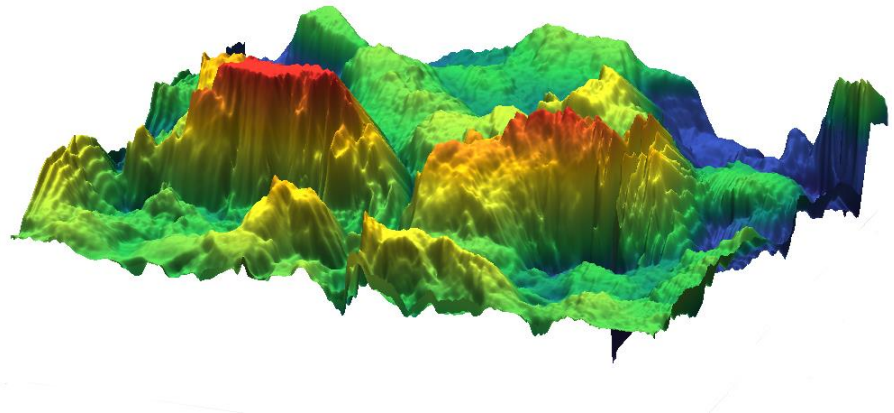
Potential Connection of Borrelia Infection and Breast Cancer



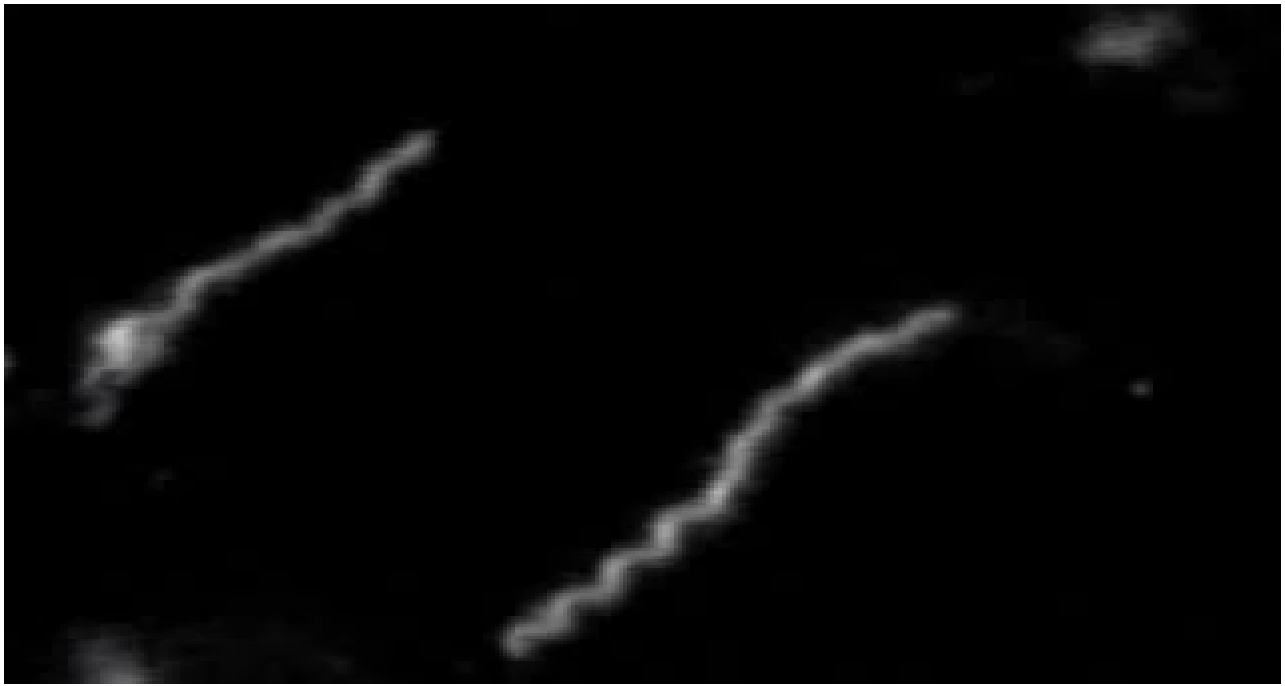
EVA SAPI Ph.D.
UNIVERSITY OF NEW HAVEN

Borrelia burgdorferi the spirochete that causes Lyme disease

- In 1982, the etiologic agent of Lyme disease was discovered by Willy Burgdorfer who isolated spirochetes belonging to the genus *Borrelia* from the mid-guts of *Ixodes* ticks.
- He showed that these spirochetes reacted with immune serum from patients that had been diagnosed with Lyme disease. Subsequently, the etiologic agent was given the name ***Borrelia burgdorferi.***

Borrelia burgdorferi , FA stain (CDC)

Let’s look at *Borrelia* exposed to penicillin



Youtube video

The most recognized forms of *Borrelia burgdorferi*

- Spirochetes
- Round bodies (cysts, granules)
- Attached biofilm

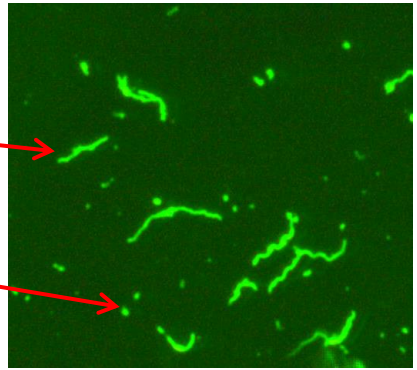


Photo by Pabbati N MS

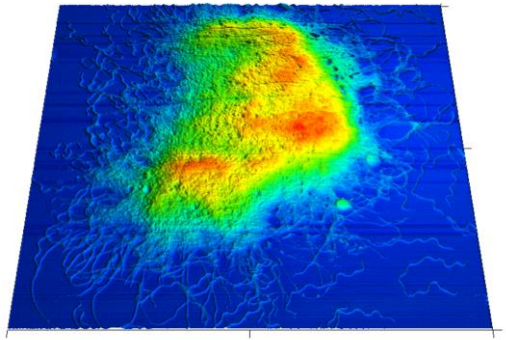
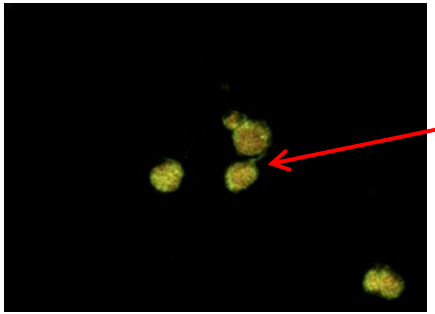


Photo by Luecke DF MS



Persisters

Photo by Socarras K MS

Infection and Drug Resistance

Dovepress
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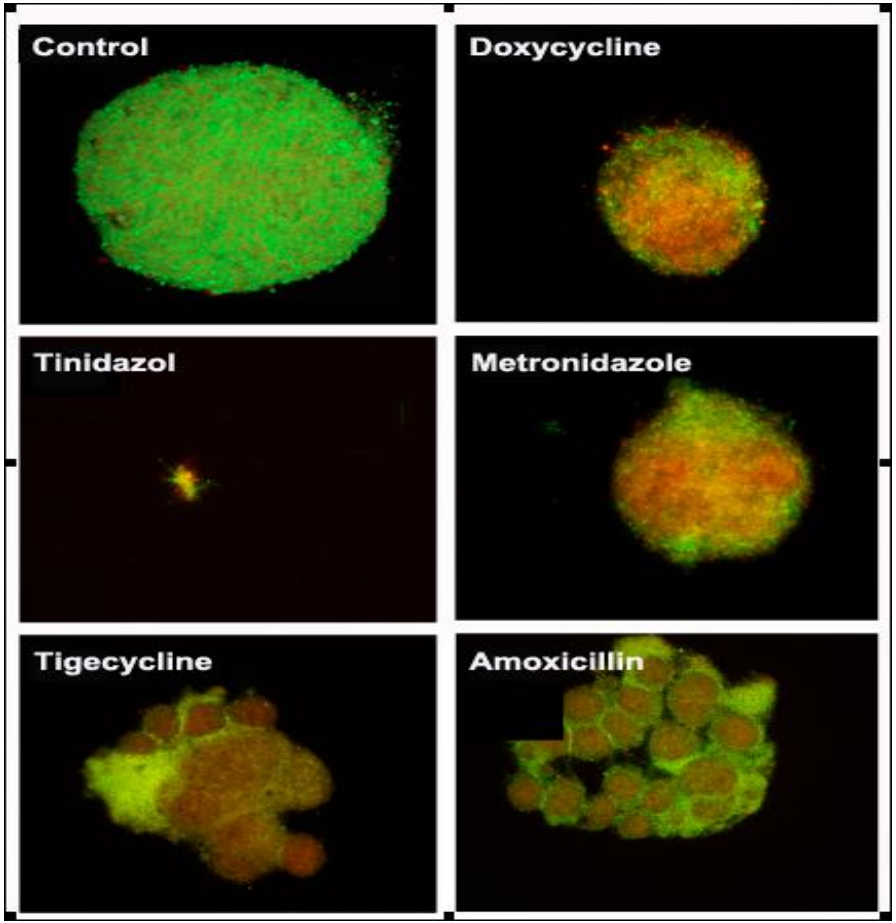
ORIGINAL RESEARCH

Evaluation of in-vitro antibiotic susceptibility of different morphological forms of *Borrelia burgdorferi*

Table 1 MIC and MBC determination by different methods[a]

Antibiotics	Microdilution method/literature data (MIC) µg/mL	Our data (MIC) µg/mL			Microdilution method/literature data (MBC) µg/mL	Our data (MBC) µg/mL		
		Microdilution method	Direct cell counting	BacLight™ staining		Microdilution method	Direct cell counting	BacLight™ staining
Doxycycline	0.06–2.00	0.4	>25	>25	0.25–6.40	25	>200	>200
Tigecycline	0.006	0.015	>5	>5	0.05	0.125	>10	>10
Amoxicillin	0.03–2.00	0.3	>100	>100	<0.03–32.00	5	>200	>200
Metronidazole	0.06–32.00	0.3	>250	>250	>4	10	>500	>500
Tinidazole	–	0.09	>62.5	>62.5	>128	10	>250	>250



Red stain: Dead
Green stain: Viable

Effect of antibiotics on the aggregates of Borrelia measured LIVE/DEAD staining
Sapi E et al 2011

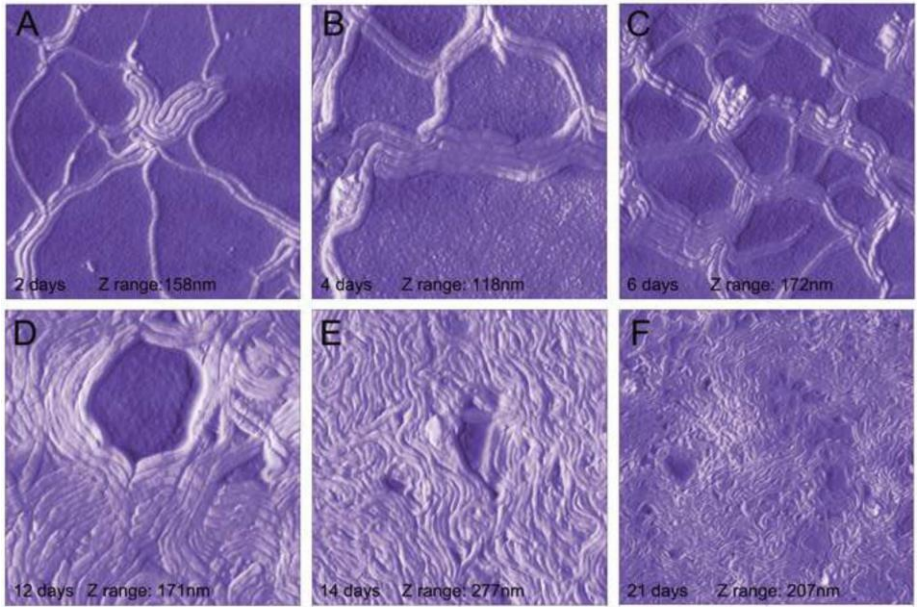
What is biofilm?

- collections of microorganisms (bacteria, yeasts or protozoa) that form on a hard surface (exception floating biofilms)
- examples: plaque that forms on teeth and the slime that forms on surfaces in watery areas (shower)
- surrounded by slimy secretions: mucoid polysaccharide structure which attaches the community to a surface
- estimated that over 90% of bacteria live in biofilm (late Costerton WJ)

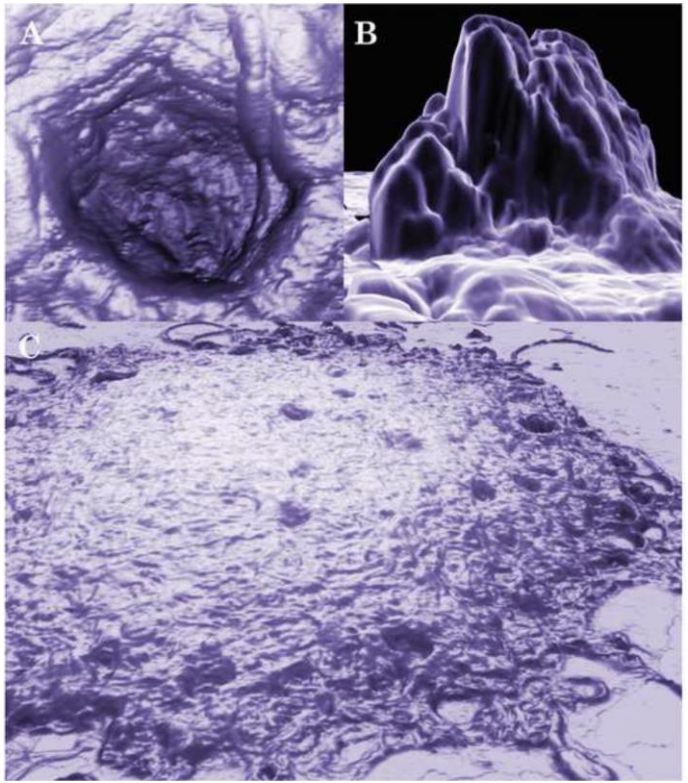
Problems caused by biofilms

- Can form almost anywhere that water is present, including catheters, kitchen counters, showers, water pipes etc.
- Damage to industrial equipment
- Contamination of food, pharmaceutical and medical products
- Energy loss through inefficient energy transfer
- Medical infections and super-resistant to antibiotics

