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MICROSCOPIC PICTURE AND WATER CONTENT IN DENTIN ANALYSIS OF BONDING AGENT BOND TO DENTIN SURFACE
An Evaluation of Various Humidity

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ABSTRACT

The bond strength of bonding agent (HEMA based) in dentin surface can occur if collagen fibril is in permeable condition. This condition is influenced by the humidity in teeth surface, water content in dentin and the bonding resin insertion to dentin tubule. This research is aimed to identify the microscopic picture of tags resin and hybrid layer thickness and water content in dentin in relation to humidity in dentin surface. Bovine incisor was cut and shaped with diamond disk until reaching dentin area. After cutting was completed, dentin was cleaned and soaked into physiologic salt for 24 hours. Then it was dried with tissue paper for 5 minutes and put into desiccators for 1 hour. Soon after it is completed, it is measured and put into oven in 70°C to reach constant weight. From this process, we could reveal water content in dentin. Sample preparation for SEM: Cavity preparation was made to the tooth in labial area in circle shape with 5 mm diameter, 2 mm depth. Then etching was done with 37% phosphoric acid for 15 seconds and washed with 20 cc distilled water, and dried with cotton pellets. Then, it was put into desiccators for 1 hour. After it was completed, self cured acrylic ingredient was applied. In the middle of cavity, the tooth was cut. The cut tooth is examined for the hybrid layer thickness and tags resin length with scanning electronic microscope. The result data were analyzed with one-way ANOVA test at 95% confidence level and continued with LSD test. This research result showed that in 60% water content humidity of dentin was at the lowest point and 90% was at the highest point. At 60% to 90% humidity, mean score of hybrid layer, tags resin length showed there was no significant difference. In conclusion, the higher the humidity of ground teeth area, the higher the water content in dentin. There was no bonding microscopic picture between bonding dentin (HEMA) and collagen in humidity increasing.

Keyword: *dentin bonding agent, humidity, water content, tags resin, hybrid layer*

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INTRODUCTION

Along with the development of restoration technology, composite resin agent is mostly used to fix the teeth. In dentin area dentists usually use mediator agent before applying composite resin that contains bonding dentin liquid in hydrophilic character. One step of bonding dentin resin application (HEMA) in tooth dentin surface is drying procedure. This step is based on total etched technique. The purpose is to loose water residue used to wash dentin surface after acid etched (Anusavice 2003). The washing is to loose created salt resulting from the reaction of acid etching and dentin mineral. Nevertheless, some researchers showed that in drying procedure, dentin surface can be left neither in overdry nor overwet condition (Bracket et al. 2006; Nakabayashi & Pashley 1998). If the dentin surface is overdry, the dentin collagen will collapse, so that bonding resin is difficult to bond with collagen. Conversely, if dentin is overwet, bonding resin (HEMA) is difficult to bond with dentin collagen because there are so many water molecules around the collagen fibril. Thus, it needs optimal dentin surface to achieve maximum bonding between bonding resin agent and dentin collagen.

Usually dentin bonding agent is derivate of methacrylate and polimerable resin (Anusavice 2003; Craig et al. 2002; Jacques & Hebling 2005). This agent is mostly HEMA-based because HEMA has good chemical and physical characteristic (Besnault & Attal 2001; Finger & Tani 2002; Hishiyama et al. 2003; Xu et al. 1997). Good solid characteristic in dentin bonding agent in relation to low viscosity, so it can increase surface energy. HEMA is firstly used as agent to increase dentin bonding to repair adaptation inter-surface from resin restoration (Fusayama et al. 1979; Xu et al. 1997). After dentin is etched and dentin collagen fibril network is open, HEMA will be physically and chemically bound with the collagen fibril (Boracci & Ferrari 2002; Nishiyama et al. 2000; Nishiyama et al.

