Exogenous factors influencing voice prosthetic biofilm

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2004

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Chapter 2

Prevention and treatment of mixed species biofilms on tracheo-oesophageal voice prostheses in patients following total laryngectomy: the role of probiotics

Lifetime and design features of tracheo-oesophageal voice prostheses

The optimal functioning of the tracheo-oesophageal voice prosthesis depends on several internal and external factors, which will determine the efficacy of alaryngeal voice production and valve closure during swallowing. Basically, accurate occlusion of the tracheostoma is necessary to build-up sufficient intratracheal pressure to allow air passage through the one-way valve system into the proximal oesophagus. Improper tracheostoma closure or limited pulmonary capacity may interfere with this basic procedure.¹ This may be part of patient’s acuity to produce speech or other technical problems (manual dexterity problems, tracheostoma size and shape, fistula location). Local problems may be troublesome, such as excessive production of tracheal secretions and crust formation. Also, functional and structural problems located in the pharyngo-oesophageal segment can interfere with functional alaryngeal voice production or insertion of the voice prosthesis. Strictures of pharynx and oesophagus, hypertonicity, or spasm of the upper oesophageal sphincter will limit optimal tracheo-oesophageal speech.² These problems may also influence the condition of the oesophageal flange and valve due to deformation of the silicone rubber components or hinder proper opening and closure. Swallowing disturbances will force patients to modify their diet by using more fluids and soft blended foods. Fluids will increase the chance of leakage through the valve system. Several diverticulae and niches in the pharyngo-oesophageal segment will decrease the clearance of foods in the surroundings and will result in the build-up of deposits of foods around the oesophageal flange. Unfortunately, critical analysis of habitual diets of the laryngectomees has not been performed yet due to the immense multi-
factorial dimensions of both inter- and intra-individual factors in time. But, certain foods may act on the functioning of the valve system. The prosthesis-related factors depend on the size of the shaft and the dimensions of the valve system. Some prosthesis-designs are more prone to increased phonatory resistance due to a bulky shaped valve part and hinge system or internal leakage due to improperly constructed hinged valve sensitive for insufficient closure, which is prone to mechanical forces in the oesophagus.

During normal use, the ‘wear and tear’ of the valve system is variable and depends on various mechanical, chemical, and microbial factors. Airflow through the prosthesis may attribute to auto-cleansing of the valve part, but frequent opening and closure may alter the intrinsic characteristics of silicone rubber devices (e.g. delayed closure). The smoothness of the valve design may determine the ease of accumulation of foods and other substances at the valve slit or hinge. So, the prosthesis with the lowest airflow resistance may be technically convenient for the patient (low phonatory efforts), but may be prone to internal fluid leakage.

Further development and modification of critical design features requires intensive research on the biofilm formation. Design features and valve components of Groningen, Provox II, Blom-Singer indwelling, and VoiceMaster voice prostheses were determined in an artificial throat study while biofilm was grown under dynamic growth conditions. Obstruction of the semicircular slit-valved Groningen prosthesis leading to increased airway resistance was caused not only by a build up of deposits on the oesophageal flange and valve hat, but also by accumulation of deposits on the semicircular valve seating. The hinged flap valved Provox II prosthesis failed to close sufficiently because of biofilm formation on the valve seating. These findings may facilitate future design modifications, which will limit biofilm formation on critical sites of the valve system, while valve opening is not hampered for optimal aerodynamic functions necessary for tracheo-oesophageal speech.

To determine which bacterial or yeast strains, isolated from explanted voice prostheses, contribute most to increases in airflow resistance of silicone rubber voice prostheses, biofilms consisting of either a bacterial or a yeast strain were determined in an artificial throat model. The effects of these biofilms on airflow resistances were determined by calculating the difference in airflow resistance of the individual voice prosthesis as covered with a 7-day-old biofilm with the situation prior to biofilm formation. Conspicuously, voice prosthetic biofilms formed by the bacterial strains *Staphylococcus aureus* GB 2/1 and *Rothia dentocariosa* GBJ 41/25B and their excreted organic matter showed larger increases in airflow resistance (more then 30 cmH₂O s/L) than biofilms formed by Candida species. This is contrary to the literature, where there seems to be agreement that Candida species are mainly responsible for clinical failure of silicone rubber voice prostheses.

