We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Scanning Electron Microscopy (SEM) and Environmental SEM: Suitable Tools for Study of Adhesion Stage and Biofilm Formation

Soumya El Abed^{1,2}, Saad Koraichi Ibnsouda^{1,2},
Hassan Latrache³ and Fatima Hamadi³

¹Laboratory of Microbial Biotechnology, Faculty of Science and Technics, Fez,

²Regional University Center of Interface, University Sidi Mohamed Ben Abdellah, Fez,

³Laboratory of Valorization and Security Food Products,
Faculty of Science and Technics, Beni Mellal,
Morocco

1. Introduction

For most of the history of microbiology, microorganisms have primarily been characterized as planktonic, freely suspended cells and described on the basis of their growth characteristics in nutritionally rich culture media. The discovery of microorganisms, 1684, is usually ascribed to Antoni van Leeuwenhoek, who was the first person to publish microscopic observations of bacteria. The direct quantitative recovery techniques showed unequivocally that more than 99.9% of the bacteria grow in biofilms on a wide variety of surfaces. Although the most common mode of growth for microorganisms on earth is in surface associated communities (Stoodley et al., 2002; Sutherland, 2001), the first reported findings of microorganisms “attached in layers” were not made until the 1940s. During the 1960s and 70s the research on “microbial slimes” accelerated but the term “biofilm” was not unanimously formulated until 1984 (Bryers, 2000). Biofilm has three-dimensional (3D) structured, heterogeneous community of microbial cells enclosed in an exopolysaccharide matrix (also called glycocalyx) that are irreversibly attached to an inert or living surface. As establish, biofilm formation has a serious implications in public health and medicine. In the case of human health, a number of microbial infections are associated with surface colonization not only on live surfaces (sinusitis, pulmonary infection in cystic fibrosis patients, periodontitis, etc. (Hall-Stoodley et al., 2004) but also on medical implants (contact lenses, dental implants, intravascular catheters, urinary stents) etc. (Donlan, 2001; Hall-Stoodley et al., 2004). Biofilms affect heat exchangers, filters, etc. because they induce biocorrosion and biofouling, producing damages on metallic surfaces and the efficiency loss in industrial set-up (Dunne, 2002; Garret et al., 2008). However, biofilms have also useful applications in bioremediation (Vidali, 2001) of different environments (microorganisms degrade and convert pollutants into less toxic forms) and biolixiviation (bacteria can efficiently dissolve minerals used in industry, to obtain copper and gold).

In order that we may gain a greater insight into the ecology of the microorganisms that exist in biofilm, it is necessary not only to be able to isolate them by traditional culture methods but also to have some understanding of the way in which these individual microorganisms interact in situ in their environment. Different microscopic techniques for biofilm monitoring including Scanning Electron microscopy (SEM) have been proved to be suitable tools in order to follow the study of adhesion stage and biofilm formation. Scanning electron microscopy as a specialized field of science that employs the electron microscope as a tool and uses a beam of electrons to form an image of a specimen allowing imaging and quantification of surface topographic features.

The scope of this chapter is to illustrate the importance of scanning electron microscopy and environmental scanning electron microscopy in biofilm examination and control. Furthermore, although we are conscious about the vast variety of biofilms in natural, clinical and industrial environments, this chapter will mainly concentrate on imaging application of SEM and ESEM biofilms.

2. Step of biofilm formation

Planktonic cells are able to attach on the surfaces and form biofilm through a process that include several steps:

Fig. 1. Schematic illustrations of biofilm formation and development. (Filloux & Vallet, 2003).

2.1 Attachment/colonization

The primary adhesion stage constitutes the beneficial contact between a conditioned surface and planktonic microorganisms. During the process of attachment, the organism must be brought into close proximity of the surface, propelled either randomly or in a directed fashion via chemotaxis and mobility (Prakash et al., 2003). This step is reversible and it is characterized by a number of physicochemical variables that defines the interaction between the microbial cell surface and the conditioned surface of interest (An et al., 2000; Liu et al., 2004; Singh et al., 2002).

2.2 Irreversible adhesion

The second step is the irreversible adhesion during which bacteria start to express adhesion protein such as curli or fimbriae to adhere to the surface. Microorganisms starts to produce intercellular connections (intercellular curli for example) and a polymeric matrix, usually

called extracellular polymeric substances (EPS). This matrix is a complex hydrogel embedding the bacteria community and building up in three dimensions. The backbone of this gel is mainly composed of polysaccharides produced by bacteria (such as colanic acid, chitosan, alginate), other components such as enzymes, DNA, RNA, nutrients, proteins, surfactants (Flemming et al., 2007). The exact role of the matrix is not yet completely elucidated but it has been demonstrated that the matrix acts as a protective layer (Fux et al., 2005) and is microenvironment-conservative (Beech, 2004).

After the adherence of microorganism to the inert surface, the association becomes stable for micro-colonies formation (Bechmann & Eduvean, 2006; O'Toole et al., 2000). The microorganism begin to multiply while sending out chemical signals that intercommunicate among the bacterial cells. In this way, the bacteria multiply within the embedded exopolysaccharide matrix, thus giving rise to formation of a micro-colonies (Prakash et al., 2003).

2.3 Maturation of biofilm

Once bacteria have irreversibly attached to a surface, the process of biofilm maturation begins. The overall density and complexity of the biofilm increase as surface-bound organisms begin to actively replicate and extracellular components generated by attached bacteria interact with organic and inorganic molecules in the immediate environment to create the glycocalyx (Carpentier & Cerf, 1993). The maturation of biofilm generate many process already having taken place, such as quorum sensing (Nadell et al., 2008), gene transfer (Molin, 2003), persister development (Lewis, 2005) etc. All of these processes contribute to the community life of the biofilm and play an important role in biofilm survival and biofilm spreading, since they allow also detachment of biofilm parts and release of free bacteria, which is the most common way for biofilm to spread (Kaplan et al., 2003).

