“The majority of bacteria exist in nature attached to a substratum”

MacEachran, D.P. O’Toole, G.A.

The Biofilm Mode of Life, 2007 p23.
Biofilms of *Borrelia burgdorferi*

And Clinical Implications for

Chronic Borreliosis

Alan B. MacDonald, MD,

July 7, 2008

University of New Haven

Lyme Disease Symposium

New Haven, Conn
Clinical Implications of Biofilms of Borrelia burgdorferi

Biofilms of Borrelia burgdorferi in human tissue provide microscopic proof of persistence of spirochetes in cases of chronic Lyme borreliosis. Biofilms of borrelia, by definition, explain persistence of infection after antibiotic therapy and recurrence of disease symptoms in chronic Lyme borreliosis.
Dr Eva Sapi

The first to recognize that *Borrelia burgdorferi*

Could exist in Biofilm Communities
Common shared properties in "mature " Biofilms

“The microcolony structure observed in established Mature biofilms is strikingly similar across mono-and Multispecies biofilms, across different habitats, as well as for Different organismal levels”

Kjelleberg, S., and Givskov, M.
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Perfect spirochete
Routes to the formation of Biofilms

Multiple, Parallel pathways to Biofilm Formation
Borrelia burgdorferi in culture – Coexistent cystic form and Spiral forms
A vocabulary of words and images

Borrelia of the *Spiral* type

Borrelia of the *Cystic* type

Borrelia of the *Granular* type

Borrelia of the *Cell wall deficient* type
Mixtures of Borrelia types may be found in Borrelia biofilms

Some Borrelia biofilms may contain a majority of spiral Borrelia, while others may contain a majority of granular or Cystic Borrelia.

Biofilms may contain different species of pathogens

(For example Borrelia and Babesia, or other multiorganism combinations)
Spiral Borrelia
Separate Cystic forms of Borrelia burgdorferi Without extracellular Matrix
Cystic Borrelia without granules inside

Cystic Borrelia with Granules inside

Cystic form of B31 in human plasma previously filter sterilized with 0.2 micron filter
Granular Borrelia
Evolving from spiral borrelia
Segmentation of DNA inside of the spirochetal cylinder
Granular forms
Of Borrelia in
Brain tissue
Cell wall deficient Borrelia
Cell wall deficient form of Borrelia burgdorferi
Membrane material separating from Borrelia burgdorferi
Membrane material separating from Borrelia burgdorferi
Cell wall deficient forms of Borrelia burgdorferi
Biofilm: A *community of microbes*
enveloped in a protective Extracellular matrix
“Biofilm” is the Extracellular material which holds the communities of Bacteria together in a sessile community

The biofilm composition is often mucopolysaccharide material.

Some biofilms (Pseudomonas species) are composed of Extracellular DNA.

Other biofilms may incorporate Flagellae, Fimbriae, Pili into the biofilm
Figure 11.1 Biofilm-specific traits and their role in antipredator resistance.
Planktonic microbes

Motility
Provided
By Flagellae

Attachment to surface
Provided by Flagellae

Two functions of Flagellae:

*Propulsion*
*Adhesion to surface*
Attachment of early biofilm – Reversible and Irreversible
Growth of Biofilms
Regeneration of Planktonic microbes within the biofilm
Detachment

3

Diagram showing the process of detachment.
Regional ASPECTS

Zonation

Figure 2: Three hypotheses for mechanisms of antibiotic resistance in biofilms

- **Slow penetration**: Antibiotic (yellow) may fail to penetrate beyond the surface layers of the biofilm.
- **Resistant phenotype**: Some of the bacteria may differentiate into a protected phenotypic state (green).
- **Altered microenvironment**: In zones of nutrient depletion or waste product accumulation (red), antibiotic action may be antagonised.

