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카르보디이미드 반응과 실란 커플링을 이용한 모발강화 효과

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Hair Strengthening Effect of Silane Coupling and Carbodiimide Chemistry

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요 약: 화학적으로 손상된 모발은 모발 자체의 물리적 특성이 약해져서 일상생활 중에 외부 자극에 취약하다. 본 연구의 목적은 모발 케라틴 단백질간의 화학적 결합이 모발의 인장강도를 회복이 반복적인 빗질 하에서 더 이상의 모발 악화를 방지하는지 여부를 결정하는 데 있다. 손상된 모발은 펌 시술을 통해 얻었다. 펌 손상 모발을 관능성 가교제인 3-aminopropyltriethoxysilane (APTES)을 이용하여 실란 커플링 및 카르보디이미드 반응을 통해 모발 내부에 가교 결합이 형성되도록 처리하였다. 인장 강도, 영률 및 고원 응력(plateau stress)을 포함한 물리적 특성을 측정하여 내부 가교 결합의 효과를 확인하였고, 가교 결합의 존재는 Fourier transform infrared (FT-IR) 분광법으로 확인하였다. 모발 절단 및 갈라짐의 정도는 건조 상태 모발의 반복적 빗질 시험으로 평가하였다. 결과적으로 화학적으로 손상된 모발의 물리적 성질은 내부 가교 결합으로 회복되었다. APTES의 실란 커플링 및 카르보디이미드 반응의 결합은 FT-IR 스펙트럼으로 확인하였다. 열을 가하면서 반복적으로 빗어 낸 후 모발의 절단 및 갈라짐 방지가 확인되었다. 인간의 모발은 펌 시술을 포함한 화학적 손상으로 약화될 수 있으므로 이러한 특성을 복원하는 것은 헤어 케어 업계의 주요 과제다. 본 연구에서는 화학적 결합을 통해 손상된 모발의 내부에 가교 결합 형성이 모발의 건강을 회복시키는 강력한 방법이 될 수 있음을 시사한다.

Abstract: Chemically damaged hair is vulnerable to external stimuli in daily life due to the weakened physical properties of the hair strand itself. The purpose of this work was to determine whether chemical conjugation between hair keratin proteins restores tensile strength and thus results inpreventing further deterioration under repeated combing. A model damaged hair tress was produced by a typical perm-process. Then, it was internally crosslinked by the bifunctional cross-linker (3-aminopropyl)triethoxysilane (APTES), *via* both silane coupling and carbodiimide chemistry. Physical properties, including tensile strength, Young's modulus, and plateau stress, were measured to verify the effect of internal cross-linking, and the existence of crosslinking was verified by Fourier transform infrared (FT-IR) spectroscopy. The degrees of hair breakage and split ends were evaluated by repeated combing–drying tests. Physical properties of chemically damaged hair were restored by internal crosslinking. Successful crosslinking of APTES *via* both silane coupling and carbodiimide chemistry was verified by FT-IR spectra. Prevention of breakage and split ends after repeated combing with heat was observed. Human hair can be weakened by chemical damage including perm-processing, so restoring such properties is a major issue in the hair care industry. This work shows that internal crosslinking of damaged hair *via* chemical conjugation would be a potent method to restore the healthy hair.

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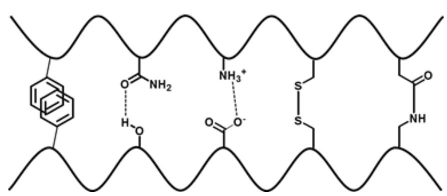
1. Introduction

Human hair is one of the most important parts of the human body because of its aesthetic qualities and its protection against external damage. In most vertebrates, hair, a fiber-reinforced nanocomposite, covers the outermost layer of the body for protection[1,2]. Hair is a crucial factor that contributes to the recognition of a person, and it strongly influences physical attraction in general[3]. Additionally, for centuries, certain decorations of hair have represented social positions and individual identities. Several studies have reported the negative psychological effects of alopecia[4-6]. Human hair is composed of the cuticle, the cortex, and the medulla; the major parts are the cuticle and cortex[7-9].