2.4 Detachment and dispersal of biofilm cells

As the biofilm gets older, cells detach, disperse and colonize a new niche. This detachment can be due to various factors including, fluid dynamics and shear effects of the bulk fluid (Brugnoni et al., 2007). At some point of biofilms may partially dissolve releasing cells that more away to other where a new cycle begins (Prakash et al., 2003; Singh et al., 2002).

3. Imaging application

SEM is a well-established basic method to observe the morphology of bacteria adhered on a material surfaces, the morphology of the material surface, and the relationships between them (Peters et al., 1982). SEM has been used for enumeration of adhered bacteria or tissue large number of samples. It is as a key technique that provides also information about the morphology of biofilm, presence of EPS and the nature of corrosion products (crystalline or amorphous).

3.1 SEM applied of adhesion stage

Microbial adhesion is the first step of the formation of biofilm and an extremely complicated process that is affected by many factors. In this regard, detailed investigation of microbial

adhesion involved in the developmental process from single sessile bacteria to multicellular biofilm is crucial to elaborate strategies to control biofilm development. Moreover, submicrometer-scale cell surface polymers and appendages, such as curli, flagella, and exocellular polymers, have been shown to play essential roles during cell adhesion and biofilm formation (Busscher et al., 2008; Dufrêne, 2008; Rodrigues & Elimelech, 2009). A SEM image of such a curli is depicted in Figure 2.

Fig. 2. SEM images of *E.coli* K-12 MG 1655 ompR234 producing curli (Olsen et al., 1989)

Adhesion phenomena has been evaluated as function of substratum, liquid medium, carbone source, pH and hydrodynamics parameters including flow rate. Many of the conclusions about biofilm development, composition, distribution, and relationship to substratum have been derived from scanning electron microscopy (Bragadeewaran et al., 2010; Herald & Zottola, 1988; Pinna et al., 2000). We report here several investigations made in our laboratory used scanning electron microscopy to study adhesion phenomena. Hamadi et al., (2005) have investigated the adhesion of *Staphylococcus aureus* ATCC 25923 to glass at different pH values using scanning electron microscopy and image analysis with the Mathlab® program is shown in Figure 3.

The surface topography has been widely discussed as a parameter influencing microbial adhesion. In this regard, experiments made by Kouider et al., (2010) using SEM to determine the effect of stainless steel surface roughness on *Staphylococcus aureus* adhesion shown that adhesion level was found to largely depend on the substrate roughness with maximum at $R_a = 0.025 \text{ m}$ and minimum at $R_a = 0.8 \text{ m}$. Mallouki et al., (2007) have studied the anti-adhesive effect of fucans by SEM and a MATLAB program to determine the number and characteristics of adhered cells.

3.2 SEM applied of biofilm formation

Scanning electron microscopy (SEM) is a useful technique for the investigation of surface structure of biological samples (Duckett & Ligrone, 1995; Minoura et al., 1995; Motta et al., 1994). For instance, much of the current knowledge about biofilms is due to the advances in imaging studies, especially the SEM. Early microscopic techniques used in biofilm monitoring, mainly applied during the 1980s, include scanning electron microscopy. SEM has been previously used to show a clear visualization of bacteria within a biofilm and is capable of demonstrating even a single bacterium and the relation of the biofilm to the underlying surface.

Fig. 3. SEM images of *S. aureus* adhered to glass as a function of pH (Hamadi et al., 2005)

Biofilm morphology and mass are important characteristics that control the kinetics of substrate removal by biofilms. SEM is a powerful technique for revealing the fine structure of living systems and has been applied to biofilms (Eighmy et al., 1983; Richards and Turner, 1984; Weber et al., 1978). It has also been of special importance in elucidating biofilm structure for understanding the physiology and ecology of these microbial systems (Blenkinsopp & Costerton 1991). For example, electron-microscopic studies proved that the biofilm is composed of bacterial cells “wrapped” in a dense “glycocalyx”, i.e. exopolysaccharide matrix (Blenkinsopp & Costerton, 1991; Eighmy et al. 1983). In medical applications, for example, Storti et al., (2005) used scanning electron microscopy and reported that the extracellular biofilm matrix appears as an amorphous material on the catheter surface. In the same context, scanning electron microscopy (SEM) images of matrix-enclosed microbial assemblages on leaf surfaces (Surico, 1993) have led some authors to suggest that biofilms occur in the phyllosphere (Beattie and Lindow, 1995). Morris et al., (1997) have been to observe microbial biofilms directly on leaf surfaces. Bacterial aggregates in the phyllosphere have been observed previously with SEM (Surico,1993), but most have been very small (less than 20 mm long) or have lacked an obvious exopolymeric matrix (Surico,1993). Previous studies have claimed to demonstrate the presence of biofilms in situ on plant aerial surfaces using SEM (Gras et al., 1994).

Biofilm thickness is also especially important for calculation of heat exchange or diffusion rates of antimicrobials or nutrients through a biofilm and for evaluation of the mechanical properties of a biofilm (Korstgens et al., 2001). As reported elsewhere, SEM sample (freeze-dried cross-section of Foley bladder catheter) revealed the thickness of biofilm and also the layers of embedded of slime by different strains and species of bacterial cells (Ganderton et al., 1992).

