Preservation of 5300 year old red blood cells in the Iceman

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Changes in elasticity and structures of red blood cells (RBCs) are important indicators of disease, and this makes them interesting for medical studies. In forensics, blood analyses represent a crucial part of crime scene investigations. For these reasons, the recovery and analysis of blood cells from ancient tissues is of major interest. In this study, we show that RBCs were preserved in Iceman tissue samples for more than 5000 years. The morphological and molecular composition of the blood corpuscle is verified by atomic force microscope and Raman spectroscopy measurements. The cell size and shape approximated those of healthy, dried, recent RBCs. Raman spectra of the ancient corpuscle revealed bands that are characteristic of haemoglobin. Additional vibrational modes typical for other proteinaceous fragments, possibly fibrin, suggested the formation of a blood clot. The band intensities, however, were approximately an order of magnitude weaker than those of recent RBCs. This fact points to a decrease in the RBC-specific metalloprotein haemoglobin and, thus, to a degradation of the cells. Together, the results show the preservation of RBCs in the 5000 year old mummy tissue and give the first insights into their degradation.

Keywords: ancient erythrocytes; haemoglobin; protein degradation; Iceman; atomic force microscope; Raman spectroscopy

1. INTRODUCTION

Examining mummies with sensitive analytic tools enables the reconstruction of their ancestry and genetic relationships [1,2], diet, diseases [2], living conditions, state of preservation and the mummification processes [3]. While many studies provided molecular evidence for the presence of infectious diseases in ancient populations, leading to deep insights into the evolution of such diseases [4,5], only a few reports on the recovery of blood from mummified bodies are available. Previous investigations, based on optical or electron microscopy data, postulated that blood remains or fragments could be preserved in mummies as old as 2000 years [6–10]. Although molecular verification of blood findings was not performed, detection of blood components was of major interest because it could give new perspectives on the lives and fates of our ancestors. Blood can indicate the general health status of an individual and it can be analysed to detect pathological conditions or to provide valuable information in forensic crime scene investigations.

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Here, we report the direct detection of red blood cells (RBCs) in tissue samples from the Iceman with an atomic force microscope (AFM) and Raman spectroscopy. Single and clustered RBCs were found, and their characteristic Raman spectra were obtained. The spectra contained Raman bands of proteinaceous remnants, most likely fibrin, which indicates the formation of a blood clot. The Raman spectra, however, also document a degradation of the cells. Their spectral intensity was approximately an order of magnitude weaker than that of recent RBCs. Additional elasticity measurements on the cells imply a loss in RBC stability—which also points to degradation.

2. MATERIAL AND METHODS

Iceman tissue was obtained by punch biopsies from the stab trauma to the right hand (sample A) [18] and from the wound under the left spine scapulae on the Iceman’s back (sample B) [15]. The extracted tissue was rehydrated for 48 h in a 9.5 parts formaldehyde (2%) and 0.5 parts Brij 35 solution, and subsequently fixed with 4 per cent formaldehyde (formalin) for 2 h, dehydrated in an ascending alcohol series and embedded into paraffin wax. Histological specimens were obtained by cutting 2–4 μm thick transverse sections and transferring them onto glass slides. Before AFM analysis, the paraffin was dissolved in xylene. Finally, the sections were rehydrated with a descending alcohol series, rinsed with ultrapure water and dried under ambient conditions [14]. As a reference, a recent human tissue sample, taken from a volunteer and processed in the same manner as the Iceman samples, was used. Additionally, fresh capillary whole blood was drawn from the fingertip of a volunteer, applied to a glass slide and left to dry for 6 h. Furthermore, a glass slide was coated with a meshwork of fibrin, an essential protein formed during the clotting process. The preparation of fibrin was carried out following the protocol of Riedel et al. [20].