Peptide bonds are also important to the structure of human hair. They provide hydrogen bonds between the C = O and the N-H of two different peptide chains. Ionic interactions between acidic and basic amino acids are also a part of the intermolecular bonding in hair as shown below[10]. These chemical bonds determine the physical properties of human hair, including tensile strength.

Hair can be damaged by various things. For instance, the hair cuticle and cortex are severely damaged during the physicochemical reactions of beauty treatments such as haircuts, shampooing, hair drying, combing, permanent waving, bleaching and hair coloring. Environmental stresses, including humidity, ultraviolet radiation, seawater, and chlorinated wate are other causes of damage to human hair in daily life. Natural aging is another unavoidable origin of hair damage. Thibaut and coworkers[11] performed a physical, chemical, biochemical, and microscopic evaluation of very long virgin hair (> 2.4 m) without any history of chemical treatment such as bleaching, coloring or permanent waving. Progressive deterioration was observed from the outside to the inside of the hair, and a gradual abrasion of the cuticle layer was detected in the longitudinal direction for over one meter, as measured by luster, hydrophobicity and friction, which was strongly correlated with decreased levels of ceramides and 18-methyl eicosanoic acid (18-MEA). The reduced mechanical and optical properties of the hair caused by natural aging originated from loss of KAPs.

Beauty treatments such as permanent waving, coloring and bleaching are representative methods that cause chemical damage to human hair by denaturation of hair keratin. Korte and coworkers[12] reported that a hydrophilic surface is generated by the elimination of the hydrophobic top layer and the formation of cysteic acid (SO_3^-) residues during the hair bleaching process, as measured by dynamic chemical force microscopy (dCFM) and infrared (IR) spectroscopy. Moreover, not only discoloration of melanin but also reduction of tensile strength is caused by hair bleaching through the destruction of disulfide bond and cavitation in the cuticle layer. The potential strengthening effect on damaged hair of the β-sheet motif of human γ D-crystallin protein from the cytosol of lenticular fiber cells was presented by Ribeiro *et al.*[13]. Studies from Cheng and Syed showed that the breaking load and tensile strength of hair after treatment with UV irradiation, bleaching or permanent waving were decreased by chemical damage as shown below[14,15].

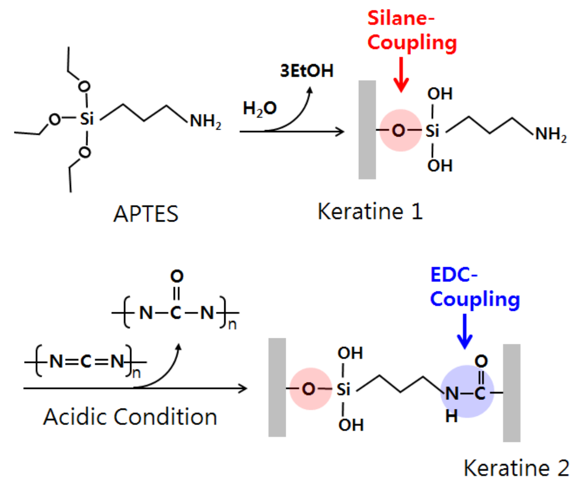
Chemical damage weakens the physical properties of hair fibers by an elution of internal proteins, and it leads to intense breakage during the combing process in addition to a rough feeling of the hair tip. Therefore, from the perspective of beauty, a dominant function of hair, the prevention and restoration of damaged hair are the most valuable techniques in the hair care industry.

