-176.

[8] Rault, I., Frei, V., Herbage, D., Abdul-Marak, N. and Hue, A. (1996). Evaluation of different chemical methods for cross-linking collagen gel, films and sponges, *Journal of Materials Science Materials in Medicine*, Vol.7, pp. 215-221.

[9] Marios, Y., Chakfe, N., Deng, X.Y., Mario, M., How, T., King, M.W. and Guidoin, R. (1995). Carbodiimide cross-linked gelatin-new coating for porous polyester arterial prosthesis, *Biomaterials*, Vol.16, pp. 1131-1139.

[10] Biswal, D.R. and Singh, R.P. (2004). Characterisation of carboxymethyl cellulose and polyacrylamide graft copolymer, *Carbohydrate Polymers*, Vol.57, pp. 379-387.

[11] Wang, M., Xu, L., Hu, H., Zhai, M., Peng, J., Nho, Y., Li, J. and Wei, G. (2007). Radiation synthesis of PVP/CMC hydrogels as wound dressing, *Nuclear Instruments and Methods in Physics Research B*, Vol.265, pp. 385-389.

[12] Moseley, R., Walker, M., Waddington, R.J. and Chen, W.Y.J. (2003). Comparison of the antioxidant properties of wound dressing materials-carboxymethycellulose, hyaluronan benzyl ester and hyaluronan, towards polymorphonuclear leukocyte-derived reactive oxygen species, *Biomaterials*, Vol.24, pp. 1549-1557.

[13] Yuan, N.Y., Lin, Y.A., Ho, M.H., Wang, D.M., Lai, J.Y. and Hsieh, H.J. (2009). Effects of cooling mode on the structure and strength of porous scaffolds made of chitosan, alginate, and carboxymethyl cellulose by the freeze-gelatin method, *Carbohydrate Polymers*, Vol.78, pp. 349-356.

[14] Uda, H., Sugawara, Y. and Nakasu, M. (2006). Experimental studies on hydroxyapatite powder-carboxymethyl chitin composite: injectable material for bone augmentation, *Journal of Plastic, Reconstructive & Aesthetic Surgery*, Vol.59, pp. 188-196.

[15] Radmacher, M., Fritz, M., Kacher, C.M., Cleveland, J.P. and Hansma, P.K. (1996). Measuring the Viscoelastic Properties of Human Platelets with the Atomic Force Microscope, *Biophysical Journal*, Vol. 70, pp. 556-567.

[16] Domke, J. and Radmacher, M. (1998). Measuring the Elastic Properties of Thin Polymer Films with the Atomic Force Microscope, *Langmuir*, Vol. 14, pp. 3320-3325.

[17] Li, L.P., Herzog, W., Korhonen, R.K. and Jurvelin, J.S. (2005). The role of viscoelasticity of collagen fibers in articular cartilage: axial tension versus compression, *Medical Engineering & Physics*, Vol. 27, pp. 51-57.

[18] Probe unique features of contact cantilever NSC36 from Park Systems Corp., Suwon, Korea.

[19] Rotsch, C., Jacobson, K. and Radmacher, M. (1999). Dimensional and mechanical dynamics of active and stable edges in motile fibroblasts investigated by using atomic force microscopy, *Proceedings of the National academy of Sciences of the United States of America*, Vol. 96, pp. 921-926.