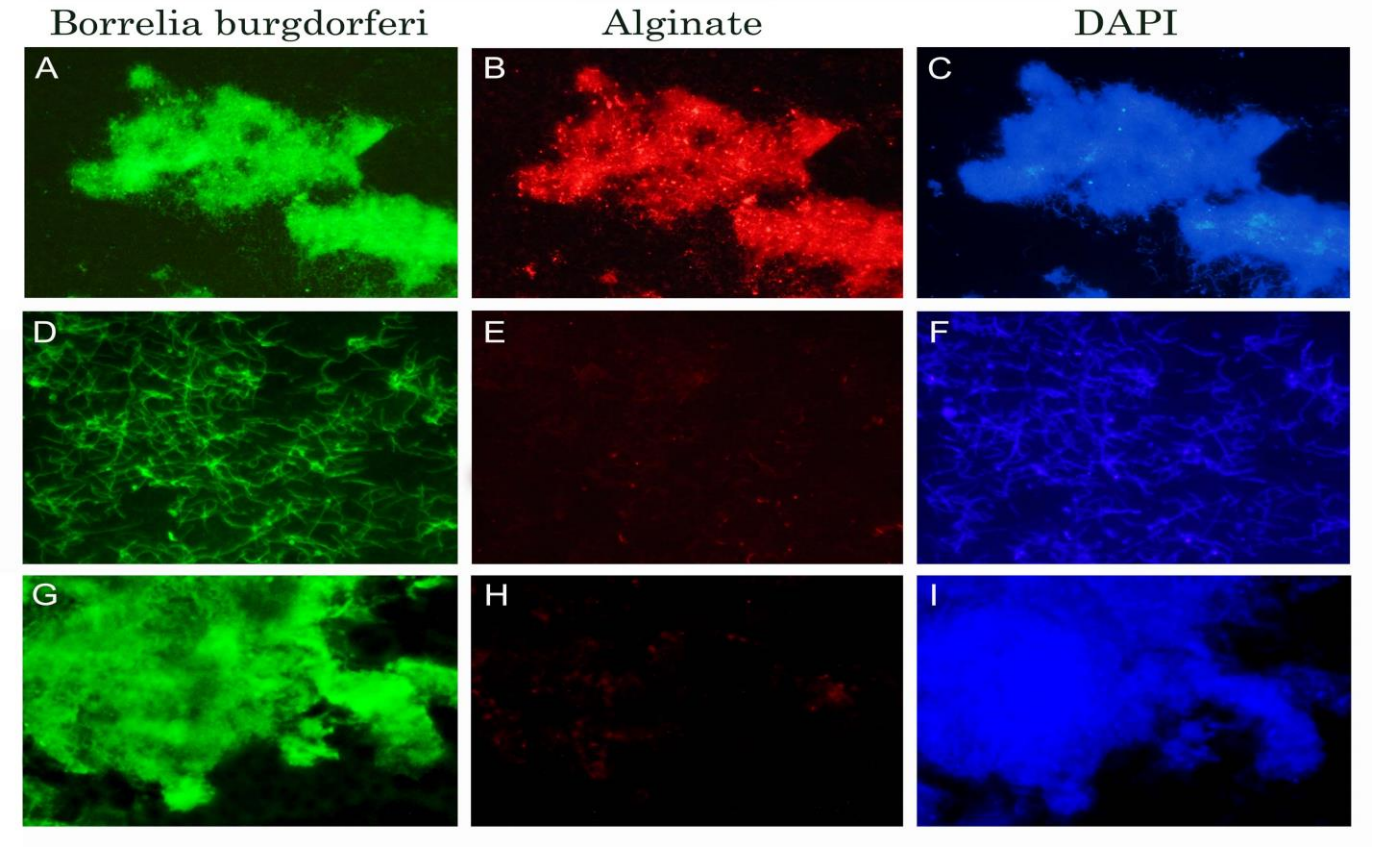
Borrelia biofilm



Sapi E et al PLoS ONE 2012



Alginate on the surfaces of *Borrelia* aggregates



Sapi E et al 2012 PLoS ONE

Can Borrelia biofilms exist in vivo?

- *Double immunohistochemical staining with Borrelia and biofilm specific markers*
- *In situ hybridization confirmation experiments*
- *PCR/Whole genome sequencing confirmation*

Samples:

  - Skin biopsies from *Borrelia* infected skin lesions
    - *(Dr. B. Zelger)*
  - *Autopsy tissues from a Lyme disease patient*
    - *(Dr. K. Leigner and Dr J. Goldman)*

Original article

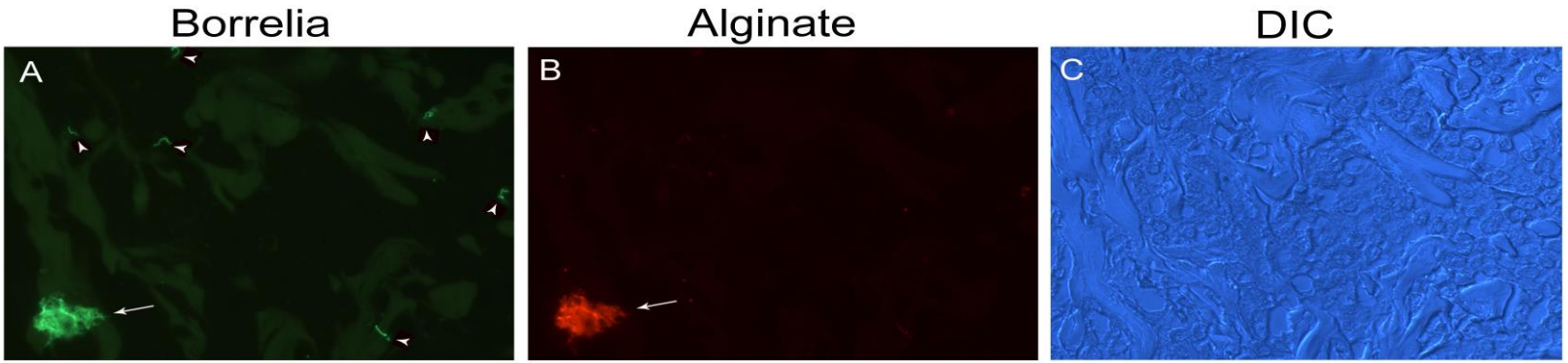
European Journal of Microbiology and Immunology
DOI: 10.1556/1886.2015.00049

EVIDENCE OF *IN VIVO* EXISTENCE OF *BORRELIA* BIOFILM IN BORRELIAL LYMPHOCYTOMAS

E. Sapi[1,*], K. Balasubramanian[1], A. Poruri[1], J. S. Maghsoudlou[1], K. M. Socarras[1], A. V. Timmaraju[1], K. R. Filush[1], K. Gupta[1], S. Shaikh[1], P. A. S. Theophilus[1], D. F. Luecke[1], A. MacDonald[1], B. Zelger[2]

[1]Department of Biology and Environmental Science, University of New Haven, West Haven, CT 06516, USA
[2]Department of Dermatology and Venereology, Medical University Innsbruck, Innsbruck, Austria

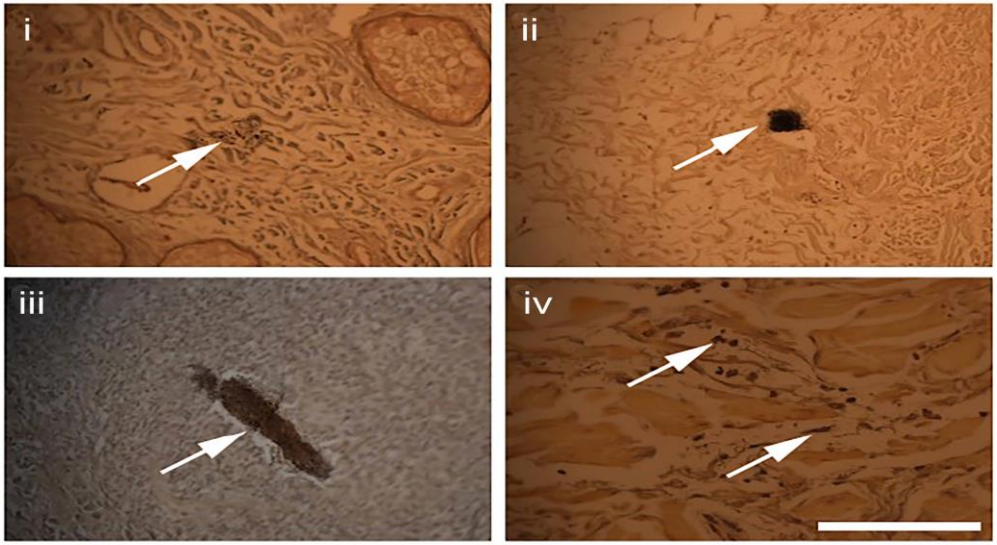
Double immunostaining: Borrelia and alginate antibody from Skin biopsies from Borrelia Lymphocytoma



