M. Pfeifer

Blood as a Soil on Surgical Instruments: Chemical Profile, Cleaning, Detection

Classified as medical devices, surgical instruments require standardised and validated reprocessing. The procedure conventionally employed here is cleaning and disinfection in a washer/disinfector followed by sterilisation in an autoclave. Since the organic contamination of surgical instruments encountered in practice can be very pronounced, cleaning constitutes the basis for the subsequent reprocessing steps of disinfection and sterilisation. In this respect, blood represents the most common and hence the most important form of contamination.

Keywords: Blood coagulation, fibrin fibres, denaturation, residual soil, hydrolysis

1 Introduction

By virtue of the exceedingly complex composition and broad spectrum of functions of blood, enormous difficulties continue to be faced when trying to reproduce this fluid as a substitute for donor blood (1). But if one views blood as a cleaning problem when reprocessing surgical instruments, the majority of its biological functions can be disregarded. The only aspect meriting attention in this context is the process of blood coagulation with the end product fibrin. Since, to perform their preordained tasks, all blood components are necessarily present in a water-soluble form, the insoluble fibrin fibres formed during coagulation are particularly relevant for cleaning.

Of importance concomitantly are the chemical properties of the proteins, since most of the compounds found in the blood are such proteins. Even though approx. 45% of the blood is composed of red blood cells (erythrocytes), these need not be dealt with separately because, during the cleaning process, they very easily release through haemolysis the protein haemoglobin which accounts for up to 95% of their contents (2).

1.1 Properties of the Proteins

Proteins are generally composed of 20 different ammo acids, linked together by means of very stable peptide bonds. Encountered here are molecular masses ranging from 10,000 to several million. Due to this complexity, the structure of these compounds is very complex, thus explaining the high sensitivity to chemical and even physical influences.

Despite the high molecular masses, the blood proteins are water-soluble compounds. The ionizable side chains as well as the spatial arrangement (tertiary structure) of the peptide chain itself play an important role. But it is precisely the tertiary structure of the proteins that is amenable to change mediated by external influences: this process is called denaturation.

Unfolding of the polypeptide chain detracts in most cases from solubility. In the case of blood proteins on a surface, this process results in hardening (coagulation) due to the formation of high-molecular aggregates. Temperatures between 60-70^{o} C suffice for complete denaturation. An important consideration here is that denaturation is very dependent on the presence of water. For example, in a dry state casein forfeits its solubility only after 6 hours at 130^{o} C, whereas blood immediately coagulates on immersion in boiling water (3).

Other denaturing factors are marked acidic or alkaline reactions of the solution, organic solvents. UV light and chemicals, such as e.g. urea or guanidine. Furthermore, aldehydes can also cause proteins to undergo direct chemical change (4). But the agent inducing the most hardening action is glutaraldehyde due to cross-linking of proteins (5).

1.2 Blood Coagulation

Blood coagulation induces solidification of the liquid blood in the blood clot, thus sealing traumatised blood vessels. This ultimately entails the formation of insoluble fibrin fibres from the soluble fibrinogen present in the blood. This

multi-step process, in which almost 20 different substances are involved, can be activated by two different pathways (6):

The extrinsic pathway triggers coagulation due to the vascular injury itself. This form of activation is effected within the space of seconds.

The intrinsic pathway is initiated by contact with an unphysiological surface. This pathway is activated only within a few minutes.

During the most important step here, thrombin cleaves two fibrin peptides from fibrinogen. Hitherto, the latter had prevented fibrinogen aggregation thanks to their highly negative charge and due to occupation of binding sites. The fibrin monomers thus formed polymerise and are further stabilised by coagulation factor XIII (cross-linking due to isopeptide bonds). A network of fibrin fibres, enclosing the blood cells, is thus formed (6).

2 Materials and Methods

The soil exclusively employed for the investigations was fresh, coagulable human blood. Dosing only during the first four minutes after withdrawal of the blood ensured that coagulation would take place on the test bodies, thus making it possible to take account of the important fibrin portion. The test bodies used were stainless-steel sheets (stainless steel material 1.4301) measuring 20 x 70 mm.

Since the lack of standardisation of human blood precludes the procurement of exact reproducible findings, quantitative results were largely omitted. Hence what was investigated here was the chemical profile of blood in respect of cleaning and of detection possibilities of any residues. Quantitative investigations, as required for verification and optimisation of cleaning processes, must hence be conducted with a standardised test soil.

3 Results

3.1 Solubility

Figure 1 shows protein detachment (in an immersion experiment without mechanical cleaning component) of a dried blood soil at 18 ^{o}C in demineralised water. Here it is demonstrated that after approx. 8 minutes no further reduction is made in the remaining residue. The residual white layer is composed of insoluble fibrin fibres, which account for approx. 4% of the total protein content of this blood sample. This confirms that the red blood cells easily release their well-soluble haemoglobin content. Translated into a practical setting, this means that already pre-cleaning with clean water can remove the greatest part of contamination.

Conversely, a problem is caused by the fibrin fibres which cannot be dissolved even with surfactants, which, not least, becomes obvious in the fact that in the production of fibrin preparations sodium dodecyl sulphate is used for their cleaning (7). The fastest way to dissolve fibrin is by using an alkaline detergent at as high a temperature as possible. By means of hydrolytic cleavage of peptide bonds this generally degrades insoluble proteins to smaller and hence more soluble products.