Particles with the approximate size and shape of RBCs were identified with an inverted optical microscope (Axiovert 135; Zeiss, Oberkochen, Germany). Then, high-resolution images were taken with a NanoWizard-II AFM (JPK Instruments, Berlin, Germany). The AFM was operated in the intermittent contact mode. Silicon cantilevers (BS Tap 300; Budget Sensors, Redding, CA, USA) with nominal spring constants of 40 N m–1, and the loading force for each measurement was limited to 500 nN. Four recent RBCs and two corpuscles extracted from the arrowhead wound sample on the back of the mummy (sample B) were tested. Only (putative) RBCs that were lying flat on the glass substrate were analysed to ensure good mechanical contact with the substrate. The numerical value for Young’s modulus E was obtained from fitting a Hertzian model [21] on the force curves. Sneddon’s extension [22,23] of the Hertzian model was used to calculate the deformation δ of the flat elastic sample surface penetrated by a rigid spherical indenter (AFM tip) of radius R. The spherical indenter geometry was assumed because the indentation depth of the AFM tip into the sample was small compared with the tip radius. The samples’ Young’s modulus was calculated from

\[ E = \frac{F(1 - \nu^2)}{(a + R^2/a)} \delta - aR^2 \]  

(2.1)

with the sample deformation given by

\[ \delta = \frac{a}{2} \ln \left( \frac{R + a}{R - a} \right). \]  

(2.2)

with F the applied force, a the radius of the contact area between the tip and the sample, and ν the Poisson ratio of the material analysed. The Poisson ratio was set to 0.5, assuming an incompressible material.

For the molecular analysis, a confocal Raman spectroscope (WITec alpha 300 R; WITec GmbH, Ulm, Germany; excitation wavelength 532 nm) was used. To avoid photodegradation, laser power was limited to 1.0 mW. The spectrometer was operated with an 1800 g mm–1 grating. The spectral resolution was 1 cm–1 per CCD-pixel. Three different positions were analysed for each sample, and at least three single spectra, with 180 s of integration time, were taken at each position. Owing to the confocal set-up of the microscope, Raman spectra were collected from a sample area with 300 nm diameter and a focal depth of approximately 1 μm.

3. RESULTS

One corpuscle with a structure likely to be a RBC [24] was found in the hand wound tissue of the Iceman (figure 1d), and two single corpuscles were detected within the arrowhead wound sample. Sample B furthermore showed an agglomeration of several randomly distributed particles (figure 1f). The selected corpuscles exhibit a discoidal, concave surface with a diameter of approximately 1 μm. The mean cell diameter of the ancient corpuscle. The cells are also similar when comparing the mean area and volume of the ancient corpuscles, Raman spectra contained Raman bands of proteinaceous remnants, most likely fibrin, which indicates the formation of a blood clot. The Raman spectra, however, also document a degradation of the cells. Their spectral intensity was approximately an order of magnitude weaker than that of recent RBCs. Additionally, elasticity measurements on the cells imply a loss in RBC stability—which also points to degradation.

\[ \delta = a \ln \left( \frac{R + a}{R - a} \right). \]  

(2.2)

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measurements were performed. The spectral fingerprint region of the particle in Iceman sample A was compared with that of an air-dried recent whole blood sample and with that of recent single RBCs. The recent single RBCs were subjected to the same sample preparation as that of the mummy tissue before examination. All Raman spectra shown in figure 2 exhibit distinct bands at 1586, 1395, 1308 and 747 cm⁻¹, which are assigned to the stretching vibration modes n₃₇, n₂₀/n₂₉, n₂₁ and n₁₅ of porphyrin. Porphyrin is the characteristic building unit of the major RBC protein haemoglobin [25–27]. Furthermore, bands that are typical for other protein components, such as the twisting deformation mode of methylene at approximately 1230 cm⁻¹, were present. The spectrum of Iceman sample A additionally shows two small peaks at approximately 1665 and 1248 cm⁻¹ and two prominent bands at 1446 and 1002 cm⁻¹. The latter are assigned to the proteinaceous deformation vibration of methyl δ(CH₃) and methylene δ(CH₂) molecules and to the vibration mode of phenylalanine [14,28]. The two small peaks at 1665 and 1248 cm⁻¹ are assigned to the amide I (C=O stretching) and the amide III (C−N stretching and N−H in plane deformation modes) groups [29]. A detailed band assignment is shown in table 2. Comparing the intensities of the spectra, a strong decrease in scattering efficiency can be observed for the ancient particle. The intensity of the Raman spectrum of Iceman sample A is approximately an order of magnitude weaker than that of the recent blood samples, although the ratio between the band intensities within the spectrum remained largely unchanged.

When the fingerprint region from 700 to 1720 cm⁻¹ in the particles found in Iceman sample B was analysed, hardly any Raman bands indicated the presence of RBCs (figure 3). A characteristic spectrum of the corpuscles in sample B is shown in figure 3c. The first strong Raman band observed is the amide I peak at approximately 1656 cm⁻¹, corresponding to the C=O stretching vibration ν(CO) typical for proteins. Further prominent bands appear between 1620 and 1600 cm⁻¹, originating from the C=C vibrational stretch mode ν(CC) of the amino acids tyrosine or phenylalanine. Similar to Iceman sample A the corpuscles in sample B feature

Table 1. Dimensions of recent RBCs and ancient corpuscles.

