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Design and validation of a dynamic flow model simulating encrustation of biomaterials in the urinary tract

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Abstract

A number of models exist for assessing encrustation on biomaterials employed as devices in the urinary tract. However, static urine models are suitable only for assessment of biomaterials residing in the bladder and the dynamic models available suffer from a number of disadvantages, notably their complexity and limitation to short-term assessment. The dynamic model described herein is a relatively simple design incorporating the ability to assess a large number of biomaterials in replicate fashion and over long periods of time. The biomaterials tested in the dynamic model conform to the urethral catheter and ureteral stent devices that experience urine flow within the urinary tract. The model was initially validated using Percuflex as a test biomaterial. The mass of calcium and magnesium, representing hydroxyapatite and struvite encrustation, respectively, on Percuflex was detected by atomic absorption spectrometry. No significant differences in encrustation levels were detected either between vessels or between biomaterial positions on any mandrel within the vessels, indicating the suitability of the dynamic model for reproducible determination of biomaterial encrustation. The dynamic model was then used to compare the encrustation of biomaterials commonly employed in urinary-tract devices, namely polyurethane, Percuflex and silicone. Calcium and magnesium levels on polyurethane and Percuflex were shown to be statistically similar, whereas silicone exhibited significantly reduced encrustation. When, subsequently, comparisons were made of biomaterial encrustation between the dynamic model and a static model, calcium and magnesium levels arising from the latter model were significantly higher on each of the biomaterials. However, the same rank order of encrustation resistance was observed for the biomaterials in both models, with silicone performing better than polyurethane or Percuflex. The prediction of in-vivo performance based on in-vitro models of encrustation is often difficult, although the model described provides a more accurate method for assessing the potential of novel and existing biomaterials for use in urinary medical devices requiring flow of urine.

Introduction

Ureteral stents and urethral catheters are urinary medical devices that are employed to facilitate urine drainage from the kidney to the bladder and from the bladder to outside of the body. Whereas urethral catheters are primarily used whenever mobility is compromised or if there is damage to the physiology of the bladder or urethra, ureteral stents are employed whenever there is obstruction of urinary flow in the upper urinary tract (Gorman & Tunney 1997; Jones et al 1997; Gorman & Jones 2003). Typical indications for the use of ureteral stents include carcinomas, retroperitoneal fibrosis and obstructive uropathy (Jones et al 1997).

There is a wide range of polymeric materials from which ureteral stents and urethral catheters are fabricated, including latex, silicone, silicone co-polymers and polyurethane (Tunney et al 1996a; Gorman & Jones 2003). Despite the diversity in the chemical and mechanical properties of these biomaterials, currently available urethral catheters and ureteral stents are prone to the build-up of blocking encrustations on their surfaces, which compromises urinary drainage and results in pain and distress in the patient and ultimately device removal (Keane et al 1994; Gorman & Jones 2003). Due to the importance of encrustation on the performance of urinary medical devices,

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the conditions that are encountered by urinary tract prostheses in-vivo resulting in encrustation have been investigated by many workers in the field (Gorman & Tunney 1997; Gorman & Jones 2003). Some models have tested biomaterials in relatively static urine (from healthy subjects) to which infecting microorganisms had been added (Hesse et al 1989). Others have employed infected-patient urine (Gleeson et al 1989) or infected artificial urine (Schmitz et al 1993) and some have employed artificial urine designed to mimic infected urine (Cox et al 1987, 1988; Tunney et al 1996b). Urine presents in the urinary tract either as a relatively static body, as in the bladder, or as a flowing fluid in the ureters and urethra. The greater portion of the lengths of ureteral stents and urethral catheters are subject to urine flow, therefore it would be useful to have a model to mimic the conditions that these devices encounter. Models with urine movement, or dynamic models, have been described in the literature (e.g. Gleeson et al (1989) developed a dynamic model but the slow turnover rate of urine in the reaction vessel and the use of urine infected with *Proteus vulgaris* to induce deposition of encrusted material were considerable disadvantages). As an infecting organism is used, encrustation studies are limited to several days after which time the medium is no longer able to sustain growth or viability of the infecting organism. Another *Proteus* strain, *P. mirabilis*, was used as the infecting organism in the dynamic model described by Sarangapani et al (1995). In this case the reaction vessel employed was quite complex and could accommodate a few samples only. These models, therefore, are unsuitable to study build-up of encrustation on materials intended to fabricate stents or catheters for long-term use in the urinary tract. An ideal dynamic model would therefore incorporate a simple reaction vessel, which could accommodate several materials with several replicates. Use of artificial urine would be preferable, thereby decreasing problems encountered with infected urine. The system should also be able to run for up to six months, as this is the time scale desired by manufacturers of devices (stents) in the upper urinary tract. In light of this, the aims of this study were to design, develop, validate and test a novel dynamic encrustation model. Validation of reproducibility was performed using one commercially available material, Percuflex, a thermoplastic block copolymer. The dynamic model was then tested using three commercially available urinary device materials, polyurethane, Percuflex and silicone. A comparative study was then undertaken to assess the level of encrustation obtained on the three materials in a relatively static model.