Hair sample	Breaking load (N)
Virgin	94.13
UV irradiation	76.95 (96 h)
Bleaching	75.22 (12% H_2O_2)
Permanent waving	89.25 (pH 7)
	78.53 (pH 9)
	76.28 (pH 10)

Hair sample	Tensile strength (10^3N-m)
Untreated	1.21
Hair color (30 vol. developer)	1.14
Acid wave	0.99
Hair relaxer (NaOH)	0.78
Hair relaxer (guanidine)	0.70
Hair bleach (30 vol. developer)	0.48

Song and coworkers[16] recently reported that internal crosslinking between amine, thiol or hydroxyl residues of hair proteins and polycarboxylic acids such as malonic acid, malic acid, succinic acid, adipic acid, tartaric acid, and 1,2,3,4-butanetetracarboxylic acid (BTCA) at 180 ℃, catalyzed by sodium hypophosphite (SHP), could enhance the tensile strength and the maintenance of a permanent wave. Additionally, a successful reinforcement of hair by treatment and internal reaction of (3-aminopropyl) triethoxysilane (APTES) *via* sol-gel condensation between hydroxyl groups of hair proteins and silanol moieties of APTES was reported by Hallegot *et al.*[17].

Carbodiimide compounds, represented by 1-ethyl-3-dimethylaminopropyl carbodiimide hydrochloride (EDC-HCl), are some of the major reagents for the coupling reaction between carboxylic acid and amines to form amide bonds, known as carbodiimide chemistry. These compounds are widely utilized in biochemical fields for protein conjugation, surface modification, fluorescence tagging, or structure formations. For instance, Hartrianti and coworkers[18] made a biocompatible, physically and mechanically controllable keratin-alginate sponge by crosslinking human keratin to alginate *via* carbodiimide chemistry with EDC-HCl and discussed its potential for commercialization. Li *et al.*[19] controlled the strength and resistance against enzymatic digestion of the collagen from the porcine acellular dermal matrix (PADM). According to the authors, the PADM was re-constructed by conjugation between free amines and carboxylic acids of collagen fibers *via* carbodiimide chemistry using EDC-HCl and NHS, and it was readily available for biomedical application by a low-cost and non-toxic method.



Scheme 1. Proposed reaction mechanism of strengthened keratin fibers through silane coupling and carbodiimide chemistry.

Herein, we report a method to restore the physical properties and to prevent the further deterioration of chemically damaged hair. The silane group of APTES was conjugated with the hydroxyl group of human hair keratin *via* silane coupling, while the amine group on the opposite site of APTES was conjugated with a carboxylic group of keratin *via* carbodiimide chemistry. Internal crosslinking of hair proteins restored the physical properties of chemically damaged hair. A detailed mechanism is illustrated in Scheme 1.

2. Materials and Methods

2.1. Materials

1-Ethyl-3-dimethylaminopropyl carbodiimide hydrochloride (EDC-HCl, 99%), *N*-hydroxysuccinimide (NHS, 98%), sodium hydroxide pellet (97%), and other solvents were purchased from Sigma Aldrich (St. Louis, Missouri

(USA)). (3-Aminopropyl)triethoxysilane (APTES, 99%) was purchased from KCC (Korea). Ammonium thioglycolate (50% w/w) was purchased from Daejung Reagents (Korea). Sodium laureth sulfate (SLES, 37% w/w) was obtained from LG Household & Health Care (Korea). Virgin hair was purchased from Beaulax (Japan), and damaged hair tress was purchased from Phoenix Korea (Korea).

2.2. Instrumental Methods

Hair cross-sections were measured with a laser scan micrometer (LSM-501S/6200, Mitutoyo, USA). Tensile strength, Young's modulus, and plateau stress were measured with a tensile tester (MTT 175, Dia-Stron, UK). FT-IR spectra were measured from a FT-IR spectrometer (Avatar 320, Nicolet, USA). Repeated combing tests for hair breakage and split-end tests were conducted by an automated combing machine.

