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NOTES

A Sensor To Detect the Early Stages in the Development of Crystalline *Proteus mirabilis* Biofilm on Indwelling Bladder Catheters

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A simple sensor has been developed to detect the early stages of urinary catheter encrustation and avoid the clinical crises induced by catheter blockage. In laboratory models of colonization by *Proteus mirabilis*, the sensor signaled encrustation at an average time of 43 h before catheters were blocked with crystalline biofilm.

The care of many elderly and disabled patients undergoing long-term bladder catheterization is complicated by the encrustation and blockage of their catheters (3, 15). The problem is unpredictable and can result in emergency referrals of patients with urinary retention or incontinence owing to catheter obstruction (5). The main cause of catheter encrustation is infection by urease-producing organisms, particularly *Proteus mirabilis* (6, 7). These organisms colonize the catheter, forming a biofilm (11, 13). The bacterial urease generates ammonia from urea, and the urine becomes alkaline. Under these conditions, crystals of calcium and magnesium phosphate are formed and a crystalline biofilm develops, which eventually blocks the flow of urine from the bladder (1, 2, 8).

Our aim was to develop a simple sensor to signal an early warning of impending catheter encrustation and blockage. The idea was to produce sensors by impregnating polymeric materials with pH indicators. The sensors, located in the catheter drainage system, continuously monitor the pH at the surface of the catheter over a 7-day period (the interval after which the urine drainage bags are usually replaced). Bromothymol blue (BTB) was chosen as the indicator because of its transition from yellow to dark blue over the pH range 6 to 8. A change from an acid to an alkaline reaction at the sensor surface signals infection and biofilm formation by *P. mirabilis*. The appearance of the signal indicates that action should be taken to avoid an acute clinical episode. Our objectives were to (i) develop polymers suitable for the manufacture of sensors, (ii) test their ability to signal catheter encrustation in laboratory models, and (iii) identify the optimum location for the sensor in the drainage system.

The sensors were prepared by dissolving cellulose acetate (Acordis Ltd., Coventry, United Kingdom) in acetone and adding a mixture of BTB (Sigma-Aldrich, St. Louis, Mo.) and

sulfuric acid. Under these conditions, the BTB becomes covalently bound to the cellulose acetate. Polyethylene glycol was added as a plasticizer and to control the rate of movement of ions through the matrix, thus controlling the response rate of the material to changes in pH. The mixture was spread over a glass plate to allow the polymer mixture to set. Strips (2 cm long by 1 cm wide by 1 mm thick) of the calcium acetate-BTB polymer were then prepared, washed in water to remove residual acid, and stored at 4°C.

The bladder model has been described previously (14). It consists of a glass chamber maintained at 37°C by a water jacket. Each model was sterilized by autoclaving, and then a 4.7-mm all-silicone catheter (Bard Ltd., Crawley, United Kingdom) was inserted into the chamber through its base. The catheter retention balloons were inflated with water and the catheters connected to drainage tubes and bags in the normal way. Sterile urine was pumped into the chambers at a rate of 0.5 ml/min, so that residual volumes collected below the catheter eyeholes before flowing through the drainage tube to the collection bags. The artificial urine used in the experimental work was based on that devised by Griffith and colleagues (4). Its composition and method of sterilization have been described previously (14). Sets of

TABLE 1. Comparison of the times required for sensors placed in a urine bag and at the catheter-drainage tube junction to produce the signal indicating the presence of *P. mirabilis* in catheterized bladders

Expt	Time (h) at which the signal from the sensor was recorded		Time (h) to catheter blockage
	Catheter-tube junction	Urine bag	
1	20	12	64
2	26	16	51
3	21	10	49
4	22	11	57
Mean value	22.25	12.25	55.25

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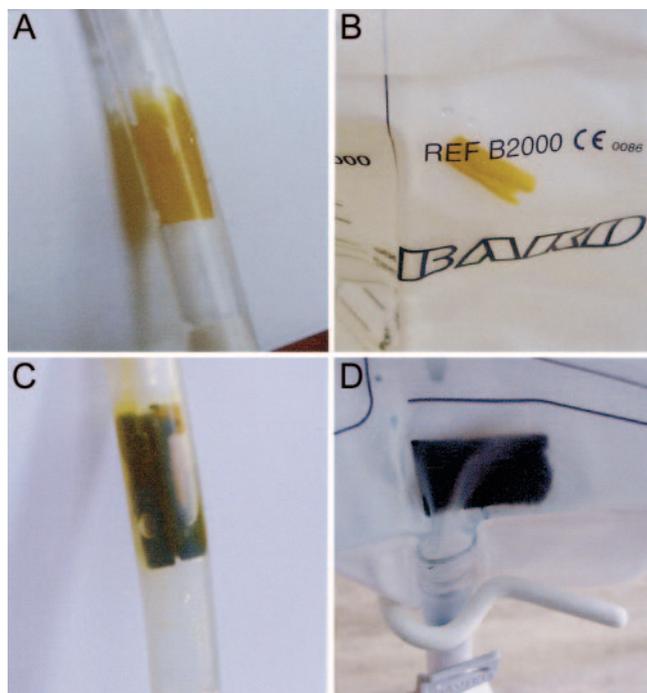