Materials and Methods

Chemicals

Calcium chloride hexahydrate, magnesium chloride hexahydrate, potassium dihydrogen orthophosphate and urea were purchased from BDH Chemicals Ltd (Poole, Dorset, UK). Chick ovalbumin, grade II crude dried egg white, potassium dihydrogen orthophosphate and urease (type IX from Jack beans) were purchased from Sigma Fine Chemicals Ltd (Poole, Dorset, UK).

Biomaterials

The biomaterials examined in the encrustation study were: polyurethane french size 6, lot no. 112800, supplied by Cook Urological (Spencer, IN); Percuflex french size 6, lot no. 641721 from Boston Scientific Ltd (Galway, Ireland); silicone extruded tubing from Surgitek Ltd (Racine, WI).

Artificial urine

The composition of the artificial urine was similar to that used by Tunney et al (1996b) but with a reduced albumin level of 0.04% w/v to avoid problems of tubing blockage. The composition of artificial urine and urease solutions (% w/v) was as follows: solution A — potassium dihydrogen orthophosphate 0.76, magnesium chloride hexahydrate 0.36, urea 1.60; solution B — calcium chloride hexahydrate 0.53, chick ovalbumin 0.04; urease solution — Jack bean urease Type IX 0.125.

In brief, solutions A and B were prepared, stored and added to the reservoir separately to avoid precipitation of brushite ($CaHPO_4.2H_2O$) which would decrease the molarity of the artificial urine with respect to calcium and phosphate ions. Infection-induced deposition of encrusted material is thought to be initiated by the urea-splitting effects of the enzyme urease, produced by bacteria such as *P. mirabilis*. An increase in pH is caused by ammonia release as shown by $(NH_2)_2CO + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2$.

Daily removal of 1 L of the reservoir contents was performed using a siphon pump. This volume was replaced with 500 mL of A and 500 mL B, pre-warmed to 37 °C to avoid temperature fluctuation in the vessel. Twice weekly, 160 mL of solution was removed from the reservoir and replaced with 160 mL of urease solution. The pH of the final solution was approximately 8.0.

Dynamic flow model

The dynamic encrustation model consisted of a purpose-designed glass reaction vessel (AGB Scientific, Carrickfergus, N. Ireland) linked to a pumping system to circulate artificial urine through the vessel containing biomaterial samples attached onto stainless steel mandrels, as depicted in Figures 1A and 1B. A more detailed description of the glass reaction vessel, including dimensions, is presented in Figure 2. Artificial urine was pumped from a 5-L reservoir of artificial urine through silicone tubing (i.d. 7.7 mm, wall thickness 1.3 mm) using a Watson-Marlow pump (model 502S). The vessels were filled initially with urine using a plastic syringe. Gate clamps were used to temporarily seal the tubing leading from the vessels to prevent backflow of urine as the vessels were clamped into position. The inlet tube, pump manifold tubing and tubing leading to the vessels were filled with urine from the reservoir by turning on the pump. The gate