Sapi E et al 2016

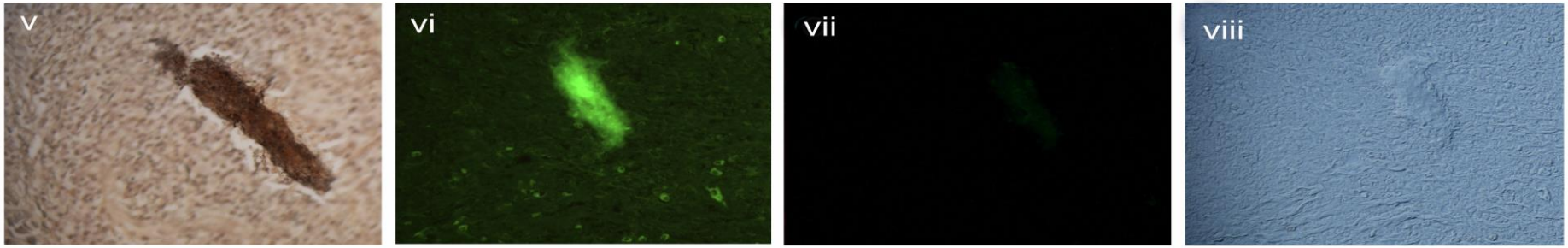
A

DIETERLE SILVER STAINING

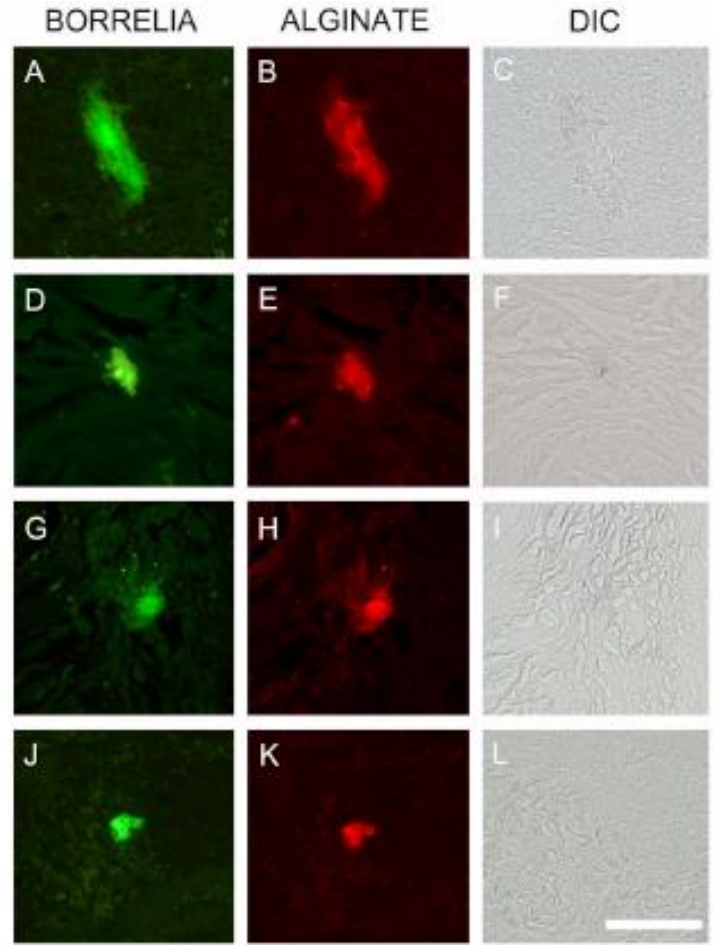


Sapi E et al 2016

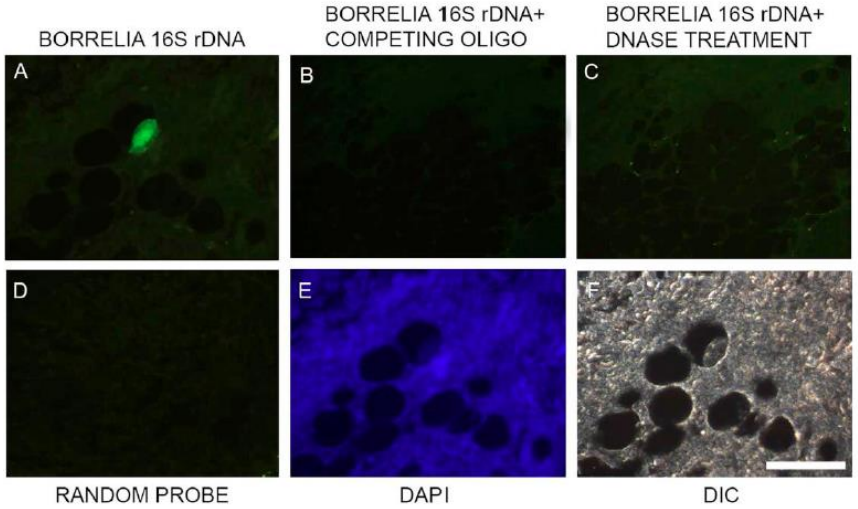
B

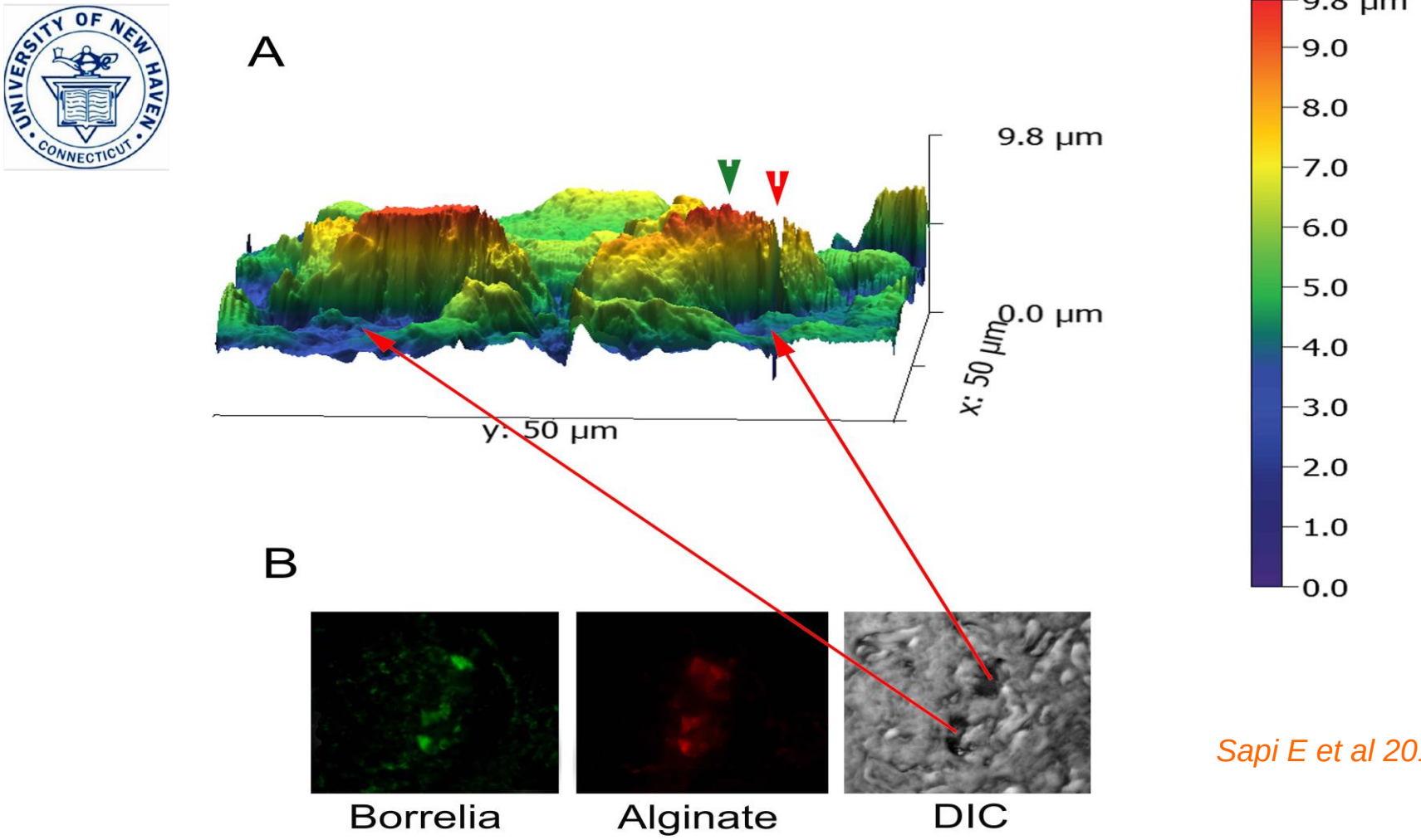


Additional IHC and *in situ* hybridization data



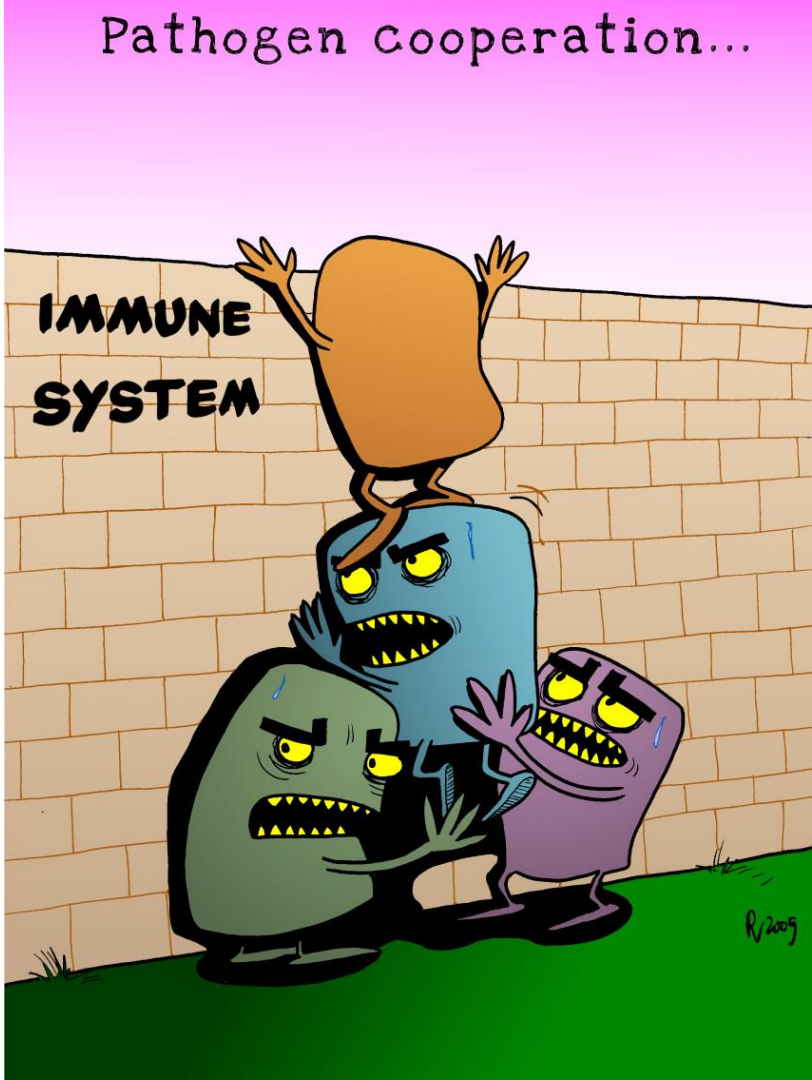
Sapi E et al 2016





Sapi E et al 2016

So could other pathogens exist in these tissues?



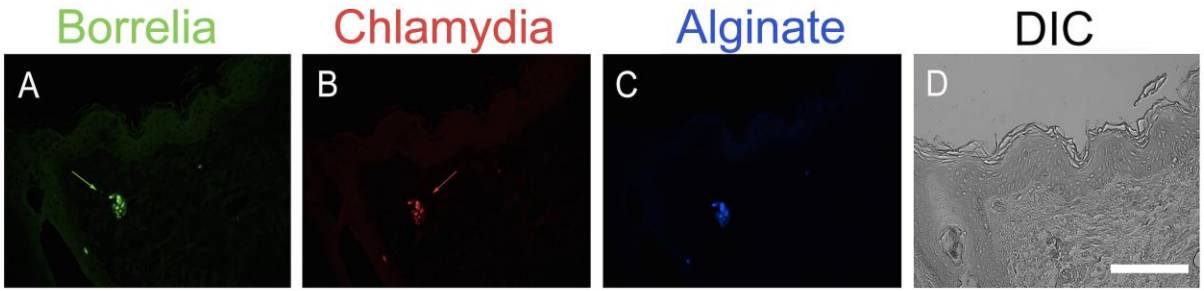
Velica, DevianArt

Metagenomic analyses for Borrelia Lymphocytoma Tissues

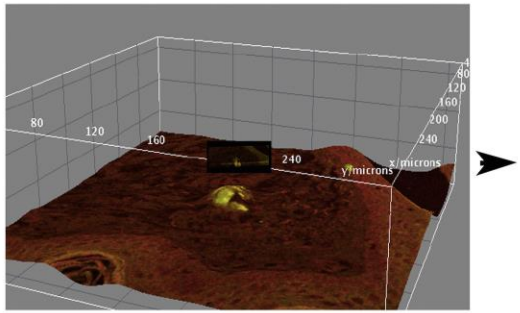
Reads *Borrelia burgdorferi* sensu lato,
Reads for *Chlamydia spp*

Genomic DNA analyzed by whole genome sequencing method (Perkin Elmer). Reads were aligned to human reference sequences using Burrows-Wheeler aligner software (http://bio-bwa.sourceforge.net)
After Sequence Alignment/Map (http://samtools.sourceforge.net) tool was used to filter out reads not mapped to human. The remaining reads were aligned to bacterial reference genomes using Blast program (NCBI).

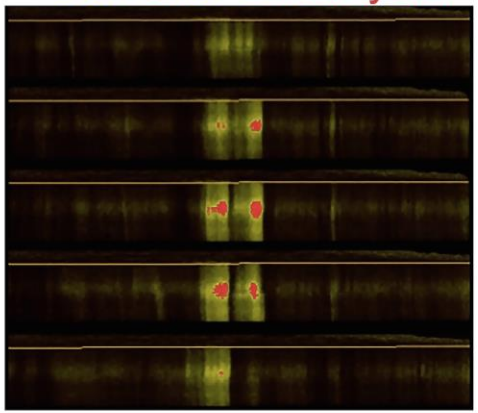
Sapi E et al 2019



Immunohistochemistry



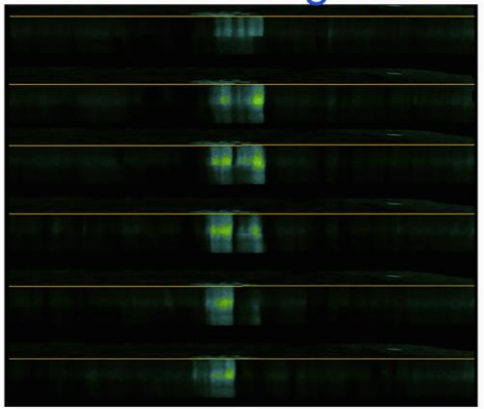
CONFOCAL MICROSCOPY



Z STACKS

TOP

BOTTOM



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Sapi E et al 2019

Mixed Biofilm in Other Infected Skin Tissues

healthcare

MDPI

Article

Mixed *Borrelia burgdorferi* and *Helicobacter pylori* Biofilms in Morgellons Disease Dermatological Specimens †

Marianne J. Middelveen [1], Katherine R. Filush [2], Cheryl Bandoski [2], Rumanah S. Kasliwala [2], Anthony Melillo [2], Raphael B. Stricker [3,*] and Eva Sapi [2]

Mixed Biofilm in Infected Skin Tissues

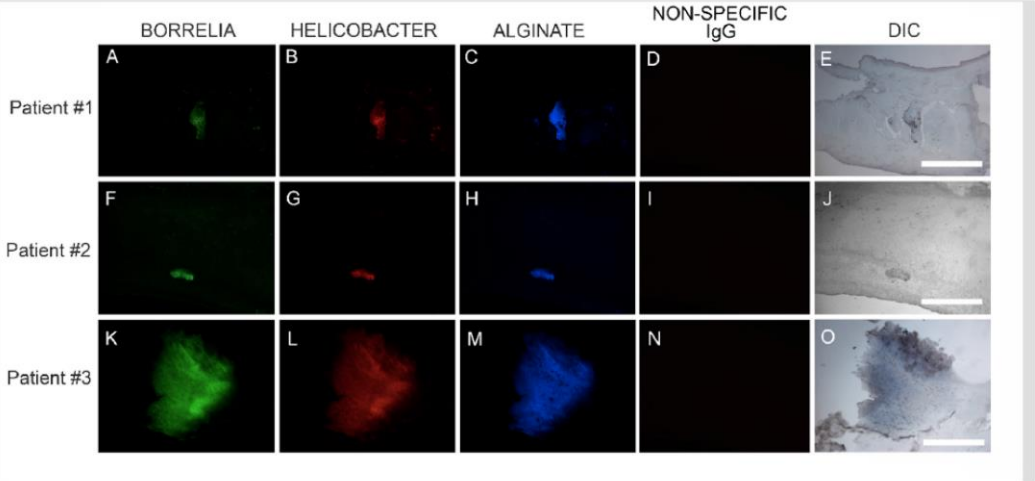
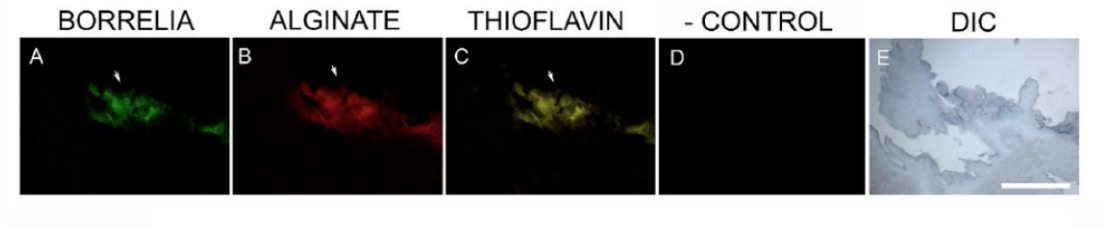


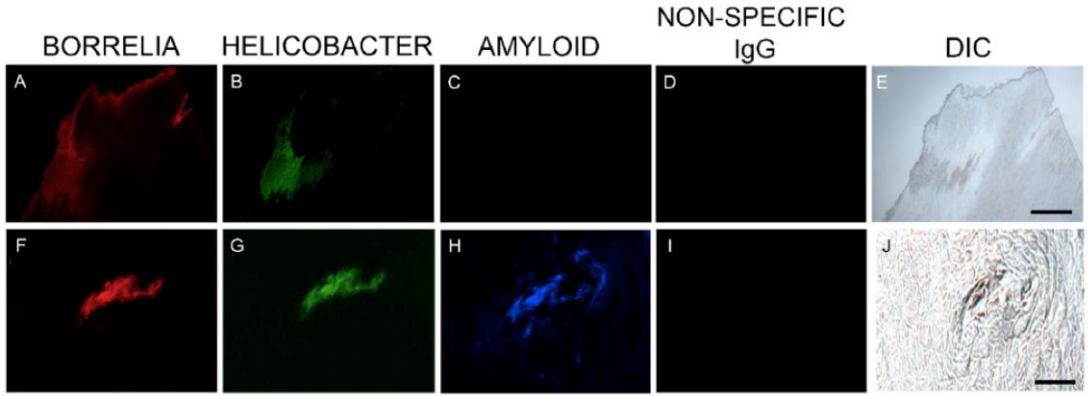
Figure 4. Representative IHC images showing biofilm aggregates in MD skin sections stained for *Borrelia*, *Helicobacter* and alginate. IHC detection was performed as described in the Methods section. Panels A, F and K show skin sections treated with anti-*Borrelia* monoclonal antibody (green). Panels B, G and L show skin sections treated with anti-*Helicobacter* antibody (red). Panels C, H and M show skin sections treated with anti-alginate antibody (blue). Panels D, I and N show negative control sections treated with non-specific IgG. Panels E, J and O show sections imaged with DIC. Images were taken at 200× magnification. Scale bar = 100 μm.