2002). Chemical bonding between HEMA and collagen is covalent bonding. It creates chemical interaction to bond nitrogen atom of collagen and carbon atom of aldehyde resin (Anusavice 2003). Generally, the successful dentin bonding agent bond to dentin collagen depends on several factors. They are low monomer viscosity, monomer type and concentration, acid applied as conditioner, temperature and humidity around collagen fibril (Besnault & Attal 2001; Breschi et al. 2002; Nakabayashi & Pashley 1998; Perdigao & Lopes 2001). Finger & Tani (2002) made a research about the influence of humidity to bond strength of dentin bonding agent in dentin surface in vitro. They said that mouth humidity depends on the use of rubberdam. If it is used, the humidity is influenced by dentist room humidity, which is 50% with 23°C temperature. But if rubberdam is not used, then the humidity will be 80% - 94%. The above research is compatible with Chiba et al (2004) experiment, showing that bond strength of dentin bonding will decrease as the humidity used in the experiment increases. The research was in 60% - 90% humidity with 25^0C temperature.

Water content in dentin is important for chemical and physical characteristic of dentin collagen (Nakabayashi & Pashley 1998; Reis et al. 2004; Wang & Spencer 2003). They said that by the existence of water molecule around collagen, there will be hydrogen bonding in collagen fibril or among collagen fibril. This hydrogen bonding will make optimum appearance of collagen fibril. Furthermore, this fibril would be easily bound to HEMA resin. Conversely, if there is only few water molecule, hydrogen bonding will be cut which may cause collapsed fibril, and inter fibrillar close contact. In this circumstances, inter peptide bonding will decrease and matrix collagen will decay, so that it is no longer permeable for HEMA resin.

Nakabayashi & Pashley (1998) said that the collapse process of collagen network is known as passive theory. Passive theory is demineralized dentin collagen network floating or suspended in the water. Each fibril will separate one another by water molecule filling the inter fibril room where it is previously filled with apatite crystal. In drying process, water that hold collagen network will be eliminated and it will cause bonding fibril collagen. Page (1997) in his analysis discuss that water comprises 70% of the net weight of a cell and an important factor of cell life. We use water in the process of life as reactant and available source to provide H_3O^+ and OH^- ion, dissociation or reaction with acid and base.

The influence of wet dentin to bond strength varies. A study on HEMA resin to dentin surface was done by Reis et al (2004). They used wet and dry dentin surface. The result showed that water-based resin needed more dry dentin surface and acetone-based resin loose easily. So, dentin surface will dry soon. Dentine bonding agent bond with collagen fibril will make layer called hybrid layer (Anusavice 2003; Nakabayashi & Pasley 1998). A lot of researchers argue that this layer thickness affects bond strength of dentin bonding with collagen fibril and the bonding resin insertion into dentin tubules (resin tags) can also affect bond strength of resin bonding to tooth surface (Breshi et al. 2002; Cho & Dickens 2004). The purpose of this research was to reveal the microscopic picture and water content analysis in dentin after dentin bonding agent is applied. The examination will be done as the various humidity influences the tooth surface

MATERIALS AND METHODS

Acid etched agent was Ivoclar Vivadent (Schaan/ Liechtenstein) with composition of 37% phosphoric acid in water, alcohol polyvinyl and pigment. Bonding agent: Voco (Germany) with composition: Bis – GMA, HEMA, BHT (butylated hydroxyl toluene), acetone and organic acid, Primer liquid with mixed bonding resin in a bottle. The type of self-curing acrylic resin was Vertex - Dentimex, Holland. Available tooth is incisor bovine taken from butchery at Pegirian Surabaya. Tooth is shaped and cut with diamond disk under flowing water. Available tools to cut and smooth the surface tooth: diamond disk, diamond burs, emery paper no. 400 and no. 1000 (Fuji Star, Japan). The equipment to measure dentin water content was Electronic Moisture Balance, MOC EB-280 model (Shimadzu, Co. Japan). To measure humidity, we used desiccators with vacuum, air thermometer and hygrometer (Haar-Synth, Hygro, Germany); compressor/air suction Schuco (USA). While tags resin length and hybrid layer thickness was measured with scanning electron microscope (SEM) with type: JOEL, JSM T-100, Japan.

The steps of research on water content in dentin were as follows: Bovine tooth was cut with diamond disk and shaped with diamond bur into the dentin. During the cutting and shaping process dentin was always under flowing water. After cutting, dentin was cleaned and soaked in physiologic salt liquid for 24 hours. After that, it was dried with tissue paper for 5 minutes. Next, the specimen was put into the desiccators for 1 hour. The classification of research group was as follows: Group I was put into desiccators with 60% humidity, Group II 70%, Group III 80%, and Group IV 90%, while Group V served as control. The treatment was done inside the research room (65%).

