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SURFACE MORPHOLOGY OF UNUSED AND USED HYDROMER^{R}-COATED INTRAVENOUS CATHETERS

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Abstract

Hydromer^{R}-coated polyurethane (Erythroflex)^{R} catheters, unused, or intravenously inserted for 2-20 days, were studied by scanning electron microscopy (SEM). Both unfixed and fixed (2% glutaraldehyde in phosphate buffer), and air-or critical-point dried (CPD) specimens were investigated. The catheter segments were sputter-coated with approx. 20 nm gold and studied at an accelerating voltage of 20 kV. The specimens were examined for surface depositions, thickness and structure of the Hydromer^{R} layers, and occurrence of adhering and embedded bacteria.

The outer Hydromer^{R} layer showed, in the unused specimens, scratches and fissures, as well as adhering foreign bodies. In used specimens, the layer was swollen, with cracks (like "dried earth"), and, occasionally, amorphous substances and coccoid bacteria were seen adhering. Damage to the layer, or even its total disappearance was also noted in some specimens.

The inner (luminal) Hydromer^{R} layer was, in unused specimens, clean and slightly wavy. In used catheters, it was thicker, possibly swollen, with small, isolated or agglomerated protrusions, like a "lunar landscape". Adhering platelets and amorphous substances were also occasionally seen.

The results suggest that the Hydromer^{R} is a fragile material in both its dry and wet forms. Thus, the Hydromer^{R}-coated catheters should neither be stored in flexible packs, nor inserted by the Seldinger technique. The findings do not support the belief that the Hydromer^{R}-coating can prevent either thrombus formation, or intraluminal occlusion of the in-situ catheters.

KEY WORDS: Intravenous catheters, Catheter structure, Surface morphology, Coated substrate having a low coefficient of friction, Hydrophilic catheter-coating, Hydromer^{R}-coating, Hydrogels, Poly-N-vinyl-pyrrolidone, Polyurethanes

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Introduction

Poly-N-vinyl-pyrrolidone (P-NVP)-coated polyurethane catheters have a very low friction coefficient when wetted with an aqueous liquid[11]. Such catheters (e.g. CardioSearch Hydromer^{R}-Erythroflex^{R}) have, in some studies, been associated with a lower degree of platelet adhesion and thrombus formation than that of conventional materials such as polyvinylchloride, silicone elastomer and polyurethanes, both in vitro and in vivo[3]. It was therefore concluded by Crowley and Borrow[4] that Hydromer^{R}-untreated polyurethane catheters should not be used because of high thrombogenic activity. It was also postulated by CardioSearch[3] that Hydromer^{R} acts like a sponge, drawing water from body fluids, providing an effective barrier between the blood and the catheter.The platelets should therefore not so readily adhere, and a reduction in the formation of the fibrin sleeves, and mural or occlusive thrombi should also be expected.

There have also been reports on negative properties of P-NVP used as an antithrombogenic coating. Hydrophilic polymers are brittle in the dry state[10], and fissures and cracks possibly occurring in the dried P-NVP-layer may represent areas of high surface energy serving as stimuli for blood coagulation[5]. P-NVP is also extremely soluble in water[8], and a higher degree of adsorption of plasma proteins (particularly fibrinogen) onto the surface of P-NVP-coated silastic catheters compared with the uncoated ones was also reported[9]. The latter facts thus bring into question the short-and long-term antithrombogenic effects of P-NVP-coating when used on intravascular catheters.

The present paper reports on SEM-studies performed on unused and used P-NVP-coated intravenous catheters (Hydromer^{R}-Erythroflex^{R}) to better understand the relevance of the above mentioned findings for the clinical use of these devices.

Materials and Methods

Ten mm specimens (cut with razor blades) of unused CT-11615-D-Hydromer^{R}-Erythroflex^{R} venous catheters were treated as follows:

Unused catheters had been stored in either plastic bags or in the original, rigid blister-

packages. They were inserted into the superior caval vein using the Seldinger technique. The catheters were moistened with isotonic saline to facilitate insertion. They were kept in place for 2 to 20 days. Before removal, the orifice in the skin at the insertion site was enlarged with a scalpel. Two of the 5 catheters, indwelling 2 and 6 days, were flushed with 20 ml of isotonic saline after withdrawal. Parts of these catheters were cut with a razor blade and air-dried without fixation. Other parts were fixed and air-dried. The 3 other catheters, indwelling 6,7, and 20 days, were flushed with 20 ml of air, fixed, and critical-point dried.

Fixation was performed by immersing the specimens for 24 h in 2% phosphate buffered glutaraldehyde (pH = 7.3). The fixed specimens were carefully rinsed with sterile, distilled water. After rinsing, some specimens were air-dried in a desiccator for 3 days, whereas other were dehydrated for critical-point drying. Dehydration was performed by passing the samples through a graded series of ethanol solutions (50%, 70%, 80%, 85%, 90%, 20 min in each; 95%, 30 min; 99.5%, 2 changes of 30 min). Critical-point drying was performed for one hour with liquid CO_2 using a Polaron critical-point drier.