In general, other application of SEM techniques may be mentioned. Akernan et al., (1993) used scanning electron microscopy of nanobacteria - novel biofilm producing organisms in

blood. Indeed, nanoscale characterization of *Escherichia coli* Biofilm formed in the glass surface using scanning electron microscopy has been reported by Lim et al., (2008). He showed reticular structures on the surface of biofilms. The reticular structures consist of nanopores having diameter ranging from 14 nm to 100 nm.

Scanning electron microscopy (SEM) is one of the many methods available for the visual the effect of antibacterial or antifungal on biofilm development (Camargo et al., 2005; McDowell et al., 2004; Sasidharan et al., 2010; Sevinç & Hanley, 2010; Zameer & Gopal, 2010; Zeraik & Nitschke, 2010). Sasidharan et al., (2010) used SEM for studied The effects of potential antifungal extracts from natural sources in *Candida albicans* biofilm (Figure.4).

Fig. 4. Scanning electron micrograph reduction in *Candida albicans* biofilm after 36 h treatment. (a) Control and (b) Cassia spectabilis extract treated *C. albicans* cells.

3.3 Advantages and disadvantages of SEM

In part, it is true that Scanning electron microscopy (SEM) present a many advantages, the more important are: (i) higher resolution of visualization microbial biofilms (Walker et al., 2001) than other imaging techniques, typically 3.5 nm, (ii) able to measure and quantify data in three dimensions. However, this technique utilizes graded solvents (alcohol, acetone, and xylene) to gradually dehydrate the specimen prior to examination, since water of hydration is not compatible with the vacuum used with the electron beam. While any pretreatment can alter specimen morphology, drying appears to significantly alter biofilms due to EPS polymers collapsing (Fassel & Edmiston, 1999; Little et al., 1991). The dehydration process results in significant sample distortion and artifacts; the extracellular polymeric substances, which are approximately 95% water and the liquid loss led them to appear more like fibers surrounding the cells than like a gelatinous matrix (Characklis & Marshall, 1990). Several ultrastructural studies have used conventional scanning electron microscopy (SEM) to investigate the glycocalyx, but these studies (Costerton et al., 1981; Fassel et al., 1991; Marshall et al., 1971) were hampered by low resolution and also by the inability to use low voltages (<5 keV), which yield increased information from small topographical features (Pawley & Erlandsen, 1989).

Typically, SEM imaging requires a high vacuum, $\leq 10^{-8}$ Torr (reviewed in Stewart, 1985), having first been chemically fixed, dehydrated, and coated with a conductive material (e.g. gold) to prevent charge buildup from the electron beam. Few biological specimens tolerate

these conditions without rapid collapse (Heslop-Harrison, 1970) and fewer still survive (Read & Lord, 1991). Uncoated non-conductors build up local concentration of electron, referred to as-charging- that prevent the formation of usable images. Energy X-ray Spectroscopy (EDS) can be used to determine the elemental composition of surface films in the SEM, but EDS analyses must be completed prior to deposition of the thin metal coating. EDS data are typically collected from an area, the specimen must be removed from the specimen chamber and coating with a conductive layer, and returned to the SEM.

To allow observations under the high vacuum conditions of SEM, many preparations of biological samples have been developed, e.g., glutaraldehyde fixation, negative staining, the Sputter-Cryo technique, and coating with gold or osmium (Allan-Wojtas et al., 2008; Hassan et al., 2003; Lamed et al., 1987). Moreover, these preparations have some positive effects on the biological sample; for instance, they enhance contrast, reduce damage, and are uncharged up by the electron beam.

4. Biofilm formation: Environmental Scanning Electron Microscopy (ESEM)

A new SEM technique is now available which allows overcoming these obstacles. a modified, low-vacuum scanning electron microscopy technique for biofilm monitoring that enables imaging of hydrated specimens, termed environmental scanning electron microscopy (ESEM) also called variable pressure SEM (VP-SEM), was introduced in the mid-1990s (Little et al., 1991). The environmental SEM (reviewed in Stokes & Donald, 2000) uses a series of pressure limiting apertures (Muscariello et al., 2005) while preventing gas leakage from the specimen chamber, which can be maintained at 1–20 Torr. The ESEM is based upon the gaseous detection device (GDD). The main feature distinguishing ESEM from conventional SEM is the presence of a gas in the specimen chamber. Gases may include nitrous oxide, helium, argon and other, but water vapour is the most efficient amplifying gas found and the most common gas used in ESEM. The ionization GDD uses the ionization of the gas for the detection of secondary electrons from the specimen surface. It is a conical electrode about 1 cm in diameter that is positioned with the apex downward and concentric with the beam at the bottom of the pole piece. Secondary electrons emitted from the sample collide with water molecules in the chamber producing additional electrons and positive ions. The positive ions are attracted to the sample surface and eliminate the charging artifacts. A proportional cascade amplification of the original secondary electron signal results. With the GDD both secondary and backscattered electron images can be produced. Detailed technical explanations about this device can be found elsewhere (Danilatos, 1990).

The balance of gas flows into and out of the ESEM sample chamber determines its pressure.

The multiple apertures are situated below the objective lens and separate the sample chamber from the column. This feature allows the column to remain at high vacuum while the specimen chamber may sustain pressures as high as 50 Torr. The temperature and humidity of the sample can also be manually controlled to provide a suitable environment for maintaining the biological samples in their natural state.