Soon after being removed from desiccators, and measured (for example A mg), then they were put into

oven with 70°C temperature and then measured until reaching constant weight (B mg). Heating time was about 40-55 minutes. Water weight, C = (A – B) mg, so that dentin water content was (C : A) x 100%. The measurement of humidity inside the desiccators was as follows: to arrange 90% humidity inside the desiccators (at the base part) 150 cc water was filled in, and then calibrated hygrometer was installed. In this condition, hygrometer humidity was about 94-95%. After that, from the pipe above desiccators, the air was pumped out with air suction to reaching 90% humidity and the pipe was directly closed. For 80% humidity, the air inside desiccators was pumped out to achieve 80% humidity. To make the process goes faster, we put activated silica gel, and the air was pumped out to achieve 60% humidity.

The SEM (Scanning Electron Microscope) examination was as follows: In labial incisor round preparation was shaped, 5 mm in diameter, 2 mm depth into dentin. Then, the available tooth is washed with sterile distilled water spray using 20 cc injection syringe and dried with cotton pellets. The next step the acid was etched (37% phosphoric acid) for 15 second and washed with distilled water from 20 cc injection syringe. Then, it was dried with cotton pellet smoothly and put into desiccators for 1 hour. For group I: 60% humidity, group II: 70% humidity, group III: 80% humidity, group IV: 90% humidity and group IV (control): 65% humidity. Soon after they were taken out from desiccators, the available tooth was given with dentin bonding liquid (Voco) and subsequently filled with self-cured acrylic. Then the tooth was cut by making surrounded patch in the middle of cavity preparation with diamond disk. Through the patch, tooth was cut with chisel and hit with the hammer to become two parts. Before the cutting, tooth was shaped to reach the expected size: having a diameter of less than 10 mm and 1-2 mm thickness. The intended section was sprayed with 20 cc sterile distilled water and left dry in room temperature. Unintended section was marked with marker. Then the tooth was ready to be examined with SEM.

Examination process in SEM: The examined tooth is put into holder (stub) with aluminum powdered special glue (epoxy resin). The tooth was left to dry within 1 day. Holder is cylindric in 10 mm diameter and 5 mm height. Dentin was layered by gold using vacuum evaporator for about 1 hour. Vacuum pressure was about 10^{-5} – 10^{-5} torr. The measurement of resin tags and hybrid layer of SEM can be seen in Figure 1.

A – B space in negative film and printed photo (positive) was measured. For example A – B negative film = 30 mm and printed photo spaced 54 mm, then the difference was 54 : 30 = 1.8. Enlargement (v) was used for example in 3500 times. Enlargement (v) = 3500, means 3500 x 10 μm = 35.000 μm = 35 mm. So, 10 μm would be equal with 6.3 mm. The result was stated in micron (the experiment steps were based on Adioro, 2006). The resulted data were analyzed statistically with one-way ANOVA test at 95% confidence level, and followed up with LSD test.

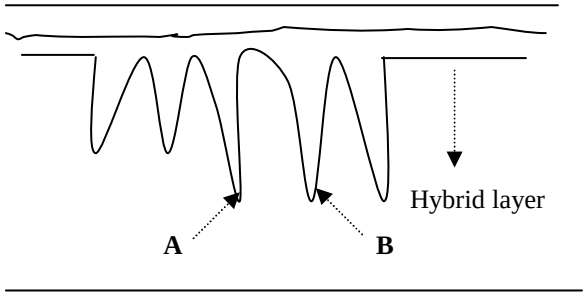

Figure 1. Scheme of tags resin inside dentin tubule A & B is tag corner

RESULTS

Water content measurement in dentin was aimed to know dentin water weight percentage before and after heating to dentin mass with electronic moisture balance. The result of dentin water and SD percentage are shown in Table 1.

Table 1. Mean and standard deviation of water content in dentin (%)

Humidity	N	Mean (%)	SD
60%	5	2.1000[a]	0.3391
70%	5	3.2400[b]	0.4219
80%	5	4.0800[c]	0.3033
90%	5	4.6000[d]	0.3082
control (65%)	5	2.7600[e]	0.1631

Note: ANOVA F : 40.966, p > 0.001 superscript with different letter shows differences at $\alpha = 0.05$.