After drying, the specimens were mounted on stubs using a liquid carbon glue and coated with approx 20 nm of gold in a sputter-coater (Edwards S150, Edward's High Vacuum, Crawley, Sussex, G.B.) equipped with a water cooled stage. Sputtering was performed in 3 intervals, 30 sec each.

During all steps of preparation, the specimens were very carefully handled to prevent accidental surface damage.

The specimens were studied in a combined scanning and transmission electron microscope (JEOL 100 CX) used in the scanning (SE) mode, accelerating voltage 20 kV. Plane surface views of outer and inner surfaces and cross-sections were examined. Selected, representative, standardized areas were photographed at the same magnification.

The tip of the catheter kept in vivo for 20 days was cultured for bacteriological diagnostics. It was found colonized by Staphylococci epidermidis.

Results

Unused specimens

The Hydromer^R-Eryhtroflex^R catheter consisted of a core (Erythroflex^R carrying polymer) in which electron dense crystals of barium sulfate were embedded. These crystals were often found to be located in "lacunae" in the polymer, giving it a "Swiss-cheese"-like appearance. The outer and the inner layers were coated by P-NVP (Hydromer^R) (fig. 1).

The outer Hydromer^R layer of unused specimens stored in pliable, plastic bags was covered by a large number of foreign bodies and presented deep cracks and fissures, occasionally reaching down to the core polymer (figs. 2 a,b,c). In specimens preserved in rigid packages, the number of adhering foreign bodies was very low, and scratches were only superficial (fig. 2 d). The inner Hydromer^R layer was found to be clean and slightly wavy (figs. 3 a,b).

Used specimens

The structure of the core, as judged by SEM of cross-sections, appeared similar in unused and used specimens.

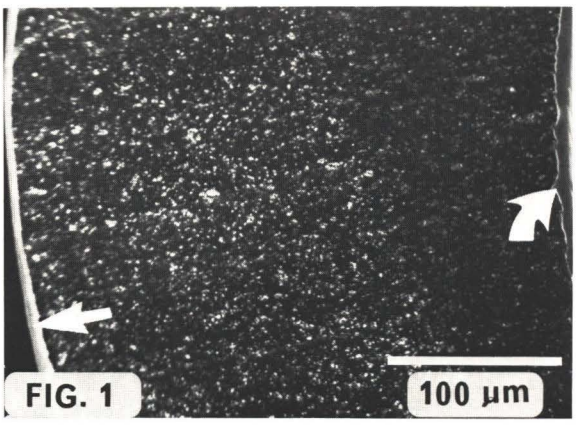

Fig. 1. Unused Hydromer^R-Erythroflex^R catheter. Cross-section. Unfixed, air-dried specimen. Note the "core" ("carrying-polymer",i.e., Erythroflex^R) and the outer (straight arrow) and inner (curved arrow) Hydromer^R layers.

The outer Hydromer^R layer was thicker than that in unused specimens. Cracks were also more frequent in both air-dried (fig. 4 a) and CPD-specimens (fig. 4 b) giving them an appearance like "dried earth". After indwelling up to 7 days, foreign bodies were infrequent on the surfaces, whereas on specimens taken from the 20-day catheter coccoid structures and an amorphous substance were observed (fig. 4 c). The Hydromer^R layer was at least 5-6 µm in most specimens, even in those indwelling for 20 days, but, in some cases, its patchy degradation (fig. 4 d), or even total disappearance (fig. 4 e) was seen.

The inner Hydromer^R layer presented, in some specimens, small, bulging protrusions, occurring isolated or in groups, giving it a "lunar landscape" appearance (fig. 5 a). Platelets were found adhering to the inner surface of one sample (fig. 5 b). The inner Hydromer^R layer appeared swollen in most specimens (fig. 5 c), and the thickness was always the same as, or greater than that of the unused specimens, even after 20 days indwelling. The luminal, cross-sectioned area was in some specimens significantly reduced due to the presence of an amorphous substance adhering to the surface (fig. 5 d).

Methods of preparation

With all methods used, smooth and even Hydromer^R layers were seen (particularly on the luminal walls), as well as areas of altered surface morphology exhibiting cracks, reduction of thickness, and peeling. Among possible artifacts can be mentioned occurrence of "bubbles", bursting under the electron beam in fixed, air-dried specimens, and a "honey-comb"-like pattern of Hydromer^R flakes in the CPD-ones.

For studies of the Hydromer^R layer itself, as well as of crystals and proteins adhering to it, unfixed and air-dried specimens showed the most consistent morphology.If, however, the relationship between the catheter surface and formed elements, such as blood-cells and bacteria was to be studied, fixation was found to be necessary, and, in this case, fixation had to be combined with an efficient method of drying, such as critical-point drying.

