

## **Biofilms of Borrelia burgdorferi in Chronic Cutaneous Borreliosis**

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We propose the hypothesis that Borrelia burgdorferi can form biofilm structures in lymphocytomas and acrodermatitis 4chronica atrophicans based on Eisendle K et al<sup>1</sup> findings published in Am J Clin Pathol 2007; 127:213-222. This elegant 5 and novel immunohistochemical study used focus floating microscopy to demonstrate the presence of Borrelia in these 6 cutaneous lesions. The findings of this paper suggested that Borrelia can grow in a "medusa colony" or in a "granular colony with a reddish veil" (Figures 5A and 6F in this article). Our close examination of these pictures revealed striking similarity to previously published biofilm pictures<sup>2</sup> and our preliminary findings on specific Biofilm-like colony 1 formation of Borrelia burgdorferi when cultured in the presence of human plasma (see Figure 1).

Further justification of this hypothesis will require the detailed characterization of these structures for typical biofilm properties such as identifying multiple organisms in the colonies which are located in a specialized polysaccharide matrix, morphological diversity of microbes in biofilm due to specialization of individual bacteria induced by the recently discovered quorum sensing mechanisms and of course the occurrence of these structures in chronic infections.

Bacterial biofilms are responsible for several chronic diseases (e.g. periodontitis and chronic lung infection in cystic fibrosis patients) that are very difficult to treat because they show much greater resistance to antibiotics (up to 1000-fold) than their free-living counterparts<sup>3</sup>. The biofilm resistance is very unique in a sense that it requires multiple mechanisms such as incomplete penetration of the antibiotics into the matrix, inactivation of antibiotics by altered chemical microenvironment within the biofilm (low pH, anaerobic condition etc) and an altered, highly protected phenotypic system of the resistant bacteria population<sup>4</sup>.

<sup>2</sup>ff Borrelia bugdorferi is indeed capable forming biofilm, it will change the way how we think about Lyme disease especially in patients where it seems to be persistent disease despite the long term antibiotics treatment<sup>5</sup>. The elucidation of the molecular mechanisms responsible for the switch from free living (planktonic) growth to a biofilm phenotype and the development of resistance to antibiotics should provide novel therapeutic targets for chronic Lyme disease.

In summary, we suggest that chronic cutaneous Borreliosis conditions such as lymphocytomas and acrodermatitis chronica atrophicans need further investigation to see whether these could be infections of the Borrelia biofilm type.

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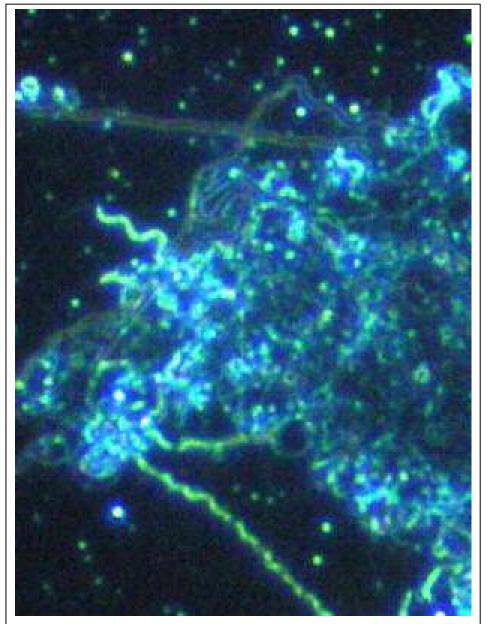
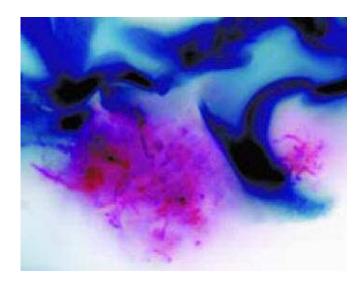
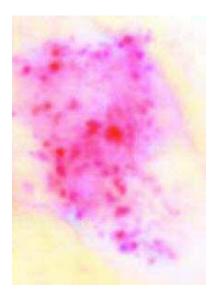


Figure 1 Borrelia burgdorferi in pure culture from American Type Culture Collection (35210) in BSK-M medium (Sigma Aldrich), darkfield 1000x original magnification. Conventional spiral forms merge with biofilm elements including cystic rounded forms, tubular elements and dot-like punctate granular forms in a gel matrix.



For Comparison Eisendle Image 5 91x71mm (96 x 96 DPI)



For Comparison Eisendle Image 6 from Acrodermatitis Chronica Atrophicans  $54x75\text{mm}\ (96\ x\ 96\ \text{DPI})$