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Applications from bacterial adhesion and biofilm studies in relation to urogenital tissues and biomaterials: A review

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SUMMARY

The urogenital tract, particularly of the adult female, is the habitat for many species of microorganisms. These populations are in a state of flux, are susceptible to disruption by antibiotics and spermicides, and are exposed to many different biomaterial substrata. Infections of the genital area and bladder are common, and are invariably initiated by microbial adhesion to surfaces. This review examines the actual and potential applications to industry and to patients emerging from the study of bacterial adhesion to surfaces.

INTRODUCTION

The ability of bacteria to adhere to host and foreign surfaces represents an important initiating stage in the process of healthy colonization as well as in causation of infections in the urinary tract [51]. The organisms found naturally on the urethral and genital mucosa are in a state of flux, and can be disrupted by antibiotics, spermicides and incoming transient virulent organisms. The importance of adherence has been particularly well documented in relation to urinary tract infection (UTI), a common disease among adult women and seniors, with 10 million symptomatic and an estimated 10 million asymptomatic cases reported in North America in 1992. The use of biomaterials further complicates the clinical problem by providing a means (through physical force disrupting the urethral bacteria, providing a substratum for bacterial colonization, and interfering with the cleansing, flushing effect of micturition) whereby bacteria can enter the bladder and infect the host. In 1992, the number of urogenital devices used in North America was substantial: 74 billion tampons, 58 billion diapers, 33 billion feminine pads, 1.6 billion incontinence pads, 16 million catheters, 0.25 million ureteral stents and 0.2 million diaphragms [48]. The severity of the problem and the size of the market have led to concentrated efforts to develop improved diagnostic, therapeutic and preventive methods. Much research has focused on understanding and subsequently interfering with the process of bacterial adhesion to surfaces as a means of preventing infection. The following sections discuss briefly the most recent advances in this field. The review confines itself to practical discoveries which influence the detection, prevention and treatment of catheterized and non-catheterized urinary tract infections.

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1. Understanding pathogenesis

Bacterial adhesins. The large gathering of scientists and clinicians in Sweden for the Fourth International Symposium on Pyelonephritis provided an excellent focus for the advances in the area [24]. The depth of material presented on bacterial adhesion emphasized the importance of this process. By adhering to the urogenital epithelium, microbes gain a foothold into the bladder and kidney, and resist being dislodged and washed out during voiding. This initiating step is mediated by specific (adhesin-receptor) and nonspecific (electrostatic, hydrophobicity) factors.

Uropathogens express different types of surface structures, some termed fimbriae or pili, which can mediate binding to host receptors. As many as 75% of cystitis strains of *Escherichia coli* possess the ability to express type 1 fimbriae, which bind to mannose receptors on the uroepithelium and to Tamm Horsfall protein, the major urinary mucus [21]. The fimbrial expression can be regulated by the organisms by random and nutritionally-based phase variation, and this variation appears to be important in the initiation of some bladder infections [22,31,50]. In vitro data indicates that type 1 fimbriated strains are less adherent to uroepithelial cells [50], yet they are capable of initiating renal scarring [85]. The most important causative organism in kidney infection appears to be type P fimbriated *E. coli* [70], with the protein adhesin being located on the fimbrial tip [32]. However, as many pyelonephritis strains also express type 1 fimbriae and as non-fimbriated organisms can adhere well

to uroepithelial cells, the importance of P fimbriae expression alone may have previously been over-estimated. This finding of phase variation and non-fimbriated adherence of uropathogenic *E. coli* was first reported in 1984 [50], but has more recently been verified by the isolation and characterization of non-fimbriated uropathogenic *E. coli* [28]. Other fimbriated organisms, such as type 1c and S-fimbriated *E. coli* have also been identified [45,73]. Certainly, urinary tract infections can be caused by a wide variety of organisms in a number of ways.

Two practical applications have been explored from the adherence investigations. The first utilizes the receptor-specific sites for bacterial adhesins in an agglutination test for detection of the pathogens [13]. However, it is uncertain whether this test will alter diagnosis and therapy, and as it fails to detect other uropathogens, it is unlikely to be widely used. It is also doubtful that the detection of P fimbriated *E. coli* can be used as a localization test per se [29].