Middelveen MJ et al 2019

Amyloid Changes in Mixed Borrelia Biofilms



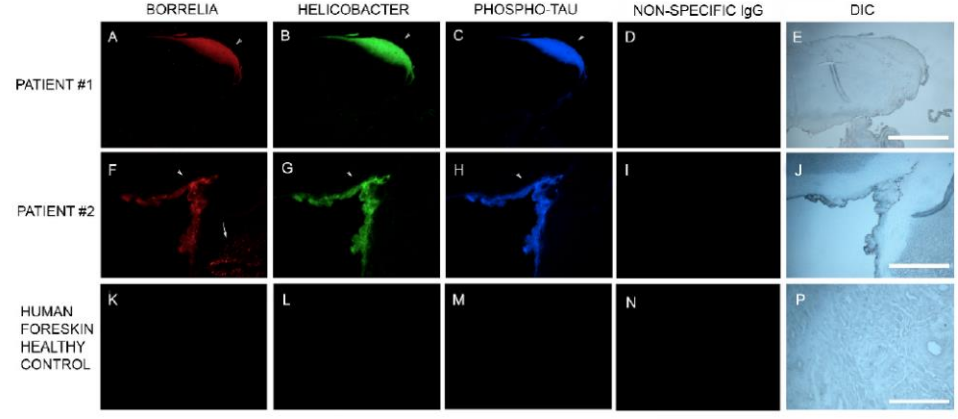
(A)



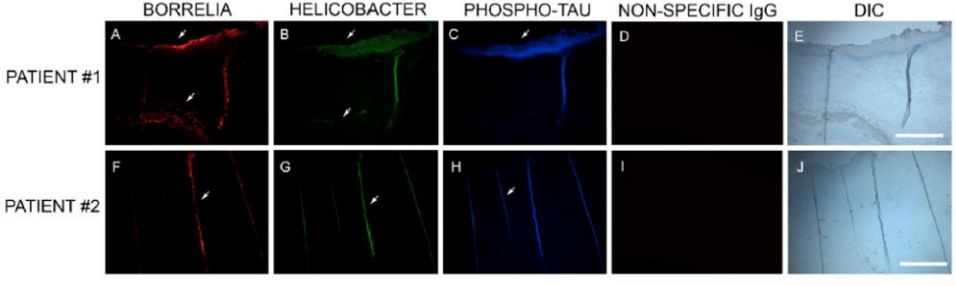
(B)

Middelveen MJ et al 2019

Mixed Biofilm and Phospho-Tau in Infected Skin Tissues



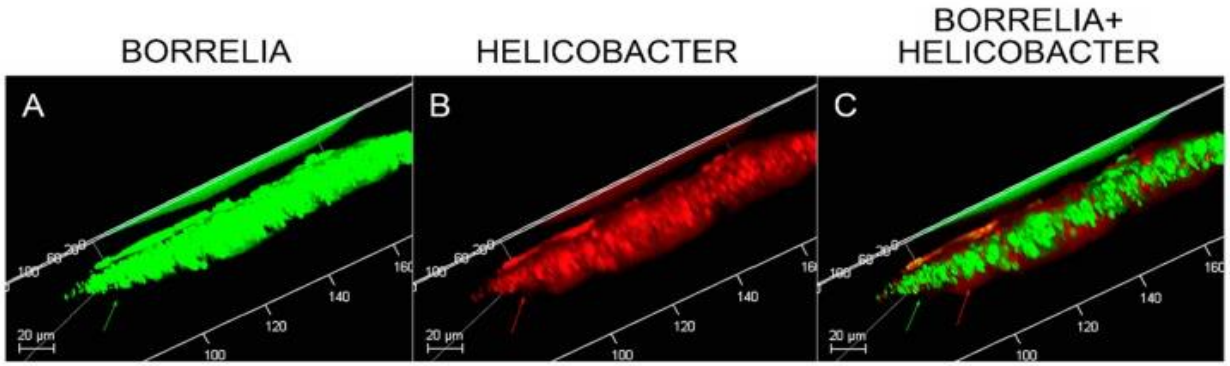
(A)



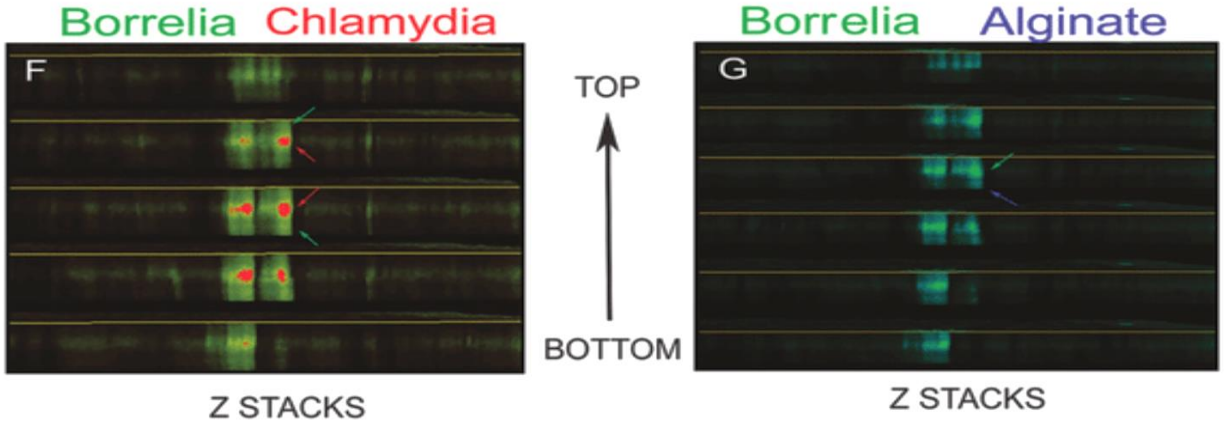
(B)

Middelveen MJ et al 2019

Borrelia in Mixed Biofilms

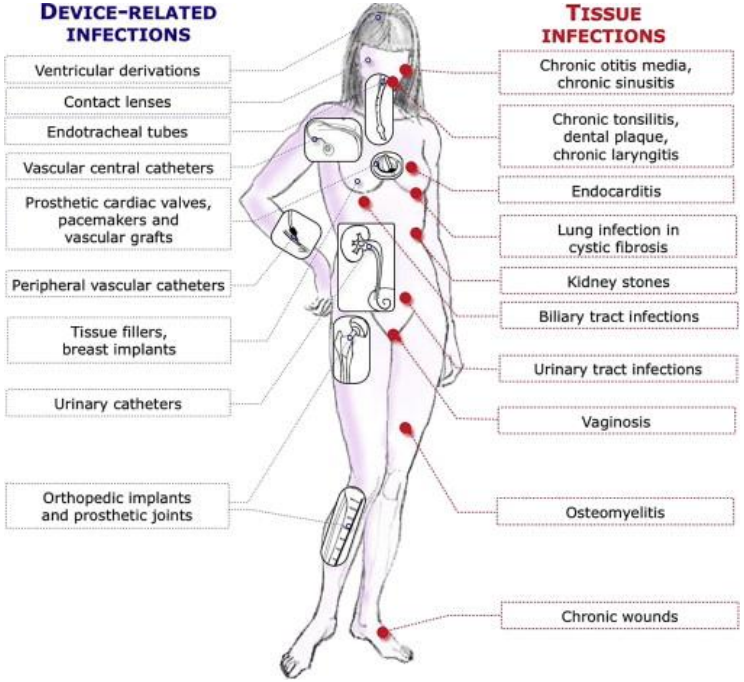


Middelveen MJ, Filush KR, Bandoski C, et al. Mixed *Borrelia burgdorferi* and *Helicobacter pylori* Biofilms in Morgellons Disease Dermatological Specimens. *Healthcare (Basel)*. 2019;7(2):70. Published 2019 May 17. doi:10.3390/healthcare7020070



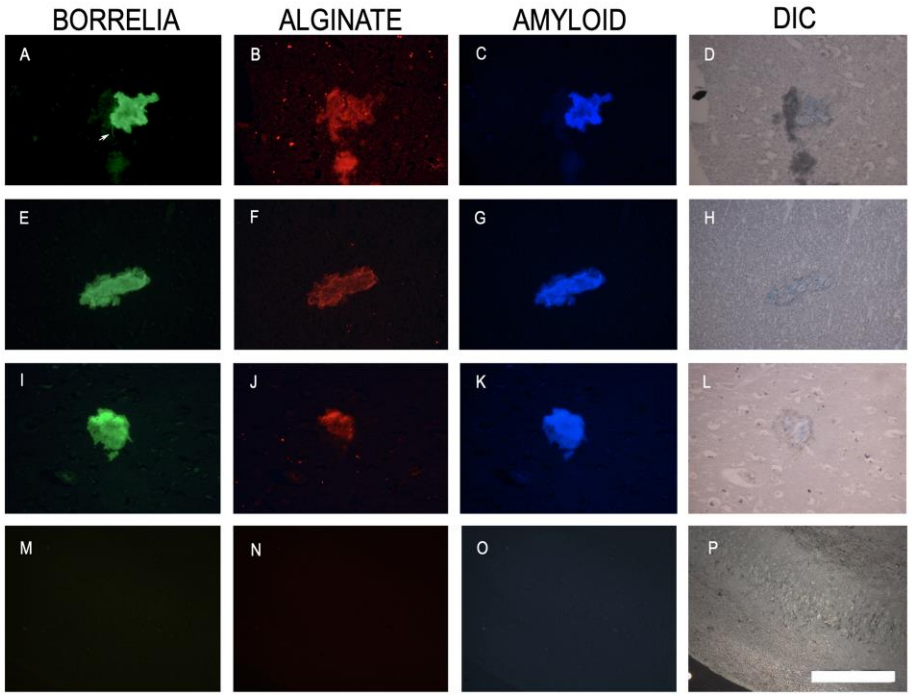
Sapi E, Gupta K, Wawrzeniak K, et al. *Borrelia* and *Chlamydia* Can Form Mixed Biofilms in Infected Human Skin Tissues. *Eur J Microbiol Immunol (Bp)*. 2019;9(2):46–55. Published 2019 Apr 11. doi:10.1556/1886.2019.00003

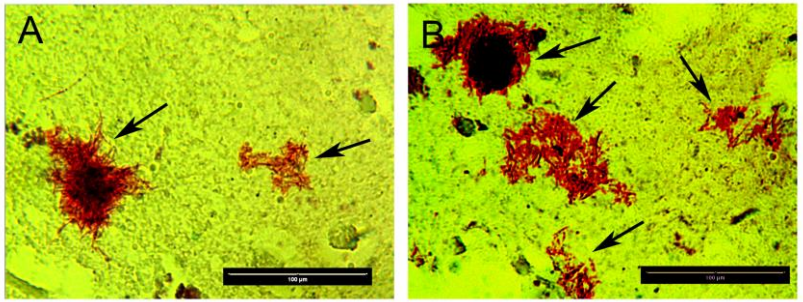
Where else you can find Borrelia biofilm in the human body?

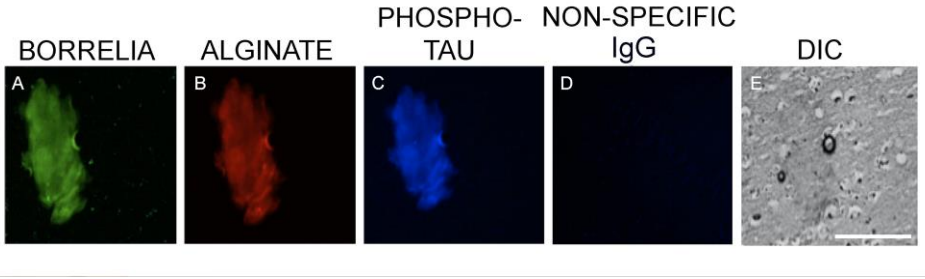


Hoiby N et al 2014

Borrelia Biofilm in Human Brain from in a Lyme disease /Alzheimer patient Correlates with Amyloid Changes







Senejani et al 2022

Autopsy tissues from Columbia University
Dr. James Goldman and Dr. Kenneth Liegner

- A 53-year-old woman with a 16-year history of Lyme disease who initially presented with headaches, fevers, fatigue, memory loss, cranial nerve palsies and gait disturbance and later developed progressive spastic quadraparesis and died despite the multiple antibiotic treatment
- Autopsy tissues from liver, heart, brain, kidney, lung, lymph node arrived from Columbia and the study was funded by:

GLOBAL LYME ALLIANCE
Conquering Tick-borne Disease through Research & Education

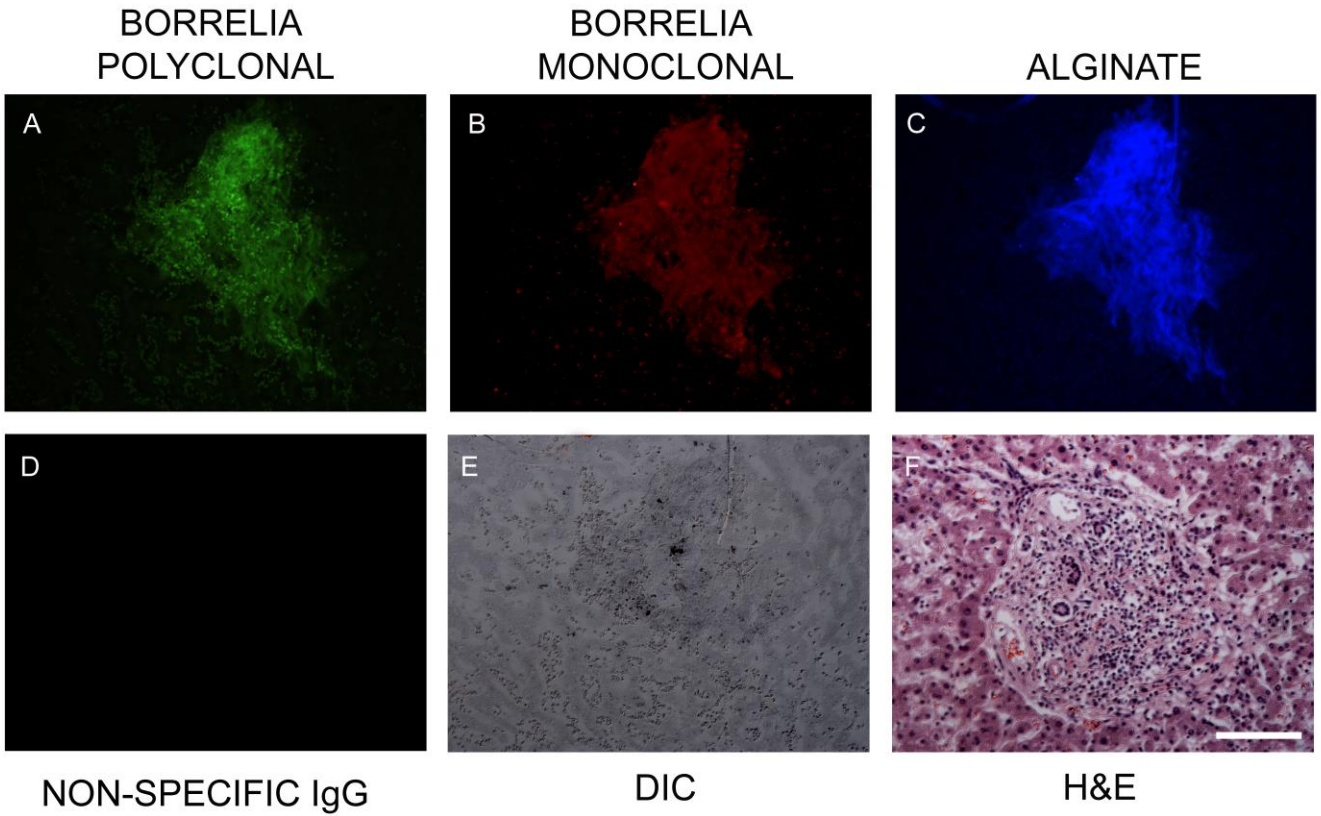
CULTURE-CONFIRMED TREATMENT FAILURE OF CEFOTAXIME AND MINOCYCLINE IN A CASE OF LYME MENINGOENCEPHALOMYELITIS IN THE UNITED STATES.

