Biodynamics of Biofilm Formation on Nasogastric Tubes in Elderly Patients

Arthur Leibovitz MD[1], Yehuda Baumoehl MD[1], Doron Steinberg PhD[2] and Refael Segal MD[1]

[1]Shmuel Harofeh Hospital, Geriatric Medical Center, Beer Yaakov, Israel
Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel
[2]Biofilm Laboratory, Institute of Dental Research, Hebrew University-Hadassah Faculty of Dentistry, Jerusalem, Israel

Key words: biofilm, nasogastric tube, elderly, long-term care

Abstract

Background: We previously reported on the high propensity of pathogenic oral flora in the oropharynx of nasogastric tube-fed patients, and subsequently showed biofilm formation on the NGTs of these patients. There is a close relationship of biofilm and oropharyngeal colonization with pathogenic bacteria, aspiration pneumonia and antibiotic resistance.

Objectives: To investigate the time relation between the insertion of a new NGT and formation of the biofilm.

Methods: We examined sequential samples on NGTs that were forcibly pulled out by the patients themselves during any of the 7 days after insertion. Scanning electron micrography and confocal laser scanning microscopy were used for biofilm detection.

Results: Biofilm was identified on 60% of the five samples of day 1 and on all the samples of the following days, by both microscopic methods.

Conclusions: Biofilms form within a single day on most NGTs inserted for the feeding of elderly patients with dysphagia. Further research should be devoted to prevention of biofilm formation on NGTs.

IMAJ 2005;7:428–430

For Editorial see page 467

The oropharynx is a well-known potential reservoir for colonization by pathogenic microorganisms. It is the only readily accessible site in the body that has hard non-shedding surfaces conducive to microbial colonization. These allow the accumulation of secreted microbial products and the formation of oral biofilms that serve as culture media for bacteria [1,2]. Within the oral cavity, distinct habitats – cheeks, palate, tongue, periodontal region, teeth – provide different ecologic niches. These facilitate the growth of a variety of microorganisms, resulting in the establishment of diverse biofilm communities [2,3].

Biofilms are biological systems that have a high level of organization where bacteria/fungi form structured coordinated communities [3]. The ecologic advantages for the bacteria include protection from a hostile environment, nutrient availability, metabolic cooperation, and acquisition of new genetic traits [3]. Unfortunately, biofilm-originated bacteria are notoriously difficult to eliminate and treat [4,5], being up to 1,000-fold more resistant to antibacterial treatment than the same organism grown in a planktonic environment [6]. Medical devices and prostheses are highly susceptible to harboring bacteria, and the role of biofilms in the contamination of these implants has been well established [3,7]. Among the implants prone to contamination are catheters (venous, urinary) as well as devices such as prosthetic valves and tracheostomy tubes [8]. Overall, it is thought that at least 60% of all nosocomial infections are due to biofilms [3]. Nowadays, increasing numbers of the frail elderly population with oropharyngeal dysphagia are fed by either percutaneous enterogastric or nasogastric tube. The decision to initiate enteral feeding as well as the choice between NGT and PEG involve medical, emotional and ethical issues. Feeding by NGT is still widely practised in Israel [9].

In a recent study [10], we documented bacterial biofilm formation on NGTs in elderly patients. We also showed that these biofilms are related to pathologic colonization of the oropharynx. The potential clinical implications of such a reservoir of pathogens may be far reaching. First, there is the heightened risk of aspiration pneumonia and systemic infections, as well as the creation of "reservoirs of resistance" due to the "antibiotic pressure" to which these patients are subjected due to frequent clinical infections [11]. The presence of biofilm would certainly facilitate and augment this antibiotic resistance [12]. Furthermore, since these elderly long-term care patients are often transferred to general hospitals [13], they may well serve as vectors of resistant organisms in other medical settings. Clearly, biofilm formation on NGTs has serious clinical implications.

The purpose of this study was to define the biodynamics of biofilm formation on NGTs, i.e., the time interval between insertion of the tube and emergence of the biofilm.