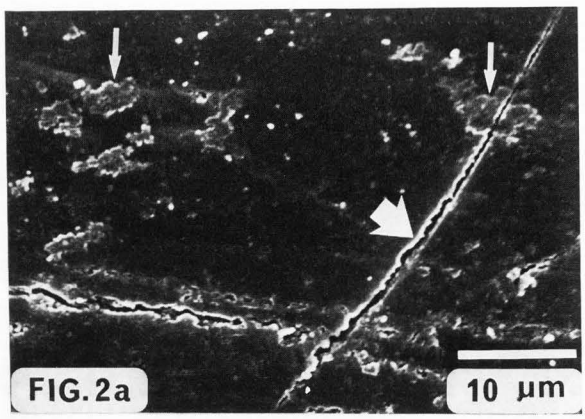

Fig. 2a. Unused specimen stored in a plastic bag. Outer surface, flat overview. Unfixed, air-dried specimen. Note discontinuities and cracks (large arrow) in the surface, and foreign bodies (small arrows) adhering to it.

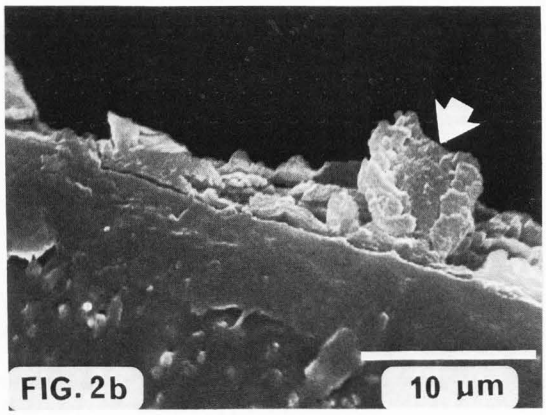

Fig. 2b. Unused specimen stored in a plastic bag. Cross-section. Unfixed, air-dried specimen. Note the outer Hydromer^{R} layer covered by foreign bodies (arrow) and the "cheese-like" appearance of the core polymer.

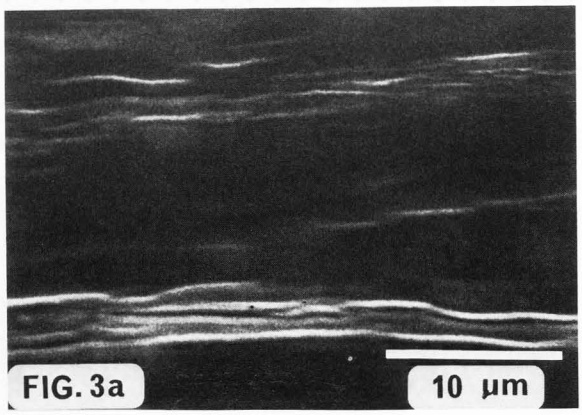

Fig. 3a. Unused specimen from original package. Inner surface, flat overview. Unfixed, air-dried specimen.Note slightly wavy, but basically clean and even Hydromer^{R} layer.

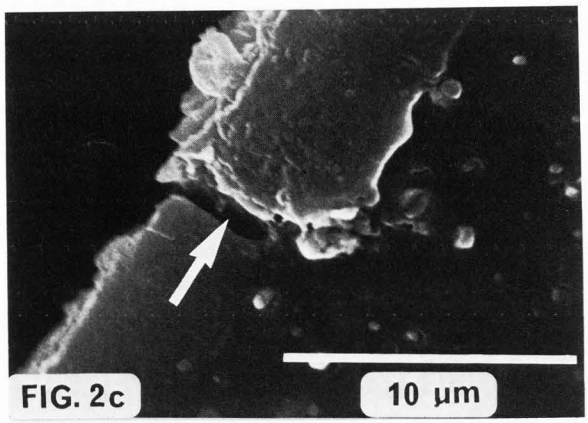

Fig. 2c. Unused specimen stored in a plastic bag. Cross-section. Unfixed, air-dried specimen. Deep fissure (arrow) in the outer Hydromer^{R} layer, extending down to the "core".

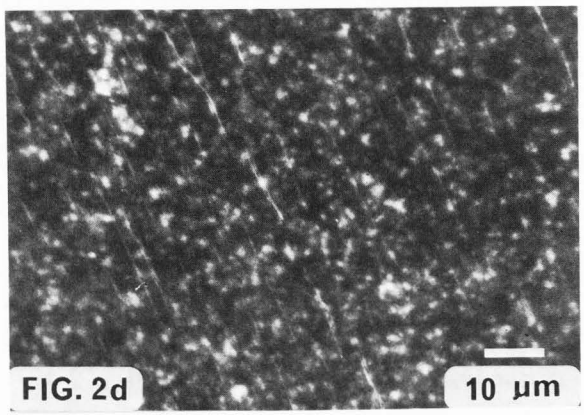

Fig. 2d. Unused specimen from original package. Outer surface, flat overview. Unfixed, air-dried. Note superficial scratches, but no foreign bodies. White dots represent barium sulfate crystals embedded in the core, under the Hydromer^{R} layer.