Kenneth B. Liegner, Carl E. Rosenkilde, Grant L. Campbell*, Thomas J. Quan, and David T. Dennis, Armonk, NY, USA, Mount Kisco, NY, USA, and Centers for Disease Control, Fort Collins, CO, USA.

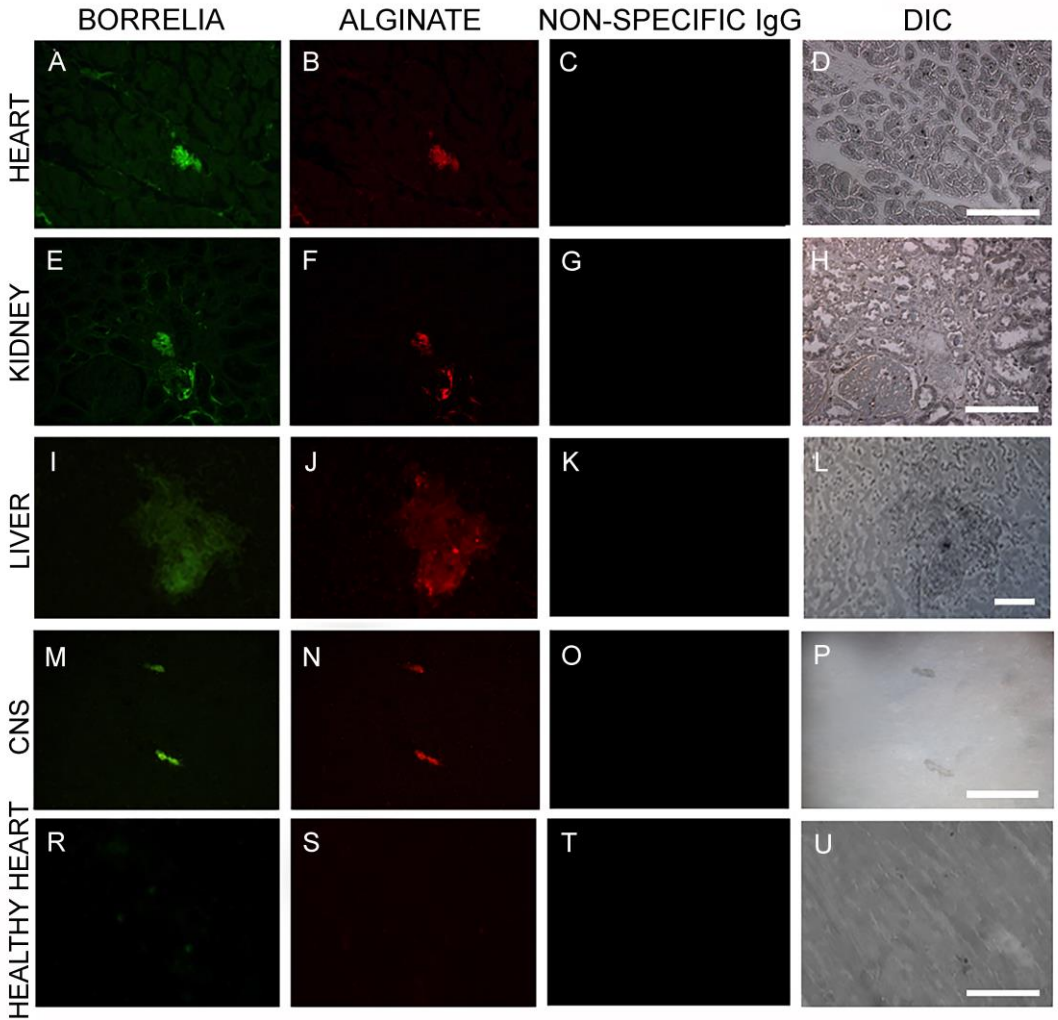
In 1987, a 37-year-old woman living in Westchester County, NY, developed spastic paraparesis, bilateral Babinski reflexes, and cranial nerve and bulbar dysfunction characterized by dysphagia, dysphonia, diplopia, absent gag reflex, and dysfunction of bowel and bladder control. CSF contained 19 WBC/mm^3 (86% lymphs). A test for antibodies to *Borrelia burgdorferi* (*Bb*) in serum was negative. No etiology was established despite an extensive workup. Symptoms and signs reportedly worsened gradually from 1988 to present. There was a past history of splenectomy for idiopathic thrombocytopenic purpura diagnosed in 1975. In 1989, the right frontal region and right basal ganglia were abnormal on brain MRI. In January 1990, CSF contained 6 WBC/mm^3 (93% lymphs), but no oligoclonal bands or myelin basic protein. Paired CSF and serum tests for antibodies to *Bb*, and PCR for *Bb*-specific oligonucleotides in CSF, were negative. An empiric 21-day course of cefotaxime (3 g/12 hr i.v.) was given in January, 1990 with no clear clinical benefit. Following treatment, CSF contained 9 WBC/mm^3 (93% lymphs). Four months of minocycline (200 mg/day p.o.) begun in November, 1990 also yielded no clear clinical benefit. In December, 1990 a T-cell stimulation test with *Bb* antigens was strongly positive. In December, 1991 CSF contained 6 WBC/mm^3 (89% lymphs) and elevated IgG. Paired serum and CSF samples were strongly positive for antibodies to *Bb*, with a CSF-to-serum index of 1.04. Culture of this CSF specimen in BSK-II yielded a strain of *Bb*. Culture-confirmed treatment failures have been previously reported for three Lyme neuroborreliosis cases in Europe. The present case apparently is the first of this type to be reported from the United States.

5th International Conference of Lyme Borrelosis – 1992 Arlington VA

Liver autopsy tissues examined for Borrelia



Sapi E, Goldman J, Liegner K - unpublished data 2019



Immunohistochemical analyses of four different organs for Borrelia and for alginate (biofilm marker)
Heart, Kidney, Liver, CNS

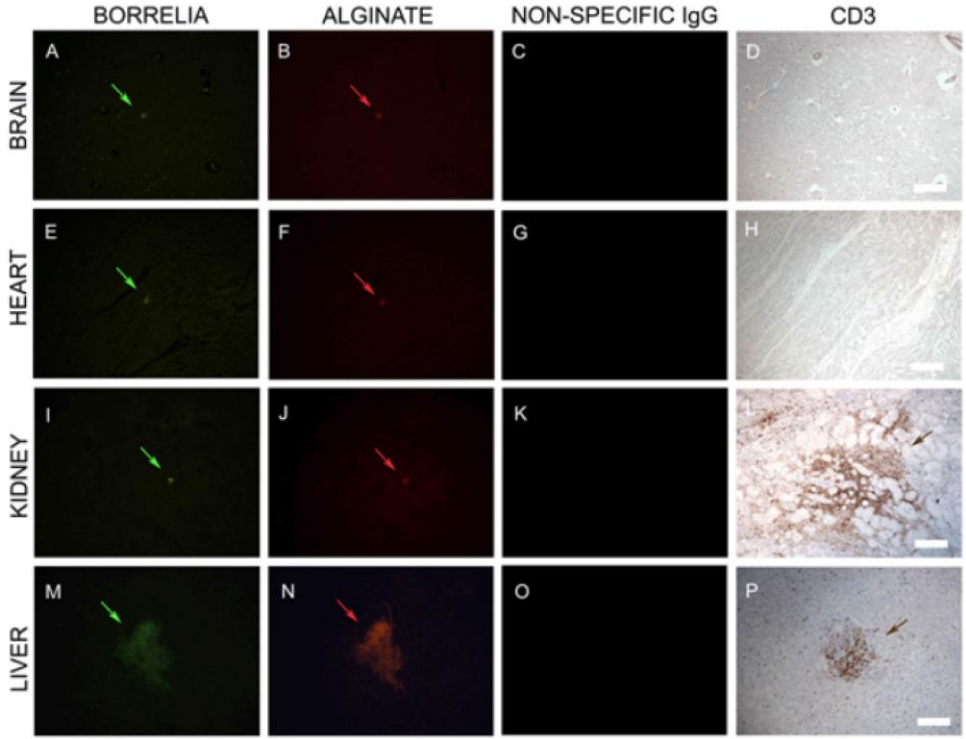
Sapi E et al 2019

Quantitative Analysis of Borrelia biofilms in liver, brain, heart and kidney tissues

Tissue	# Slides	# Biofilms/Slide	Size (μM)
Liver	150	0-6	20-300
Brain	210	0-3	20-100
Heart	130	0-4	20-100
Kidney	145	0-4	20-100

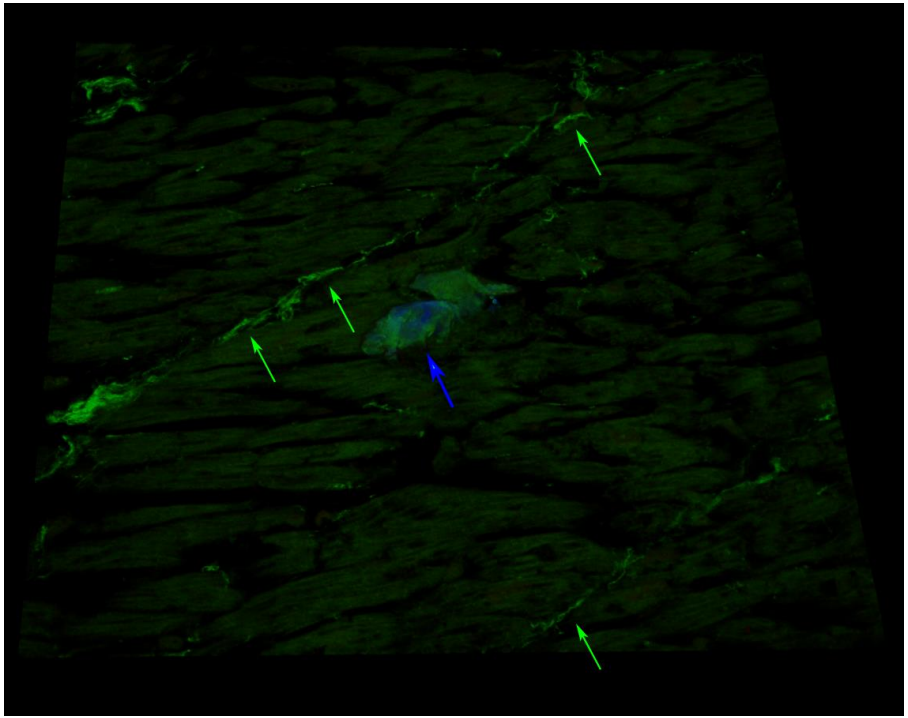
Sapi E, Goldman J, Liegner K 2019

Borrelia biofilm presence corresponded with infiltrating T cells in kidney and liver tissues – inflammation response?



Sapi E, Goldman J, Liegner K 2019

Borrelia Spirochetes and Biofilms in the Heart



Sapi E, Goldman J, Liegner K 2019

Borrelia biofilm presence corresponded with inflammatory markers

Table 2 Expression of inflammatory mediators present in brain, heart, kidney and liver tissues (+++: high expression, ++: medium expression, +: minimal expression, -: no expression).

	CD3	CD8	CD20	CRP	CXCL-9	CXCL13	MMP-9
Brain	-	-	-	++	++	-	++
Heart	-	++	++	+++	++	-	++
Kidney	+++	-	-	+	-	-	+++
Liver	+++	+++	-	++	+	+++	+++

Sapi E, Goldman J, Liegner K 2019 unpublished data

Effect of *Borrelia burgdorferi* Outer Membrane Vesicles on Host Oxidative Stress Response

by Keith Wawrzeniak, Gauri Gaur, Eva Sapi and Alireza G. Senejani *

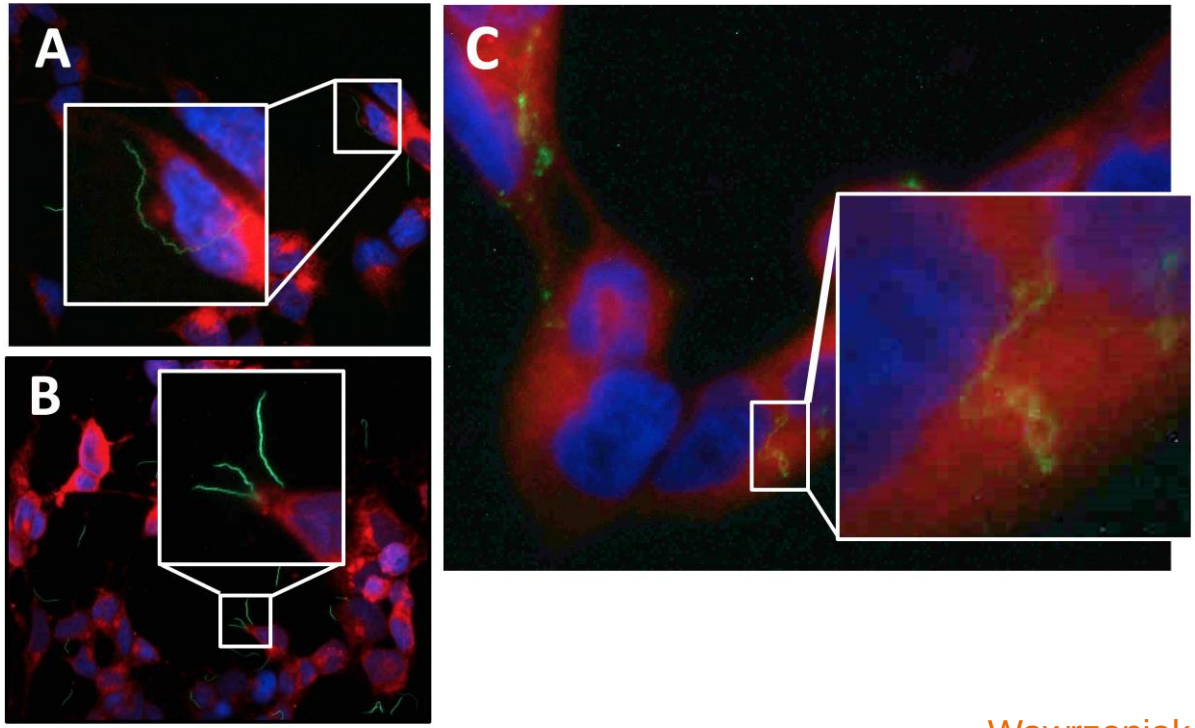
Department of Biology and Environmental Science, University of New Haven, West Haven, CT 06516, USA

* Author to whom correspondence should be addressed.