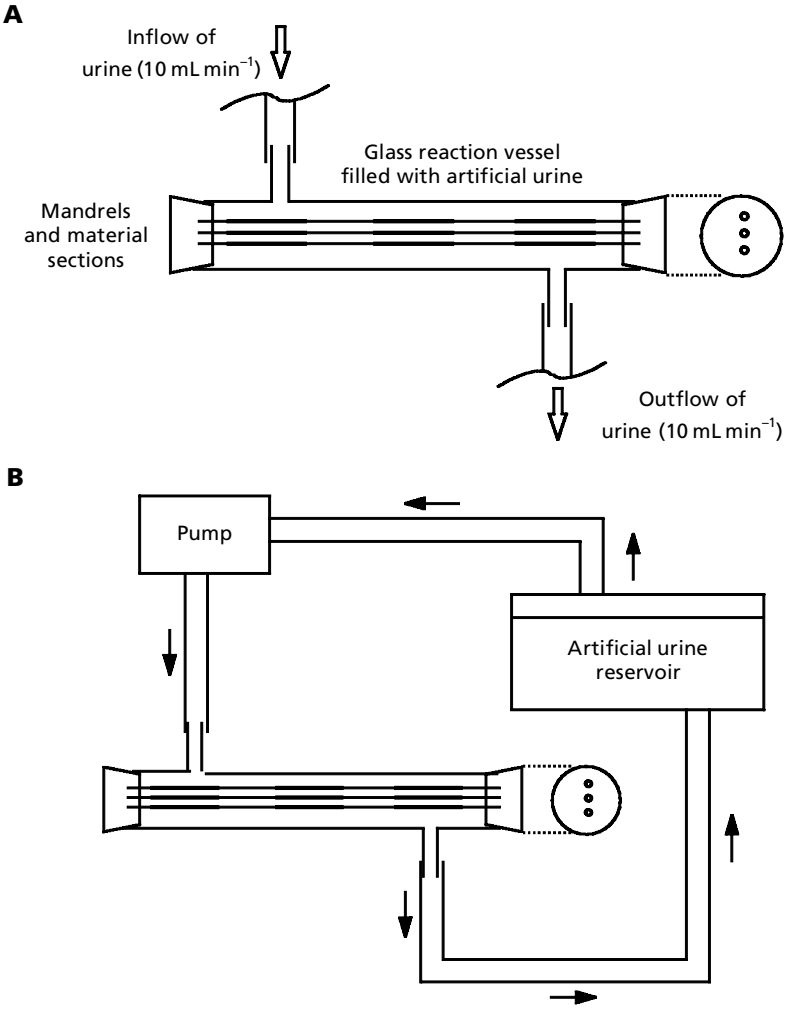

Figure 1 Diagrammatic representation of the reaction vessel (A) and the urine pumping system (B) in the dynamic encrustation model.

clamps were then removed. A pump speed of 10 mL min^{-1} was required to maintain the urine level in the reaction vessel. Urine flow rate was monitored and the pump speed adjusted if required. Several reaction vessels were clamped in parallel to a vertical steel pole. Each vessel had a separate inflow and outflow of urine and all outflowing urine was pumped back into the reservoir. Biomaterial sections to be tested were secured equidistant along each mandrel (diameter 1.2 mm; Boston Scientific Ltd., Galway, Ireland) using cyanoacrylate adhesive. A single biomaterial section may be employed (Figure 2), although in this study three sections of biomaterial (3 cm) per mandrel and 3 mandrels per vessel were tested. The mandrels were secured into rubber stoppers (size 27) at each end of the vessel. Each vessel and each mandrel within each vessel was numerically identified. The reaction vessels, pumping system and reservoir were maintaind at 37 °C to mimic physiological temperature. On a daily basis, 1 L of artificial urine was replaced in the reservoir and the operation of the model was checked to ensure reproducible flow from the outlet tube over a 5-min period. Any crystalline material deposited in the tubing was also cleared daily to prevent blockages.