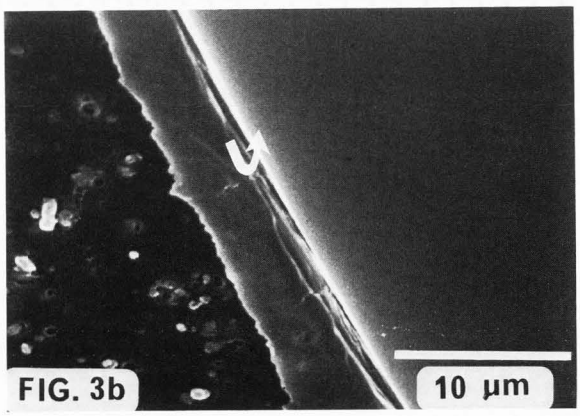

Fig. 3b. Unused specimen from original package. Unfixed, air-dried. Slanting section. Clean, inner Hydromer^{R} layer. The arrow starts from the slanting section of the inner Hydromer^{R} layer and ends at its luminal surface.

Discussion

SEM-studies of Hydromer^R-coated catheters

Unused specimens. The Hydromer^R surface was found to be brittle in its dry state. This may account for the occurrence of cracks, scratches and deep fissures observed in the outer layer, particularly in specimens stored in pliable plastic bags. The thin, dry Hydromer^R layer behaved like a rigid "sugar-glaze" applied on a flexible base (the polyurethane core). Therefore, the layer cracked when the catheter was bent, or subjected to other forms of trauma.

Adhesion of the numerous foreign bodies, sticking particularly to the outer Hydromer^R layer, may be explained by the charge (probably negative) of its surface. The foreign bodies may represent dust particles detached from the package, or even Hydromer^R fragments separated from

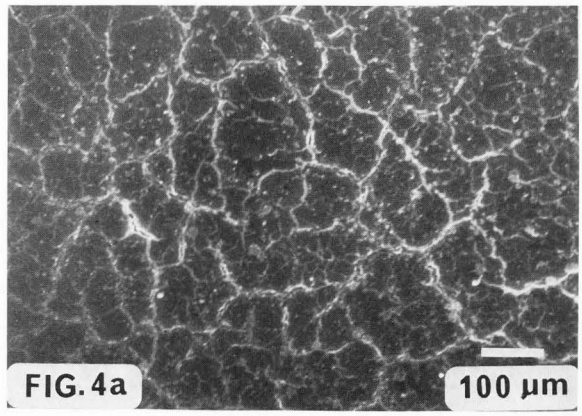

Fig. 4a. Outer Hydromer^R layer after 2 days in place. Flat overview. Fixed, air-dried specimen. Note swollen Hydromer^R layer with cracks, like "dried earth".

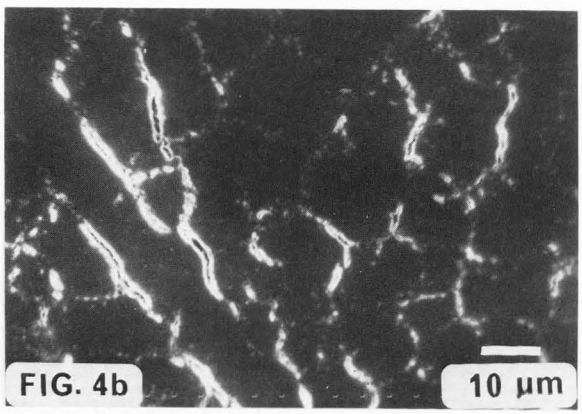

Fig. 4b. Outer Hydromer^R layer after 6 days in place. Flat overview. Fixed, critical-point dried. Note cracks in the surface giving it an appearance like "dried earth".

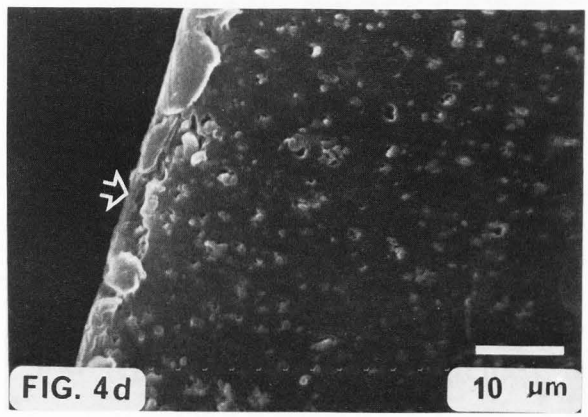

Fig. 4d. Outer Hydromer^R layer (cross-section) after 6 days in place. Fixed, air-dried. Note degradation of the Hydromer^R layer (arrow).

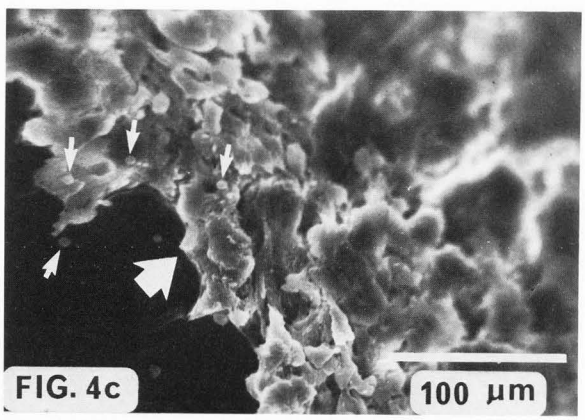

Fig. 4c. Outer Hydromer^R layer after 20 days in place. Flat overview. Fixed, critical-point dried. Note the amorphous substance (big arrow) and coccoid structures (small arrows) adhering to the surface.