The second potential application is in immunization using bacterial adhesin vaccines. This has been studied in animals with limited success against type P and type 1 fimbriated *E. coli* [1,66]. This result may not be entirely unexpected, as P fimbriae do not elicit a strong antibody response in patients with pyelonephritis [69]. Nevertheless, it has been suggested that human antibodies against P fimbriae could potentially prevent infection [16], and that maternal immunization can reduce the development of pyelonephritis in newborns [23]. Further, immunization protocols have been tested using bacterial whole cells, but the usefulness of this technique has still to be confirmed and the mechanisms for effectiveness have to be verified [87].

Host factors. It is recognized that host factors can in some cases predispose a patient to urinary tract infections. The scope of this topic itself is worthy of a full review; however, it is outside the scope and aims of the present report. Rather, an abbreviated discussion will be presented.

The suggestion that there might be a correlation between bacterial adhesion and host factors in the onset of UTI came, in part, from the observation that there is a higher receptivity to bacterial adhesion of uroepithelial cells from patients with recurrent UTI than from those without a history of infection [5]. It is further supported by the fact that in the absence of vesicoureteral reflux, patients with P1 blood group are more prone to bacterial infection, due to having receptor sites for P fimbriated *E. coli* [34]. Studies have shown that increased binding of *E. coli* to uroepithelial cells from nonsecretors (who cannot secrete A, B, or H blood group antigens in saliva, express Lewis antigens, and have Le(a+b−) erythrocytes) occurs regardless of the P blood group, suggesting that secretor individuals may produce substances which inhibit bacterial access to the host cells, and that nonsecretors are more susceptible to recurrent UTI [26,30,33,77].

The potential influence of hormones on bacterial adhesion in the urinary tract has been documented; there seems to be an increased receptivity of uroepithelial cells for *E. coli* when estrogen levels are highest [53,71]. The oral contraceptives norgestrel (0.3 mg daily) and ethinyl estradiol (0.03 mg daily) were found to increase receptivity of uroepithelial cells to *E. coli*, again providing a correlation between hormones and bacterial adhesion [76]. The application of this knowledge to patient care has not yet been forthcoming.

In regard to host defenses, voiding is clearly an important mechanism to eradicate bacteria. Other defense mechanisms in the bladder have been proposed. These include antibacterial properties of the uroepithelial cells [74], and anti-adherent components of the bladder mucopolysaccharides [68]. The urine itself has been shown to contain components, especially Tamm Horsfall protein (THP) and neutral alpha-mannosides, that are believed to inhibit S and type 1 fimbriated *E. coli*, respectively, from binding to the uroepithelium [46]. The inhibitory effects of THP were also reported to prevent *E. coli* adhesion to human kidney cells [18]. However, studies have shown that THP may also mediate bacterial binding to cells and biomaterials [19,21]. In addition, *E. coli* have been found able to adhere to mucus-coated and non-coated uroepithelial cells, and this may in fact mediate bacterial colonization [49]. The concept of blocking uropathogenic adhesion using receptor analog therapy or instilling substances such as mannose to block adhesion, has been conceived but not transferred to the clinic. There may be many reasons for this, including difficulties with delivery and the multiplicity of factors involved in the induction of infection.

Host immune defenses can clear infecting bacteria, but they can also have potentially detrimental effects to the host. The role of inflammatory mediators has received much attention lately. Renal scarring in association with chronic pyelonephritis has been shown to be related to the magnitude of the initial inflammatory response, and is influenced by the bacterial surface cationic charge and hydrophobicity [79,81,82,86].

It has been shown that membrane glycoproteins of human polymorphonuclear leukocytes (PMN) act as receptors for type 1 fimbriated *E. coli* [67], stimulate oxidative activity [3] and cause degranulation of PMN [83]. It is apparent that *E. coli* causing upper tract infection invariably express type 1 and P fimbriae. The latter do not elicit a PMN response, and it is hypothesized that the organisms' ability to phase-vary fimbrial expression can allow de-expression of type 1 fimbriae in the kidney, thereby preventing phagocytosis. The inflammatory process is enhanced by adherent bacteria [14], especially those that produce hemolysin, substances that appear to induce release of histamine and leukotriene [72]. Further innovative studies by deMan [15] demonstrated that interleukin-6 was produced within 30 min of challenge with *E. coli* into the mouse bladder. The rapid response was due to the lipopolysaccharide-induced mucosal interaction. It would be interesting to assess this interleukin-6 response in asymptomatic patients, and in those infected with Gram-positive organisms. The use of immune modifiers to treat or prevent UTI has not yet been fully exploited, but there would appear to be much potential, especially in utilizing compounds which would minimize damage to the host.