Validation of the reproducibility of the dynamic model

The reproducibility of the model was validated to ensure that the position of each biomaterial section exhibited statistically similar levels of encrustation and, in addition, that there were no differences in the mass of encrustation deposited on each material in each reaction vessel. Three sections of a model biomaterial, Percuflex, 3cm in length, were secured on each mandrel. Four vessels were set up in parallel and urine flow initiated. After two weeks, each section of Percuflex was removed from the model and the encrustation attached quantified. The mass of calcium and magnesium, representing hydroxyapatite and struvite encrustation, respectively, per unit area on each section of Percuflex was detected using a Perkin Elmer 2380 Atomic Absorption Spectrophotometer. In this, each section of encrusted Percuflex was placed in 8 mL of 1 M

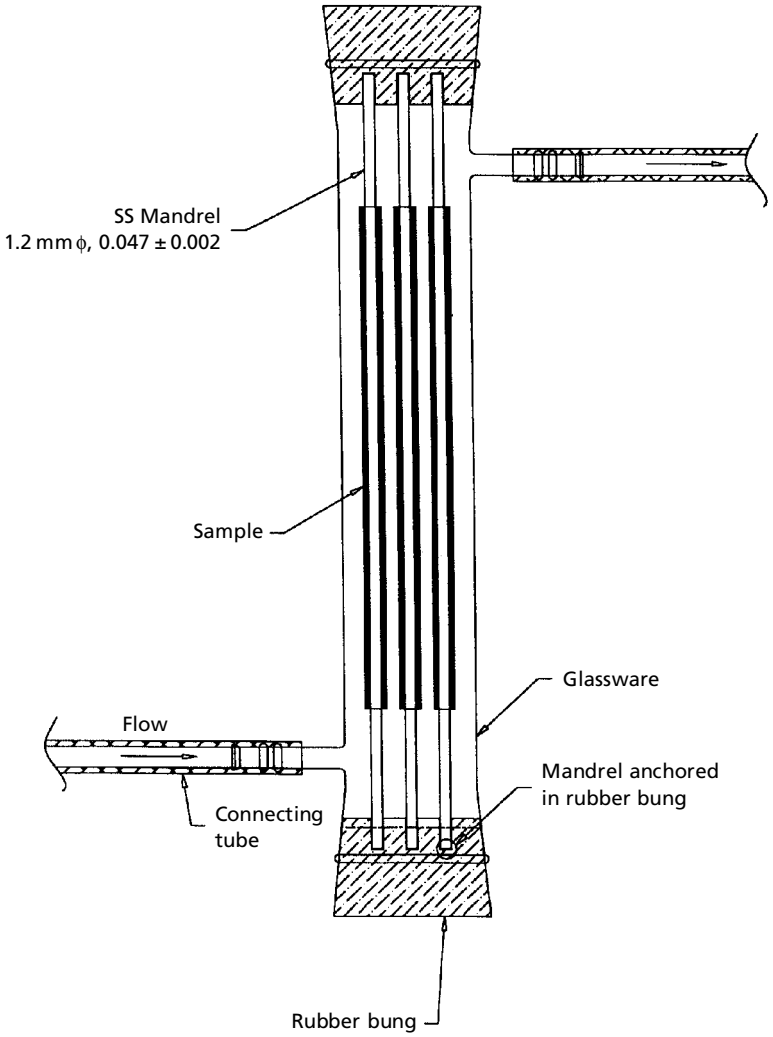

Figure 2 Engineering drawing of the reaction vessel used in the dynamic encrustation model.

acetic acid and these sections were then placed in an ultrasonic bath for four hours to remove and dissolve all encrusted deposits. The material sections were removed from the acetic acid solution. The solutions were then filtered and the mass of each element determined following reference to a previously constructed calibration curve ($r > 0.99$).

Comparative testing of the dynamic encrustation model

The performance of the dynamic encrustation model was examined in two ways. Firstly, the comparative masses of encrustation (hydroxyapatite and struvite) on biomaterials that are commonly employed in urinary tract devices were examined. In this, sections (3×3 cm) of polyurethane, Percuflex and silicone were placed on mandrels within the dynamic model as described above. After two weeks of urine flow, calcium and magnesium encrustation on each section of material were determined.