<table>
<thead>
<tr>
<th>sample</th>
<th>quantity</th>
<th>height (µm)</th>
<th>diameter (µm)</th>
<th>area (µm²)</th>
<th>volume (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>recent RBCs</td>
<td>19</td>
<td>2.0 ± 0.5</td>
<td>6.3 ± 0.4</td>
<td>31.0 ± 3.7</td>
<td>40.0 ± 12.2</td>
</tr>
<tr>
<td>ancient corpuscle</td>
<td>3</td>
<td>2.5 ± 0.2</td>
<td>6.0 ± 0.3</td>
<td>28.8 ± 3.2</td>
<td>42.1 ± 4.1</td>
</tr>
</tbody>
</table>

Figure 1. AFM images of RBCs. (a,b) Single RBCs from recent human tissue. (c) An assembly of RBCs. (d,e) Single corpuscles found in Iceman sample A and sample B are shown. An assembly of several randomly distributed corpuscles, similar to those found within the recent sample (c), are displayed in image (f). The imaged corpuscles (d–f) feature the characteristic discoid and concave surface of RBCs.

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Raman scattering. Areas with low intensity are in blue. Specific molecule vibrations are predominant in areas that correlate with the position of the ancient particles. Figure 4c,e,f illustrates the intensity distribution around the bands at 1586, 1395 and 1308 cm\(^{-1}\), which are associated with the porphyrin vibration modes \(\nu_{27}\), \(\nu_{29}/\nu_{29} \) and \(\nu_{21}\). In the wavenumber range 1370–1410 cm\(^{-1}\), no Raman scattering occurred (figure 4c). The molecule vibrations \(\nu_{27}\) and \(\nu_{21}\), however, show a moderate signal at the position of the ancient particles. The highest Raman signals are observed in the wavenumber range of the protein-specific bands, e.g. the amide I peak around 1656 cm\(^{-1}\) (figure 4b), the methyl \(\delta(CH_{3})\) and methylene \(\delta(CH_{2})\) band at approximately 1449 cm\(^{-1}\) (figure 4d) and the C–N stretch mode around 1125 cm\(^{-1}\) (figure 4g).

An analysis of the mechanical properties of RBCs can give further insights into their structural integrity. In the circulatory and cardiovascular system, they are subject to deformation owing to varying flow conditions. To withstand wear, the cells feature an elastic discoidal membrane. This structure, constituting a minimum energy configuration [32], and the dynamic remodelling of their spectrin cytoskeleton [33], enables them to pass through thin capillaries and reach tissues. Because changes in the mechanical properties of RBCs are an indicator of disease and can provide an insight into their molecular preservation, nanoindentation measurements were carried out to assess the elasticity of the fixed, ancient corpuscles. A histogram with Young’s modulus of the mummy corpuscles (grey) and the reference RBCs (black) is shown in figure 5. By way of illustration, a Lorentzian distribution is additionally fitted. For the fixed mummy samples, a mean Young’s modulus of 2.0 ± 1.0 GPa with a distribution maximum of 1.7 GPa was determined. The measurements conducted on fixed, recent RBCs yielded a mean value of 2.5 ± 1.2 GPa, and the distribution maximum was 2.3 GPa. The difference between the mean Young’s moduli is statistically significant and was analysed with the independent two-sample Student’s \(t\)-test.

4. DISCUSSION

To study the presence of human RBCs in the tissue of the 5300 year old Iceman, tissue samples extracted from two Iceman wounds were examined. Performing AFM measurements, isolated single corpuscles with the approximate size and shape of normal RBCs were identified in Iceman samples A and B. The corpuscles featured a discoidal and concave shape, which is typical for RBCs, and their morphology did not demonstrate any evidence of degradation, damage or disorder. Moreover, the dimensions of these ancient corpuscles matched those of the similarly prepared reference RBCs. Additionally, a cluster of several randomly agglomerated particles was revealed in sample B. In this sample, all particles greater than approximately 5 \(\mu\)m showed artificial interfaces, which are presumably cutting edges that arose from the preparation of the histological specimens, i.e. slicing of the tissue with a microtome. Apart from these sectioned
To further confirm the presence of RBCs, Raman spectra were taken from the Iceman samples and compared with reference whole blood and reference RBC spectra. The stretching vibration modes ν37, ν20/ν29, ν2, and ν15, which are characteristic of haemoglobin [25–27], dominated the spectrum of the corpuscle in Iceman sample A. This suggested that the ancient particle is a RBC. However, the scattering intensity of the ancient spectrum was approximately an order of magnitude weaker than that of the recent blood samples. The intensity I of a Raman band depends on

\[ I \propto N \alpha^4 \left( \frac{\partial \alpha}{\partial \eta} \right)^2, \]  