As with other implantable devices in man, biofilm formation on the prosthesis by oral and
skin bacteria and yeast will gradually take place on and into the silicone rubber. This leads to dysfunction of the valve system, causing leakage into the trachea or higher phonatory resistance with difficulty to speak, after which the prosthesis has to be replaced. This replacement procedure is uncomfortable and time consuming, and in time may lead to tissue damage of the shunt (patulous tracheo-oesophageal shunt). Also, these replacements lead to higher costs (travel expenses of the patient, medical personnel, new prostheses and instrumentation expenses).5,6

Traditionally, an indwelling prosthesis is replaced by introducing a guide-wire through the old prosthesis or fistula and slided upwards through the pharynx and out of the mouth. Dependent on the type of prosthesis, the dysfunctional prosthesis is pulled out of the fistula or partially removed through the mouth with the guide-wire. The new prosthesis attached to the connector of the guide-wire will be pulled towards the fistula by the guide-wire by using swallowing actions followed by careful introduction of the tracheal flange into the fistula. Recently, this unpleasant replacement method has been abandoned in favour of an anterograde method (front loading technique) using a special introductory system.7 Although the front-loading method is less stressful for the patient, frequent prosthesis replacements can still damage the fistula.8 Various differences in lifetime between different types of voice prostheses have been reported which may vary between 4 months to up to 10 months.8,9,10,11

Biofilm formation

Van Leeuwenhoek was the first who observed micro-organisms growing on tooth surfaces in a biofilm fashion. Later publications reported the typical assemblage of surface associated microbial cells that are enclosed in an extracellular polymeric substance (EPS) matrix, which is also highly resistant to various disinfectants. In 1978, Costeron et al.12 put forth a theory of biofilm based upon observations of dental plaque and sessile communities in mountain streams, that explained the mechanisms whereby micro-organisms adhere to living and non-living materials and the benefits they have by growing in such an ecological niche. Electron microscopy and standard microbiologic culture techniques for biofilm characterization revealed insights into the biofilm-related growth, but the utilization of confocal laser scanning microscopy to characterize biofilm ultrastructure, and genetic investigations regarding cell adhesions and biofilm formation have dramatically impacted the understanding of biofilms.

Micro-organisms exist predominantly as biofilms rather than as planktonic or free-floating cells. Biofilms develop on surfaces after attachment of certain micro-organisms. The process of attachment is known to be complex and is regulated by the available growth medi-
um, substratum, and cell surface. Once the biofilm has been established it comprises micro-
bial cells and extracellular polymeric substance (EPS) matrix in a defined architecture, which
provides an optimal environment for growth and exchange of genetic material between the
cells. Biofilms are known for their role in certain infectious diseases and in a variety of
device-related infections. Each biofilm elicits specific mechanisms for initial attachment to a
surface, development of a community structure and ecosystem, and detachment.\textsuperscript{13}

An ideal environment for the attachment and growth of micro-organisms is established by
the solid-liquid interface between a surface and aqueous medium. In case of biofilms on
voice prostheses similar effects of the substratum, conditioning films forming on the sub-
stratum of silicone rubber, hydrodynamics of the medium through the oesophagus during
swallowing, characteristics of the medium (fluids and food), and various properties of the cell
surfaces all play a role. In a biofilm the microbial cells are irreversibly associated with a sur-
face and enclosed in a matrix of primarily polysaccharide material.

Biofilm formation on voice prostheses starts developing from the first moment the device is
placed in the fistula, because it is a non-sterile environment. Depending on the environment in
which the biofilm has developed non-cellular materials may also be found in the biofilm matrix.
Usually, the environment is highly dependent on the local oropharyngeal flora and the various
components of the daily oral intake of foods. Biofilm formation on voice prostheses can be
caused by a mixture of bacterial and fungal species\textsuperscript{14,15,16} The exact composition of the biofilm

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{biofilm_steps.png}
\caption{Sequential steps in the formation of a biofilm on a voice prosthesis and subsequent biodegradation by ingrowing yeasts.}
\end{figure}
In literature, intensive studies on bacterial biofilms have been published in recent years concerning their structure and properties. However, pathogenic fungi in the genus Candida are getting more attention recently. Only a relative small number of Candida species are pathogenic for humans. Candida species are capable of causing various superficial and invasive mycotic infections. Most of these opportunistic pathogens attack immunocompromized hosts. Usually, Candida species can be present in several morphological forms in these infected tissues; oval budding yeasts, continuous septate hyphae or pseudohyphae. Nowadays, Candida-species are well recognized as important nosocomial pathogens in modern medicine due to the use of immunosuppressive and cytotoxic drugs, powerful antibiotics that suppress normal bacterial flora, and the implantation of various devices. Any surgically implanted device, such as a urinary catheter, endotracheal tube, voice prosthesis, will almost invariably be associated with infections whereby biofilms can be detected on the surface of the device. Also in case of the microbial colonization of functioning voice prostheses Candida albicans and commensal oral microflora are predominantly involved. Inoculation of the device can also occur during insertion by contamination via the skin or hands, by migration along the wound or fistula, or due to passing food and saliva.