The relative humidity in an ESEM specimen chamber can be controlled (Stokes & Donald, 2000), so ESEM is particularly useful for hydrated materials (Muscariello et al., 2005; Stokes & Donald, 2000; Stokes, 2001). A gaseous secondary electron detector (GSED) exploits the gas in the specimen chamber for signal amplification. BSED operation produces positive

ions that have the added benefit of limiting charging of non-conductive specimens (Stokes & Donald, 2000). It does not require prior fixing and staining of the biofilm, minimizes biofilm dehydration and thus preserves native morphologies including surface structures (Walker et al., 2001) and native morphologies of bacteria and biofilms (e.g. Priester et al., 2007) and is able to achieve high magnifications, comparable with SEM. Shrinkage is prevented and artefact formation is reduced.

Additional advantages of ESEM include minimal processing of samples. It results in shorter time scales and lower costs while reducing the possibility of introducing artefacts. Samples can be preserved in saline in a common refrigerator (in fresh) if examination is to be deferred a few hours (Ramírez-Camacho et al., 2008). ESEM provides spatial resolutions of 10 nm or less. Compared to SEM, ESEM produces different, perhaps complementary, information for biological specimens (Doucet et al., 2005; Surman et al., 1996). Cell structures are visible with SEM, but external polymers around cells are more apparent in ESEM (Callow et al., 2003; Doucet et al., 2005; S. Douglas & D.D. Douglas, 2001).

4.1 ESEM applied of biofilm formation

Sutton et al., (1994) used this technique to study the structure of a *Streptococcus crista* CR3 biofilm. Gilpin & Sigee (1995) showed that biological samples can be imaged in the ESEM in wet or partially hydrated states with a minimum of sample damage and changes in specimen morphology. This gave the possibility to the visualization of biofilm surfaces in their natural wet anaerobic state (Darkin et al., 2001). Recently, Schwartz et al., (2009) used ESEM imaging to obtain information about the bacterial composition, matrix composition, and spatial biofilm structures of natural biofilms grown on filter materials at waterworks.

Scanning electron microscopes are frequently equipped with an energy dispersive x-ray analyser. This equipment permits elemental analysis with a high horizontal resolution of the inspected specimens. In this same context, mineral structures formed by bacterial and microalgal biofilms growing on the archaeological surface in Maltese hypogea were studied using Energy Dispersive X-Ray Spectroscopy (EDS) coupled to Environmental Scanning Electron Microscopy (ESEM), are reported by Zammit et al., (2011). These techniques have shown that mineral structures having different morphologies and chemical composition were associated with the microorganisms in the subaerophytic biofilm (Figure.5).

Fig. 5. ESEM and EDS analysis for the system under SRB-biofilm influence. (A) SEM Image of carbon steel exposed to sterile artificial seawater (supplemented with nutrients) and with SRB, (B) EDS analysis corresponding to the ESEM smooth region.

Interestingly, Shen et al., (2011) have been proposed a novel method for measuring an adhesion force of single yeast cell based on a nanorobotic manipulation system inside an environmental scanning electron microscope (ESEM) and Dubey & Ben-Yehuda (2011) report the identification of analogous nanotubular channels formed among bacterial cells grown on solid surface. They demonstrate that nanotubes connect bacteria of the same and different species, thereby providing an effective conduit for exchange of intracellular content.

5. Conclusion

Scanning electron microscopy is a key tool to study the effect of physicochemical properties on adhesion phenomena (pH, roughness, topography, temperature, etc). SEM plays also a paramount role for assessing the microbial populations, three-dimensional structure, physiology, thickness, etc.

SEM proved to be an invaluable method for ultra-structural investigation, allowing imaging of the overall appearance and/or specific features of biofilms formed in different environments, e.g. microbial colonies and individual cells, the glycocalyx, and the presence of inorganic products within the biofilm.

Surely, Scanning Electron Microscope (SEM) is a powerful research tool, but since it requires high vacuum conditions, the wet materials and biological samples must undergo a complex preparation that limits the application of SEM on this kind of specimen and often causes the introduction of artifacts. The introduction of Environmental Scanning Electron Microscope (ESEM), working in gaseous atmosphere, represented a new perspective in biofilm monitoring with high resolution without prior fixing and staining.

ESEM could be useful as a complementary technique to help in the characterization of the structure and architecture of biofilms. In fact, ESEM could reveal the exact topography of intact, live and fully hydrated biofilms, with a higher magnification than the other microscopy techniques. In general, a combination of several techniques is to be recommended when investigating biofilms as the different techniques offer distinctly valuable information about different aspects of biofilm development.

6. References

- Akernan, K.K. Kuronen, Ilpo. Olavi Kajander, E. (1993). Scanning electron microscopy of nanobacteria - Novel biofilm producing organisms in blood. *Scanning*, Vol.15, Supplement III.
- Allan-Wojtas, P. Hansen, L.T. & Paulson, A.T. (2008). Microstructural studies of probiotic bacteria loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation. *LWT-Food Science and Technology*, Vol.41, No.1, (January 2008), pp.101-108.
- An, Y. H, Dickinson, R. B. & Doyle, R. J. (2000). Mechanisms of bacterial adhesion and pathogenesis of implant and tissue infections. pp. 1-27. In An, Y. H. & Friedman, R. J. (ed.), *Handbook of bacterial adhesion: principles, methods, and applications*. Humana Press, Totowa, N.J.
- Bacteria to Polystyrene Surfaces: Effect of Temperature and hydrophobicity. *Current of Microbiology*, Vol.61, (December 2010), pp.554-559.