2.3. Preparation of Chemically Damaged Hair

Virgin hair and the damaged hair tress were used after being washed with 15% (w/w) SLES two times and kept overnight at 25 ℃ and 50% RH. Chemically damaged hair was obtained by typical perm-processing. In brief, 2x hair weight of 1 M ammonium thioglycolate (pH 9.5 by NaOH) was added to pre-washed virgin hair, followed by incubation at 25 ℃ for 10 min and washing with tap water for 5 min at flow rate of 35 mL s^{-1} and towel-drying. Then, 2x hair weight of 3% (w/w) H_2O_2 (pH 7.0 by NaOH) was added, followed by incubation at 25 ℃ for 10 min, washing with 0.1x hair weight of 15% SLES two times, drying and incubation at 25 ℃ and 50% RH overnight.

2.4. Silane Coupling and Carbodiimide Chemistry (APTES/EDC-HCl) of Damaged Hair

To restore the physical properties of chemically damaged hair, 0.5 g of perm-processed hair was immersed in 10% APTES (phosphate buffer, pH 4.5) for 15 min and washed for 1 min to eliminate the residual reagent. After towel-drying, the hair tress was immersed in 1% EDC-HCl/0.1% NHS solution (phosphate buffer, pH 4.5) for 15 min to induce amide coupling by carbodiimide chemistry, then it was washed for 1 min and towel-dried. To activate silane coupling, the hair tress was dried at 80 ℃ for 2 min. As a control experiment, hair was immersed in the same APTES solution, washed, and towel-dried. To ensure the successful conjugation, FT-IR spectra of damaged hair, APTES-only, and APTES/EDC-HCl treated hair were analyzed.

2.5. Physical Properties of Hair

Physical properties of virgin, damaged and treated hair were measured after incubation at 25 ℃, humidity 50% RH for at least 24 h from final treatment to ensure the equilibrium of water contents. The hair cross-section of each strand was gauged, and the elastic gradient, plateau load, and break load of the strand were measured. Tensile strength, Young's modulus and plateau stress were calculated. The results from virgin hair and damaged hair were regarded as 100% and 0%, respectively to calculate the restoration rate after treatment.

2.6. Prevention of Hair Breakage and Split Ends

To evaluate the practical meaning of chemical conjugation, the prevention of hair breakage and split ends were evaluated. Briefly, 5 g of non-APTES- or APTES/EDC-HCl-treated damaged hair tress was combed for 60 min (60 times/min) with continuous heat treatment with a hair dryer. For the hair breakage experiment, broken hair tips were collected and weighed. For the split-end study, the number and the length of split ends were measured. The result from non-treated hair was regarded as 100% to calculate the prevention rate after treatment.

2.7. Statistical Analysis

For tensile strength, young's modulus, and plateau stress, p-value was obtained by performing t-test to compare the experimental results of the damaged hair to the results of treated hair. For hair breakage, the weigh of broken hair over the weigh of total tress was calculated and compared, and for hair split ends test, the significant difference of the lengths of split ends between those of

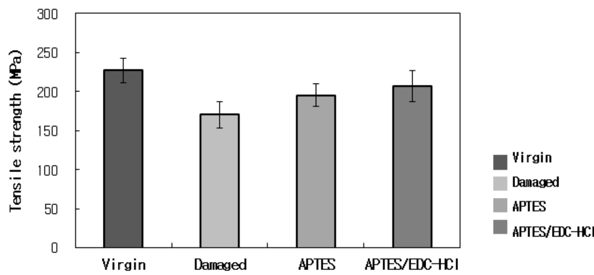


Figure 1. Tensile strength of virgin, damaged, and silane coupling/carbodiimide treated hair.

non-treated and treated hair was evaluated after outlier removal.