The presence of particular combinations of bacterial and yeast strains in voice prosthetic biofilms has been suggested to be crucial for causing valve failure. A possible difference in biofilm composition of patients requiring frequent versus infrequent prosthesis replacements was determined in Groningen voice prostheses, which were removed because of increased airflow resistance or leakage of food or liquids through the prosthesis. The failing voice prostheses were subdivided into a short lifetime group, corresponding with an implantation-period less than 4 months and an extended lifetime group, comprising an implantation-period over 9 months. The bacterial strain Rothia dentocariosa and the yeast strains Candida albicans I and Candida tropicalis turned out to be the predominant strains isolated from biofilms on voice prostheses in the short lifetime group, while in the extended lifetime group R. dentocariosa was found with a fourfold lower isolation frequency and C. albicans I was found with a twofold lower isolation frequency. C. tropicalis was absent in the extended lifetime group.

**Drug resistance of biofilms**

Microbial biofilms seem to protect their micro-organisms from the effects of antimicrobial agents such as antibiotics, antifungics, antiseptics and biocides. Compared to planktonic living bacteria, biofilm related micro-organisms are 10 – 1000 times more resistant to antibiotics. Possibly some newer antifungal drugs which could interfere with the glucan synthesis in the Candida cell walls could be an effective target for biofilms if this polysaccharide is
present in the biofilm matrix.\textsuperscript{29,30} The suggested mechanisms of biofilm resistance to antimicrobial agents are related to restricted penetration of drugs through the biofilm matrix, phenotypic changes caused by decreased growth rate or limited nutrient sources, or expression of resistance genes induced by surface contacts.\textsuperscript{31} However, a multitude of mechanisms are involved in the operation of micro-organisms which vary per biofilm and are dependent on the administered antimicrobial agents.\textsuperscript{31}

An enhanced antimicrobial resistance of mixed fungal and bacterial biofilms is suggested in polymicrobial biofilms by the development of an increased matrix viscosity.\textsuperscript{32} The extensive interspecies interactions can modulate the action of antibacterial agents, while bacteria can affect the activity of antifungal agents in biofilms.

**Artificial throat**

Ideally, a system designed to model biofilm formation on an indwelling medical device would be one that simulated, as closely as possible, the conditions the device was exposed to in the patient. In the literature various procedures have been described to characterize the properties of bacterial and fungal biofilms.\textsuperscript{33} By using catheter discs, acrylic denture strips or microtitre plates biofilm growth can be monitored quantitatively while excellent correlation with biofilm dry weight is achieved. Unfortunately these methods only measure biofilm formation under static incubation conditions. In vivo, biofilms are subjected to liquid flows. These conditions can be partly mimiced by using sophisticated flow systems such as cylindrical cellulose filters or perfused biofilm fermenter, which can accurately control biofilm growth rate.\textsuperscript{34}

Further understanding of the process of development and inhibition of the colonization of surfaces requires comprehensive clinical studies. However, in vivo research of the biomaterials (e.g. voice prostheses) is difficult and time consuming. In order to simulate the natural process of biofilm development under dynamic nutrient conditions, an artificial throat was developed to determine the process of biofilm formation on voice prostheses.\textsuperscript{35} Biofilm developed on Groningen button voice prostheses in vitro could not be distinguished from that formed over several months in vivo. This method incorporated the ‘feast and famine’ cycle as a standardized approach for studying functional and structural aspects of all commercially available indwelling and non-indwelling voice prostheses, including the Groningen button and Provox II prosthesis under various laboratory conditions.
Prevention of biofilm formation

Since the deposits on dysfunctional prostheses have been regarded as potential hazardous factors for their function and device life, research has been focused on the composition of these deposits which contained a wide mixture of oropharyngeal micro-organisms. The ingrowing yeasts on the silicone rubber surfaces were primarily considered as pathogenic microbes, which could colonize and destruct the material and the valve function. Once the prostheses are colonized irreversible damage to the valve occurs and mechanical cleansing will not be effective anymore.3

Although typical biofilm formation will evolve on the surface of the silicone devices as well as by ingrowth of yeasts into the material (consumption of silicone by fungi), some Candida biofilms are reported to be limited to the surface of the devices.38 In these cases mechanical cleansing, using a brush or airflow (Provox flush®), or the use of antimicrobial rinses might be useful.