(4.1)

where N is the number of scattering molecules within a sample, I0 is the intensity of the excitation laser, \( \partial \alpha/\partial \eta \) is the change in polarizability of the exited molecules and f is the frequency of the excitation laser. Because the intensity and the frequency of the laser were kept constant during the measurements, and because the bands in the ancient particle spectrum can be clearly assigned to molecule vibrations with defined polarizability, the change in the scattering intensity was most probably caused by the reduced number of scattering molecules within the ancient particle. This fact indicates decomposition of the Iceman RBC, in association with the degradation of the majority of the RBC-specific haem compounds and hence the reduction in scattering molecules. RBC degradation is mainly caused by the action of reactive oxygen species (ROS) such as superoxide \( \mathrm{O}_2^- \) radicals that are released during the autoxidation of oxygen-loaded haemoglobin (oxyHb) [34]. During the dismutation of superoxide, hydrogen peroxide (\( \mathrm{H}_2\mathrm{O}_2 \)) can also be generated [35]. Both ROS cause oxidative stress within the RBC, which eventually leads to the decomposition of proteins due to fragmentation of their peptide chains. The effectiveness of ROS is governed by the oxyHb autoxidation rate of approximately 0.5–3% per day [35,36] and the vast amount of molecular oxygen that can be bound within a single RBC. Besides the action of ROS, damage of the RBC can also be induced by freezing and thawing, as observed in cryopreservation procedures. Thereby RBC injury can be attributed to events such as intracellular and extracellular ice formation, excessive cell shrinkage, osmotic stress or dehydration [37]. Such mechanisms affect the RBC on the microscopic scale, i.e. the protein content and the molecular composition.

The ancient RBC spectrum also showed bands at 1665, 1446, 1248 and 1002 cm\(^{-1}\). These were assigned to the amide I band typical for proteins, the

### Table 2. Raman peak assignment of Iceman sample A.

<table>
<thead>
<tr>
<th>whole blood wavenumber (cm(^{-1}))</th>
<th>red blood cell wavenumber (cm(^{-1}))</th>
<th>Iceman sample A wavenumber (cm(^{-1}))</th>
<th>assignment</th>
<th>mode</th>
<th>literature</th>
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<td>1665</td>
<td>amide I</td>
<td>ν(CO)</td>
<td>[30]</td>
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<td>1565</td>
<td></td>
<td>ν(C=C)as</td>
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<td>[25]</td>
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<td>[25]</td>
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<td></td>
<td></td>
<td></td>
<td>γ15</td>
<td>[25–26]</td>
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with considerable similarities to the spectrum of fibrin. The corpuscles from sample B strongly differ from that of sample A. It has features such as collagen (see the electronic supplementary material). Fibrin is the end product of a complex cascade of coagulation reactions that are initiated at the moment of vascular injury. In the first step, platelets become activated and then become adherent to the damaged vessel wall. They form a primary haemostatic plug. Meanwhile, the enzyme thrombin is produced. Thrombin then cleaves fibrinogen and catalyses the polymerization of fibrin, which subsequently generates a meshwork around the platelet plug and reinforces the haemostatic clot decomposed over time, leaving behind only RBCs and possibly some fibrin fragments. Although our results show more complex RBC spectra, they are in agreement with measurements on recent dried human blood spots conducted by Virkler et al. [41], who showed that dried whole blood is chemically heterogeneous, and its Raman spectrum could be a linear combination composed mainly of the two Raman active components: haemoglobin and fibrin. The clotting process that occurs while blood dries explains the spectral combination. One hypothesis was that fibrin was formed during the clotting, and that it explained the spectral combination. One hypothesis was that fibrin was formed during the clotting, and that it was therefore found in a large concentration in the dried blood spots.