FIG. 1. Appearance of sensors located at the catheter-drainage bag junction (A) and in the drainage bag (B) at 0 h, at the catheter-drainage bag junction at 26 h (C), and in the drainage bag at 16 h (D) after inoculation with *P. mirabilis*. The catheter became blocked at 51 h. The sensor registers intermediate colors between yellow and blue, but these are transitory. The positive reactions are indicated by the dark-blue colors shown in panels C and D. Other colors are considered to indicate negativity.

models were primed with urine (30 ml), which was then inoculated with 100 µl of a 4-h culture of 2×10^8 CFU/ml of *P. mirabilis* B2, a strain that had been isolated from a patient's encrusted catheter. After an hour to allow the test organism to establish itself in the bladder urine, the urine supply was switched back on, and the models operated until the catheters became blocked. The times required for catheters to become blocked were recorded. Under these conditions, the pH of the urine in the bladder rises from 6.1 to >8.0 in under 12 h.

Initial experiments were performed using noninfected models in which sensors placed at the catheter-drainage

tubing junction were exposed to flow regimens of phosphate buffers at pH 6.2 and 8.0. Spectrophotometric analysis (at 450 nm) showed that in all cases, <10% of the indicator eluted from the polymer over 8 days. To test the ability of the sensors to signal *P. mirabilis* infection, models were supplied with urine and inoculated with *P. mirabilis*. Sensors were inserted into the drainage system at the catheter-drainage tube junction and in the urine bag. The times required for sensors to signal and catheters to become blocked in four replicated experiments are presented in Table 1. The appearance of the sensors at various times is illustrated in Fig. 1. It is clear that sensors located in the bags gave stronger and earlier signals. The bag also has the advantage of being in a more conspicuous site.

In a further series of experiments, at the time the sensors were judged to be giving a strong signal, the urine supply was switched off and the catheters and sensors removed. Sections were cut from each catheter and, together with the sensor, viewed directly with a JEOL 5200 scanning electron microscope (Jeol, Ltd., Tokyo, Japan) under the low-vacuum setting. The micrographs (Fig. 2) illustrate that at the point when the sensor gives the signal, encrustation has already started at the catheter eyehole and in the central channel. The deposits visible on the catheters and sensors are typical of the poorly crystallized apatite that forms early in the development of the crystalline biofilm (10). The observation that the sensor in the bag signaled encrustation at an average time of 43 h before the catheter became blocked offers an opportunity for action to avoid clinical crises induced by blockage.

The main route of infection of the catheterized urinary tract is through the urethra along the outer surface of the catheter. Infections, however, can occur from contaminants in the urine drainage bag ascending through the catheter lumen into the bladder (9). Locating the sensor device in the urine bag could thus detect some infections at a very early stage, before they have reached the bladder.

In conclusion, the results show that the cellulose acetate-bromothymol blue sensor is capable of giving a clear, simple signal of infection of the catheterized urinary tract by *P. mirabilis*. The device could be inserted into all types of drainage systems. The optimum location is the urine bag. It signals the early stages of catheter encrustation in time to allow action to avoid the clinical crises induced by catheter blockage. The sensor would have greater value if we had an effective strategy

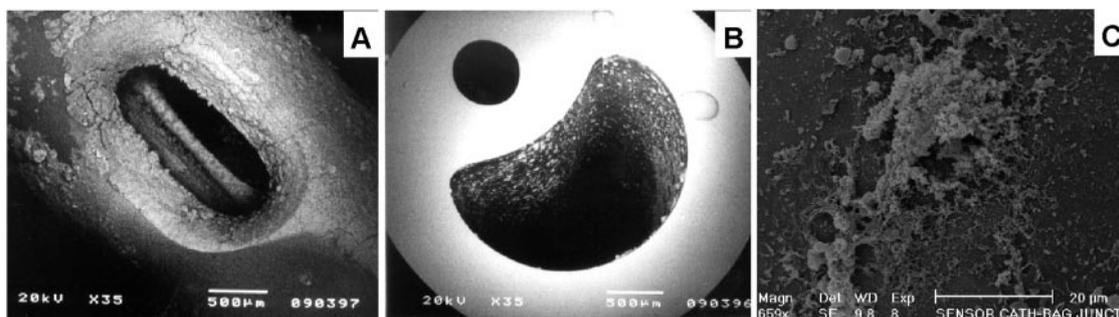


FIG. 2. Scanning electron micrographs showing the early stages of encrustation on a catheter (A and B) removed at 12 h from a model colonized by *P. mirabilis* when a signal was recorded from the sensor. Encrustation was also present on the sensor at this time (C).

to inhibit the encrustation process that could be implemented in those cases where the sensor gives its signal. Such a strategy has been developed (12). It involves inflating the catheter retention balloon with a solution of triclosan, rather than water. We are now planning a clinical study to test the efficacy of the sensor-modulator system in patients.

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