Patients and Methods

This prospective study was conducted in the four skilled nursing wards of a 396-bed geriatric hospital. Skilled nursing wards are licensed to provide care to nursing patients with severe bed sores, advanced cancer, hemodynamic instability, or NGT feeding. The indication for enteral feeding was oropharyngeal dysphagia of at least grade 3, according to the Functional Out-

NGT = nasogastric tube

PEG = percutaneous enterogastric

come Swallowing Scale, FOSS [14]. Maintenance of oral hygiene in these enteral tube-fed patients involved cleansing the oral cavity three times a day with lemon-glycerin wadding sticks impregnated with a solution of glycerine citric acid, lemon flavoring and sodium benzoate 0.1%. The NGTs in use in our hospital are made of polyvinyl chloride (Duodenal Levin Tube, Maersk Medical, Lynge, Denmark).

Many of these patients were (at the beginning of this period) what we call NGT self-removers. Any patient who forcibly pulled out a first-ever introduced NGT feeding tube during the first 7 days of its insertion was included in the study. Excluded were patients with advanced cancer or those who had received antibiotic treatment up to 2 weeks prior to the study. Thirty-five NGT samples, 5 for each of the 7 post-NGT insertion days, were collected.

Samples from the oropharyngeal section [Figure 1] of the extricated NGT were prepared and examined by scanning electron micrography and confocal laser scanning microscope. For examination by SEM, the samples were fixed overnight with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, washed with the same buffer, dehydrated in increasing concentrations of ethanol, dried with a critical point drier, and coated with gold (Polaron-sem coating unit E5100, Thermo VG Scientific, Beverly, MA, USA). The outer surface of the samples was examined by a Jeol-840A scanning electron microscope (Jeol USA, Peabody, MA).

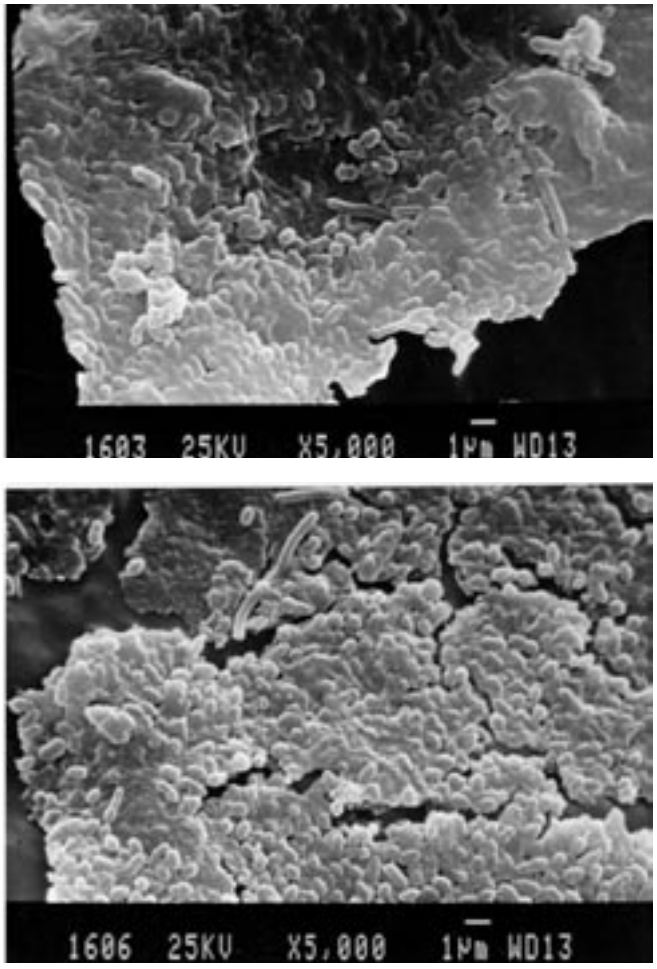

Figure 1. Representative SEM biofilms on nasogastric tubes showing bacterial organisms after 1 day (upper figure) and 2 days (lower figure)

For examination by CLSM, samples were fixed with formaldehyde, washed three times with ddH_2O, and stained with propidium iodide 1:100 for 30 minutes in dark conditions. Next, the samples were washed again with ddH_2O and then observed through a CLSM as follows: we used a Zeiss LSM 410 confocal laser scanning system attached to Zeiss Axiovert inverted microscope with a 10*/0.3 Plan-Neofluar lens, and excited red fluorescence of PI with a helium-neon laser (543 nm excitation line with 570 nm long-pass filter). In each experiment, exciting laser intensity, background level, contrast and electronic zoom size were maintained at the same level. At least two random fields for each experiment were taken and scored. Z-series of optical sections were acquired at 5 µm interval spacing steps from the surface through the vertical axis of the specimen by a computer-controlled motor drive. Entire series of confocal images were assembled in an integral image processor and projected into a single in-focus three-dimensional image using the Zeiss image analysis software. Red color indicates bacteria, while very high concentrations of bacteria are expressed as white spots. The black areas indicate the absence of bacteria.