Finally, when analysing the Raman spectra of the pre-processed tissue, the effect of the sample preparation must be taken into account. The ancient and the recent RBC samples were processed into histological sections, i.e. the tissue was fixed with formaldehyde, embedded into paraffin and cut with a microtome. The paraffin was then dissolved with xylene, and the sample was subsequently rehydrated in a descending alcohol series. The dehydration effects of alcohol were found to cause degradation of RBCs because it weakens the membrane–water interactions of the cell membrane [42]. This can lead to the loss of some cellular components and can therefore cause changes in the Raman spectra. Dehydration and the effect of fixatives, such as formaldehyde and glutaraldehyde, on RBC Raman spectra were also reported by Agha-Khiavi et al. [25]. In their study, they showed that the Raman spectra of RBCs, which were fixed in a formaldehyde–glutaraldehyde mixture and embedded into paraffin, were very similar to the spectra of air-dried RBCs. This indicates that the processing of dried RBCs into histological tissue sections does not cause additional alterations of the RBC Raman spectra. Furthermore, because the reference RBCs and Iceman samples were processed equally, preparation-induced

deformation vibration of methyl $\delta(\text{CH}_3)$ and methylene $\delta(\text{CH}_2)$, the amide III band that represents peptide bonds and the vibration mode of phenylalanine. All these bands are common in proteins such as collagen or fibrin [30,31,38,39], and the amide III band is also occasionally observed in the spectra of air-dried RBCs and met-RBCs, as discussed by Asghari-Khiavi et al. [25]. Compared with reference protein spectra and those of the particle found in Iceman sample B (figure 3), the protein band at 1665 cm$^{-1}$ is very faint in the ancient RBC spectrum, whereas the other protein bands are more pronounced. Thus, the Raman spectrum of Iceman sample A is a complex composition of haemoglobin and other protein spectra.

In summary, the altered spectral signature and intensity in comparison with data of recent RBCs were most probably caused by a modification of the haemoglobin and, consequently, the porphyrin. The proteinaceous compounds may have been less strongly affected. Some molecule vibrations could have also been impaired by the effect of blood clotting and the formation of proteins such as fibrin.

Changes in molecular structure owing to protein compounds are even more evident in the corpuscle from Iceman sample B. Although the corpuscle morphology resembles the structure of regular RBCs, the Raman spectra significantly differed from the reference RBC spectra. The corpuscles mostly lacked the previously mentioned porphyrin modes and predominantly showed bands characteristic of fibrin or other proteins such as collagen. The Iceman red blood cells (2586)

Figure 3. Raman spectra of the corpuscle in Iceman sample A (a), a fibrin meshwork (b), and the corpuscles found in the Iceman tissue sample B (c). The spectrum obtained from sample B strongly differs from that of sample A. It has features with considerable similarities to the spectrum of fibrin.
differences are rather unlikely. Therefore, we conclude that the processing of the tissue had little influence on the comparative spectroscopic examination.

Deformation and failure phenomena of hierarchical protein materials are observed in physiologically extreme conditions and in the progression of disease [43]. The structural proteins and thus the shape, molecular structure and the elasticity of RBCs are also prone to disease-specific alterations [44]. Reduced mechanical deformability, together with increased RBC membrane stiffness, are reported in infection with the malaria parasite [45–47]. A similar phenotype appears in blood disorders such as sickle-cell disease, hereditary elliptocytosis or hereditary spherocytosis, in which the deformability of the RBC membrane is reduced and the cell shape is strongly altered [47–49].

Owing to the pre-processing of the tissue samples, however, we cannot draw conclusions on any disease-specific mechanical changes of the RBCs. Nevertheless, a relative comparison between equally processed recent and mummy RBCs helps us to assess the degree of tissue preservation. AFM nanoindentation measurements revealed changes in the mechanical behaviour of the RBCs. Young’s modulus of the ancient RBCs in sample B was 2.0 ± 1.0 GPa, whereas the modulus of the equally processed recent RBCs averaged 2.5 ± 1.2 GPa.

Cross-linking owing to fixation with formaldehyde [50], age [51] or disease-specific influences would lead to an increased membrane stiffness of RBCs, and, thus, to an increase in Young’s modulus. Our measurements on ancient RBCs, however, show a decrease in Young’s modulus associated with a lower stiffness of the ancient RBCs. Together with the reduced Raman scattering intensity, the softening indicates a degradation of the RBCs. Possible degradation processes include scaffold damage due to crystallization of ice during freezing, irradiation with UV light or wound healing-specific transformation processes that occur during the stages of blood clot degradation [52].