Several clinical studies were conducted to evaluate the effects of antimycotic drugs on the lifetime and function of different types of voice prostheses. A reduction of both the oropharyngeal flora as well as on the voice prostheses was determined by most of these studies. Bauters et al.41 assessed the colonization of tracheo-oesophageal voice prostheses by albicans and non-albicans Candida species and determined their susceptibility for three antimycotics that are frequently used for prophylaxis or treatment of oral candidiasis (i.e., miconazole, fluconazole, and nystatin). The predominant species isolated were Candida albicans (40%), Candida glabrata (30%), Candida krusei (15%), and Candida tropicalis (5%). A broad range of minimal inhibitory concentrations of the isolates was observed for miconazole and fluconazole. But, a uniform sensitivity with minimal inhibitory concentration values for nystatin was found for all isolates. Nystatin oral therapy significantly prolonged the lifespan of the indwelling Blom-Singer voice prosthesis in case of fungal colonization. After Nystatin therapy was stopped, the influence on lifetime persisted for the next inserted prosthesis.42

A consequence of continuous application of antimycotic drugs might be further medicalization of the patient, but frequent replacement of these prosthetic devices by medical professionals may substantially increase medical costs. Also, by using antifungal medication multiresistant bacteria and yeasts may develop in the complex mixtures of biofilms.

One of the crucial facts in patients after laryngectomy is the effect of radiotherapy on the surrounding tissues after and before surgery. The mucosa is changed by the destructive effects of radiotherapy, while the salivary glands are also sensitive to irradiation. In relation to biofilm several important functions can be defined for saliva. On all oral surfaces con-
ditioning films are formed, which contribute to selection for microbial adherence. It also serves as a medium for transporting planktonic bacteria within and between the mouth and the pharynx up to the oesophagus. Reattachment to surfaces may be prevented by salivary agglutinins, while attachment of dead cells may occur due to microbial proteins. These observations were primarily based upon laboratory studies. The effects of differences in salivary function on oral biofilms are yielding inconsistent results in clinical studies. Some clinical studies suggest that salivary proteins do influence the quantity and composition of streptococci in saliva. These findings may be important to devise ways to enhance salivary function in persons who are in higher risk due to deficiencies in this property.

In a retrospective analysis performed on 101 patients after total laryngectomy a relationship between voice prosthetic lifetime and the irradiation dose applied to the neck node levels (fields of the neck) in which the major salivary glands are partially included was established. Also, a possible relationship between voice prosthetic lifetime and the irradiation dose applied to the primary tumour site was studied. Irradiation of extensive neck fields, including the submandibular glands, did not influence the voice prosthetic lifetime after total laryngectomy. However, primary tumour doses exceeding 60 Gray significantly shortened the mean voice prosthetic lifetime per patient.

After irradiation the production of salivary proteins with antibiotic capacities decreases and infections may occur. To investigate whether synthetic salivary antimicrobial peptides have an inhibitory effect on the growth of bacteria and yeasts, the antimicrobial activities of six synthetic salivary peptides (histatin 5, dhvarl, dhvar4, dhvar5, lactoferrin b 1730 and cystatin S1-15) were determined for different oropharyngeal yeast (four) and bacterial (eight) strains and for a “total microflora” isolated from explanted voice prostheses using agar diffusion tests. Dhvar4 was the only one that was active against all micro-organisms tested, including the total microflora. Therefore, the synthetic salivary peptide dhvar4 may represent a useful drug, as an alternative for antibiotics and antymycotics employed in various ways to prolong the lifetime of voice prostheses in laryngectomees.