3. Results and Discussion

3.1. Hair Strengthening

Generally, tensile strength represents the physical properties of hair and indicates the level of damage to hair. Tensile strength of virgin, damaged, APTES-treated, and APTES/EDC-HCl treated hair was measured (Figure 1). As previously described, the tensile strength of hair was severely deteriorated by perm-processing; the 227 MPa tensile strength decreased to 171 MPa after perm-processing. However, it was restored by 64.7% by treatment with APTES/EDC-HCl, calculated by restoration rate (%) = (tensile strength of virgin hair-tensile strength of treated hair) / (tensile strength of virgin hair - tensile strength of damaged hair) × 100. In contrast, the restoration rate after treatment with APTES was just 44.26%. When compared to the tensile strengths of fibers from hair treated with APTES, those treated with APTES/EDC-HCl showed a significant difference (p = 4.2E-2), meaning that carbodiimide successfully enhanced the tensile strength further.

Young's modulus is another important factor to evaluate the physical properties of fibers. Like tensile strength, decreased Young's modulus is a typical feature of hair damage. Young's modulus was decreased after perm-processing from 3122 MPa to 2456 MPa (data not shown). Although treatment with only APTES restored

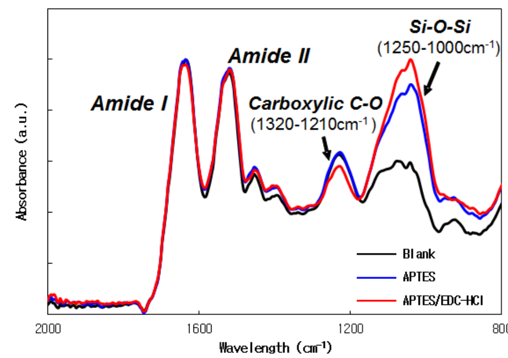


Figure 2. FT-IR spectra of blank, APTES, and APTES/EDC-HCl treated hair tress.

the value up to 98.39%, the restoration rate of Young's modulus after treatment with both APTES and EDC-HCl was over 100%, as calculated by restoration rate (%) = (Young's modulus of treated hair − Young's modulus of damaged hair) / (Young's modulus of virgin hair - Young's modulus of damaged hair) × 100.

Similar to the previous physical properties, plateau stress indicates the damage rate of hair. The plateau stress levels of virgin, damaged, APTES-treated, and APTES/EDC-HCl-treated hair are measured (data not shown). The restoration rate, calculated as (plateau stress of virgin hair-plateau stress of treated hair) / (plateau stress of virgin hair − plateau stress of damaged hair) × 100, of APTES only was approximately 79%, but both silane coupling and carbodiimide chemistry recovered over 100% of virgin hair. Taken together, these data show that dual crosslinking of APTES, not only silane coupling but also carbodiimide chemistry, is important to restore the physical properties of damaged hair to a normal state.

3.2. FT-IR Analysis

A gradual restoration effect on physical properties was observed after treatment with APTES and EDC-HCl, as expected. To ensure the crosslinking of APTES, Fourier-transform infrared (FT-IR) spectra of control-, APTES- and APTES/EDC-HCl-treated hair were obtained. As shown in Figure 2, a strong peak of Si-O-Si (1100-1250 cm^{-1}) appeared from both APTES-and

Table 1. Prevention of Hair Split Ends by Silane Coupling/ carbodiimide Treatment

	Number of split ends (n)	Total length (μm)	Average lenth (μm)
Blank	22	255267	11603
APTES	14	57585	4113
APTES/EDC-HC1	12	40484	3774

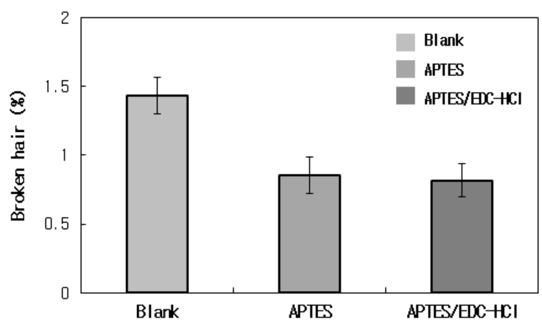


Figure 3. Hair breakage rate of blank, APTES, and APTES/EDC-HCl treated hair.