Antibiotics **2020**, *9*(5), 275; https://doi.org/10.3390/antibiotics9050275

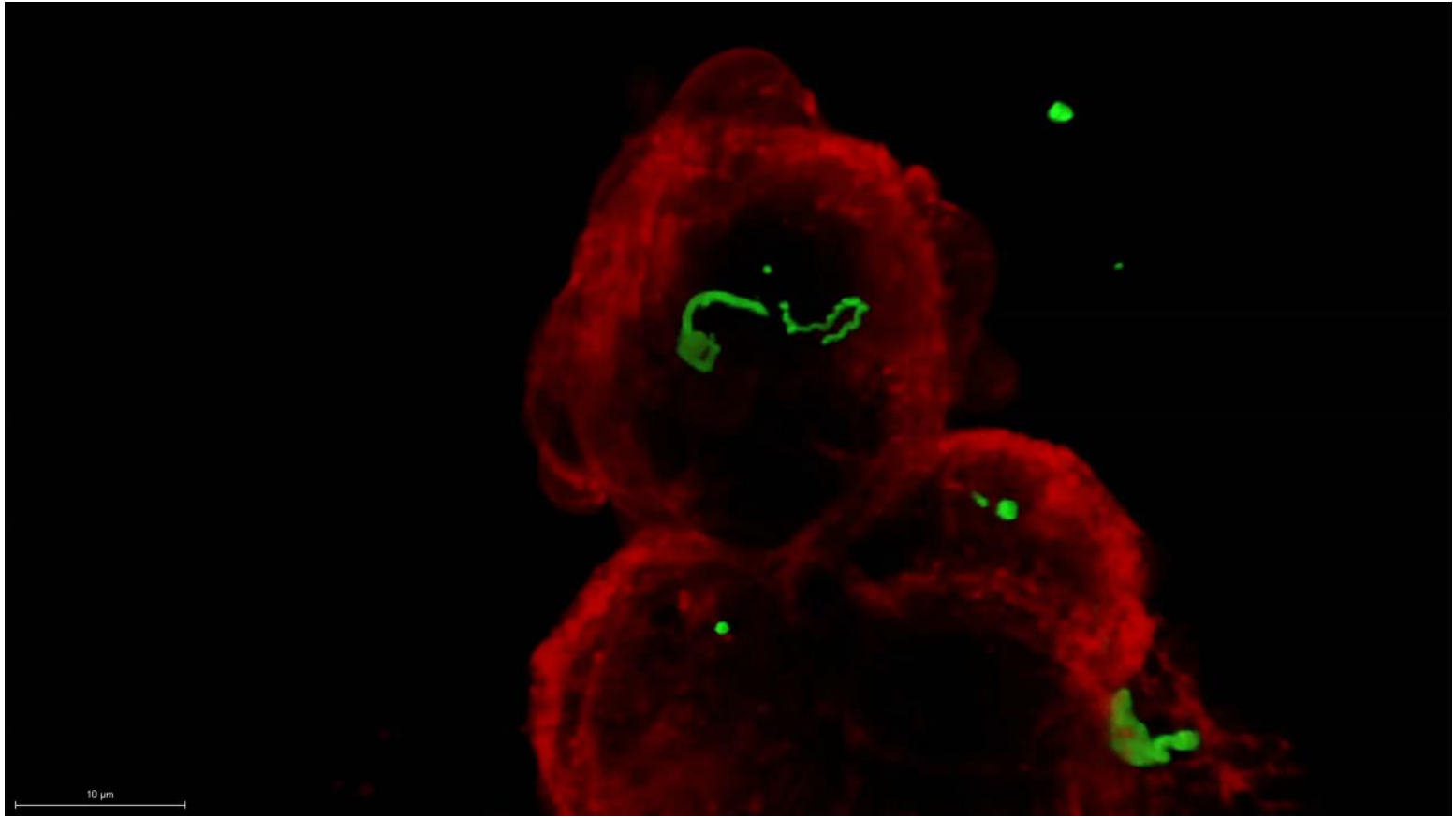
"In summary, these results show that Borrelia burgdorferi OMVs serve to directly counter superoxide production in BE2C neurons, thereby 'priming' the host environment to support B. burgdorferi colonization"

Borrelia-host interaction



- Neuron cells (BE2C) infected with Borrelia at
- 30 min (A),
- 2-hour (B), and
- 24-hour (C) time points.

Wawrzeniak K et al 2020


10 µm

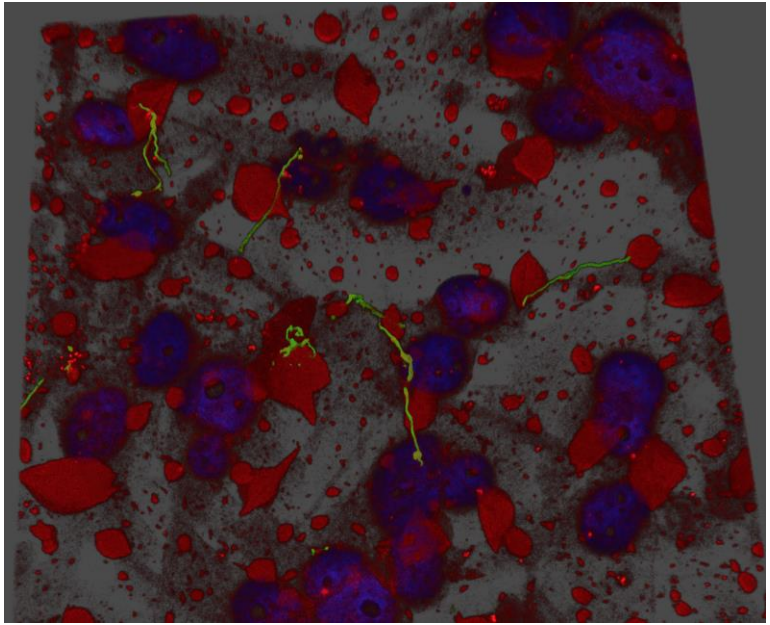
Where else can we find Borrelia – *Neoplastic Tissues?*

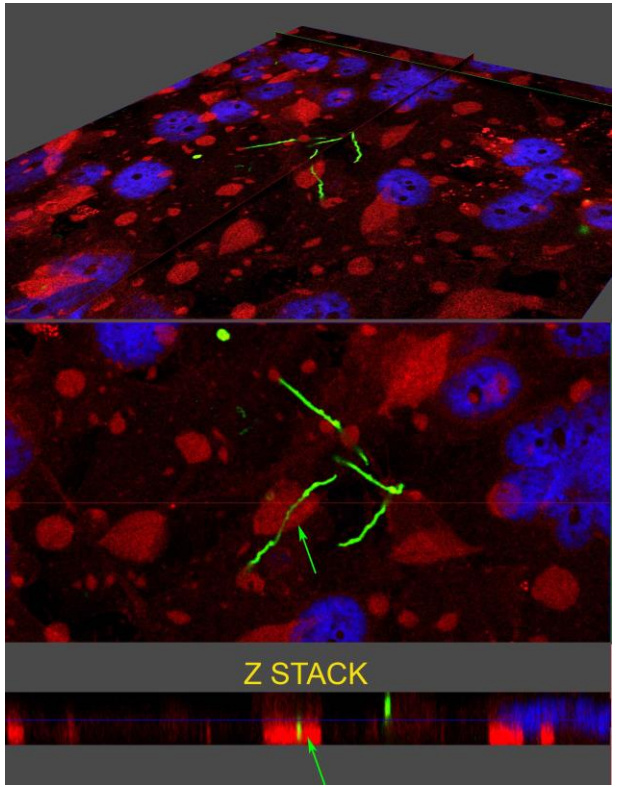
Breast cancer pilot study

- OBJECTIVE: 200 invasive breast cancer samples and 20 from healthy breast tissues evaluated for Borrelia burgdorferi presence by immunohistochemical methods
- RESULTS: Significant numbers of invasive breast ductal carcinoma and lobular invasive breasts carcinomas were positive for the presence of *Borrelia burgdorferi* spirochetes and biofilms while all of the fibroadenomas or the healthy control tissues were negative.

Gauri G and Sapi E 2021 unpublished data

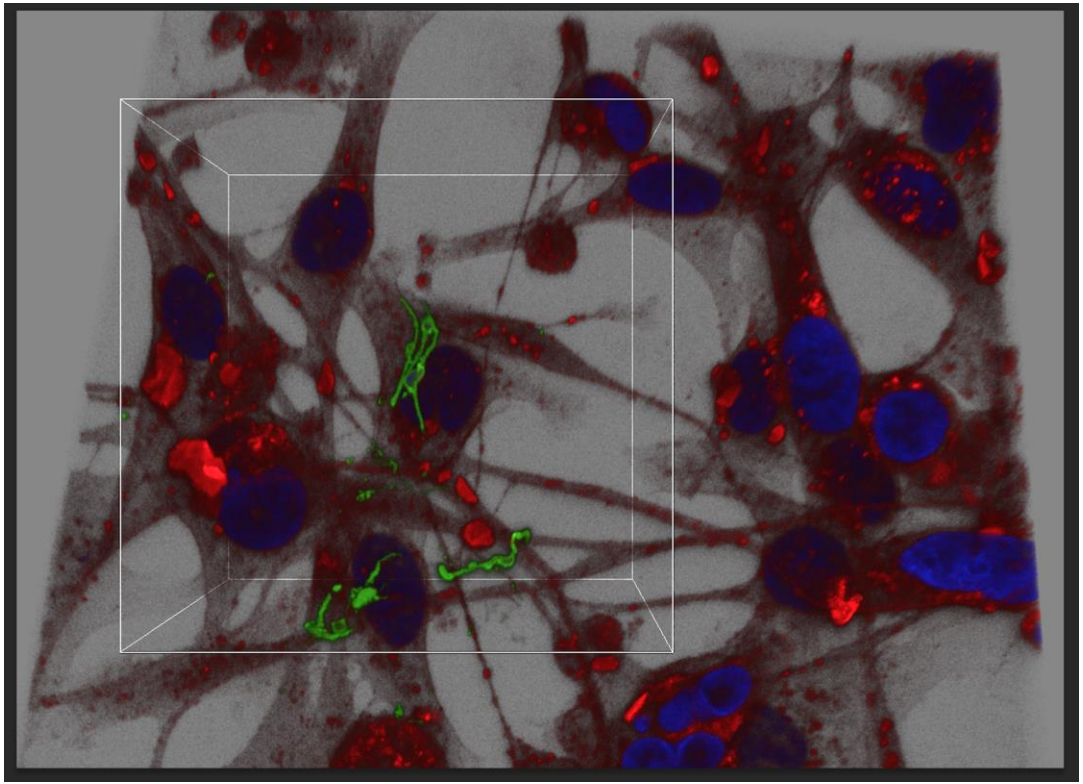
Borrelia inside of breast cancer cells?