Secondly, the encrustation deposited on each of the biomaterials (polyurethane, silicone) in the dynamic model was compared with the encrustation arising in the encrustation model developed by Tunney et al (1996b). Artificial urine of the same composition was used in each case. In contrast to the dynamic model, the biomaterials in this model were suspended in artificial urine in a 5-L tank. Limited urine movement in the tank is provided by Teflon-coated magnetic stirrers and is, therefore, termed a static model for the purposes of comparison in this paper.

Scanning electron microscopy

Scanning electron microscopy was employed to visually examine the morphology of the encrustation deposited on the surfaces of the various biomaterials using both the dynamic and static encrustation models. Samples were mounted on specimen stubs, coated using a high-vacuum

gold-evaporation technique and viewed using a JEOL 6400 scanning electron microscope.

Statistical analysis of results

The effects of position of the biomaterial on each mandrel and the vessel number on the masses of calcium and magnesium deposited on each section of Percuflex were statistically examined using Friedman's test. The levels of encrustation (calcium and magnesium) on each biomaterial following assessment using the static and dynamic encrustation models were statistically compared using the Kruskal-Wallis test. Post-hoc comparisons of individual means were performed using Nemenyi's test (Jones 2002). Each experiment was repeated three times and in all cases $P < 0.05$ denoted significance.

Results

Validation of the reproducibility of the dynamic model was performed to ensure that sections of the same biomaterial became similarly encrusted when placed at any mandrel position in any reaction vessel. The results of this validation will dictate whether the model can be used to compare the encrustation performances of urinary biomaterials. Importantly, no significant differences in encrustation levels were detected either between reaction vessels or between the positions of the biomaterial on any mandrel, indicating the suitability of the dynamic model for reproducible determination of biomaterial encrustation (Tables 1 and 2). With respect to calcium encrustation, the mean (± s.e.) of mass deposited per unit area of material for each vessel ranged from 41.44 ± 1.60 to 43.60 ± 0.47 $\mu g\ cm^{-2}$, whereas the mean of deposited calcium associated with each position on the mandrel ranged from 41.93 ± 1.67 to 42.45 ± 1.37 $\mu g\,cm^{-2}$. Similarly, the mean of magnesium deposited per unit area of material for each reaction vessel ranged from 22.20 ± 0.31 to 23.46 ± 1.21 $\mu g\ cm^{-2}$, whereas the mean of deposited magnesium associated with each position ranged from 22.12 ± 0.39 to 23.03 ± 0.95 $\mu g\,cm^{-2}$.

Table 1 The effect of reaction vessel and position of biomaterial (Percuflex) on the mandrels within each reaction vessel in the dynamic encrustation model on the subsequent mass of calcium encrustation.

Vessel no.	Mass ($\mu g\,cm^{-2}$) of calcium encrustation on each biomaterial section positioned on each mandrel at:		
	Position 1	Position 2	Position 3
1	40.16 ± 3.01	43.24 ± 3.61	40.92 ± 3.69
2	43.69 ± 4.19	42.01 ± 4.04	43.09 ± 3.94
3	42.96 ± 4.14	40.99 ± 2.06	44.01 ± 3.58
4	40.91 ± 2.98	43.11 ± 4.16	41.77 ± 3.09

Data are means ± s.d.

Table 2 The effect of reaction vessel and position of biomaterial (Percuflex) on the mandrels within each reaction vessel in the dynamic encrustation model on the subsequent mass of magnesium encrustation.

Vessel no.	Mass ($\mu g\ cm^{-2}$) of magnesium encrustation on each biomaterial section positioned on each mandrel at:		
	Position 1	Position 2	Position 3
1	22.61 ± 2.05	22.35 ± 1.68	21.15 ± 1.88
2	23.22 ± 2.61	22.00 ± 2.87	23.86 ± 2.36
3	21.89 ± 2.27	22.57 ± 1.84	21.91 ± 2.66
4	23.88 ± 2.23	21.89 ± 2.86	22.88 ± 2.69

Data are means ± s.d.