The incidence of pathological gastro-oesophageal reflux and laryngo-oesophageal reflux in head and neck are common in cancer patients. Reflux may have a serious impact on the mucosal surfaces and may contribute to extensive mucositis during irradiation in the head and neck area. Secondary Candida mucositis infections are common and require effective antymycotic therapy. The possible role of gastro-oesophageal reflux in candida biofilms on dysfunctional voice prostheses was suggested in a study in which the gastric contents showed stomach matched cultures from the prostheses. Also the prosthesis lifetime was markedly increased in patients with reflux complaints who were treated with anti-reflux medication.
Modification of biomaterials
Alternatives for biofilm prevention were sought in the development of modifications of the prosthetic materials, especially coatings. The application of surface modified silicone rubber devices led to remarkable results in laboratory circumstances. Although some promising surface modifications are available yet, definite manufacturing is troublesome due to technical difficulties and potential side effects, which may occur during long term application.19

Also the surface material affects biofilm formation; the use of latex or silicone elastomers increase biofilm formation, while polyurethane or 100% silicone substantially decrease biofilm formation in a disc model.52 Moreover, in vivo host proteins from serum or saliva will be rapidly absorbed and create conditioning films, which promote biofilm formation.53

Dairy
In the Codex Alimentarius of 1992 yoghurt is defined as a coagulated milk product after fermentation of lactic acid in milk by Lactobacillus bulgaricus and Streptococcus thermophilus.54 Also other fermenting organisms can be combined with L. bulgaricus and S. thermophilus in yoghurt or fermented dairy products to produce lactic acid. Although milk and yoghurt have similar vitamin and mineral compositions, yoghurt has less lactose and more lactic acid, galactose, peptides, free amino acids, and free fatty acid than does milk.55 Varying reports of the therapeutic efficacy of lactic acid bacteria may be related to the different fermenting organisms and the experimental procedures. Most studies indicated that their potential therapeutic effects are due to changes in the microecology of the gastrointestinal tract. The increase of lactic acid bacteria may suppress the growth of pathogenic bacteria that contributes to reduction of infections.56

However, these organisms must be able to colonize the human intestine and resist the influence of gastric acid. The conventionally used micro-organisms for yoghurt fermentation are less resistant for gastric acid than the lactic acid bacteria that colonize the human intestine. The inhibitory effects of these fermenting bacteria against disease causing bacteria are due to metabolites of lactic acid fermentation: organic acid and bacteriocin.57,58 Also, consumption of yoghurt with lactic acid bacteria can reduce antibiotic induced alterations of the intestinal microflora.59

The therapeutic and preventive effects of yoghurt and other dairy products containing lactic acid bacteria, which are commonly used to produce fermented foods and milk products, have been studied extensively on diseases as cancer, infection, gastro-intestinal disorders, and allergic disorders.54 The immune system is an important contributor to all of these diseases; an immunostimulatory effect of fermented foods has been suggested and investigated. Although several studies provide a strong indication of such an enhanced
immune response, the hypothesis needs to be substantiated in well designed evidence based studies. In general the results are limited by problems with study design, lack of appropriate controls, inappropriate route of administration, limited duration, and lack of human studies.

Anecdotal evidence from patients and their support groups suggested that the use of buttermilk and Turkish yoghurt had a positive effect on the lifetime of voice prostheses. This was evaluated in experiments using the artificial throat. Prostheses removed from the artificial throat in the control group were covered with a thick biofilm. Scanning electron microscopy showed microcolonies growing into the silicone rubber, similar to the ingrowth observed on explanted Groningen buttons. The simulated consumption of buttermilk in the other artificial throat almost fully prevented the formation of a biofilm on the prostheses during the experimental period. These in vitro experiments in the artificial throat demonstrate that the deterioration of voice prostheses can be limited by the daily intake of buttermilk through its inhibitory effects on biofilm formation. Since the biofilms grown in the artificial throat were similar to those found on dysfunctional prostheses removed from laryngectomized patients a link to an alternative approach for biofilm prevention and or treatment was established.

Probiotics

The major consumption of probiotics by humans is in the form of dairy-based foods containing mainly lactobacilli and/or bifidobacteria, while probiotics are also available as powders or tablets. In order to exert the maximum probiotic effects of bifidobacteria or lactobacilli it is important that viable bacterial numbers in the food and the faecal recovery of the administered bacteria are high. Although consumption of supplements containing pure probiotics to reach the required level may be more convenient, food is a better choice due to the synergistic effect between components of food and probiotic cultures. Dairy products containing probiotics also provide a number of high quality nutrients including calcium, protein, bioactive peptides, sphingolipids, and conjugated linoleic acids. Buffering of the gastric acidity by food may also enhance the recovery of probiotics. Very important for a good compliance of patients who should use these products for a long period is incorporating foods with probiotics as a lifestyle habit based on one’s inherent need to eat.

