Biofilms of *Borrelia burgdorferi*
And Clinical Implications for
Chronic Borreliosis

Alan B. MacDonald, MD,

May 17, 2008
University of New Haven
Lyme Disease Symposium
New Haven, Conn
Copyright notice

All of the Images in this presentation are Copyright by various authors, Details available upon request
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
In Situ DNA hybridization
Alexa Fluor (red) Fluorochrome

Alzheimer
Hippocampus
1000x Oil immersion

Oligonucleotides
BBO 147 (Fla B)
B. burgdorferi
Cell wall deficient Borrelia
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.
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
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
Communities of Pure Borrelia burgdorferi

Biofilm composed of Cystic forms

Cystic Biofilm VARIANT
Attachment 1
Growth 2
Detachment 3
Cell wall deficient Borrelia in a biofilm community
Image A: Acrodermatitis chronica atrophicans of left leg characterized by ill-defined, hyperpigmented, and atrophic patch (note prominent veins).

B: Histologic examination (H&E, x10) reveals a dense lichenoid and middermal perivascular infiltrate with hints of follicle formation (C, H&E, x100) composed of lymphocytes, some plasma cells, and an increase of fibroblasts between fibrrosclerotic collagen bundles (D, H&E, x200).
Dr K. Eisendle
Acrodermatitis Chronica Atrophicans
Immunohistochemistry

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

Morphea – with biofilm-like “clump” of Borrelia
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 – *Borrelia burgdorferi* biofilm 2008
Borrelia hermsii in transit to spherical form after penicillin treatment
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
Cystic Form emerging from Borrelia burgdorferi DNA Stain
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

granules within the cyst

CYST FORM

GRANULAR CHANGE IN SPIROCHETE
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.
Group of Cystic B31
Cystic borrelia bu
Unstained slides wi
A gift from
Rocky Mtn Lab. Nationa
and In
disease
DNA stain by Al
Copyright
all rights res
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.
Borrelia biofilm works in progress

--Quorum sensing in Biofilms

---Viable but non-cultivatable Borrelia in Biofilm communities
Thank you for your Kind attention.

This research was made possible from support from:

Turn The Corner Foundation
The Lyme Disease Association
Time for Lyme Foundation
Original Isolate of Borrelia burgdorferi, 1981
Image from the Yale Journal of Biology and Medicine