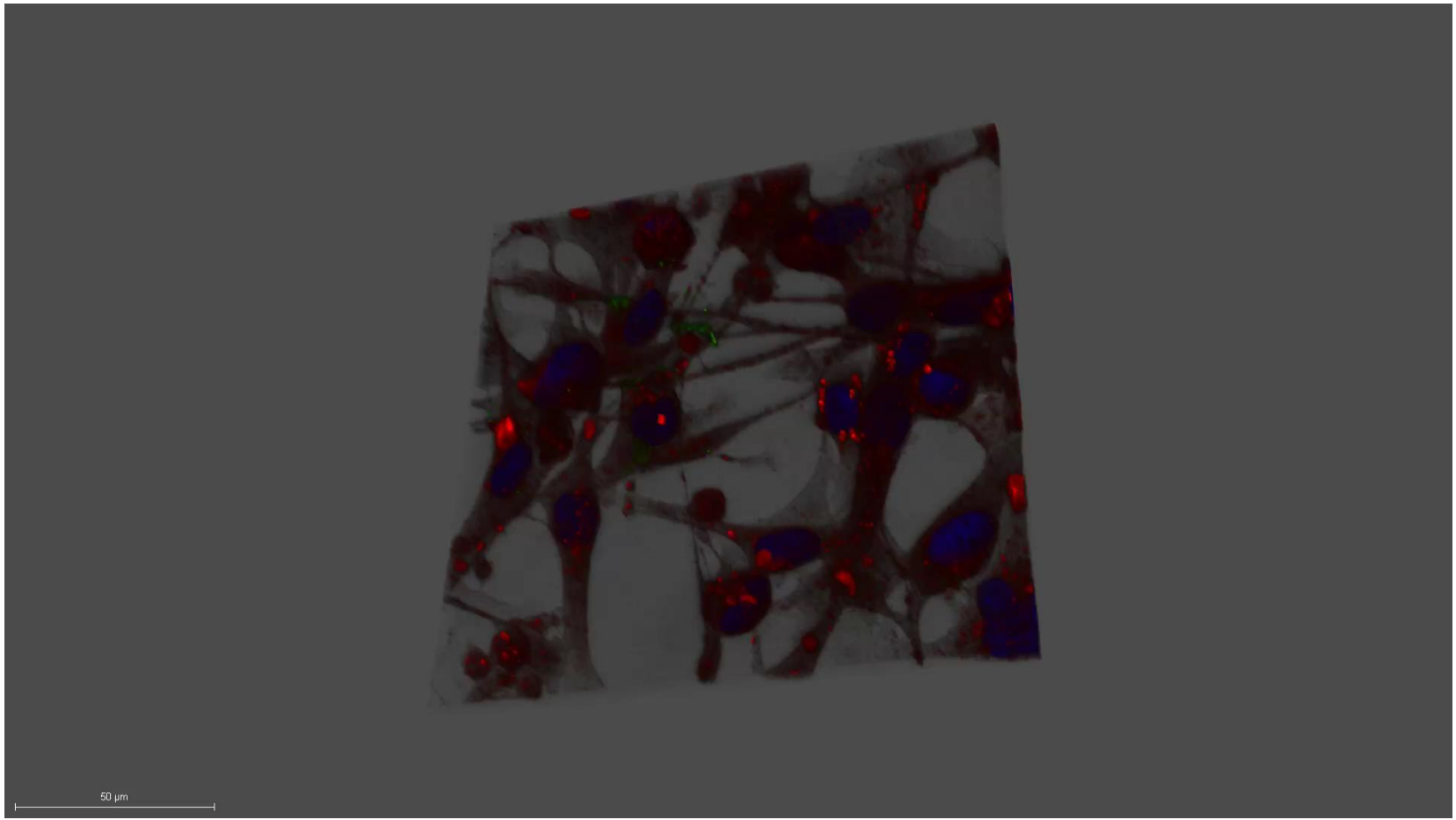


Gauri G and Sapi E 2020 unpublished data

Borrelia inside of breast cancer cells



Gauri G and Sapi E 2022


50 µm

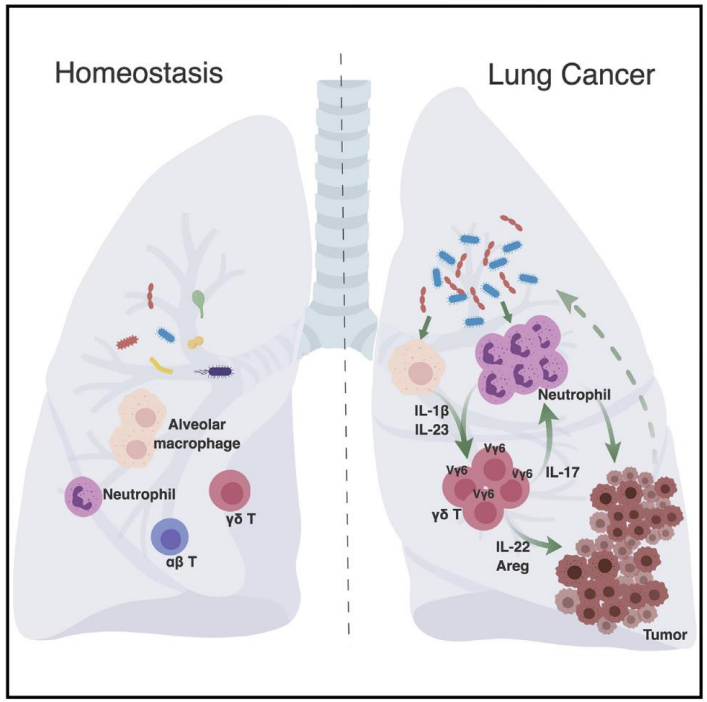
According to American Cancer Society - 15% of all cancers are caused by infectious agents

- *Helicobacter pylori* - Gastric cancer
- *Streptococcus bovis/gallolyticus* - Colorectal carcinoma
- *Salmonella typhi* – Gallbladder carcinoma
- *Chlamydia pneumonia, Mycoplasma* sp. - Lung cancer
- *C. pneumonia, C. trachomatis, C. psittaci* - Pulmonary Mucosa-Associated Lymphoid Tissue (MALT) lymphoma
- *Mycoplasma* sp., *C. trachomatis* - Ovarian cancer
- *Staphylococcus epidermidis, Escherichia sp. Bartonella sp.* – prevalent in breast cancer

Cell

Commensal Microbiota Promote Lung Cancer Development via γδ T Cells

Graphical Abstract



Authors

Chengcheng Jin, Georgia K. Lagoudas, Chen Zhao, ..., Paul C. Blainey, James G. Fox, Tyler Jacks

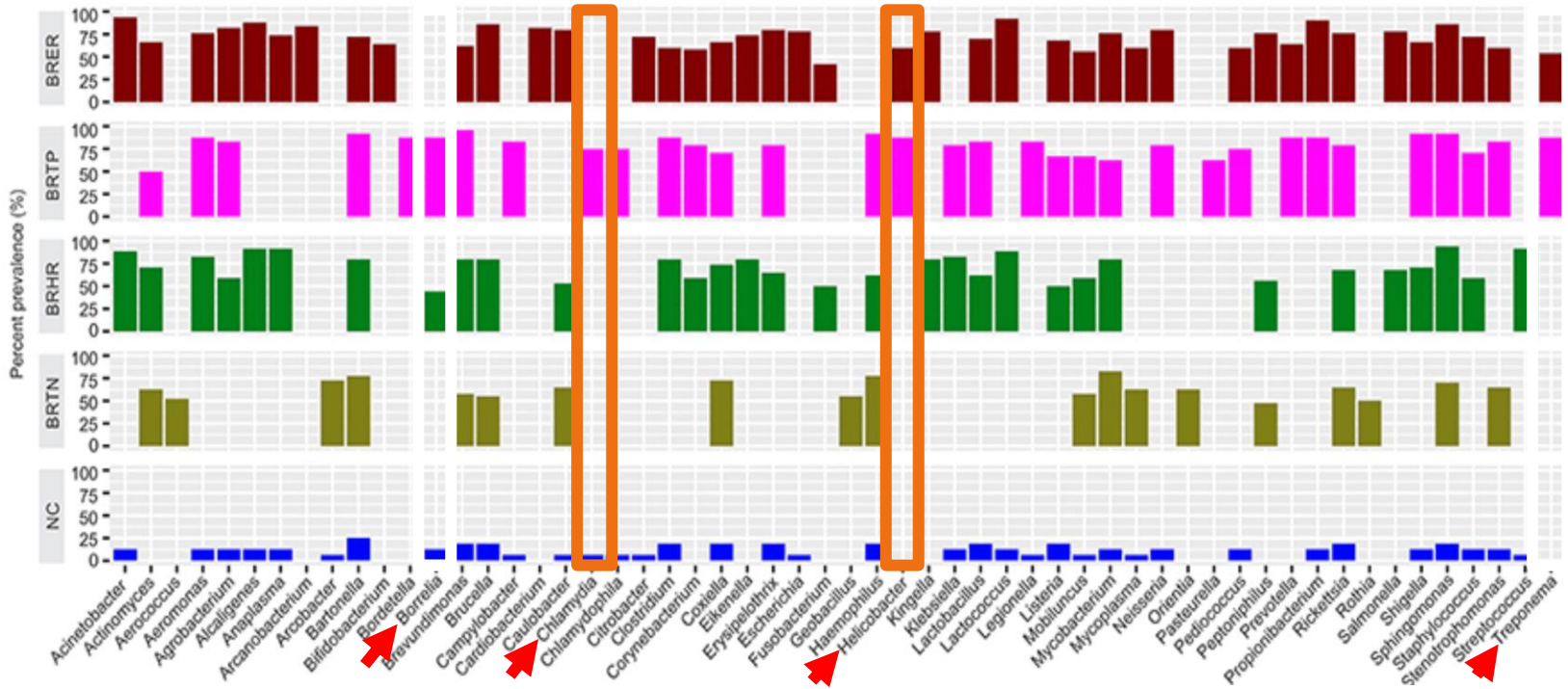
Correspondence

tjacks@mit.edu

In Brief

Lung cancer development is associated with increased bacterial burden and altered bacterial composition in the lung. Depletion of microbiota or blockade of the downstream cellular or molecular immune mediators significantly suppress lung tumor growth.

Jin et al., 2019, Cell 176, 998–1013



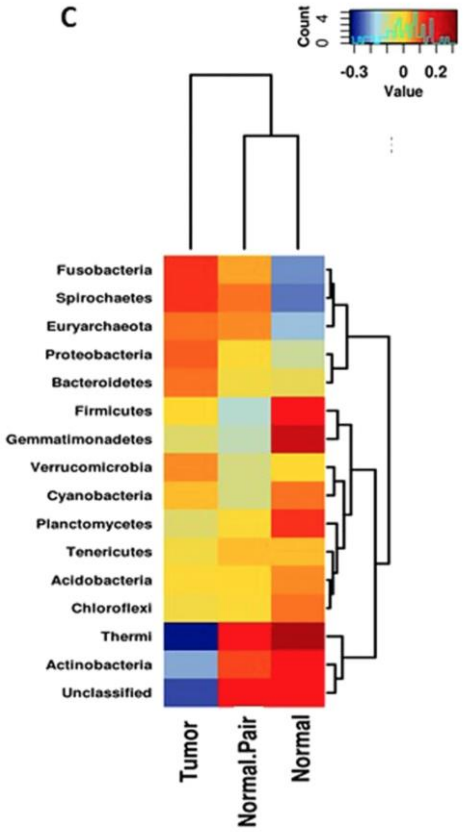
Banerjee S., Tian T., Wei Z., Shih N., Feldman M.D., Peck K.N., DeMichele A.M., Alwine J.C., Robertson E.S. Distinct microbial signatures associated with different breast cancer types. Front. Microbiol. 2018;9:951. doi: 10.3389/fmicb.2018.00951

- *Spirochete bacteria like Borrelia and Treponema associated with different types of breast cancer*

Distinct microbial communities that differ by race, stage, or breast-tumor subtype in breast tissues of non-Hispanic Black and non-Hispanic White women

Alana Smith, Joseph F. Pierre, Liza Makowski, Elizabeth Tolley, Beverly Lyn-Cook, Lu Lu, Gregory Vidal & Athena Starlard-Davenport

Scientific Reports **9**, Article number: 11940 (2019) | Cite this article

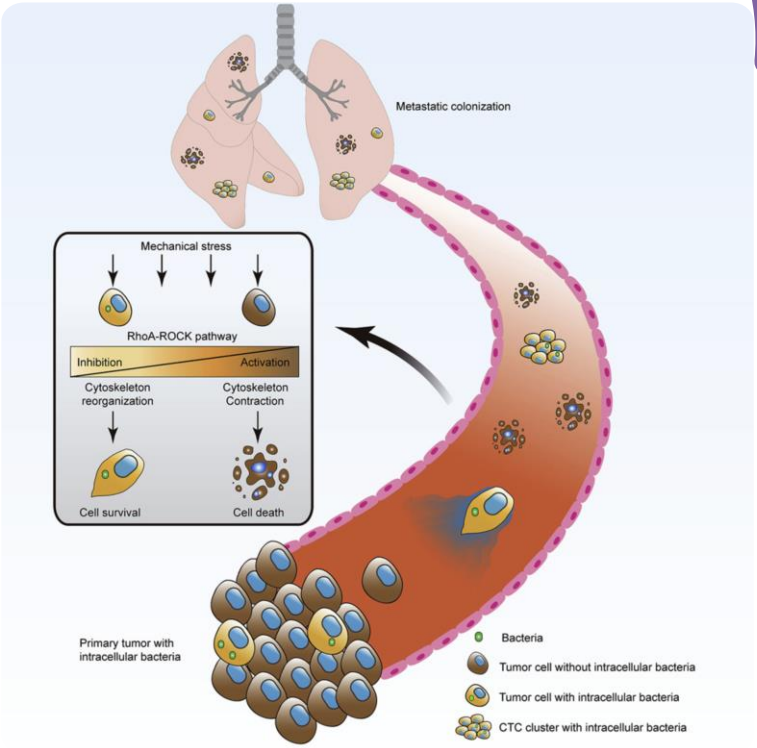


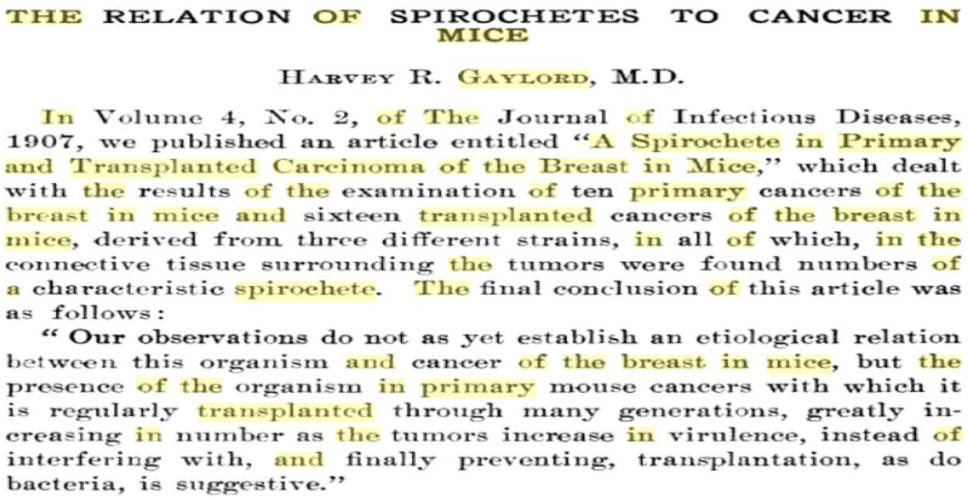
Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer

Fu A et al - Cell 185, 1356–1372, 2022

Bacteria living inside tumor cells promote cancer metastasis by helping cancerous host cells against mechanical stress in the bloodstream, in turn promoting cell survival during tumor progression.

Antibiotics treatment reduced tumor metastasis more than 3-fold!





THE RELATION OF SPIROCHETES TO CANCER IN MICE

HARVEY R. GAYLORD, M.D.

In Volume 4, No. 2, of The Journal of Infectious Diseases, 1907, we published an article entitled "A Spirochete in Primary and Transplanted Carcinoma of the Breast in Mice," which dealt with the results of the examination of ten primary cancers of the breast in mice and sixteen transplanted cancers of the breast in mice, derived from three different strains, in all of which, in the connective tissue surrounding the tumors were found numbers of a characteristic spirochete. The final conclusion of this article was as follows:

"Our observations do not as yet establish an etiological relation between this organism and cancer of the breast in mice, but the presence of the organism in primary mouse cancers with which it is regularly transplanted through many generations, greatly increasing in number as the tumors increase in virulence, instead of interfering with, and finally preventing, transplantation, as do bacteria, is suggestive."