Many studies of probiotics on the physiologic effects show an effective dose of $10^9$-$10^{10}$ organisms per day. This corresponds to an intake of approximately a litre of acidophilus milk per day. The consumption of more than one probiotic strain may exert a synergistic effect, so that a smaller absolute volume is required. Also, simultaneous consumption may enhance the lifespan and activity of the probiotics. Since probiotics do not permanently adhere to the intestine, daily consumption is the best way to maintain their effectiveness. A fermented
soymilk containing *Bifidobacterium breve* or Yakult-fermented soymilk showed to be an excellent vehicle for live bifidobacteria. In volunteers with low levels of resident bifidobacteria feeding of fermented soymilk significantly increased the number of total bifidobacteria.62

A number of studies suggest that lactic acid bacteria can decrease the incidence, duration and severity of some gastric and intestinal illness. *L. acidophilus* may reduce the symptoms of small bowel bacterial overgrowth.63 There is also evidence that probiotic bacteria inhibit gastric colonization and activity of *Helicobacter pylori*. *L. salivarius* was found to inhibit *H. pylori* colonization in vitro64, while an inhibition of *H. pylori* was shown in humans consuming *L. johnsonii*.65 In a clinical trial by Wendakoon et al. the efficacy of an especially designed yoghurt product containing specific probiotics was conducted on the eradication of *H. pylori*.66 The yoghurt contained three Lactococcus species (i.e. *L. acidophilus*, *L. casei*) and a commercially available starter culture (*L. acidophilus*, *L. bulgaricus*, *Streptococcus thermophilus*). All of these cultures were found to have an effective inhibitory potential on the growth of *H. pylori* in vitro. Although the designed fermented milk containing lactobacilli was effective in the inhibition of *H. pylori* in vitro, eradication of this infection in 27 subjects was not achieved by consuming this product based upon a urea breath test.

The adhesion of probiotic bacteria to mucosal surfaces is considered as a first step in preventing attachment of pathogens by physically blocking receptors for specific adhesin analogues or by steric hinderance. Therefore, probiotics are defined as living micro-organisms, which upon ingestion in adequate numbers exert positive health effects beyond inherent basic nutrition.67 Besides preventing colonization by pathogens probiotics may strengthen the epithelial barrier to prevent pathologic translocation of the epithelium by promoting accelerated epithelial repair. This may be advantageous in patients undergoing radiotherapy to limit radioinduced damage to the mucosal epithelium.68 The effects are suggested to relate to the molecular mechanisms of probiotic and epithelial crosstalk to enhance mucosal immune responsiveness and inhibit pathogen colonization.

The dominant presence of lactobacilli in the urogenital microflora of healthy women and the absence of lactobacilli during urogenital tract infections has drawn attention to these species.69 The strains have not been associated with disease and have been regarded as non-pathogenic members of the intestinal and urogenital flora. *Lactococcus rhamnosus* GR-1 was found to be the best of 34 isolated strains to adhere to squamous and transitional uroepithelial cells, competitive exclusion of pathogens, and production of inhibitors of uropathogen growth.70 Another characteristic of lactobacilli explaining their probiotic actions was the production of biosurfactant by some 15 strains.71 Hydrogen peroxide-producing strains are believed to be important in vaginal colonization, while lactic acid was shown to be more potent in inhibiting the growth of uropathogens as *Gardnerella vaginalis*.72 A decrease of the
frequency of urinary tract infections was shown in a study in which *L. rhamnosus* GR-1 was given weekly as a suppository. The viable counts of lactobacillus recovered from vaginal swabs increased during therapy and especially in the periods of decreased UTI’s. From these studies it was also concluded that a low vaginal pH (<5) was not sufficient alone to prevent infections.73

**Future implications of probiotics**

Over a wide range of clinical conditions there will be a considerable potential for the benefits of probiotics. New food products as well as disease-specific medical foods will come on the market to accelerate and identify the right strains for optimal benefit of the desired effects. Originating as food supplements, probiotic micro-organisms are now most often administered orally and offer an attractive alternative for treatment of intestinal disorders. A better understanding of the mechanisms by which these micro-organisms act has now opened up possibilities for designing new probiotic strains. Through genetic engineering, it is possible not only to strengthen the effects of existing strains, but also to create completely new probiotics. These need not necessarily be composed only of bacterial products but can also include elements of regulatory systems or enzymes derived from a foreign-human-source. However, the problems with dosage and viability of probiotic strains, industry standardization and potential safety issues must be overcome. If designed carefully and with absolute attention to biological safety in its broadest sense, the development of genetically modified probiotics has the potential to revolutionize alimentary health.74
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