APTES/EDC-HCl-treated hair as a result of silane coupling. The Si-O-Si intensity of APTES-treated hair was relatively lower, which may imply less durability after washing. On the other hand, the intensity of C-O (1210-1320 cm^{-1}) was decreased after treatment with EDC-HCl, indicating that some portion of carboxylic acid groups was transformed into another functional group. Therefore, the overall result verifies the successful conjugation of APTES *via* silane coupling and carbodiimide chemistry.

3.3. Prevention of Hair Breakage

Damaged hair is extremely vulnerable to external forces such as repeated combing due to its weakened physical properties, so prevention of hair breakage is an important issue in hair care. The hair breakage was evaluated against damaged and treated hair after 60 min of extreme damage with combing and heat treatment (Figure 3). Without any treatment, 1.43% (w/w) of hair was broken by repeated combing and heat. On the other hand, hair breakage was reduced to 0.86% and 0.82% of breakage after treatment with APTES and APTES/EDC-HCl, respectively. Thus, the restoration of physical properties is strongly related to the prevention of hair breakage by repeated combing.

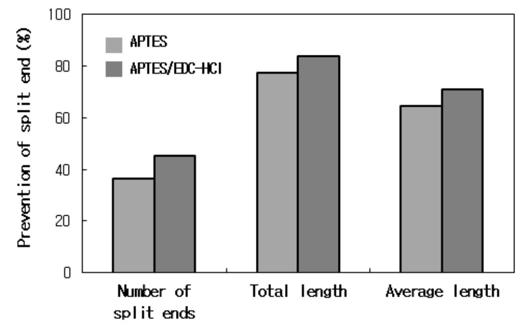


Figure 4. Prevention of split end of APTES- and APTES/EDC-HCl treated hair. Prevention of split end (%) = (number of split ends of Blank-number of split ends of Treated hair) / (number of split ends of Blank), (total length of Blank-total length of Treated hair) / (total length of Blank), (average length of Blank-average length of Treated hair) / (average length of Blank).

3.4. Prevention of Split Ends

To confirm the ability of APTES/EDC-HCl to prevent split ends, the number of split ends from damaged hair and treated hair was counted, and the lengths were measured (Table 1). As shown in Figure 4, the average length of split ends was 4.11 mm and 3.77 mm for APTES and APTES/EDC-HCl, respectively. There was no statistical difference between the lengths of split ends of non-treated hair and those of APTES-treated hair (p = 9.4E-2). However, there was statistical difference between those of non-treated hair and those of hair treated with APTES/EDC-HCl (p = 2.3E-4). This indicates that the silane-coupling and carbodiimide reactions dramatically prevents hair split ends.

4. Conclusions

In this study, the effect of silane coupling by APTES and carbodiimide chemistry by EDC-HCl on human damaged hair was studied. The effects of APTES/EDC-HCl on physical properties, including tensile strength, Young's modulus, and plateau stress, and the practical outcomes, such as prevention of breakage and split ends, were evaluated. All of the physical properties were weakened by perm-processing but restored after treatment with APTES/EDC-HCl. Moreover, both hair breakage and split ends were prevented. Consequently, the results from this study strongly suggest that internal crosslinking would be a powerful strategy to restore and strengthen damaged hair.