Table 1 shows that higher humidity at dentin surface causes higher water content in dentin. To find whether the data was normally spreading, we performed one sample Kolmogorov-Smirnov test. It was found that dentin water content at 60% humidity had p value = 0.974 (p > 0.05); 70% humidity had p value = 0.0999 (p > 0.05); 80% humidity had p value = 1.000 (p > 0.05); 90% humidity had p = 0.946 (p > 0.05) and control group (65%) had p value = 0.861 (p > 0.05). P value for all groups was higher than 0.05, indicating that dentin water content had normal distribution.

To find whether dentin water content in experiment groups was homogeneous, we conducted Levene's homogeneity test. From the measurement the p value = 0.874 ($p > 0.05$). It means that dentin water content 60% to 90% humidity was homogeneous, because dentin water content variable had normal distribution. To identify differences among the groups, one way ANOVA test measurement was done. As the result, the p value = 0.001 ($p < 0.05$), showing a distinctive differences among the groups at 60% to 90% humidity. To find differences in every group LSD test was performed. If we compared dentin water content in experiment groups one with another, there were distinctive differences ($p < 0.05$). This condition occurred in all groups of 60% to 90% humidity (Table 2).

Table 2. LSD test among dentin water content experiment group in different humidity.

Humidity	60%	65%	70%	80%	90%
60%	------	+	+	+	+
65%	+	-------	+	+	+
70%	+	+	-------	+	+
80%	+	+	+	-------	+
90%	+	+	+	+	-------

Note : +) there is significant differences at $\alpha = 0.05$
-) there is no difference

In this study magnification used was from 1,000 to 10,000 times. The analyzed subjects were dentin hybrid layer and tags resin lengths. Dentin hybrid is a layer formed from chemical and physical interaction between resin bonding agent and dentin collagen fibril (Nakabayashi and Pasley 1998). In SEM examination, the layer is seen as a whiter layer (more solid) at interface between resin bonding agent and dentin surface (hybrid layer thickness is between 1 μm to 5 μm). Table 3 listed dentin hybrid thickness measurement result (hybrid layer thickness mean and deviation standard).

The highest hybrid layer thickness mean score is well written at control group (65%) that is 5.3 ± 5.6 μm. SD value was high enough since the resulted data had a high space variation, which was minimally 1.5 μm and maximally 11.8 μm. The dentin hybrid layer thickness mean value decreased at 90% humidity group (3.1 ± 0.5 μm) ; 70% humidity (2.6 μm ± 2.0 μm) ; 60% humidity (2.1 ± 0.7 μm) and 80% humidity group (1.4 ± 0.3 μm). Levene's homogeneity test showed that dentin hybrid layer thickness was not homogeneous ($p = 0.05$). To find the dentin hybrid layer thickness data spreading then we carried out normal distribution test. Significant value of 60% to 90% humidity group showed p value of > 0.05, indicating that dentin hybrid layer thickness had normal distribution. To know the difference of every dentin hybrid layer thickness, we analyzed with ANOVA test. The resulted data shows that p value = 0.398 ($p > 0.05$). It means that if the comparison of dentin hybrid layer thickness at 60% to 90% humidity would show no distinctive differences. To prove the dentin hybrid layer thickness difference in each experimental group, Dunnett T3 test was done. From the resulted data, there was distinctive difference only between 80% humidity group and 90% humidity group, while the other experimental groups showed no distinctive difference. SEM measurement to resin tags length, mean and resin tag SD and mean can be seen in Table 4.

Table 3. Mean and SD of dentin hybrid layer thickness (μm)

Treatment	N	Mean (μm)	SD
60%	4	2.1500^{a}	0.7594
70%	4	2.6500^{a}	2.0404
80%	3	1.4000^{a}	0.2646
90%	4	3.1250^{ab}	0.4787
65%	3	5.3000^{a}	5.6560

Note: ANOVA F = 1.099, p = 0.398 superscript with different letter shows significant differences at $\alpha = 0.05$.