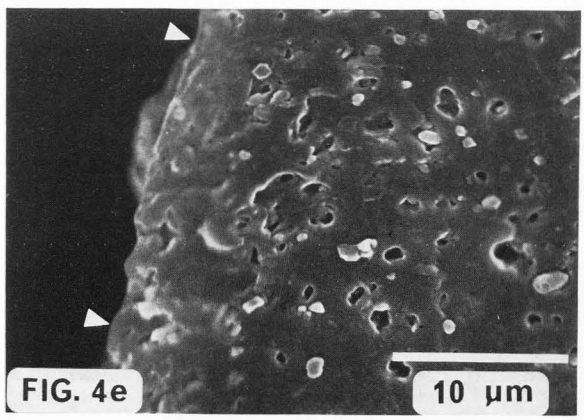

Fig. 4e. Outer Hydromer^R layer (cross-section) after 6 days in place. Fixed, air-dried. Note disappearance of Hydromer^R layer and exposure of uncoated "core"-polymer (arrows).

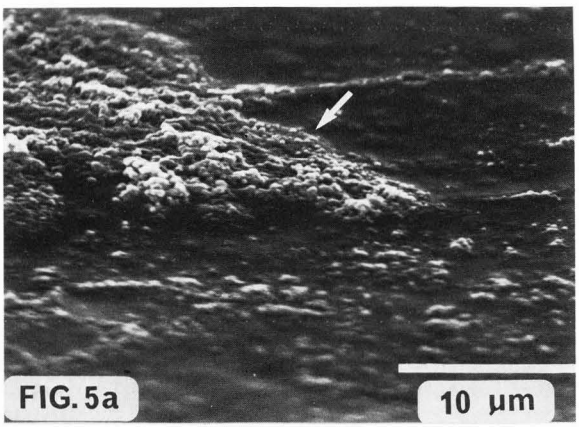

Fig. 5a. Hydromer^R layer (view of inner surface) Fixed, air-dried. Note small, bulging protrusions giving the surface a "lunar landscape" appearance (arrow).

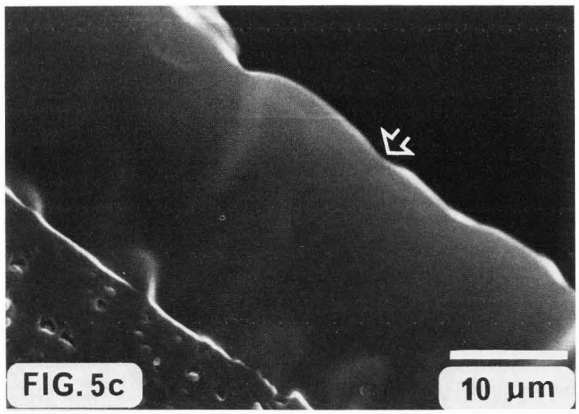

Fig. 5c. Inner Hydromer^R layer (cross-section) after 2 days in place. Fixed, air-dried. Note the swollen Hydromer^R layer (arrow).

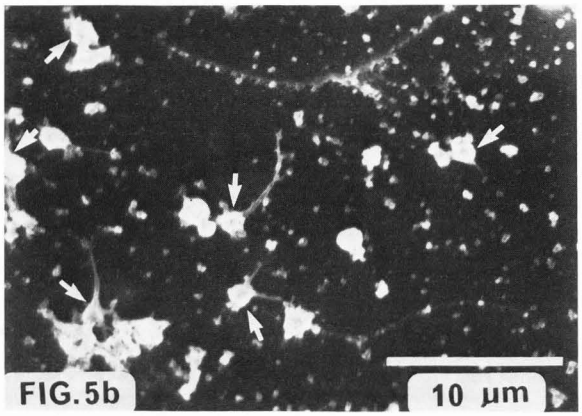

Fig. 5b. Inner Hydromer^R layer after 7 days in place. Flat overview. Fixed, critical-point dried specimen. Note platelets (arrows) adhering to the surface.

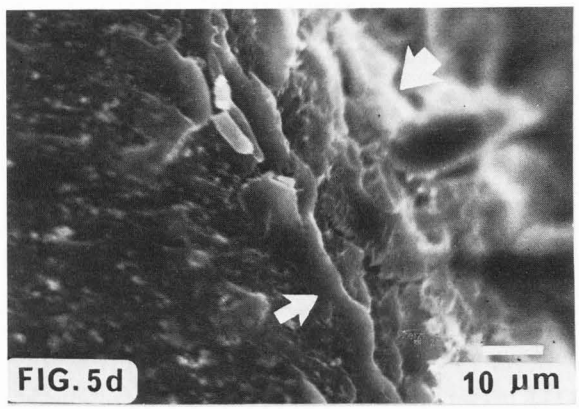

Fig. 5d. Inner Hydromer^R layer (slanting section) after 20 days in place. Fixed, critical point dried specimen. Note the Hydromer^R layer (small arrow), and the amorphous substance adhering to it (big arrow) reducing catheter lumen.

the layer during preparation (cutting of the specimens). The foreign bodies shown did not originate from the coating process. This view is supported by the clean, even surface of the inner Hydromer^R layer. This had not been in contact with package, nor had it been submitted to any trauma during preparation and mounting of the specimens.