Figure 2 shows the hydrolytic kinetics (detachment from a stainless steel surface) of fibrin, in one instance at 100 ^{o}C in 1 molar sodium hydroxide solution (procedure for elution of residual soils) and once at 65 ^{o}C in 0.1 molar sodium hydroxide solution (similar conditions as in automated reprocessing). In neither case are the proteins completely decomposed to amino acids. This would require at least 5 hours of heating with reflux in 5 molar sodium hydroxide solution (8). But the hydrolysis products of the proteins are in solution.

3.2 Repercussions of Denaturation or of Chemical Change

Is has already been mentioned that heat in conjunction with water results in coagulation of blood proteins. Figure 3 illustrates the quantity of blood protein remaining on the stainless-steel surfaces after a 10-minute immersion in demineralised water at 50-80 ^{o}C. Noticeable here is that even at 80 ^{o}C. approx. one third of the proteins are detached from the surface. The remaining residues, however, were in all cases hardened due to coagulation and evidence increased adhesion to the surface. Hence, in automated reprocessing the temperature employed for pre-cleaning should never exceed 40 ^{o}C.

In a dry state proteins are largely insensitive to thermal denaturation. Accordingly, dried blood can be easily heated for

10 minutes up to 100 C, without the proteins forfeiting their water solubility. Much more resistant soils are obtained due to the effects of aldehydes contained in disinfectants, which easily react with the amino groups of the proteins (9). Glutaraldehyde hardens blood proteins so much that, during hydrolysis at 65 ^{o}C in 0.1 molar sodium hydroxide solution, even after two hours no solution can be observed. The elution procedure (In NaOH/100 ^{o}C/lh) used here for determination of residual proteins leads, depending on the layer thickness, to quantitative elution only after approx. 50 minutes. Conversely, iodine-and alcohol-based disinfectants generate only a slight change, which does not essentially hamper reprocessing with alkaline detergents.

3.3 Detection Methods

Myriad detection methods are described in the literature (10). In all these determination methods it is, however, essential to quantitatively bring the residual soil to solution. Since in the case of a residual soil in washer/disinfectors, water-soluble, surfactant-soluble and easily hydrolysed proteins have been removed and the remaining proteins have been hardened due to the thermal final disinfection and heat denaturation, simple elution, for example with a surfactant solution or with a sodium hydroxide at room temperature, does not suffice. Moreover, methods used to determine the free amino groups of proteins are too imprecise, since for example after chemical changes induced by aldehydes, no amino groups can be detected any more. Concomitantly, the number of free amino groups changes depending on the hydrolytic cleavage of a protein.

UV absorption has proved to the method of choice, which is based on the light absorption of aromatic amino acids. This method has the advantage of enabling hydrolysis to be employed as an elution method, since absorption at 240 nm is not dependent on the degree of hydrolysis. Accordingly fibrin fibres, denatured proteins and even proteins that have been cross-linked by glutaraldehyde can be quantitatively acquisitioned. In addition, haemoglobin as the chief constituent of the blood can be separately acquisitioned due to the maximum absorption of its hydrolysis products which is 388 nm.

Residual proteins can also be well recognised visually. As illustrated in figure 4, even 0.001 mg (lug) of haemoglobin can be easily seen on a stainless-steel surface. The haemoglobin had been distributed by dosing aliquots of 10 ul of a corresponding standard solution, and thus takes up a surface of approx. 15 mm^2. Of course, visual inspection does not permit either quantitative or qualitative classification of a residual soil.

4 Discussion

The experiments conducted here give an insight into the manifold investigation methods, attributable to the ultra complexity of the chemistry of the proteins. For example, denaturation is also largely a function of the pH value, while in practice various pH values can be encountered due to neutral, alkaline and acidic detergents. More thorough, quantitative investigations should be conducted in all these domains, using a standardised test soil, since only in this manner exact, reproducible and comparable results can be obtained.

It has been concomitantly revealed that elution with sodium dodecyl sulphate (SDS) does not suffice for quantitative determination of residual proteins in prac-tice. Conversely, in laboratory experiments a high recovery rate can be obtained by mechanical fragmentation of test bodies. Determination of free amino groups with o-phtaldialdehyde (OPA) also entails problems, since their number in the case of chemically transformed and partially hydrolysed proteins is no longer proportional to the baseline protein quantity. Using hydrolysis and measurement of the UV absorption, residual proteins can, conversely, be reliably and quantitatively acquisitioned.

References/Literatur

1. Spektrum der Wissenschaft 1998 (4): 56-61.
2. Mutschler E: Arzneimitteiwirkunyen, Lehrbuch der Pharniakoloigie und Toxikologie, 6.. vollig neu bearbeitete und erweiterte Auflaye: 353-354.
3. Joly M: A Physio-chemical Approach to the Denaturation of Proteins 1965: 162.
4. Fraenkel-Conratu H. Oclott HS: J. Amer. Chem. Soc. 1948:70:2673.
5. Rompp Chemie Lexikon, 9.. erweiterte und neubearbeitete Auflaye: 1607.
6. Voet D. Voet JG: Biochemie. 1 Korr. Nachdruck, 1994:1100-1109.
7. Duhamel RC. Meezan E. Brendel K: A simple procedure for the purification of bovine fibrin from clottet blood by

the use of detergents. Prep. Biochem. 1980; 10 (1):43-58.
8. Neurath H: The Proteins. Composition. Structure and Funktion. Second Edition. Volume 1: 35.
9. Fraenkel-Conratu H. Oclott HS: J. Amer. Chem. Soc. 1948:70: 2673.
10. Rompp Chemie Lexikon. 9., erweiterte und neubearbeitete Auflage: 3655.

HOME