Substratum
Review

Antibiotic resistance of bacteria in biofilms

Philip S Stewart, J William Costerton

Bacteria that adhere to implanted medical devices or damaged tissue can encase themselves in a hydrated matrix of polysaccharide and protein, and form a slimy layer known as a biofilm. Antibiotic resistance of bacteria in the biofilm mode of growth contributes to the chronicity of infections such as those associated with implanted medical devices. The mechanisms of resistance in biofilms are different from the now familiar plasmids, transposons, and mutations that confer innate resistance to individual bacterial cells. In biofilms, resistance seems to depend on multicellular strategies. We summarise the features of biofilm infections, review emerging mechanisms of resistance, and discuss potential therapies.

As an example of sequelae of biofilms, let us consider...
Altered MicroEnvironment in Biofilms and Antibiotic Resistance

Failure of Antibiotic to *penetrate* the Biofilm

*Differentiation* of Bacteria within the Biofilm - Dormant State and Altered Genetics

Bacterial Heterogeneity in Biofilms

Accumulation of Molecules in the biofilm which *antagonize the Antibiotic action*
Communities of pure Borrelia burgdorferi (corkscrew/spiral)

Spiral Biofilm VARIANT
Communities of pure *Borrelia burgdorferi*

Mixed Cystic and Spiral VARIANT

Biofilm of B31
Communities of Pure Borrelia burgdorferi

Biofilm composed of Cystic forms

Cystic Biofilm VARIANT
Group of Cystic B31
Figure 8.1 Model of staphylococcal virulence gene expression *in vitro* and *in vivo*. Expression
Cell wall deficient Borrelia in a biofilm community
Granular borrelia in a biofilm community
Biofilm of Borrelia burgdorferi with internal “empty spaces” showing one type of organization in a Multicellular community
Biofilm of Borrelia burgdorferi
Biofilm of Borrelia burgdorferi
Biofilm of Borrelia burgdorferi
Biofilm of Borrelia burgdorferi
Biofilm of Borrelia burgdorferi
Biofilm of Borrelia burgdorferi
Biofilm of Borrelia burgdorferi
Evolution of Cystic borrelia
From spiral
Evolution of Cystic borrelia
From spiral
Evolution of Cystic borrelia
From spiral
Evolution of Cystic borrelia
From spiral
A membrane bound “bridge” connecting two biofilm units of Borrelia burgdorferi
Figure 4.3 Horizontal confocal laser scanning microscope sections in a 2-day-old DDAB-stained biofilm formed by Gfp-tagged *P. aeruginosa* PA01. The images show the fluorescent bacteria (A), the fluorescent extracellular DNA (B), and an overlay of the two (C). Reproduced from *Mol. Microbiol.* 59:1114–1128 with permission from Blackwell Publishing.
Cell wall deficient form of Borrelia burgdorferi – Membranes without cell walls
Biofilm of Borrelia burgdorferi
Two groupings of Borrelia burgdorferi in Skin – Biofilms
Image 38A, Acrodermatitis chronica atrophicans of left leg characterized by ill-defined, hyperpigmented, and atrophic patch (note prominent veins). B, Histologic examination (H&E, ×10) reveals a dense lichenoid and middermal perivascular infiltrate with hints of follicle formation (C, H&E, ×100) composed of lymphocytes, some plasma cells, and an increase of fibroblasts between fibrosclerotic collagen bundles (D, H&E, ×200).
Dr K. Eisendle
Acrodermatitis Chronica Atrophicans
Immunohistochemistry

“Granular forms of B burgdorferi in a “colony”
With a “Reddish veil”

A colony of granular Borrelia burgdorferi
- Reclassified as a Biofilm unit in Skin of ACA

Morphea – with biofilm-like “clump” of Borrelia
Image from 1981-
What is the source?