- Beattie, G.A. Lindow, S. E. (1995). The secret life of foliar bacterial pathogens on leaves. *Annual Reviews of Phytopathology*, Vol.33, (September 1995), pp.145–17.
- Bechmann, R.T. & Eduvean, R.G.C. (2006). AFM Study of the colonization of stainless steel by *Aquabacterium commune*. *International Biodeterioration & Biodegradation*, Vol.58, No.3-4, (October-December 2006), pp.112-118.
- Beech, I. (2004). Biocorrosion: towards understanding interactions between biofilms and metals. *Current Opinion in Biotechnology*, Vol.15, No. 3, (Jun 2004), pp.181-186.
- Blenkinsopp, AS. & Costerton, JW. (1991). Understanding bacterial biofilms. *Trends in Biotechnology*, Vol.9, No.1, (January 1991), pp. 138–143.
- Bragadeeswaran, S. Balasubramanian, ST. Raffi, SM. & Rani, Sophia S. (2010). Scanning electron microscopy elemental studies of primary film. *World Applied Sciences Journal*. Vol.10, No.2, pp.169-172.
- Brugnoni, L-I. Lozano, J-E. & Cubitto, M-A. (2007). Potential of yeast isolated from apple juice to adhere to stainless steel surfaces in the apple juice processing industry. *Food Research International*, Vol.40, No.3, (April 2007), pp. 332–340.
- Bryers, JD, (2000). Biofilms: an introduction, in *Biofilms II: process analysis and applications*, In: Bryers, JD, (Ed.), 3-11, Wiley-Liss, New York.
- Busscher, H. J. van de Belt-Gritter, B. Dijkstra, R. J. B. Norde, W. & van der Mei, H. C. (2008). *Streptococcus mutans* and *Streptococcus intermedius* adhesion to fibronectin films are oppositely influenced by ionic strength. *Langmuir*, Vol24, N .19,(August 2008),pp.10968–10973.
- Callow, J.A. Osborne, M.P. Callow, M.E. Baker, F. & Donald, A.M. (2003). Use of environmental scanning electron microscopy to image the spore adhesive of the marine alga *Enteromorpha* in its natural hydrated state. *Colloids Surface B: Biointerfaces*, Vol.27, No.4, (Jun 2003), pp.315–321.
- Camargo, G.M.P.A. Pizzolitto, A.C. & Pizzolitto, E.L. (2005). Biofilm formation on catheters used after cesarean section as observed by scanning electron microscopy. *International Journal of Gynecology and Obstetrics*, Vol.90, (August 2005), pp.148 – 149.
- Carpentier, B. & Cerf, O. (1993) Biofilms and their consequences, with particular reference to hygiene in the food industry. *Journal of Applied Bacteriology*, Vol.75, No. 6, (March 1993),pp.499-511.
- Characklis, W. G. & Marshall, K.C. (1990). Biofilms: a basis for an interdisciplinary approach, pp. 3–15. In: Characklis, W.G. & Marshall, K.C. (ed.), *Biofilms*. John Wiley & Sons, New York, N.Y.
- Costerton, J.W. Irvin, R.T. Cheng, K-J. (1981). The bacterial glycocalyx in nature and disease. *Annual Reviews of Microbiology*, Vol.35, (October 1981), pp.299–324
- Costerton, JW, Stewart, PS. & Greenberg, EP. (1999). Bacterial biofilms: a common cause of persistent infections. *Science*, Vol.284, No.5418, (May 1999), pp. 1318-1322.
- Danilatos, G.D. (1990). Theory of the gaseous detector device in the environmental scanning electron microscope. *Advances in Electronics and Electron Physics*, Vol.78, pp.1-102.
- Darkin, M.G. Gilpin, C. Williams, J.B. & Sangha, C.M. (2001). Direct wet surface imaging of an anaerobic biofilm by environmental scanning electron microscopy: application to landfill clay liner barriers, *Scanning*, Vol.23, No .5,pp. 346–350.
- Donlan, RM. (2001) Biofilms and Device-Associated Infections. *Emerging Infectious Diseases*, vol.7, No .2, (March-April 2001),pp.277-281.
- Doucet, F.J. Lead, J.R. Maguire, L. Achterberg, E.P. & Millward, G.E. 2005. Visualisation of natural aquatic colloids and particles - a comparison of conventional high vacuum and environmental scanning electron microscopy. *Journal of Environmental Monitoring*, Vol.7, No.2, (January 2005),pp.115–121.