Table 4. Tags resin length mean and SD (μm)

Treatment	N	Mean (μm)	SD
60%	4	4.4750^{a}	2.0614
70%	3	2.2000^{b}	0.2646
80%	3	2.4333^{bc}	2.0820
90%	3	3.6333^{abc}	0.1528
65%	3	3.9000^{abc}	0.3606

Note: ANOVA F = 2.621, p = 0.093. Superscript with different letter shows there is significant difference at $\alpha = 0.05$

Table 4 shows that the highest tags resin length mean is at 60% humidity (4.5 ± 2.1 μm). Then it decreased at 65% humidity (control) = 3.9 to 4 μm and 70% humidity = 2.2 ± 0.3 μm. 80% and 90% humidity experiment groups are 2.4 ± 2.1 μmm and 3.6 ± 0.1 μm. To analyze whether resin tag length data was normal, we conducted normal distribution test. 60% to 90% humidity experiment group had p value > 0.05, meaning that the data was normal. To know the homogeneity of the data, homogeneity test was done. It shows that p value of tags resin length was 0.144 ($p = 0.05$). It means the data was homogeneous. ANOVA measurement analysis shows the difference every tags resin length group. ANOVA test result showed p value = 0.093 (p >

0.05). It means that every tags resin length group shows no significant differences at 60% to 90% humidity. With LSD test, it showed that among 60% humidity group and 70% humidity group there was distinctive difference (p value = 0.020), so was 60% and 80% humidity group. But in the other group there was no significant difference.

DISCUSSION

As the result, dentin water content at 60% to 90% humidity at 25°C (constant temperature) significantly increased in relation of humidity increase. It shows that if dentin is in increasing humidity, the more dentin water content it would have. In this research, dentin water content ranged 2.1% - 4.6% weight. This was different from Cohen and Burns (2002) statement who found that dentin water content was ± 10% weight. This difference was because of the examined teeth in Cohen and Burns (2002) were vital teeth, while this study did not use vital teeth, although after being taken from the mouth, they were directly soaked in physiologic salt liquid. In vital tooth, dentin liquid composition is controlled by odontoblast cells. In non vital tooth, odontoblast cells is decaying, there is a protein deposit which can hold dentin tubule lumen. It effects to the decreasing volume of dentin water content (Ozok et al. 2002).

The loss of dentin liquid can be caused by available tooth shaping. Formed heat because of high speed of screwing make a lost of dentin liquid, although the handpiece is hung above the hole to spray the water. So, when tooth root was being cut, heat was also formed. Water molecule can create fibril to expand and it becomes permeable to HEMA monomer. Collagen fibril space is also in maximum condition, so it can penetrate HEMA agent well, and HEMA has good mechanical retention. By the increase of humidity, dentin water content increases along with the increase of water volume. Water molecules around collagen fibril are numerous. It makes HEMA agent bonding ability with the collagen to decrease. It is proved with the quick lost of water around collagen fibril, so that it can disturb collagen network conformation and, therefore, gives bad adhesion effect to resin bonding agent (Feninat et al. 2002). Furthermore, the existence of right water volume will hold intermolecular room among triple helix collagen. Hydrogen bonding will form acceptor peptide group inside collagen. This bonding avoid inter chain contact directly and form compact collagen and survived collagen chain conformation.

If we analyze SEM picture above, dentin hybrid layer thickness does not show regular pattern. It is because of unstitch cutting line during cutting of the tooth, seeing object for tooth is not vertical and the destruction occurring during sample preparation. In this research humidity did not affect the hybrid layer thickness. Hybrid layer thickness is influenced by acetone concentration in dentin bonding agent (Cho & Dickens 2004). They used 27%-69% acetone concentration weight. The result showed that the increased acetone concentration can cause over evaporation, so it facilitated the destruction in hybrid layer.

Survey and analysis result to the tags resin length statistically showed there was no significant difference in dentin hybrid layer thickness. Tags resin length mean at 60% humidity was the highest, then it decreased at 65% humidity (control) and 70% humidity into 3.9 ± 0.4 μm and 2.2 ± 0.3 μm. At 80% and 90% humidity resin tags increased to be 2.4 ± 2.1 μm and 3.6 ± 0.1 μm. From tags resin length pattern, it can be assumed that humidity does not influence the resin tag length. This result is also supported by Nishiyama et al (2003) and Wang and Spencer (2003).