Used specimens. The outer and the inner Hydromer^R layers of the catheters inserted into human veins appeared to have absorbed water from the blood and infusate, respectively. The flaking of the outer Hydromer^R layer, giving it an appearance like "dried earth", was thought to be the result of the return to a dehydrated state from a wet state. This is a well-known phenomenon frequently seen when preparing biological specimens (Albrecht et al.[1]; Hayat[7]).

The amorphous substance adhering to the outer Hydromer^R layer probably represents plasma proteins, a hypothesis supported from a parallel study, in which Hydromer^R-coated catheters soaked for 6 days in sterile human plasma at 37° C exhibited a similar morphology (Curelaru et al., to be published). The coccoid structures adhering to, and embedded within, the Hydromer^R of the specimen which had been in vivo for 20 days were considered to represent Staphylococci epidermidis, since such bacteria were cultured from the tip of the catheter.

It appears that the thickness of the main part of both the outer and the inner layers of Hydromer^R is maintained after at least 20 days indwelling. In some specimens, minor areas having a layer thinner than before insertion was observed. It is possible that this is due to Hydromer^R dissolution into the ethanol used for dehydration. P-NVP is extremely soluble both in water and in polar and non-polar solvents (Hoffman et al.[8]). The even more localized areas of damage found in some specimens appeared to have been induced by direct mechanical action. Manipulations during the insertion were the most likely cause, since the catheters were moistened manually with

a wet piece of cloth. The outer layer may also have been damaged during threading through the skin and tissues of the bodies.

The inner surface exhibited depositions of unidentified material, giving it a "lunar landscape"-like appearance. This material probably represented infusate precipitates (amino acids from the Vamin^R solution, and fat from the Intralipid^R), but bacteria may also have been present, as may certain elements of the blood. One example of the latter was the presence of platelets, seen in one specimen. The discovery of deposited platelets suggests that the Hydromer^R reduces (Borrow and Crowley[2]) but does not completely inhibit platelet adhesion to the catheter. The material may also, to some extent, be due to an extracellular slim substance produced by some bacteria, such as coagulase-negative Staphylococci[12], Pseudomonas aeruginosa[6], and fungi, such as Candida albicans.

Methodological considerations

P-NVP is, in swollen form, a gelatinous substance lacking protruding, electron dense material. During sputter-coating, however, the gold preferably precipitates onto such structures. This explains the depositions of only scarce gold particles on the Hydromer^R. At a flat view, a clean Hydromer^R-coated surface therefore exhibited a "see-through"-effect, where barium sulfate crystals of the catheter core were visible. Fixation with biological fixatives, such as glutaraldehyde, was found to be associated with some undesirable results concerning the morphology of the Hydromer^R itself. This was seen both with unused specimens and specimens which had been kept in solutions, such as physiological saline, for 6 days. Fixation and air-drying caused "bubbles" in the layer, bursting under the electron beam. This probably resulted from insufficient drying where fluid was still present within the Hydromer^R. Fixation and critical-point drying also gave Hydromer^R images very different from those of unused, unfixed, dry specimens: the layers were thinner, and the surfaces often resembled a "honey-comb". These artifacts probably result from the combined chemical and physical effects of fixation in glutaraldehyde and the dehydration, including immersion in both ethanol and liquid CO_2.

Fixation is necessary, however, to preserve blood cells and bacteria present on the intravenously indwelt specimens. As mentioned earlier, it should be combined with critical-point drying, to obtain complete drying. A serious drawback with critical-point drying is that adherent particles may be rinsed away during dehydration in all the changes of ethanol solutions, whereas nothing is lost when air-drying is used.

Concerning suitably preparational methods, the conclusion should be that there is yet no optimal preparational scheme for SEM of biologically used Hydromer^R-coated polymers. A recommendation is therefore to become familiar with the typical artifacts resulting from each method of preparation, and to use more than one method, combining the valid information obtained from each of these into a more complete picture.

Practical implications

Storage. The damage to hydrogel layers observed in specimens stored in pliable bags strongly suggests that the Hydromer^R-coated catheters should be preserved in rigid packages.

Catheter patency. The Hydromer^R-coating did not prevent occlusion of central venous catheters while indwelling. This has also been confirmed in a clinical study (Hultman et al., to be published).