2. Treatment with antimicrobial agents

It is outside the scope of this review to discuss treatment of UTI in depth. However, some studies now correlate antibiotic administration with effects on bacterial adhesion.

Exposure to fluoroquinolones has not, so far, been found to alter the bacterial morphology and function [27]. However, one study showed that sub-lethal doses of pefloxacin, a fluoroquinolone, did alter fimbriation and reduced bacterial adhesion to uroepithelial cells [17]. The prescription of low-dose trimethoprim-sulfamethoxazole (TMP-SMX) for long term prevention of recurrent UTI has proved fairly effective [44]. It appears that part of this efficacy may be due to trimethoprim decreasing *E. coli* P fimbriae expression and bacterial adhesion [12]. However, because bacteria are likely to utilize many mechanisms to adhere, multiply and infect the host, it appears less likely that new antibiotics which only target adhesins, can have practical use.

Once bacteria are adherent and in a biofilm mode on bladder cells, it can be very difficult to eradicate them. This is perhaps best illustrated in relation to spinal cord injured patients. Studies involving twenty-three patients has shown that dense biofilms are adherent before, during and after antibiotic therapy [55,60, unpublished data]. Two potential industrial applications have come from this work. The first stems from a correlation between a bacterial adhesion count of >20 organisms per bladder cell and the presence of at least one sign and one symptom of UTI. If larger studies confirm these findings, diagnostic kits could be developed to more accurately detect bacterial biofilm formation and infection, and to perhaps indicate when treatment might be given. The development of such a system is particularly important as existing tests are not able to detect adherent biofilms. Secondly, there is in vitro evidence to show that fluoroquinolones, especially perhaps ciprofloxacin, have the ability to penetrate biofilms [64]. This not only has potential applicability for tissue-related infections, but also for prosthetic devices where bacterial biofilms have been shown to be highly resistant to antibiotics, for example, adherent to ureteral stents [57]. It is clear that present day antimicrobial agents have not been tested and approved for use against biofilms, thus raising another two questions: should all antibiotics be re-tested for penetration of biofilms? Should only those with efficacy be approved?

3. Prevention of UTI

Currently, there are three practical methods of managing and preventing recurrent UTI, all utilizing antibiotics: (i) Long term, low dose antibiotics, such as TMP-SMX and nitrofurantoin. (ii) The use of single, post-coital antibiotics in cases related to intercourse. (iii) Self medication following development of symptoms and a positive dip-stick reaction which detects nitrites and leukocytes in the urine.

The development of an alternative therapy has recently been explored in Toronto. The work involved bacterial interference concepts which are perhaps better known historically for the use of *Staphylococcus* sp. to prevent infection in the nasopharynx of newborns [78,80]. However, unlike the work in the nasopharynx, the latest studies with lactobacilli have led to several human trials, the granting of a Canadian Patent and the development of a technology which has the potential for industrial exploitation.

In brief, the studies originated from clinical and microbiological observations that lactobacilli dominate the urethra and vagina of healthy adult females, whereas uropathogens dominate infected patients. This implied a possible role for the indigenous flora in preventing UTI. After extensive in vitro studies, specific lactobacilli strains were identified and selected for their ability to adhere to uroepithelial cells, inhibit the growth of uropathogens and competitively exclude uropathogenic adhesion [7,8,36,56,62]. Successful testing in animals led to human trials using intravaginal instillation of lactobacilli. The initial study with a single strain, *L. casei* GR-1, in a douche proved safe and had an encouraging outcome in slowing the expected time to next infection [4]. The preparation of a freeze-dried gelatin vaginal suppository and the addition of a second adherent strain, *L. fermentum* B-54, which was shown to kill enterococci in vitro [37], resulted in a 66% or more reduction in the rate of UTI in eight patients over one year [6]. Further studies on 55 patients showed a reduction in UTI rate of between 73–81% using weekly lactobacillus or lactobacillus growth factor suppositories (unpublished results). Another study also showed that lactobacilli given post-antibiotics could somewhat restore the normal urogenital flora and potentially reduce the risk of recurrent infection (although the trial of 40 patients was not large enough to make definitive conclusions) [54]. It is envisaged that further studies will be necessary using a manufacturing plant approved by government regulatory agencies, and that patient groups will include those given lactobacilli, those receiving no therapy and those given daily low doses of antibiotics. It is anticipated that the lactobacilli therapy will prove effective, even though the precise mechanisms of action have not yet been fully identified in vivo. It would seem to be an excellent business opportunity to bring a safe, natural biological product onto the market, which can reduce health care costs and provide some degree of relief to the millions of women who acquire recurrent urogenital infections.