Further comparative investigation of encrustation on a range of biomaterials within the dynamic model showed that the masses of calcium and magnesium on polyurethane and Percuflex were statistically similar, whereas the level of encrustation on silicone was significantly lower (Figure 3). Following two weeks immersion in the dynamic encrustation model, the mass of calcium per unit surface area on polyurethane, Percuflex and silicone was 42.60 ± 3.10, 43.16 ± 3.51 and 26.61 ± 1.91 $\mu g\,cm^{-2}$, respectively, whereas the mass of deposited magnesium on these three materials was 25.13 ± 1.96, 27.10 ± 2.97 and 20.60 ± 1.53 $\mu g\,cm^{-2}$, respectively. In addition, the resistance of each biomaterial to encrustation, as determined using the static encrustation model, is presented in Figure 3. The mass of encrustation (both calcium and magnesium) deposited on each material was significantly greater when evaluated using the static model. For example, the mass of calcium (per unit surface area) on silicone as assessed using the dynamic and static models was 26.61 ± 1.91 and 70.11 ± 5.19 $\mu g\,cm^{-2}$, respectively. Similar differences were observed for polyurethane and Percuflex. Furthermore, while differences in the levels of encrustation on each biomaterial were observed with each method, the same rank order of encrustation resistance was observed for biomaterials in both models, with silicone performing better than polyurethane or Percuflex. Figures 4A and 4B show scanning electron micrographs of encrustation present on the surface of Percuflex. In these, both struvite (coffin shape) and hydroxyapatite (powders) may be observed. Importantly, no differences in the morphological appearance of struvite and hydroxyapatite were apparent following deposition in either the static or dynamic encrustation models.

Discussion

The extent to which different urinary biomaterials will encrust in-vivo is a problem that needs to be addressed if newer biomaterials that offer increased resistance to encrustation are to be developed for clinical use. It is

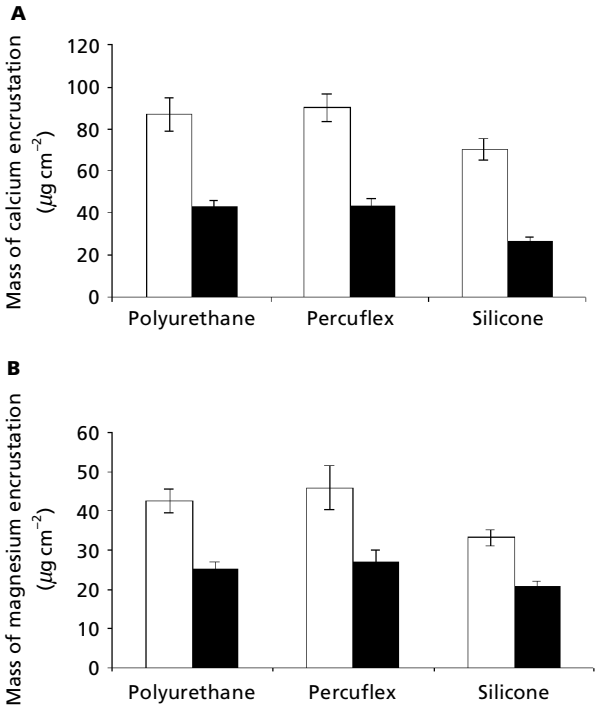

Figure 3 Graphical representation of the mass (±s.d.) of calcium (A) and magnesium (B) deposited on polyurethane, Percuflex and silicone, as assessed using the dynamic (closed columns) and static (open columns) encrustation models.