Hypo/athrombogenicity of Hydromer^R-coated surfaces. Hydromer^R-coated intravenous catheters have been claimed to have a very low degree of thrombogenicity. In a pilot study on Hydromer^R-Erythroflex^R catheters inserted via the basilic and cephalic veins of humans, we found fibrin sleeves occupying more than 50% of the vein lumen, as judged by 3 "pull-out"-phlebographies taken at catheter withdrawal (Curelaru et al., to be published). It thus appears that the Hydromer^R coated catheters are, in fact, thrombogenic. The impurities present at the hydrogel-blood interface, the fissures and the possible peeling-off of the Hydromer^R may result in the formation of nidi stimulating adhesion of plasma proteins and platelets. To get a better understanding of the practical-clinical properties of these catheters, a series of randomized, double-blind studies are at present in progress at our institutions.

Method for insertion of the catheters. The Seldinger technique appeared less suitable for the insertion of the Hydromer^R-coated catheters. With this technique, the inner Hydromer^R-layer may be injured by the guide wire, and the outer layer by manipulations during the insertion. Bending the catheter may result in cracking of the Hydromer^R, and touching of the surface with surgical gloves may lead to deposition of foreign material onto the coating substance. In other experiments, we found that the dry Hydromer^R-coated catheters elicited a resistance similar to that of plain polyurethane (0.62 vs 0.68 N) when threaded through a 0.3 mm thick latex membrane (Lundström et al., to be published), whereas with wet Hydromer^R the friction was very much reduced (0.04 N). Clinically, insertion of dry, Hydromer^R coated, soft polyurethane catheters appeared impossible (Hultman et al., to be published). Prewetting of the catheter was therefore necessary, although it may render the Hydromer^R even more fragile and likely to be damaged during threading through the tissues.

Conclusion

The findings of this study question the claimed superiority of the Hydromer^R-coated polyurethane as compared to plain polyurethane for use as central venous catheters.

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Discussion with Reviewers

Reviewer IV: Air-drying biological specimens and polymers, especially swollen polymers take on a finely cracked appearance like "dried earth". In addition, extensive surface cracking can occur leading to separation of polymer coating from the substrate and removal of adherent cells...These artifacts are avoidable by either freeze drying (with a freeze-drier designed explicitly for this purpose) or critical-point drying. Where these apparatus are not available, drying from organic solvents of low surface tension may be acceptable, although this technique is not entirely free of artifacts (see text ref. 1). In our laboratory we have observed all these artifacts: crystals, dried-earth, deep fissures, cracks, and stripping of coatings, on all manner of polymers and especially with polyurethane and hydrogels. We have found that critical-point drying with sieve dried CO_2 following ethanol dehydration through absolute (i.e. sieve-dried) ethanol is the preferred procedure for preparation of hydrogels and urethanes. I question the validity of the results due to the drying problems as discussed.

Authors: 1. Flushing of the catheters with saline and air-drying was an accepted method for the study of plastic intravenous catheters (Heideman et al.[14]; Libsack and Kollenmeyer[15]). It appears that the air-drying is not satisfactory for cell preparation, but in some limited situations (e.g. the study of rigid biological specimens) it is an acceptable method: "While air-drying from aqueous suspensions can be used for some exceptionally rigid biological specimens, it is generally not satisfactory for most cell preparation" (see text ref. 1). The primary aim of our study was to investigate the surface structure of the Hydromer^R in both its dried and swollen form. The unused catheters (non-fixed specimens) already had dried Hydromer^R-coating. The problem created by air-drying remained for the used Hydromer^R-coated catheters (with the Hydromer^R in swollen form). However, by using air-drying we were able to "grasp" the appearance of the swollen Hydromer^R. We would point out that we were unable to grasp a similar image in more than one hundred micrographs performed with critical-point drying method with Hydromer^R-coated catheters immersed for 6 days at 37° C in sterile, distilled water, isotonic and hypertonic saline, blood, plasma, as well as in the used catheter specimens indwelling in patients from 6 to 20 days.

2. Comparison of our results obtained by fixation in glutaraldehyde and air-drying with the results of other authors (e.g. Rosenbauer and Herzer[19]; Rosenbauer[18]), obtained by other methods (e.g. fixation in glutaraldehyde, dehydration with acidified DMP, and drying at critical-point) indicates very similar structure appearances for identical details of identical catheter brands.

3. Freeze-drying. We have not used this method for the following reasons: a) We do not possess a freeze-drying apparatus, and this is a strong argument to use air-drying, which--in such conditions--becomes an acceptable method..."Generally, air evaporation technique cannot equal critical-point or freeze-drying procedures with respect to the preservation of delicate biological structures and should be avoided. However, this may in some limited situations provide an acceptable alternative especially where large numbers of cells must be processed, as in screening studies, or where the apparatus for freeze-drying or critical-point dehydration is not available". (text ref. 1). b) It was reported (Rosenbauer and Herzer[19]; Rosenbauer[18]), in similar studies with unused and used, plastic, intravenous catheters, that the "freeze-drying of bigger samples can not be recommended". Specimens of 1 to 3 mm in thickness require approx. 3 days to be dried by the freeze-drying method (text ref. 7). c) The freeze-drying introduces its own artifacts: i. Ice-crystals artifacts with both fixed, and unfixed specimens: " The major disadvantage of the freeze-drying technique is the introduction of ice-crystals, which invariably damage the specimen to various degree" (text ref. 7). Thus, at least theoretically, the freeze-drying does not offer any advantages for the study of the swollen Hydromer^R. ii. Distortions, crackings, and breaks in the specimen surfaces. "It should be noted that the thermal stress arising from quenching may cause mechanical distortion of the specimen. During quenching, the outer part of the specimen becomes solid first while the inner part is still contracting. This may result in cracking of the specimen and/or breaks in extracellular process" (text ref. 7). d) The freeze-dried specimens are very fragile. Thus, the extremely fragile