References

1. J. McKittrick, P.-Y. Chen, S. G. Bodde, W. Yang, E. E. Novitskaya, and M. A. Meyers, The structure, functions, and mechanical properties of keratin, *JOM US.*, **64**(4), 449 (2012).
2. A. Rossi, L. Barbieri, G. Pistola, P. Bonaccorsi, and S. Calvieri, Hair and nail structure and function, *J. Appl. Cosmetol.*, **21**, 01 (January/March 2003).
3. R. L. Terry, Further evidence on components of facial attractiveness, *Percept. Mot. Skills.*, **45**(1), 130 (1977).
4. T. F. Cash, The psychosocial consequences of androgenetic alopecia: a review of the research literature, *Br. J. Dermatol.*, **141**(3), 398 (1999).
5. T. F. Cash, V. H. Price, and R. C. Savin, Psychological effects of androgenetica alopecia on women: comparisons with balding men with female control subjects, *J. Am. Acad. Dermatol.*, **29**(4), 568 (1993).
6. T. F. Cash, The psychological effects of androgenetica alopecia in men, *J. Am. Acad. Dermatol.*, **26**(6), 926 (1992).
7. T. Takahashi and S. Yoshida, A highly resistant structure between cuticle and cortex of human hair, *Int. J. Cosmetic Sci.*, **39**(3), 327 (2017).
8. C. R. Robbins, Chemical and Physical Behavior of Human Hair, 4th ed. Springer-Verlag, New York (2002).
9. J. H. Bradbury, The structure and chemistry of keratin fibers, Academic Press, New York (1973).
10. C. Popescu and H. Hocker, Hair-the most sophisticated biological composite material, *Chem.. Soc. Rev.*, **36**(8), 1282 (2007).
11. S. Thibaut, E. de Becker, B. A. Bernard, M. Huart, F. Fiat, N. Baghdadli, G. S. Luengo, F. Leroy, P. Angevin, A. M. Kermoal, S. Muller, M. Peron,, G. Provot, S. Kravtchenko, D. Saint-Leger, G. Desbois, L. Gauchet, K. Nowbuth, A. Galliano, J. Y. Kempf, and I. Silberzan, Chronological ageing of human hair keratin fibres, *Int. J. Cosmetic Sci.*, **32**(6), 422 (2010).
12. M. Korte, S. Akari, H. Kuhn, N. Baghdadli, H. Mohwald, and G. S. Luengo, Distribution and localization of hydrophobic and ionic chemical groups at the surface of bleached human hair fibers, *Langmuir*, **30**(41), 12124 (2014).
13. A. Ribeiro, T. Matam, C. F. Cruz, A. C. Gomes, and A. M. Cavaco-Paulo, Potential of human cD-crystallin for hair damage repair: insights into the mechanical properties and biocompatibility, *Int. J. Cosmetic Sci.*, **35**(5), 45 (2013).
14. S. Cheng, C. W. M. Yuen, C. W. Kan, and K. K. L. Cheuk, Analysis of keratin fibre damage under various surface treatment conditions, *RJTA*, **12**(1), 53 (2008).
15. A. N. Syed and H. Ayoub, Correlating porosity and tensile strength of chemically modified hair, *Cosmet. Toil.*, **117**(11), 57 (2002).
16. K. Song, H. Xu, K. Xia, and Y. Yang, Effects of chemical structures of polycarboxylic acids on molecular and performance manipulation of hair keratin, *RSC Adv.*, **63**, 58594 (2016).
17. P. Hallegot, G. Hussler, V. Jeanne-Rose, F. Leroy, P. Pineau, and H. Samain, Discovery of a sol-gel reinforcing the strength of hair structure: mechanisms of action and macroscopic effects on the hair, *J. Sol-Gel Sci. Technol.*, **79**(2), 359 (2016).
18. P. Hartrianti, L. T. H. Nguyen, J. Johanes, S. M. Chou, P. Zhu, N. S. Tan, M. B. Y. Tang, and K. W. Ng, Fabrication and characterization of a novel crosslinked human keratin-alginate sponge, *J. Tissue. Eng. Regen. Med.*, **11**(9), 2590 (2017).
19. J. Li, N. Ren, J. Qiu, H. Jiang, H. Zhao, G. Wang, R. I. Boughton, Y. Wang, and H. Liu, Carbodiimide crosslinked collagen from porcine dermal matrix for high-strength tissue engineering scaffold, *Int. J. Biol. Macromol.*, **61**, 69 (2013).