Bond strength of resin bonding at dentin surface can be analyzed with solidity volume tags resin as seen in SEM photo. The tags resin number in the photo is high, so that the bond strength dentin-resin is high. However, it cannot be thoroughly explained in detail because limited capability of SEM scanning. For example, low magnification (50x) clearly showed the boundary between resin bonding with dentin surface and also showed some tags resin. In magnification of 2000 or 3000, the intended spot area cannot be seen although we had searched several times. Thus, the author found difficulties in analyzing the rapidity and tags resin length.

In addition, SEM photo result is a picture of certain spot area. As an example, there were resin tags in mesial area of cavity preparation in the picture. The analyzed part was only that area. Sometimes tags resin was not found in distal area because the limitation of the tool. Another example is that in 2000 times magnification, SEM photo length was ± 120 mm and from the available measurement the SEM (as spot area) in the photo is only 33 μm. Even cavity preparation is 5 mm. The other spot area is unscanned. From the low to high magnification, there are many unseen spot area. It was possibly because the objects seen were not vertical (in relations of unstrict cutting line). The result of SEM survey here supports resin bonding bond to dentin surface. From the measurement, dentin hybrid layer thickness, resin tags length and rapidity had no relations with bond strength resin bonding agent to dentin collagen. This result is compatible with those of Nishiyama et al. (2003) and Wang & Spencer (2003). It can be explained as follows:

If we see microscopic tags resin picture or dentin hybrid layer by SEM, TEM, or optic microscopic, then the interpretation has two dimensional picture, although in reality tags and hybrid layer is three dimensional. It means the microscopic picture is not able to explain the real condition. In SEM photo, for example, dentin tubule usually in strict form, while in reality the tubule is not strict. If this area is cut in the cutting process, then the SEM seen is the survey area around cutting line. While in the other unseen area, it will be explained in Figure 2. A – C dentin tubule length. If the available teeth is cut then from the sight corner, as it seen on the picture A – B, the tubulus length while B – C is not seen. So the condition does not represent the real condition. Another explanation is the way of cutting. The way of cutting is making patch surrounding the tooth right in the middle. Then the patching line is cut with hammer. As the result the cutting line is not strict and irregular cutting line (see in Figure 3).

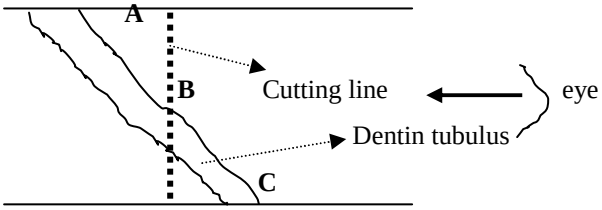

Figure 2. Diagram of dentin tubulus cutting

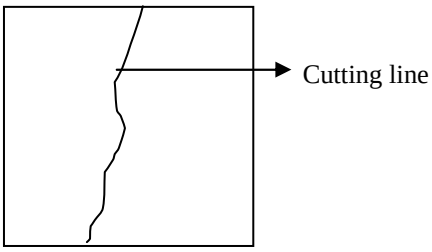

Figure 3. Irregular cutting line

If the available tooth is cut with diamond disk, then the cutting line can be strict. This cutting line is the area analyzed by SEM and the position is not vertical with sight corner. As the result, tags position or hybrid layer is not vertical with sight corner. It means there is a distortion to the tags length and hybrid layer thickness. The above argument is supported by Soetopo (1980) who said that tags length measurement result is various compared to other researchers. It is because the procedure in making available (since tags resin is easily broken), resin viscosity, surface ability to wet, surface tension and holes among the enamel surface and tags length measurement, for example with eye-piece graticule or planimetric.

Another researcher is Nishiyama et al (2000) who revealed that hybrid layer thickness did not relate to bond strength of resin bonding with tooth dentin. They showed that resin bonding strength to dentin would increase if methylene group molecule number along with the increased resin. Then in 2003, research group led by Nishiyama made similar study. As the result, although some experiment groups had the same hybrid layer thickness. However, bond strength between HEMA and resin was different. It is because of different pH HEMA liquid influences hydrogen bonding between HEMA-collagen and influences the pulling strength.

CONCLUSION

The increase of water content in the dentin is along with the increase of humidity. There is no different between hybrid layer thickness, resin tags length and resin bond strength on collagen fibril in different humidity.

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