- Douglas, S. & Douglas, D.D. (2001). Structural and geomicrobiological characteristics of a microbial community from a cold sulfide spring. *Geomicrobiology Journal*, Vol.18, No.4, (November 2001),pp.401-422.
- Dubey, G.P. and Ben-Yehuda, S. (2011). Intercellular nanotubes mediate bacterial communication. *Cell*, Vol.144, No. 4, (February 2011),pp. 590-600.
- Duckett, J.G. & Ligrone, R. (1995). The formation of catenate foliar gemmae and the origin of oil bodies in the liverwort *Odontoschisma denudatum* (Mart.) dum (Jungermanniales): a light and electron microscope study. *Annals of Botany*, Vol.76, (October 1995),pp.405-419.
- Dufrêne, Y.F. (2008). Towards nanomicrobiology using atomic force microscopy. *Nature Reviews Microbiology*, Vol.6, N .9,(September 2008),pp.674-680.
- Dunne, WM. (2002). Bacterial Adhesion: Seen Any Good Biofilms Lately? *Clinical Microbiology Reviews*, Vol.15, No.2, (April 2002), pp. 155-166.
- Eighmy, T.T. Maratea, D. & Bishop P.L. (1983). Electron microscopic examination of wastewater biofilm formation and structural components. *Applied and Environmental Microbiology*, Vol.45, No.6, pp.1921-1931.
- Fassel, T.A. & Edmiston, C.E. (1999). Bacterial biofilms: strategies for preparing glycocalyx for electron microscopy. *Methods in Enzymology*, Vol.310, pp.194-203.
- Fassel, T.A. Van Over, J.E. Hauser, C.C. Edmiston, C.E. Sanger, J.R. (1991). Adhesion of staphylococci to breast prosthesis biomaterials: an electron microscopic evaluation. *Cells Materials*, Vol.1,pp.199-208.
- Filloux, A. & Vallet, I. (2003). Biofilm: set-up and organization of a bacterial community. *Medical Science*, Vol.19, No.1, (January 2003), pp. 77-83.
- Flemming, H. Neu, T.R. & Wozniak, D.J. (2007). The EPS Matrix: The "House of Biofilm Cells". *Journal of Bacteriology*, Vol.189, No. 22, (November 2007),pp.7945-7947.
- Fux, C. Costerton, J. Stewart, P. & Stoodley P. (2005). Survival strategies of infectious biofilms. *Trends in Microbiology*, Vol.13, No. 1, (January 2005), pp.34-40.
- Ganderton, L. Chawla, J. Winters, C. Wimpenny, J. & Stickler, D. (1992). Scanning electron microscopy of bacterial biofilms on indwelling bladder catheters. *European Journal Of clinical Microbiololgy Infection Diseases*, Vol.11, No.9, (September 1992), pp. 789-796.
- Garrett, TR. Bhakoo, M. & Zhang, Z. (2008). Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science*, Vol.18, No.9, (September 2008), pp. 1049-1056.
- Gilpin, C.J. & Sigee, D.C. (1995). X-ray microanalysis of wet biological specimens in the environmental scanning electron microscope. 1. Reduction of specimen distance under different atmospheric conditions. *Journal of Microscopy*, Vol. 179, No .1,(July 1995),pp.22-28.
- Gras, M. H. Druetmichaud, C. & Cerf, O. (1994). La flore bactérienne des feuilles de salade fraîche. *Sciences des Aliments*. Vol.14, No. 2,pp.173-188.
- Hall-Stoodley, L. Costerton, J.W. & Stoodley, P. (2004). Bacterial Biofilms: from the natural environment to infectious diseases. *Nature Reviews*, Vol.2, No .2, (February 2004),pp.95-108.
- Hamadi, F. Latrache, H. Mabrouki, M. Elghmari, A. Outzourhit, A. Ellouali, M. & Chtaini, A. (2005). Effect of pH on distribution and adhesion of *Staphylococcus aureus* to glass. *Journal of Adhesion Science and Technology*, Vol.19, No.1, (November 2004), pp. 73-85.
- Hassan, A.N. Frank, J.F. & Elsoda, M. (2003). Observation of bacterial exopolysaccharide in dairy products using cryo-scanning electron microscopy. *International Dairy Journal*, Vol.13, No.9,(July 2003), pp.755-762.

- Herald, P.J. & Zottola, E.A. (1988). Scanning electron microscopic examination of *Yersinia enterocolitica* attached to stainless steel at selected temperatures and pH values. *Journal of Food Protection*, Vol.51, No.6, (Jun 1988), pp. 445–448
- Heslop-Harrison, Y.(1970). Scanning electron microscopy of fresh leaves of *Pinguicula*. *Science*, Vol.167, No .3815, (January 1970),pp.172–174.
- Kaplan, J.B. Meyenhofer, M.F. & Fine, D.H. (2003). Biofilm Growth and Detachment of *Actinobacillus actinomycetemcomitans*. *Journal of Bacteriology*, Vol.185, No.4, (February 2003), pp.1399-1404.
- Korstgens, V, Flemming, H.C. Wingender, J. & Borchard, W. (2001). Influence of calcium ions on the mechanical properties of a model biofilm of mucoid *Pseudomonas aeruginosa*. *Water Science. Technology*, Vol.13, No.6, pp.49-57.
- Kouider, N. Hamadi, F. Mallouki, B. Bengorani, J. Mabrouki, M. Zekraoui, M. Ellouali, M. & Latrache, H. (2010). Effect of stainless steel surface roughness on *Staphylococcus aureus* adhesion. *International Journal of Pure and Applied Science*, Vol.4, No.1, (August 2009),pp. 1-7.
- Lamed, R. Naimark, J. Morgenstern, E. & Bayer, E.A (1987). Scanning electron microscopic delineation of bacterial surface topology using cationized ferritin. *Journal Microbiological. Methods*, Vol.7, No.4-5,(December 1987), pp.233–240.
- Lewis, K. (2005). Persister cells and the riddle of biofilm survival. *Biochemistry (Moscow)*, Vol.70, No. 2, (February 2005), pp.267-274.
- Lim, J. (2008). Nanoscale characterization of *Escherichia coli* biofilm formed under laminar flow using atomic force microscopy (AFM) and scanning electron microscopy. *Bulletin of the Korean Chemical Society*, Vol.29, No .11, pp.2114-2118.
- Little, B. Wagner, P. Ray, R. Pope, R. & Scheetz, R. (1991). Biofilms: an ESEM evaluation of artifacts introduced during SEM preparation. *Journal of Industrial Microbiology*, Vol. 8, No.4, pp. 213-222.
- Liu, Y-Q. Liu, Y. & Tay, J-H. (2004). The effects of extracellular polymeric substances on the formation and stability of biogranules. *Applied Microbiology and Biotechnology*, Vol.65, No. 2, (Jun 2004),pp.143-148.
- Mallouki, B. Latrache, H. Mabrouki, M. Outzourhit, A. Hamadi, F. Muller, D. & Ellouali, M. (2007).The inhibitory effect of fucans on adhesion and production of slime of *Staphylococcus aureus*. *Microbiologie Hygiène Alimentaire*, Vol.19, No.55, (July 2007), pp. 64-71.
- Marshall, K.C. Stout, R. Mitchell, R. (1971). Mechanism of the initial events in the sorption of marine bacteria to surfaces. *Journal of Genetic of Microbiology*, Vol.68, No.3, (November 1971), pp.337–348
- McDowell, J.W. Daryl, B.S. Paulson, S. & Mitchell, J.A. (2004). A simulated-use evaluation of a strategy for preventing biofilm formation in dental unit waterlines. *The Journal of the American Dental Association*, Vol 135, No. 6, (Jun 2004),pp.799-805.
- Minoura, N. Aiba, S.I. Higuchi, M. Gotoh, Y. Tsukada, M. & Imai, Y. (1995). Attachment and growth of fibroblast cells on silk fibroin. *Biochemical and Biophysical Research Communications*, Vol.208, No.2, (March 1995),pp.511–516.
- Molin, S. (2003). Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. *Current Opinion in Biotechnology*, Vol.14, No. 3, (Jun 2003),pp.255-261.
- Morris, C. E, Monier, J.-M. & Jacques, M.-A. (1997). Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganism. *Applied Environmental of Microbiology*, Vol.63, No. 4, (April 1997),pp.1570-1576.