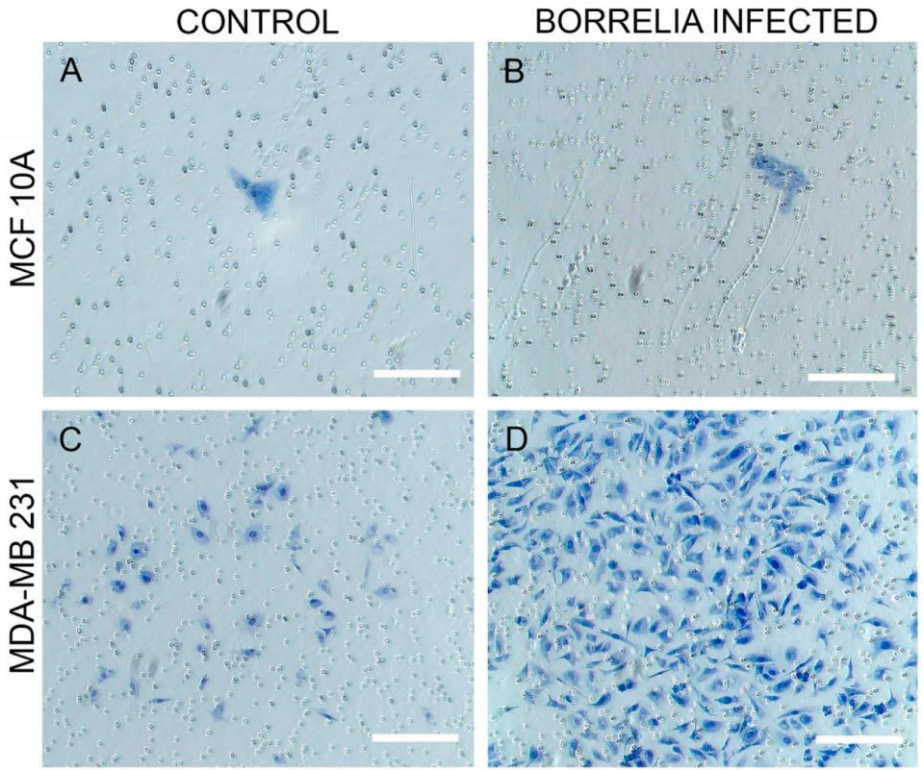
- Gaylord, 1907 – *in vivo* study suggests a potential connection between spirochetes and primary breast cancer in mice

HYPOTHESIS

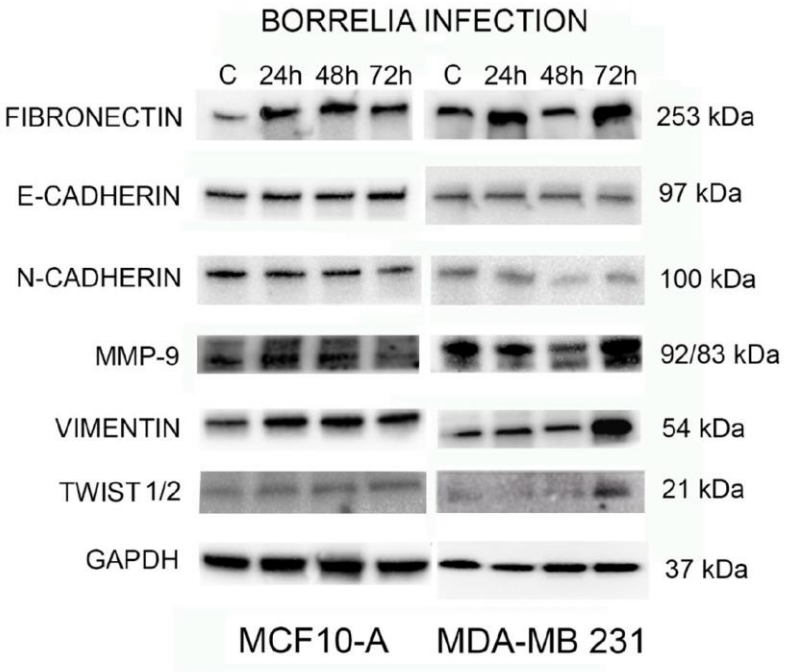
Borrelia burgdorferi plays a role in tumorigenic changes in breast epithelial cells

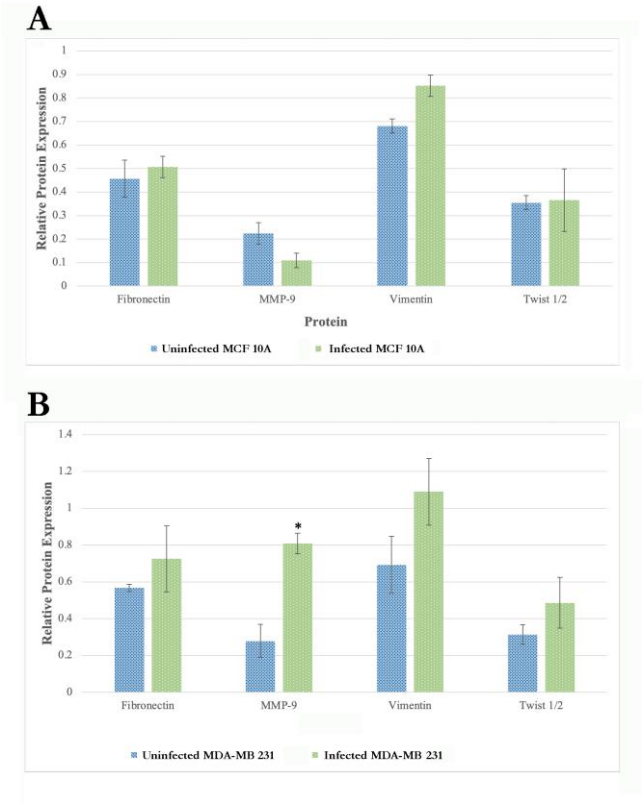
Effect of Borrelia on normal and breast cancer cells invasion

- *Gauri G et al 2021*



Effect of Borrelia infection on Breast Cancer Markers



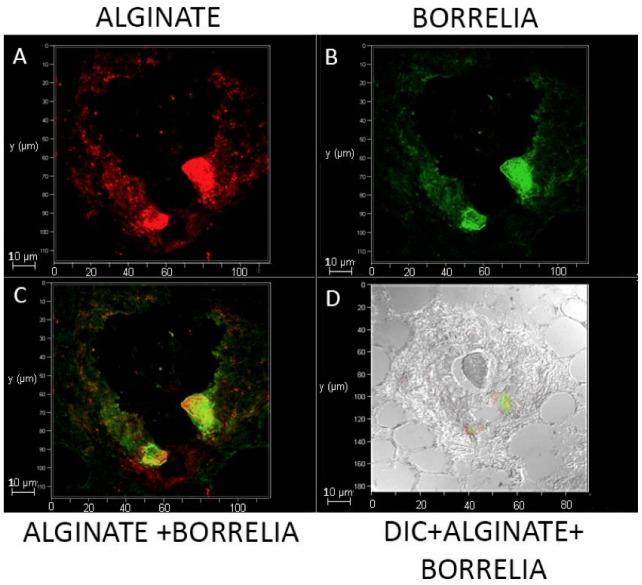


Gauri G et al 2021

Current Projects:
Identifying pathways and searching for markers in Borrelia infected cells

- Molecular analyses of Infected Breast Cancer and Normal Epithelial Cells
  - *RNAseq and tumor panel analyses*
  - *MicroRNA analyses*
  - *Tissue remodeling factors*
  - *Chemotherapeutic resistance markers*

Current Projects:
New models for antibiotic studies

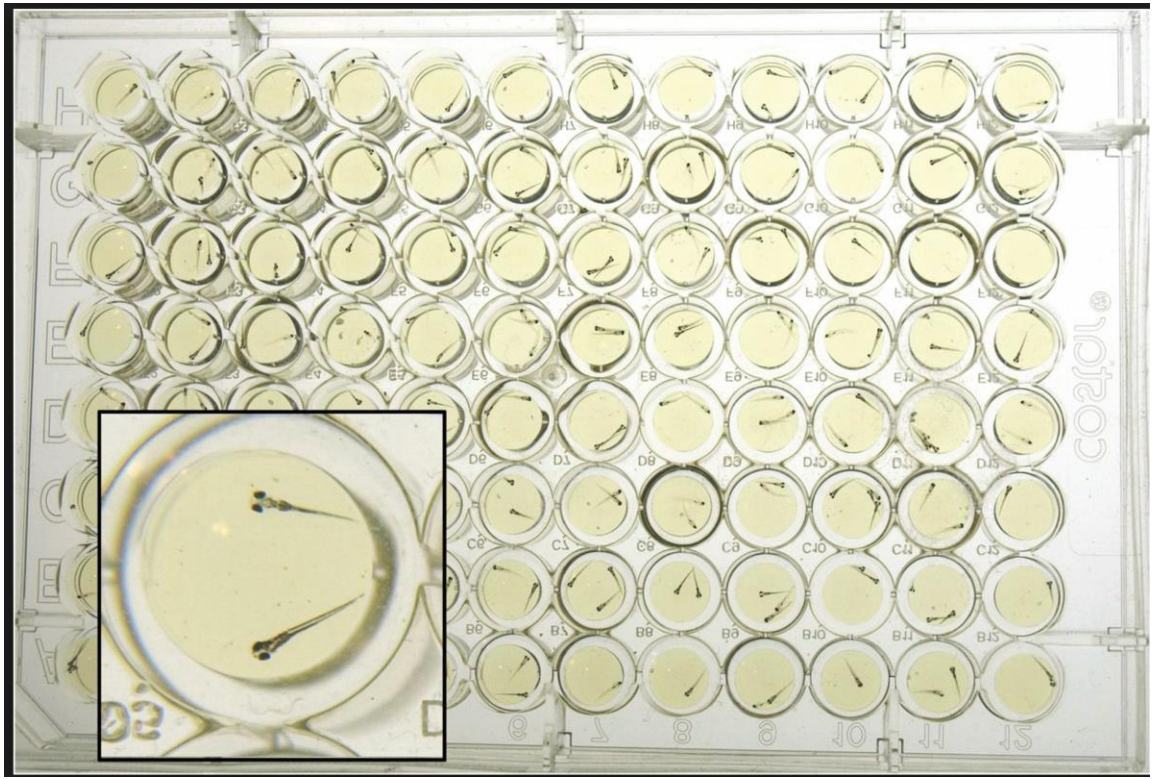


Torres J, Sapi E 2019

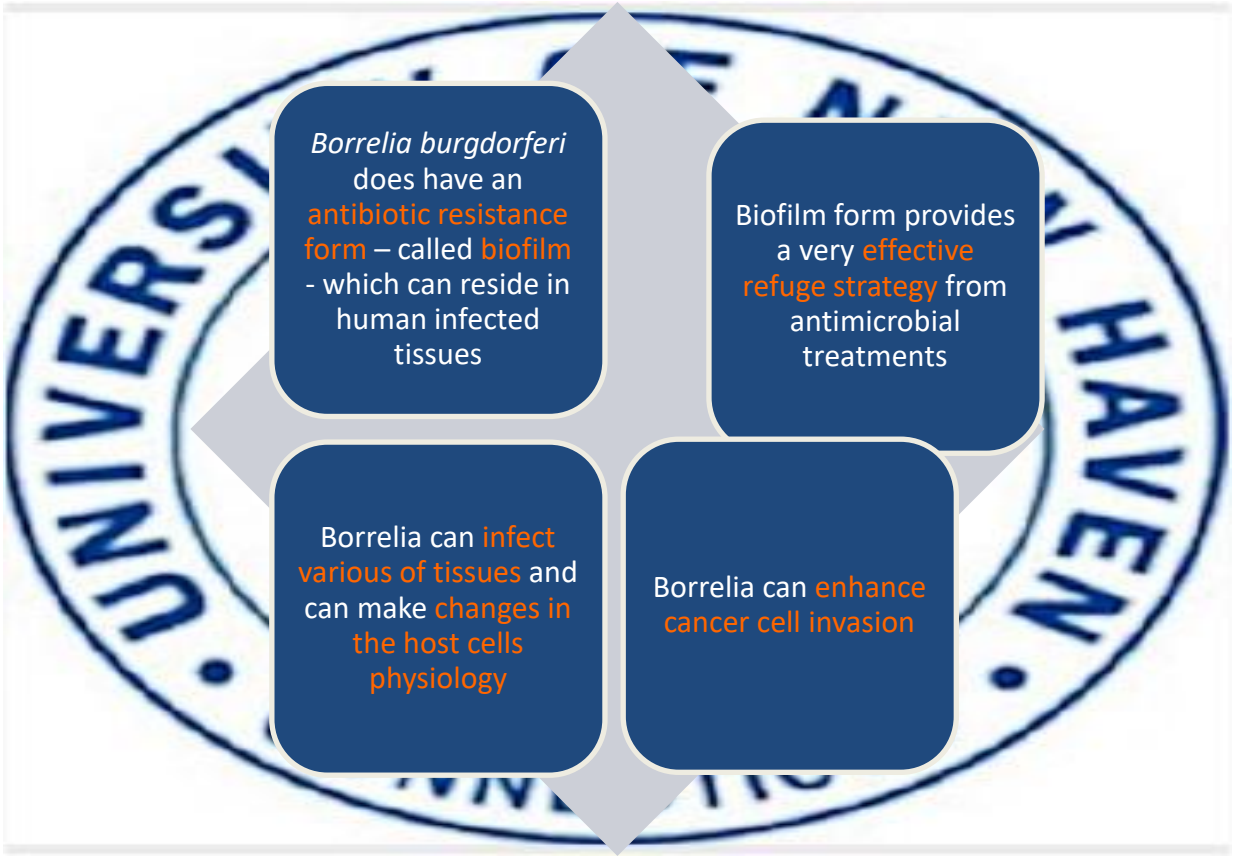
Ex vivo culture systems:
skin biopsies (see left)
heart valve cultures

Zebrafish model

Antimicrobial Testing in Zebrafish



Summary


Borrelia burgdorferi does have an antibiotic resistance form – called biofilm - which can reside in human infected tissues
Biofilm form provides a very effective refuge strategy from antimicrobial treatments
Borrelia can infect various of tissues and can make changes in the host cells physiology
Borrelia can enhance cancer cell invasion

UNH Lyme and Breast Cancer Disease Research Group

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•University of New Haven and College of A&S for funding our studies

•Philanthropic Trust , Lyme Warriers, LivLyme Foundation, Global Lyme Alliance, ILADS, Lymedisease.org, Lyme Disease Association, CT LymeRiders, for supporting our research projects

•Pink Clover Foundation and Philanthropic Trust to support our breast cancer research initiatives

•Lymedisease.org, Lyme Disease Association, Schwartz Foundation and Global Lyme Alliance for providing a “state of the art” microscopes for our morphological studies

Question?

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