Results

We examined the samples taken on days 1 and 2. Three of the five (60%) NGT samples of day 1 were already covered by biofilm 1 day (~24 hours) after their insertion. All five samples from day 2 were covered by biofilm-containing bacteria. Both methods of examination yielded similar results. Given these findings, samples from the following days were not examined.

Figure 1 shows the SEM pictures of the biofilms on the NGT samples after 1 and 2 days. The pictures of the subsequent days look similar, apart from an additional organization/stabilization of the biofilm surface. Fluorescent images taken by CLSM support our notion that biofilm formation on NGTs is a kinetic process [Figure 2].

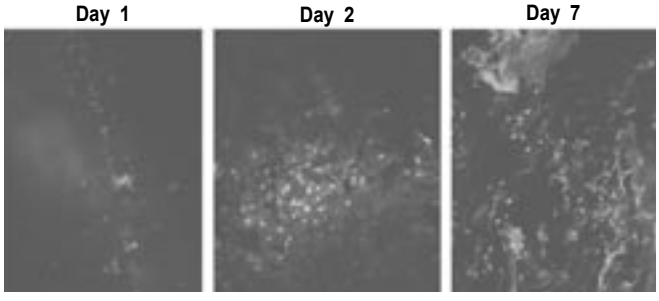

Figure 2. The sequential biofilm formation on the nasogastric feeding tube by confocal laser scanning microscopy.

SEM = scanning electron micrography
CLSM = confocal laser scanning microscope
PI = propidium iodide

Discussion

Our study demonstrates that the formation of bacterial biofilm on a newly inserted NGT for the feeding of elderly frail patients is rapid and in most cases occurs within 24 hours. To the best of our knowledge, this is the first report of this phenomenon based on *in vivo* observations in humans. These results are consistent with animal studies [15] showing that bacterial biofilm on endotracheal tubes is already well organized in a sessile antibiotic-resistant structure within 24 hours [15]. PVC tracheal tubes in humans have also been reported to rapidly form bacterial biofilms, which become a source of lower respiratory tract colonization [16,17]. Although not placed in the trachea itself, NGTs are situated in a crossroad position within the oropharynx. It follows that, being in close proximity to the trachea, lower respiratory tract colonization will likely occur. In addition, the pathogens growing in the shadow/protection of the biofilm are known to precipitate the emergence of antimicrobial resistance. Indeed, oral biofilms in the elderly are increasingly recognized as reservoirs of colonization and infection and are associated with systemic diseases [18].

As recently reported, the nature of environmental signals and the regulatory pathways that influence bacterial biofilm formation are very complex [19]. Early evidence suggested that materials like polyurethane, vialon or teflon may be less prone to bacterial biofilm formation [20]. Coating the implant interface with antibiotics or EDTA was also tried, with variable results [21,22]. A promising new approach, coating the device with the quorum-sensing inhibitor RNAIII-inhibiting peptide, was also recently reported [23].

The use of feeding NGTs for the provision of hydration, nutrition and medicines to elderly frail patients with oropharyngeal dysphagia is still widespread in Israel. Although in many cases a NGT is replaced by PEG, our study indicates that bacterial biofilms often form on the very first day of NGT insertion. This finding is also relevant to other categories of patients fed by means of a NGT, such as newborn infants and intensive care unit patients. Efforts should be devoted to the search for new ways to prevent, reduce or at least delay biofilm formation on a device located in such a critical position.