The fragmentation of the RBC cytoskeleton proteins such as the spectrin and actin filaments will cause a destabilization of the cell membrane. In addition to the aforementioned mechanisms, cleavage of membrane proteins and degradation of the cytoskeleton by the fragmentation of their protein peptide chains can also be induced by the action of ROS. The various degradation processes would ultimately lead to the softening of the RBC membrane.

Table 3. Raman peaks assigned for Iceman sample B.

<table>
<thead>
<tr>
<th>RBC wavenumber (cm⁻¹)</th>
<th>fibrin wavenumber (cm⁻¹)</th>
<th>Iceman sample B wavenumber (cm⁻¹)</th>
<th>assignment mode</th>
<th>literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1639</td>
<td>1666</td>
<td>1656</td>
<td>amide I v(CO)</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>1617</td>
<td>1605</td>
<td>v(C=−C)m, Phe, Tyr</td>
<td>[29,30]</td>
</tr>
<tr>
<td>1586</td>
<td>1581</td>
<td>1583</td>
<td>v(C=C)m, Pro, Hypro</td>
<td>[29]</td>
</tr>
<tr>
<td>1563</td>
<td>1447</td>
<td>1449</td>
<td>δ(CH₂, CH₃)</td>
<td>[29]</td>
</tr>
<tr>
<td>1441</td>
<td>1402</td>
<td>1399</td>
<td>δ(CH₂)</td>
<td>[31]</td>
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<td>1395</td>
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<td>1340</td>
<td>v₂₀, v₂₉ v(pyr quarter-ring)</td>
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<tr>
<td>1340</td>
<td>1252</td>
<td>1248</td>
<td>v₂₁ v(pyr half-ring)sym</td>
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<td>1208</td>
<td>1174</td>
<td>amide III v(CN)</td>
<td>[29,31]</td>
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<td>1157</td>
<td>prop δ(CH₂) twisting</td>
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<td>1125</td>
<td>C–H bend Tyr</td>
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<td>δ(C₆H₂)as, δ(CH₂)</td>
<td>[25,27,31]</td>
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<td>v₄₅ v(C₆C₄)₅₃₅, v(CC) aromatic ring Phe</td>
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<td>957</td>
<td>v(C₆–C₆)</td>
<td>[25]</td>
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<tr>
<td></td>
<td>937</td>
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<td>γ(C₆H₂)sym</td>
<td>[26]</td>
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<td>898</td>
<td>γ(C₆H₄), v(CC) aromatic ring Tyr</td>
<td>[28,30]</td>
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<tr>
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<td>854</td>
<td>855</td>
<td>v(CC) aromatic ring Tyr</td>
<td>[30]</td>
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<td>829</td>
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<td>aromatic ring breath</td>
<td>[30]</td>
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<tr>
<td>746</td>
<td>758</td>
<td>758</td>
<td>v(pyr breathing)</td>
<td>[25,26]</td>
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</table>
The elasticity values determined are given for fixed recent and ancient RBCs, which were prepared following the same protocol. Obviously, the preparation comprises the mechanical properties of a tissue by the formation of methylene bridges that cross-link polypeptide chains. Thus, no conclusions on health status can be drawn from the elasticity measurements. However, comparing the elasticity values of both specimens, it is clear that the ancient samples were softer. This is in line with the observation that less Raman scattering occurred in the ancient RBCs. Both effects can be explained by a degradation of the proteinaceous content of the RBCs.

In summary, the morphology and the Raman fingerprint of some corpuscles point to remnants of a haemostatic clot. This observation confirms that the Iceman sustained several injuries before his death. AFM imaging revealed RBCs with normal morphology. Blood disorders caused by RBC membrane defects, such as sickle-cell disease, elliptocytosis or spherocytosis, can thus be excluded. Nanoindentation measurements show that the elasticity of the ancient RBCs is slightly reduced, which suggests that they suffered from degradation. Complementary Raman spectroscopy also indicates a degradation of the blood cells. Nevertheless,
Figure 5. The distribution of Young’s moduli from the corpuscle of Iceman sample B and contemporary single RBCs. Young’s modulus for the mummy particles (grey) is significantly lower than Young’s modulus for the recent RBCs (black).

our examinations show an unambiguous identification of RBCs in a 5300 year old mummy.

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