Image from 1987-
What is the source?
Human Brain Culture demonstrating a Biofilm of *Borrelia burgdorferi*  
Year 1987

Tick gut Culture showing *Borrelia burgdorferi* in a Biofilm Unit  
Year 1981
For comparison –2008-- Borrelia burgdorferi biofilm grown from Pure culture from ATCC strain
Formation of Cystic and Cell wall deficient Spherical forms is initiated by Localized LOSS of Cell Wall
Cystic and spiral Borrelia burgdorferi in Aged pure culture
The In Transit concept
For Borrelia biofilms

Contribution of Borrelia DNA to the formation of Extracellular Matrix in Borrelia biofilms
Figure 1 B. Hermsii with loss of cell wall and developing spheroid form
Emerging Cystic Form attached to corkscrew shaped Borrelia Burgdorferi 
RED ARROW SHOWS FILAMENT FORM INSIDE OF CYST CURVED GREEN ARROWS SHOW CYST PERIMETER DNA STAIN
Emerging Cyst form of *Borrelia Burgdorferi*

(see rounded area of dots)

White arrows show boundaries of the emerging cystic form containing granular elements.
ATCC B31 B burgdorferi
culture aged 1 year
with diverse atypical
spirochetal and cystic forms
Figure 3 - "In transit" form of Borrelia burgdorferi. Note the "herniations of rounded cellular material not bound by the confines of the rigid cell wall of the spirochete"
Figure 5 - "In transit " form of Borrelia burgdorferi with "blush" of External DNA
Figure 7 - In Transit form of Borrelia burgdorferi with externalized cellular elements
Figure 6 externalized cellular constituents. Early biofilm form of Borrelia Burgdorferi. Note coalescence of externalized cellular constituents.
Figure 8 Early Biofilm of *Borrelia burgdorferi*. 
Paired Borrelia in ACA skin with adjacent red blush staining ?? In Transit biofilm form ??
Alzheimer’s disease – Frontal lobe Cortex – Imprint cytology showing a group of Borrelia with adjacent
Cystic borrelia bu
Unstained slides w
A gift from
Rocky Mt n Lab. National
and Int disease
DNA stain by Al
Copyright
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DNA distribution in biofilm of Borrelia burgdorferi
Original Isolate of Borrelia burgdorferi, 1981
Image from the Yale Journal of Biology and Medicine
Biofilms as primitive Multicellular systems

Micro Colony formation

Differentiation of Microbes within the biofilm

Dispersal from biofilm colonies

   Microfilm “units”

   Planktonic “units”

Nitric oxide – Signal for differentiation

   and Dispersal from biofilms
Signal transmission within Biofilms

Cell to cell communication

Cyclic diGMP (2nd messenger) [GGDEF/EAL SYSTEM]

Nitric Oxide

Peptide signaling

“melting” phenomenon – formation of Syncytial Units
Figure 5.1 Structure of the second messenger bis-(3'-5')-cyclic di-guanosine monophosphate (cyclic di-GMP).
Figure 5.3 Cyclic di-GMP as a second messenger links the perception of environmental cues.
Viable but NonCultivatable Microbes

Stationary Phase

Strains of Borrelia burgdorferi and other borrelia species

Difficult to grow in Laboratory

Viable in the human host

Not killed by antibiotics
Bacteriophage Activities within Biofilms
The Biofilm Matrix

Components

- Extracellular Polysaccharides
- Lipoproteins
- Peptidoglycans
- Extracellular DNA (Pseudomonas model)
Multispecies Biofilms –

Examples from other Species

Complex Systems of Microbes and Protozoans

Survival benefits

Exchange of DNA between Species?
Multicellular -Biofilm - Variations under the microscope

Flocks
Granules
Rounded shaped units (microcolonies)
Mushroom shaped units
Filamentous biofilms
Loose biofilm aggregates
Life Cycle Concept

For Microbes

Biofilm Life cycles

Planktonic Life cycles

Two components
Predators of Biofilms ??

Protozoans ?

Phagocytes ??

Bacteriophages??