The potential use of this therapy, once proven beyond doubt, is even more enhanced with the finding that the two *Lactobacillus* strains used in these studies resist the action of nonoxynol-9 (N-9). The N-9 is the main component of many spermicides, and a substance which invariably kills lactobacilli [38] while promoting the growth and adhesion of candida and *E. coli* [39,41]. The role of N-9 and its effect on the urogenital flora and infection has recently been reviewed elsewhere [40]. The concept of using a lactobacilli suppository to restore the patient's flora after N-9 usage has not yet been investigated.

4. Prostheses-related UTI

The extent of usage of biomaterials in the urogenital tract is quite phenomenal, and thus it is no surprise that infections do occur and can be severe, especially in conjunction with the use of diaphragms, IUDs, tampons and catheters. In addition, over 40% of users of incontinence

pads can suffer from UTI [42], and those who are catheterized are very susceptible to UTI, fever, sepsis and death [88]. Several important contributions have been made to our understanding of these infections and to finding ways to better prevent and manage them.

It has been established that bacterial adhesion to the catheter material is a vital step in the infection process [10,43]. and it appears to follow the deposition of a host conditioning film onto the device [63]. The bacterial binding to the biomaterial surface can be mediated by hydrophobic and electrostatic interactions [2], and the binding to specific adhesin-receptor sites can occur if receptors are present within the conditioning film. There is evidence to suggest that the more hydrophobic organisms attach best to hydrophobic surfaces [58], but uropathogenic *E. coli* have been shown to be relatively hydrophilic and more receptive to hydrophilic surfaces [59,61]. This implies that hydrophilic catheters may not be as resistant to bacterial adhesion as previously demonstrated [11,65]. Thus, basing the properties of a device solely on hydrophobicity will not be sufficient to ensure resistance to bacterial colonization.

Once the organisms have adhered to a device, bacterial biofilms begin to form on the biomaterial surface [47] and resist the killing effects of antibiotics [9,57]. The planktonic organisms which detach and cause infection have a 'normal' minimal inhibitory concentration (MIC) to antibiotics. However, within the biofilm, this MIC is invariably several fold greater. Often, the only solution is to remove the catheter, and it has been suggested that subsequent to this, the patient should be treated with absorbent pads and oral agents such as TMP-SMX [75].

Because of the biofilm resistance to antibiotics and the apparent ineffectiveness of TMP-SMX at penetrating tissue-associated biofilms [60], alternative approaches must be found. To date, these have included the use of intermittent catheterization, incorporation of a microbicidal outlet tube in closed drainage systems [25] and impregnation of catheters with antimicrobial substances, such as silver [35], antiseptics [84] and antibiotics [64]. These three latter methods will likely prove useful in preventing bacterial adhesion and infection over the short term, but may not be as effective when indwelling catheters and stents are inserted for more than one week. The technical key to success may be the leaching rate of the drug, and devising a way to re-adsorb an antimicrobial agent back onto the device after leaching has taken place.

Another approach being considered is to coat a urinary catheter or urethra with indigenous lactobacilli. In a series of experiments, such a coating significantly reduced the adhesion of *E. coli, S. epidermidis* and *E. faecalis* to polymers and catheters [20,52]. The latter study and another which has not been published, have shown uropathogens can also be displaced from biomaterial substrata. This presents an additional means by which infection rates could potentially be reduced, in essence by employing or encouraging non-pathogenic biofilms on the urethral and catheter surfaces. In spinal cord injured patients where bacterial colonization of the bladder and bacteriuria are inevitable and frequent, such an approach seems especially worthy of exploration.

In conclusion, new approaches to diagnosis, prevention and treatment of urogenital infections are certainly needed and several appear to be highly feasible. The new insight into biofilm infections and the potentially protective role of the normal urogenital flora, provide at least some basis from which new products could be generated.

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