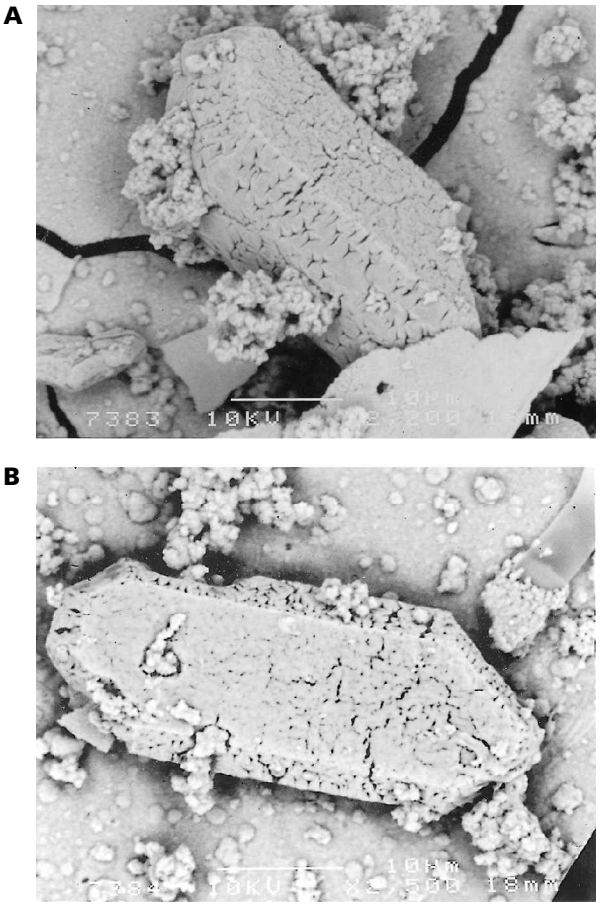

Figure 4 Scanning electron micrographs of struvite (coffin shapes) and hydroxyapatite (powder) deposited on the surface of Percuflex within the static (A) and dynamic encrustation (B) models.

difficult to develop a model that exactly mimics the in-vivo situation. Indeed, the inter-patient reactions to prosthetic devices are so erratic and difficult to predict that a model producing encrustation at a rate similar to a particular individual may be completely unlike the rate of encrustation of other individuals. Given this situation, the dynamic model described here attempts to more accurately mimic the situation in the urinary tract where urine flow, rather than static urine (bladder), presents. The design of the reaction vessel is straightforward and, accordingly, construction may be performed using a wide range of materials. In this study, grade-II glass was employed as the material of construction for the vessel due to ease and low cost of fabrication and transparency, the latter property allowing visualisation of both fluid flow and the development of encrustation on the biomaterials. However, examples of other materials from which the reaction vessel may be manufactured include metals (aluminium, 316L stainless steel), ceramics (other than glass) and polymers that possess sufficient mechanical properties and which do not include chemicals that may diffuse into the artificial urine during experimentation (e.g. polyethylene, polypropylene).

Ureteral stents and urethral catheters are continually subjected to a flow of urine and are susceptible to a build-up of encrusting deposits. The development of a reproducible method that will enable the assessment of both currently available ureteral stents and urethral catheters and, in addition, novel biomaterials designed for use as urological devices represents an advance in the design and development of medical devices. The dynamic model described within has proved to be a useful in-vitro model, allowing the assessment of biomaterials that have been processed into tubular form (e.g. ureteral stents, urethral catheters). Using Percuflex as a sample material, the reproducibility of the dynamic model was examined by quantification of the masses of encrusted deposits on the surface of individual sections placed at different positions in each of four reaction vessels. Importantly, this study illustrated that the method was reproducible in terms of both inter-reaction vessel and intra-vessel variation in encrustation deposited on the model biomaterial. This is an essential criterion in the development of a new method for the determination of encrustation on biomaterials. Furthermore, few reported methods possess the ability to examine the resistance of tubular materials, the ultimate shape of ureteral stents and urethral catheters, to encrustation and this is therefore an advantage of the current dynamic method.