References

1. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis* 2000;8(9):881–90.
2. Steinberg D. Studying plaque biofilms on various dental surfaces. In: An YH, Friedman RJ, eds. Handbook of Bacterial Adhesion: Principles, Methods, and Applications. Totowa, NJ: Humana Press, 2000:353–70.
3. Davey ME, O'toole GA. Microbial biofilms: from ecology to molecular genetics. *Microbiol Mol Biol Rev* 2000;64(4):847–67.
4. Hassett DJ, Limbach PA, Hennigan RF, et al. Bacterial biofilms of importance to medicine and bio-terrorism: proteomic techniques to identify novel vaccine components and drug targets. *Expert Opin Biol Ther* 2003;3(8):1201–7.
5. Russell AD. Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. *Lancet Infect Dis* 2003;3(12):794–803.
6. Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. *Nature* 2003;426(6964):306–10.
7. Di Filippo A, De Gaudio AR. Device-related infections in critically ill patients. Part II: Prevention of ventilator-associated pneumonia and urinary tract infections. *J Chemother* 2003;15(6):536–42.
8. Jarrett WA, Ribes J, Manaligod JM. Biofilm formation on tracheostomy tubes. *Ear Nose Throat J* 2002;81(9):659–61.
9. Clarfield AM, Paltiel A, Gindin Y, Morginstin B, Dwolatzky T. Country profile: Israel. *J Am Geriatr Soc* 2000;48(8):980–4.
10. Leibovitz A, Dan M, Zinger J, Carmeli Y, Habot B, Segal R. *Pseudomonas aeruginosa* and the oropharyngeal ecosystem of tube-fed patients. *Emerg Infect Dis* 2003;9(8):956–9.
11. Limeback H. Implications of oral infections on systemic diseases in the institutionalized elderly with a special focus on pneumonia. *Ann Periodontol* 1998;3(1):262–75.
12. Brooun A, Liu S, Lewis K. A dose-response study of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother* 2000;44(3):640–6.
13. Teresi JA, Holmes D, Bloom HG, Monaco C, Rosen S. Factors differentiating hospital transfers from long-term care facilities with high and low transfer rates. *Gerontologist* 1991;31(6):795–806.
14. Salassa JR. A functional outcome swallowing scale for staging oropharyngeal dysphagia. *Dig Dis* 1999;17(4):230–4.
15. Lyte M, Freestone PP, Neal CP, et al. Stimulation of *Staphylococcus epidermidis* growth and biofilm formation by catecholamine inotropes. *Lancet* 2003;361(9352):130–5.
16. Adair CG, Gorman SP, Feron BM, et al. Implications of endotracheal tube biofilm for ventilator-associated pneumonia. *Intensive Care Med* 1999;25(10):1072–6.
17. Gorman SP, McGovern JG, Woolfson AD, Adair CG, Jones DS. The concomitant development of poly(vinyl chloride)-related biofilm and antimicrobial resistance in relation to ventilator-associated pneumonia. *Biomaterials* 2001;22(20):2741–7.
18. Senpuku H, Sogame A, Inoshita E, Tsuha Y, Miyazaki H, Hanada N. Systemic diseases in association with microbial species in oral biofilm from elderly requiring care. *Gerontology* 2003;49(5):301–9.
19. Stanley NR, Lazazzera BA. Environmental signals and regulatory pathways that influence biofilm formation. *Mol Microbiol* 2004;52(4):917–24.
20. Lopez-Lopez G, Pascual A, Perea EJ. Effect of plastic catheter material on bacterial adherence and viability. *J Med Microbiol* 1991;34(6):349–53.
21. Hendricks SK, Kwok C, Shen M, Horbett TA, Ratner BD, Bryers JD. Plasma-deposited membranes for controlled release of antibiotics to prevent bacterial adhesion and biofilm formation. *J Biomed Mater Res* 2000;50(2):160–70.
22. Raad I, Chatzinikolaou I, Chaiban G, et al. In vitro and ex vivo activities of minocycline and EDTA against microorganisms embedded in biofilm on catheter surfaces. *Antimicrob Agents Chemother* 2003;47(11):3580–5.
24. Dell'Acqua G, Giacometti A, Cirioni O, et al. Suppression of drug-resistant Staphylococcal Infections by the quorum-sensing inhibitor RNAIII-inhibiting peptide. *J Infect Dis* 2004;15;190(2):318–20.

Correspondence: Dr. R. Segal, Shmuel Harofeh Hospital, P.O.Box 2, Beer Yaakov 70350, Israel.

Phone: (972-8) 925-8666

Fax: (972-8) 922-0672

email: shmuelh@netvision.net.il

PVC = polyvinil chloride