- Motta, P.M. Makabe, S. Naguro, T. & Correr, S. (1994). Oocyte follicle cells association during development of human ovarian follicle. A study by high resolution scanning and transmission electron microscopy. *Archives of Histology and Cytology*, Vol.57, No.4, (October 1994),pp.369-394.
- Muscariello, L. Rosso, F. Marino, G. Giordano, A. Barbarisi, M. Cafiero, G. & Barbarisi, A. (2005). A critical overview of ESEM applications in the biological field. *Journal of Cellular Physiology*, Vol.205, (Jun 2005),pp.328-334.
- Nadell, C.D. Xavier, J.B. Levin, S.A. & Foster, K.R. (2008). The Evolution of Quorum Sensing in Bacterial Biofilms, *Plos Biology*, Vol.6, No. 1, (January 2008), e14.
- O'Toole, G. Kaplan, H.B. & Kolter, R. (2000). Biofilm formation as microbial development. *Annual Reviews of Microbiology*, Vol.54, pp.49-79.
- Pawley, J.B. Erlandsen, S.L. (1989). The case for low voltage high resolution scanning electron microscopy of biological samples. *Scanning Microscopy*, 3(suppl),pp.16-173.
- Peters, G. Locci, R. & Pulverer, G. (1982). Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters, *Journal of Infectious Diseases*, Vol.146, No.4, pp.479-482.
- Pinna, A. Sechi, L.A. Zanetti, S. Delogu, D. & Carta, F. (2000). Adherence of Ocular Isolates of *Staphylococcus Epidermidis* to ACRYSOF Intraocular Lenses. *Ophthalmology*, Vol.107, No.12, (October 1982), pp. 2162- 2166.
- Prakash, B. Veeregowda, B.M. & Krishnappa, G. (2003). Biofilms: A survival strategy of bacteria. *Current Science*, Vol. 85, No. 9, (November 2003),pp.9-10.
- Priester, J.H. Horst, A.M. Van De Werfhorst, L.C. Saleta, J.L. Mertes, L.A.K. & Holden, P.A. (2007). Enhanced visualization of microbial biofilms by staining and environmental scanning electron microscopy. *Journal of Microbiological Methods*, Vol.68, No. 2, (March 2007), pp.577-587.
- Ramírez-Camacho, R. González-Tallón, A.I. Gómez, D. Trinidad, A. Ibáñez, A. García-Berrocal, J.R. Verdager, J.M. González-García, J.A. & San Román, J. (2008). Environmental scanning electron microscopy for biofilm detection in tonsils. *Acta Otorrinolaringol Esp*, Vol.59, No.1, (January 2008),pp.16-20.
- Read, N.D. & Lord, K.M. (1991). Examination of living fungal spores by scanning electron microscopy. *Experimental mycology*, Vol.15, No. 2,pp.132-139.
- Richards, S.R. & Turner, R.J. (1984). A comparative study of techniques for the examination of biofilms by scanning electron microscopy. *Water Research*, Vol.18, No.6, pp.767-773.
- Rodrigues, D.F. & Elimelech, M. (2009). Role of Type 1 Fimbriae and Mannose in the Development of *E. coli* K12 Biofilm: From Initial Cell Adhesion to Biofilm Formation. *Biofouling*, Vol.25, No. 5, (July 2009), pp.401-411.
- Sasidharan, S. Yoga Latha, L. & Angeline, T. (2010). Imaging In vitro Anti-biofilm Activity to Visualize the Ultrastructural Changes. *Microscopy: Science, Technology, Applications and Education A. Méndez-Vilas & J. Díaz (Eds.) Formatex*, 2010,pp. 622-626.
- Schwartz, T. Jungfer, C. Heißler, S. Friedrich, F. Faubel, W. & Obst, U. (2009). Combined use of molecular biology taxonomy, Raman spectrometry, and ESEM imaging to study natural biofilms grown on filter materials at waterworks. *Chemosphere*, Vol.77, No. 2, (September 2009),pp.249-257.
- Sevinç, B.A. & Hanley, L. (2010). Antibacterial activity of dental composites containing zinc oxide nanoparticles. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, Vol.94, No.1, (July 2010), pp.22-31.
- Shen, Y. Nakajima, M. Ahmad, M.R. Kojima, S. Hommac, M. & Fukuda, T. (2011). Effect of ambient humidity on the strength of the adhesion force of single yeast cell inside environmental-SEM. *Ultramicroscopy*, Vol.111, No. 8, pp:1176-1183.