In this study, the ability of the dynamic encrustation method to discern between the known resistances of various biomaterials to encrustation was investigated to

ensure that the design of the model did not influence encrustation. More specifically, the resistances of three biomaterials, silicone, Percuflex and polyurethane, to encrustation in the dynamic model were examined. Using this model it was shown that the resistance of silicone to encrustation was greatest. This observation correlated with a previous report that illustrated the marked resistance of silicone to encrustation (Tunney et al 1996a). Moreover, in this study, the levels of encrustation deposited on the various biomaterials in the dynamic model were examined and compared with those found on the biomaterials in the relatively static encrustation model (Tunney et al 1996b). In the dynamic model, less calcium and magnesium were deposited on the surface of the materials than in the static model. As the composition of the artificial urine was identical in each method, it may be assumed that the differences in the masses of encrustation associated with each method may be accredited to the fluid dynamics within the vessel. With knowledge of the flow rate of artificial urine in the dynamic model (9.26×10^{-4} kg s^{-1}) and the vessel diameter (3×10^{-2} m), and assuming that the viscosity of the artificial urine is similar to water (1.1×10^{-3} Ns m^{-2}), the Reynold's number associated with fluid flow may be calculated using equation 1 (Sinnott 1998).

$$\mathrm{Re} = 4G/\pi\eta d \quad (1)$$

Inserting the appropriate values into this equation enables calculation of Reynolds number associated with fluid flow in the dynamic model:

$$\mathrm{Re} = 4(9.26 \times 10^{-4})/[\pi(1.1 \times 10^{-3})(3 \times 10^{-2})] = 35.73 \quad (2)$$

Based on the above calculation, it may be concluded that the flow in the reaction vessel of the dynamic encrustation model is non-turbulent and therefore flow across the biomaterials is regular (Sinnott 1998). Conversely, the flow patterns in the static model are irregular, with flow adjacent to the Teflon stirrers being turbulent in nature whereas, in certain areas (adjacent to the suspended biomaterials), fluid movement is minimal. Therefore, it may be postulated that the regular flow of artificial urine across the surface of the biomaterials in the dynamic encrustation model would be expected to reduce the ability of nucleation and accumulation of encrustation on the surface in comparison with the static model. In the static model, fluid flow at the interface between the biomaterial and surrounding medium is poor and, accordingly, the residence of the artificial urine at the biomaterial surface is maximal and a greater time is available for nucleation and accumulation of hydroxyapatite and struvite on the surface of the biomaterials. In addition, unlike the static model, the constant flow of urine over the material surface in the dynamic encrustation model may dislodge some of the encrusted deposits. This will therefore reduce the mass of encrustation on the surface of the biomaterial and, furthermore, mimics more closely the in-vivo situation in the urinary tract. Although the levels of calcium and magnesium deposited were lower in the dynamic model, the rank order of material resistance to encrustation was the same as previously reported (Tunney et al 1996a), with silicone performing better than polyurethane or Percuflex. A similar rank order has also been reported using human urine supplemented with urease (Desgrandchamps et al 1997).

The extent of urine movement in previous encrustation models varies considerably, from no agitation (Hedelin et al 1991), through continuous agitation with a Teflon-coated stirrer bar (Cox et al 1988; Holmes et al 1992; Tunney et al 1996b), to a continuous flow rate of 81 mL h^{-1} (Schmitz et al 1993). The optimum level of urine agitation will be determined by the portion of the urinary tract in which the test material is destined to reside. For example, there will be more urine flow in the ureters and urethra than in the more stagnant portions of the bladder. Therefore, a material being tested for use as a short stent, residing solely in the ureter, should be tested in more dynamic conditions to mimic the flow of urine over the material surface in-vivo.

This study has proven that the dynamic model provides reproducible results in each portion of the reaction vessels and between reaction vessels when they are run in parallel. When compared with a previously described model, this novel method gave the same rank order of encrustation on each of the three biomaterials, but at a significantly lower level. This dynamic model is, therefore, presented as a reproducible method of mimicking the encrustation found in portions of the urinary tract experiencing urine movement. The dynamic model will be particularly suited to testing novel biodegradable biomaterials for use as urinary prostheses where the urine flow employed will encourage surface layers of the degradable polymer to be shed, thereby encouraging removal of encrusting deposits. Overall, the model will assist the difficult process of predicting the effectiveness of biomaterials in the clinical situation.

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