Other bacteria??
Attachment Considerations in Biofilm

Specific Adhesive proteins – bind to surfaces
Cell to Cell cohesion by Cell Binding proteins
Carbon sources at the site of attachment
Presence of mucin at site of attachment
Competition with other bacteria at attachment
Resistance to Shear Forces
Up Regulation and down Regulation of genes
Future Research in Borrelia biofilms

Mutations and Horizontal Gene Transfer in Biofilms

Transcriptome analysis in Biofilms –

Current constraints

Comparative Analysis of Events in Biofilm Life Cycles across bacterial species
Fig. 2. The segmented genome of *B. burgdorferi*. Linear plasmids are abbreviated lp and circular plasmids cp, the number represents the approximate size of the plasmid in kilobase pairs. Evidence supports that plasmids shown in red are required for infectivity or persistence in the tick or vertebrate hosts. Sizes are not drawn to scale.
Borrelia biofilm works in progress

--Quorum sensing in Biofilms

    AHL model for QS in Gram Negative bacteria

---Viable but non- cultivatable Borrelia in Biofilm communities

    Persister forms of bacteria

    Non dividing forms

    Slow to divide forms
Quorum Sensing - Chemical messenger molecules produced by a single bacterium are different (quantitatively) from those produced by a population of bacteria in a biofilm.

**Quorum Sensing Blockers**

Can we identify the Genetic underpinnings of Quorum Sensing chemical species in Borrelia and utilize these in treatment of Chronic infections?

[Examples - furanones, patulin, penicillic acid, garlic extract – as natural QS blockers in biofilm via downregulation of genes in pathogenic bacteria]
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Biofilms of borrelia, by definition, explain Persistence of infection after antibiotic therapy and recurrence of disease symptoms in chronic Lyme borreliosis.
Summation: Biofilms of Borrelia burgdorferi

1. Biofilms of Borrelia are indispensable elements for species survival in hostile environments.

2. Biofilms of borrelia provide protection to the microbes which live inside of the matrix.

3. DNA of Borrelia (externalized) constitutes a portion of the borrelia biofilm matrix?

4. Exchange of genomic material occurs between the borrelia in the biofilm.

5. Morphologic diversity of borrelia within biofilms (cyst, granular, L form, and spiral forms) is evident.
Biofilms and Chronic Infections

Randall D. Wolcott, MD
Garth D. Ehrlich, PhD

The prevailing paradigm of infectious disease is based on the work of Koch and colleagues, who more than 150 years ago isolated individual strains of bacteria and developed the pure culture method that is still used today. That work enlightened medicine by firmly establishing the germ theory of transmissible diseases and demonstrated that diseases like dysentery, tuberculosis, and anthrax are caused by microbiological agents. Hence, the field of microbiology developed around Koch’s methods with clinical microbiologists working overwhelmingly with pure log-phase cultures in nutrient-rich media because this approach provided such a powerful tool for the study of acute epidemic bacterial diseases. However, this approach that examines only planktonic bacteria (free-floating, single cell phenotype) may have limited development of a more thorough understanding of microbial processes. In most natural environments and in chronic bacterial infections, the planktonic phenotype generally exists only transiently, and usually as a minor population.

Emerging evidence describes bacterial populations as predominantly polymicrobial, sessile, community-based aggregations embedded in a self-secreted matrix that provides numerous advantages for persistence in the face of environmental and host challenges. Therefore, biofilms and the existence of a complex bacterial life cycle provide a new perspective through which to view infectious diseases. Much of the support for this perspective has come about through the application of new detection and visualization methods that have provided evidence for the theory that chronic infections are fundamentally different than acute infections, and that different interventional approaches are necessary to treat these biofilm infections more efficiently.

What is a Biofilm?

A biofilm is a thin layer of microorganisms that adhere to the surface of an organic or inorganic structure, together with their secreted polymers. Biofilms are the predominant phenotype of nearly all bacteria in their natural habitat, whether pathogenic or environmental. The biofilm provides a bulwark against environmental stressors and can include organisms from multiple kingdoms as in the case of mixed bacterial-fungal biofilms. Thirty years ago, Costerton et al. was the first to examine the attributes of biofilms, examining the extracellular polymeric substances (EPS) that holds these community bac...
Thank you for your Kind attention.

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