Hydromer^R layer could be easily damaged during the next manipulation (e.g. coating with gold). "The freeze-drying method was devised to minimize such drying artefacts, but in general the results have been disappointing, perhaps because of the movement of the phase boundaries with their inevitable disrupting tendencies. In freeze-drying, such boundaries move through the specimen twice: first, a liquid-solid boundary moves rapidly in the freezing process, and second, a solid-vapor boundary moves during sublimation of the frozen matrix" (Andersson[13]).

4. Physical dehydration of the specimens in organic solvents (e.g. acetone, alcohol, amylacetate, benzene, ethyl ether, hexane, xylene, etc.,) could alter the morphology of the swollen, as well as of the dried Hydromer^R. P-NVP, i.e. Hydromer^R, is extremely soluble in water and in many other polar and nonpolar solvents (text ref. 9). It is known that the organic solvents mentioned above physically exchange water for an organic solvent(text ref. 1) causing artifacts and increasing surface irregularities (Rosenbauer[18])... "Fracture surfaces and sharply defined contours including flaps and ridges were characteristic of the nonpolar solvent dehydrated samples" (text ref. 1).

5. Chemical dehydration using 2,2-dimethoxypropane (DMP). This method dehydrates the specimens by instant hydrolysis of DMP by water to form methanol and acetone, and was initially used for the study of biological structures (Müller and Jacks[17]). Later, Rosenbauer and Herzer[19] applied the method to the study of intravenous catheters using an acidified DMP. However, in a previous study Maser and Trimble[16] found "no difference between samples dehydrated in DMP for 5 min to 30 days and those conventionally dehydrated in ethanol or acetone". In addition, we suspected that the chemical dehydration using DMP may alter the morphology of the swollen Hydromer^R.

6. Critical-point drying. It appears that this method is not above criticism, and that it may cause its own artifacts, such as: a) Shrinkage. "It must be point out that critical point drying of specimens definitely results in varied amounts of shrinkage and possibly extraction of cellular material in some specimens"..."The amount of shrinkage occurring is under dispute and is not directly related to the softness of the tissue. Shrinkage may occur during fixation, dehydration, and critical-point drying"..."Available evidence indicates that shrinkage does occur in the intermediate and transition fluids and even during the post-critical period. The latter shrinkage is probably caused by the evaporation of residual fluid or water" (text ref. 7). b) Cracks and filaments. "Occasionally cracks in the peripheral membranes and filaments can be seen. Such cracks can be due to shrinkage or thermal expansion and contraction during critical-point drying and subsequent evaporative coating" (text ref. 1). c) Dissolution of compounds of high molecular weight. "Dense gases at or above critical pressure and at relatively low temperatures may dissolve compounds of high molecular weight" (text ref. 1). "The polyvinylpyrrolidone advantageously has an average molecular weight of at least 120,000 with the preferred average molecular weight being about 360,000" (Micklus JM and Ou-Yang TP: "Substrate having a low coefficient of friction, hydrophilic coating, and a method of making the same. U.S.A. Patent 4,119,094, Oct. 10, 1978. This patent refers to the Hydromer^R).

Reviewer V: Why did you air-dry the inner Hydromer layer of three catheters (indwelling 6, 7 and 20 days) by flushing with air before fixing and critical point drying (figs. 5b and d)?

Authors: At their withdrawal from the venous system, the catheters should be flushed with some liquid or gas in order to remove the infusate or/and blood possibly present in their lumina. In the first part of this study, we flushed the catheters with 20 ml of sterile, isotonic saline injected during approx. 30 s. However, it was suspected that crystals of sodium chloride might deposit on the inner Hydromer^R surface during drying, and alter its morphology. We believed that flushing of the catheter lumina with sterile, distilled water might cause swelling of the inner Hydromer^R layer. Therefore, in the second part of this study we chose to rinse the catheters by injecting rapidly (approx. 30 s) but gently 20 ml of air through their lumina. Thereafter, the lumina were immediately refilled with fixative. In our experience, air-drying of a Hydromer^R surface requires its storage in a desiccator for at least 48 h. Even doing so, the drying is not always complete. This was demonstrated by presence of "bubbles" in the Hydromer^R, bursting under the electron beam. Therefore, we believe that the brief (30 s) rinsing of the catheter lumina with 20 ml of air did not significantly affect the wetness of the inner Hydromer^R layer.

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