- Singh, P. K. Parsek, M. R. Greenberg, E. P. & Welsh, M. J. (2002). A component of innate immunity prevents bacterial biofilm development. *Nature*, Vol.417, No. 6888, (May 2002), pp.552-555.
- Stewart, A.D.G. (1985). The origins and development of scanning electron microscopy. *Journal of Microscopy*, Vol.139, No .2, (August 1985), pp.121-127.
- Stokes, D.J. & Donald, A.M. (2000). In situ mechanical testing of dry and hydrated breadcrumb in the environmental scanning electron microscope (ESEM). *Journal of Materials Science*, Vol.35, No .3, (December 2000),pp.599-607.
- Stokes, D.J. & Donald, A.M. (2000). In situ mechanical testing of dry and hydrated breadcrumb in the environmental scanning electron microscope (ESEM). *Journal of Materials Science*, Vol.35, No .3, (December 2000),pp.599-607.
- Stokes, D.J. (2001). Characterization if soft condensed matter and delicate materials using environmental scanning electron microscopy (ESEM). *Advanced Engineering Materials*, Vol.3, No.3, pp.126-130.
- Stoodley, P, Sauer K, Davies, DG. & Costerton, JW. (2002). Biofilms as complex differentiated communities. *Annual Review of Microbiology*, Vol.56, (January 2002), pp. 187-209.
- Storti, A., Pizzolitto, CA. & Pizzolitto, LE. (2005) Detection of mixed microbial biofilms on central venous catheters removed from intensive care unit patients. *Brazilian Journal of Microbiology*, Vol.36, pp.275-80.
- Surico, G. (1993). Scanning electron microscopy of olive and oleander leaves colonized by *Pseudomonas syringae* subsp. *savastanoi*. *Journal of Phytopathology*, Vol.138, No. 1, (May 1993), pp.31-40.
- Surman, S.B. Walker, J.T. Goddard, D.T. Morton, L.H.G. Keevil, C.W. Weaver, W. Skinner, A. Hanson, K. & Caldwell, D. (1996). Comparison of microscope techniques for the examination of biofilms. *Journal of Microbiological Methods*, Vol.25, No.1, (March 1996) pp.57-70.
- Sutherland, IW. (2001). The biofilm matrix - an immobilized but dynamic microbial environment. *Trends of Microbiology*, Vol.9, No.5, (May 2001), pp. 222-227.
- Sutton, N.A. Hughes, N. & Handley, PS. (1994). A comparison of conventional SEM techniques, low temperature SEM and the electroscan wet scanning electron microscope to study the structure of a biofilm of *Streptococcus crista* CR3. *Journal of Applied Bacteriology*, Vol.76, No .5,(May 1994),pp.448-454.
- Vidali, M. (2001). Bioremediation. A overview. *Pure and Applied Chemistry*, Vol.73, No.7, pp. 1163-1172.
- Walker, JT. Verran, J. Boyd, RD. & Percival, S. (2001). Microscopy methods to investigate structure of potable water biofilms. *Methods in Enzymology*, Vol.337, No.2001, (July 2004), pp.243-255.
- Weber, W.J.J. Pirbazari, M. & Melson, G.L. (1978). Biological growth on activated carbon: an investigation by scanning electron microscopy. *Environmental Science & Technology*, Vol.12, No. 7, (July 1978),pp. 817-819.
- Zameer, F. & Gopal, S. (2010). Evaluation of antibiotic susceptibility in mixed culture biofilms. *International Journal of Biotechnology and Biochemistry*, Vol.6, No.1, pp. 93-99.
- Zammit, G. Sánchez-Moral, S. & Albertano P. (2011). Bacterially mediated mineralisation processes lead to biodeterioration of artworks in Maltese catacombs. *Science of the Total Environment*, Vol.409, No. 14, (Jun 2011),pp.2773-2783.
- Zeraik, A.E. & Nitschke, M.(2010). Biosurfactants as agents to reduce adhesion of pathogenic

Scanning Electron Microscopy

Edited by Dr. Viacheslav Kazmiruk

ISBN 978-953-51-0092-8

Hard cover, 830 pages

Publisher InTech

Published online 09, March, 2012

Published in print edition March, 2012

Today, an individual would be hard-pressed to find any science field that does not employ methods and instruments based on the use of fine focused electron and ion beams. Well instrumented and supplemented with advanced methods and techniques, SEMs provide possibilities not only of surface imaging but quantitative measurement of object topologies, local electrophysical characteristics of semiconductor structures and performing elemental analysis. Moreover, a fine focused e-beam is widely used for the creation of micro and nanostructures. The book's approach covers both theoretical and practical issues related to scanning electron microscopy. The book has 41 chapters, divided into six sections: Instrumentation, Methodology, Biology, Medicine, Material Science, Nanostructured Materials for Electronic Industry, Thin Films, Membranes, Ceramic, Geoscience, and Mineralogy. Each chapter, written by different authors, is a complete work which presupposes that readers have some background knowledge on the subject.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Soumya El Abed, Saad Koraichi Ibensouda, Hassan Latrache and Fatima Hamadi (2012). Scanning Electron Microscopy (SEM) and Environmental SEM: Suitable Tools for Study of Adhesion Stage and Biofilm Formation, Scanning Electron Microscopy, Dr. Viacheslav Kazmiruk (Ed.), ISBN: 978-953-51-0092-8, InTech, Available from: <http://www.intechopen.com/books/scanning-electron-microscopy/scanning-electron-microscopy-sem-and-environmental-sem-suitable-tools-for-study-of-adhesion-stage-a>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen