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1 # Fibroblast Protection of *Borrelia burgdorferi* from Doxycycline, Cefuroxime and
2 # Daptomycin Combination is Eliminated by Oregano or Carvacrol Essential Oil

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17

18 ## Abstract

19 *Borrelia burgdorferi* could be occasionally recovered from patients after antibiotic treatment,
20 which indicates it may resist eradication by antibiotic and host defense mechanisms. Skin
21 fibroblast cells have previously been shown to protect the killing of *B. burgdorferi* by
22 ceftriaxone, a powerful antibiotic commonly used to treat Lyme disease. In this study, we
23 evaluated if fibroblast cells could also protect against the doxycycline+ cefuroxime+ daptomycin
24 drug combination which has previously been shown to completely eradicate highly persistent
25 biofilm-like microcolonies of *B. burgdorferi.* To do so, we utilized a GFP-labeled *B. burgdorferi*
26 for infection of murine fibroblast cells and assessed the effect of the drug combination on killing
27 the bacteria in the presence or absence of the fibroblast cells. Surprisingly, we found that
28 fibroblasts could protect *B. burgdorferi* from being completely killed by the drug combination
29 doxycycline, cefuroxime and daptomycin, which eradicated *B. burgdorferi* completely in the
30 absence of fibroblast cells. Interestingly, addition of essential oil carvacrol or oregano at 0.1%
31 could enhance the activity of the doxycycline+ cefuroxime+ daptomycin drug combination and
32 led to complete eradication of *B. burgdorferi* even in the presence of fibroblast cells. Further
33 studies are needed to determine if the essential oil drug combinations could eradicate persistent *B.*
34 *burgdorferi* infection in vivo in animal models. Our study provides a useful and convenient *ex*
35 *vivo* model for evaluating different drug regimens needed for developing more effective
36 treatment of persistent Lyme disease in the future.

37

38 Key words: Fibroblast; *Borrelia burgdorferi*; Coculture, Essential oils; antibiotics

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39

40 **Introduction**

41

42 Lyme disease is a multisystem infection caused by the spirochete *Borrelia burgdorferi* (*B.*
43 *burgdorferi*) that is transmitted by Ixodes tick bites into the skin of animals and humans (1, 2).
44 The skin of hosts is the key interface where the tick bites (3), and the epidermis with its
45 keratinocytes represents the first barrier encountered by ticks. Ticks overcome this barrier using
46 their biting mouthpieces to penetrate deeply into the skin and reach the dermis, where the saliva
47 interacts with resident cells such as dermal dendritic cells, mast cells and fibroblasts. *B.*
48 *burgdorferi* invades fibroblasts, and it is of interest to note that fibroblast cells have been shown
49 to protect *B. burgdorferi* from killing by antibiotics such as ceftriaxone(4, 5).

50

51 Antibiotics, such as doxycycline, amoxicillin, or cefuroxime, are effective for the majority of
52 Lyme disease cases (6-10). However, approximately 10–20% of the Lyme disease patients
53 treated with antibiotics still experience persisting symptoms of fatigue, muscle aches, joint pain
54 and neurologic impairment, that lasted for more than 6 months, a condition called Post-
55 Treatment Lyme Disease Syndrome (PTLDS) (7). Viable *B. burgdorferi* has been isolated from
56 the skin (11), cerebrospinal fluid (11, 12) and blood (13) of patients after antibiotic treatment for
57 Lyme disease. In various animal models, such as mice, dogs and monkeys, it has been shown
58 that the current Lyme antibiotic treatment with doxycycline or ceftriaxone are unable to
59 completely eradicate the *B. burgdorferi* (14-16). In addition, in vitro studies have also
60 demonstrated that *B. burgdorferi* could develop persister bacteria that are tolerant to or not
61 effectively killed by the current Lyme antibiotics (17-19). Furthermore, *B. burgdorferi* develops
62 variant forms such as round bodies and aggregated biofilm-like microcolonies which are
63 persisters with increasing tolerance to antibiotics when the culture grows to late stationary phase
64 or under stress conditions (20). These biofilm-like microcolony forms are not killed by the
65 current Lyme antibiotics singly or in combination but can be eradicated by persister drug
66 daptomycin in combination with doxycycline+cefuroxime (20).

67

68 In addition to chemical drugs such as antibiotics, natural products such as essential oils which
69 exert broad antimicrobial activity against bacteria, fungi, viruses and parasites (21-25) could
70 offer potential therapeutic options. The promising antimicrobial activity of essential oils could
71 also be used with antibiotics to reduce drug side effects, toxicity, resistance to single agents and
72 enhance the antibiotic activity of drugs against bacteria (21, 26). We have previously shown that
73 some essential oils had stronger activity against stationary phase *B. burgdorferi* than the current
74 antibiotics used for treating Lyme disease (27-29). Therefore, in this study, we evaluated 4 active
75 essential oils (carvacrol, cinnamon bark, oregano and clove bud, cinnamaldehyde, allspice,
76 hydacheim, myrrh, garlic and thyme white), which have been shown to have good activity
77 against stationary phase *B. burgdorferi* (27), and their combinations with cefuroxime,
78 doxycycline and daptomycin for their ability to kill *B. burgdorferi* in the fibroblast model.

79

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80 **Materials and Methods**

81 **Bacterial strain and culture condition**

82 *B. burgdorferi* strain B31 expressing the green fluorescent protein (GFP) marker (GFP *B.*
83 *burgdorferi*) was stored at −80°C, thawed at room temperature, and cultured at 33 °C in BSK-H
84 medium. The cultures were monitored by microscopy for growth and contamination. The number
85 of GFP-labeled *B. burgdorferi* was determined using a Petroff-Hausser bacteria counting
86 chamber.

87

88 **Antibiotics and essential oils**

89 Doxycycline (Dox), cefuroxime (CefU) (Sigma-Aldrich, USA) and daptomycin (Dap) (AK
90 Scientific, Inc., USA) were dissolved in suitable solvents to form 5 mg/ml stock solutions. The
91 antibiotic stocks were filter-sterilized by 0.2 µm filter and stored at −20°C. Essential oils
92 (carvacrol, cinnamon bark, oregano and clove bud, cinnamaldehyde, allspice, hydacheim, myrrh,
93 garlic and thyme white) were also dissolved in organic solvent dimethyl sulfoxide (DMSO) at
94 20%, followed by dilution to achieve desired dilutions as previously described (27).

95

96 **Cell cultures**

97 Murine fibroblast NIH/3T3 cells (ATCC) were cultured in culture bottles with the area of 25 cm^2
98 in Dulbecco's modified Eagle medium (DMEM; GIBCO) containing 10% fetal bovine serum
99 (FBS; GIBCO) at 37°C in a humidified incubator with 5% CO_2. When the confluence of cells
100 reached 70%-80%, confluent cell cultures were split with 0.25% trypsin-EDTA (GIBCO) and
101 then 3T3 cells were seeded onto 24-well plates at 37°C in a humidified incubator with 5% CO_2.

102

103 **Coculture of GFP-labeled *B. burgdorferi* and 3T3 cells**

104 When the confluence stage was reached, GFP-labeled *B. burgdorferi* at stationary phase of
105 growth (SP) was centrifuged at 10,000 rpm for 10 min and resuspended in DMEM supplemented
106 with 10% FBS. Then GFP *B. burgdorferi* was added to tissue culture plate wells containing 3T3
107 cells in a 1:10 ratio of fibroblasts against the bacteria or to wells without cells (31). Cells and *B.*
108 *burgdorferi* were cocultured for 48 h. Then the cells were then washed twice with warm PBS,
109 fresh DMEM medium containing cefuroxime, doxycycline and daptomycin at 5 µg/mL or
110 essential oils at three different concentrations 0.1%, 0.05% and 0.02% were added and the
111 cocultures were further incubated for 7 days before the effect of the treatment was evaluated by
112 microscopy or subculture study (5).

113

114 **Subculture study**

115 After the cocultures were treated with antibiotics or essential oils for 7 days as described
116 previously (5) the cells were washed once with warm PBS and lysed by adding 0.5 mL of
117 distilled H_2O for 5 min. Adherent cells were then scraped off, and the total content of each well
118 was inoculated into 1 ml of BSK medium. Cultures were incubated at 33 for 7 days or 21 days

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119 and monitored for the presence of viable *B. burgdorferi* by fluorescence microscopy. Control
120 wells without cells but with spirochetes underwent the same procedure.

121

122 **Microscopy**

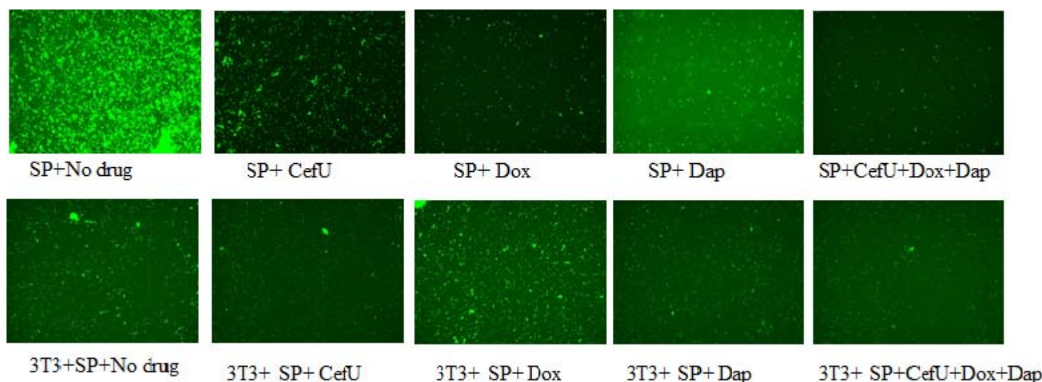
123 The subcultured GFP *B. burgdorferi* was examined using BZ-X710 All-in-One fluorescence
124 microscope (KEYENCE, Inc.). The residual cell viability reading was confirmed by using
125 fluorescence microscopy. The toxic effect of essential oils on the fibroblast cells was evaluated
126 after incubating the mixture for 7 days when the morphology of the fibroblast cells was observed
127 using phase contrast function of the BZ-X710 All-in-One fluorescence microscope (KEYENCE,
128 Inc.).

129

130 **Results**

131 **Fibroblast cells protect *B. burgdorferi* from being killed by different antibiotics including**
132 **drug combination Cefuroxime+ Doxycycline+ Daptomycin**

133 Previous studies have shown that fibroblasts provided protection against ceftriaxone [5]. Here we
134 evaluated whether a powerful persister drug combination CefU+ Dox + Dap, which is known to
135 completely eradicate *B. burgdorferi* aggregated microcolony persisters in vitro (20) could
136 eradicate *B. burgdorferi* from fibroblast cells. Surprisingly, as shown in Figure 1 and Table 1, the
137 presence of 3T3 fibroblast cells protected the *B. burgdorferi* from being completely killed not
138 only by single antibiotics doxycycline, cefuroxime, or daptomycin but also by the triple drug
139 combination Cefuroxime+ Doxycycline+ Daptomycin as shown by residual green GFP labeled *B.*
140 *burgdorferi* remaining after drug treatment for 7 days. However, we did find that Cefuroxime+
141 Doxycycline+ Daptomycin combination was more effective in killing *B. burgdorferi* than any
142 other single antibiotics (Figure 1). Nevertheless, none of them including the triple drug
143 combination Cefuroxime+ Doxycycline+ Daptomycin could completely kill *B. burgdorferi* as
144 shown by visible spirochetal growth after 21-day subculture (Table 1).



145

146 **Figure 1. Coculture of GFP *B. burgdorferi* with 3T3 cells treated with antibiotics ex vivo.**
147 [a] Representative live images of stationary phase *B. burgdorferi* treated with different antibiotics
148 at 200× magnification. *B. burgdorferi* at stationary phase of growth were added to wells
149 containing a monolayer or to wells without cells (1.5×10^6 live organisms/well) and incubated.
150 Cells and *B. burgdorferi* were cocultured for 48 h. The cells were then washed twice with warm

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151 PBS, fresh tissue culture medium containing antibiotics at 5 μg/mL was added, and the
152 cocultures were further incubated for 7 days. After 7 days, cultures were monitored for the
153 presence of viable *B. burgdorferi*. Control wells without cells but with *B. burgdorferi* underwent
154 the same procedure. Abbreviations: stationary phase culture of *B. burgdorferi* - SP, murine
155 fibroblasts NIH/3T3 - 3T3, doxycycline - Dox, cefuroxime - CefU, daptomycin - Dap.
156
157 **Table 1 Subculture the lysate of 3T3 infected with *B. burgdorferi* which were treated with**
158 **antibiotics.**

	Subculture for 21-days
SP+CefU	Growth
SP+Dox	Growth
SP+Dap	Growth
SP+CefU+Dox	Growth
SP+CefU+Dox+Dap	Growth
3T3+SP+CefU	Growth
3T3+SP+ Dox	Growth
3T3+SP+Dap	Growth
3T3+SP+CefU+Dox	Growth
3T3+SP+CefU+Dox+Dap	Growth

159 [a]After coculture of GFP *B. burgdorferi* with 3T3 cells treated with antibiotics *ex vivo* described
160 as Figures 1, the total content of each well was inoculated into 1 ml of BSK-H medium. Cultures
161 were incubated at 33℃ for 21 days. After that, cultures were monitored for the presence of
162 viable *B. burgdorferi* with microscope. Abbreviations: stationary phase culture of *B. burgdorferi*
163 - SP, murine fibroblasts NIH/3T3 - 3T3, doxycycline - Dox, cefuroxime - CefU, daptomycin -
164 Dap.

165

166 **Effect of essential oils on killing GFP *B. burgdorferi* in the fibroblast ex vivo model.**

167 We also evaluated essential oils for their activity to kill GFP *B. burgdorferi* cocultured with 3T3
168 fibroblast cells ex vivo. We tested 10 essential oils (carvacrol, cinnamon bark, oregano and clove
169 bud, cinnamaldehyde, allspice, hydacheim, myrrh, garlic and thyme white), which were shown to
170 have excellent activity against stationary phase *B. burgdorferi* in our previous studies (27, 28), at
171 three different concentrations (0.1%, 0.05% and 0.02%) for activity against GFP *B. burgdorferi*
172 cocultured with 3T3 cells. We found oregano oil and carvacrol (active component of oregano) at
173 the lower concentration of 0.1% could kill all stationary phase *B. burgdorferi*, but cinnamon bark
174 and clove bud could not eradicate all stationary phase *B. burgdorferi* (Figure 2A). At the same
175 time, carvacrol and oregano at the concentration of 0.1% reduced significantly the number of
176 stationary phase *B. burgdorferi* and showed stronger activity against *B. burgdorferi* in the
177 presence of 3T3 cells than at the lower concentrations of 0.05% and 0.02%, but still could not
178 completely eradicate all stationary phase GFP *B. burgdorferi* (Figure 2B). Meanwhile, oregano
179 oil and carvacrol had no significant effect on cell morphology. Some essential oils such as myrrh
180 and garlic had very serious toxicity effect on cell morphology (Table 2). Therefore, we combined
181 oregano oil and carvacrol with the triple persister drug regimen (Dox+CefU+Dap) to evaluate
182 whether the essential oil combinations could eradicate the persistent GFP-*B. burgdorferi* in the
183 fibroblast cells.

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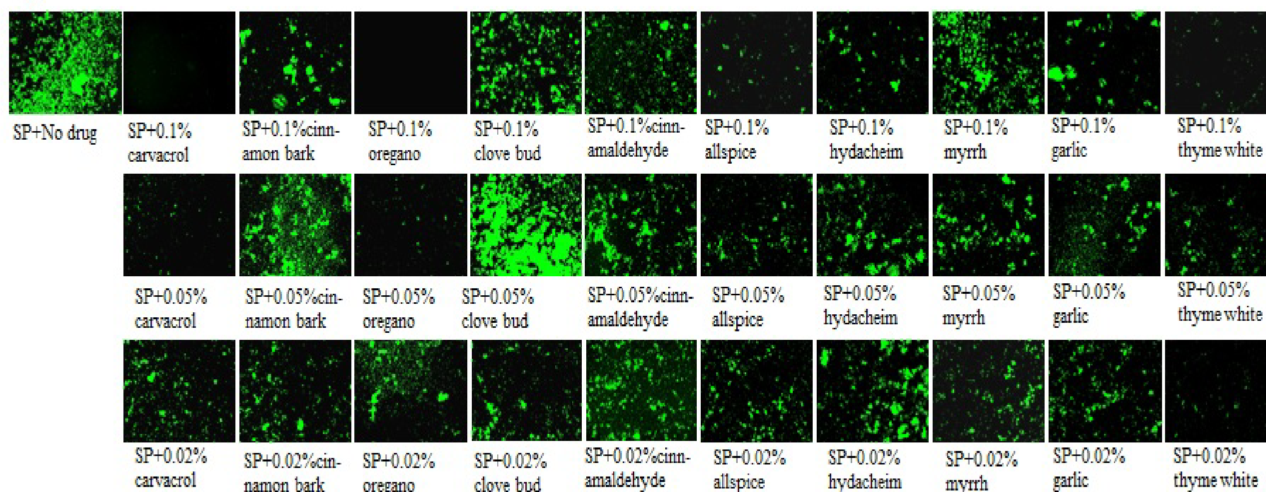

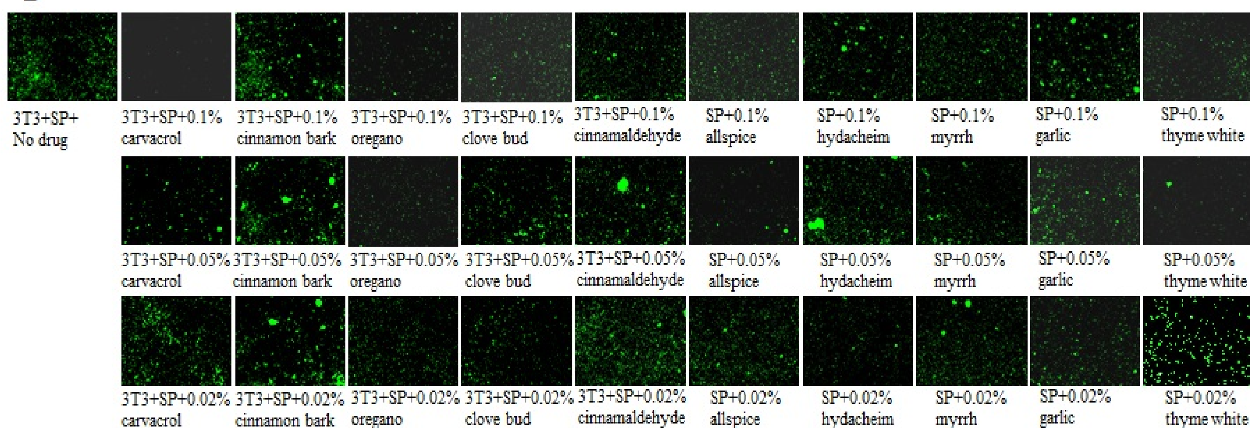

Figure 2. Coculture of GFP *B. burgdorferi* with 3T3 cells treated with essential oils ex vivo. Representative images of stationary phase *B. burgdorferi* treated with essential oils in the absence or presence of 3T3 fibroblast cells at 200 × magnification. We evaluated combination with essential oils ex vivo for activity against a 7-day-old *B. burgdorferi* stationary phase culture. *B. burgdorferi* at stationary phase of growth were added to wells containing a monolayer or to wells without cells (1.5×10^{6} live organisms/well) and incubated. Cells and *B. burgdorferi* were cocultured for 48 h. The cells were then washed twice with warm PBS, fresh tissue culture medium containing essential oils at a concentration of 0.1%, 0.05% and 0.02% were added the cocultures, and the cocultures were incubated for 7 days. After 7 days, cultures were monitored for the presence of viable *B. burgdorferi*. Control wells without cells but with *B. burgdorferi* underwent the same procedure. (A) Stationary phase culture of B. burgdorferi treated with essential oils. (B) Stationary phase culture of *B. burgdorferi* treated with essential oils. Abbreviations: stationary phase culture of *B. burgdorferi* - SP, murine fibroblasts NIH/3T3 - 3T3.

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201 **Table 2 Cytotoxicity effect of essential oils on 3T3 fibroblast cells.**

Essential oils		Cytotoxicity effect
Carvacrol	0.1%	No
	0.05%	No
	0.02	No
Cinnamon bark	0.1%	No
	0.05%	No
	0.02	No
Oregano	0.1%	No
	0.05%	No
	0.02	No
Clove bud	0.1%	No
	0.05%	No
	0.02	No
Cinnamaldehyde	0.1%	No
	0.05%	No
	0.02	No
Allspice	0.1%	No
	0.05%	No
	0.02	No
Hydacheim	0.1%	Yes
	0.05%	No
	0.02	No
Myrrh	0.1%	Yes
	0.05%	Yes
	0.02	No
Garlic	0.1%	Yes
	0.05%	Yes
	0.02	No
Thyme white	0.1%	No
	0.05%	No
	0.02	No

202 [a]3T3 cells were incubated with essential oils (0.1%, 0.05% and 0.02%) for 7 days to evaluate
203 their potential toxicity on 3T3 cells. Cell morphology was observed by phase contrast
204 microscopy.

205

206 **Essential oils in combination with the triple persister antibiotic regimen (Dox+CefU+Dap)**
207 **completely eradicate GFP *B. burgdorferi* in the fibroblast cell model**

208 To assess if essential oils could potentially enhance the activity of the persister drug
209 combination, we selected oregano oil and carvacrol at the concentration of 0.1% and combined
210 them with CefU, Dox and Dap to assess their activity on GFP *B. burgdorferi* in coculture with
211 3T3 cells. The results showed that while carvacrol, oregano or CefU+ Dox + Dap alone had good
212 activity against *B. burgdorferi* in 3T3 cells, none of them alone was able to completely eradicate

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B. burgdorferi in the fibroblast cell model. Importantly, carvacrol or oregano in combination with CefU, Dox and Dap could kill all *B. burgdorferi* cells in the absence and even in the presence of fibroblast cells (Figure 3). In the absence of fibroblast cells, there was no *B. burgdorferi* after treatment with carvacrol or oregano (Figure 3). Subculture study also confirmed the high activity of carvacrol or oregano at 0.1% combined with CefU, Dox and Dap for complete eradication of GFP *B. burgdorferi* in the fibroblast cell model as evidence by no regrowth in 21 days. However, regrowth occurred in samples treated with carvacrol, oregano or CefU+ Dox + Dap alone in the fibroblast cell model. In contrast, in the absence of the fibroblast cells, no regrowth was observed in the samples treated with carvacrol or oregano or CefU, Dox and Dap after 21-day subculture (Table 3).

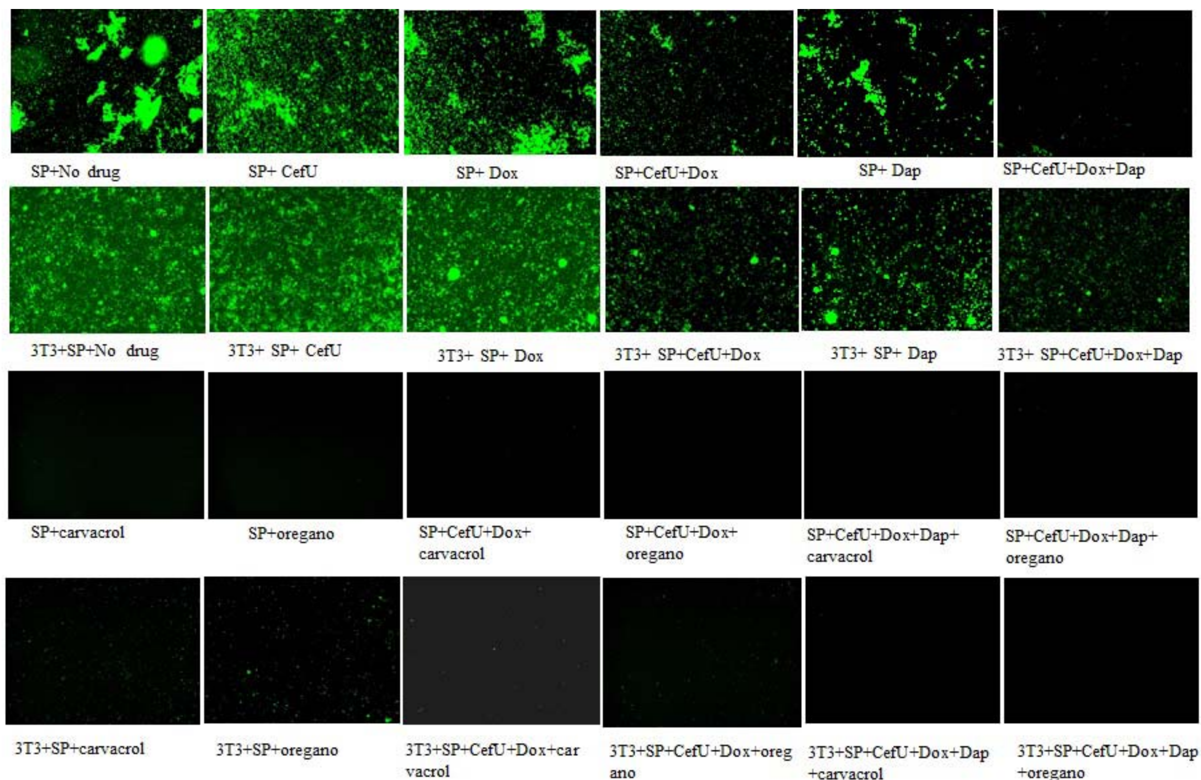

Figure3. Coculture of GFP *B. burgdorferi* with 3T3 cells treated with antibiotics and essential oils ex vivo. [a]Representative images of stationary phase *B. burgdorferi* treated with antibiotics and essential oils ex vivo at 200× magnification. We evaluated combination of antibiotics and essential oils ex vivo for activity against a 7-day-old *B. burgdorferi* stationary phase culture. Stationary phase *B. burgdorferi* cells were added to wells containing a monolayer of fibroblast cells (1.5×10^6 organisms/well) or to wells without cells and incubated at 37℃. Cells and *B. burgdorferi* were cocultured for 48 h. The cells were then washed twice with warm PBS, fresh tissue culture medium containing antibiotics at 5 μg/mL or essential oils at a concentration of 0.1% were added, and the cocultures were incubated for 7 days. After 7 days, cultures were monitored for the presence of viable *B. burgdorferi*. Control wells without cells but with *B. burgdorferi* underwent the same procedure. Abbreviations: stationary phase culture of *B. burgdorferi* - SP, murine fibroblasts NIH/3T3 - 3T3, doxycycline - Dox, cefuroxime - CefU, daptomycin - Dap.

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238

239 **Table 3 Subculture the lysate of 3T3 infected with *B. burgdorferi* which were treated with**
240 **antibiotics and essential oils.**

	Subculture for 21-days
SP+CefU	Growth
SP+Dox	Growth
SP+Dap	Growth
SP+CefU+Dox	Growth
SP+CefU+Dox+Dap	Growth
SP+Carvacrol	No growth
SP+ Oregano	No growth
SP+CefU+Dox+ Carvacrol	No growth
3T3+SP+CefU+Dox+ Oregano	No growth
SP+CefU+Dox+Dap+Carvacrol	No growth
SP+CefU+Dox+Dap+Oregano	No growth
3T3+SP+CefU	Growth
3T3+SP+ Dox	Growth
3T3+SP+Dap	Growth
3T3+SP+CefU+Dox	Growth
3T3+SP+CefU+Dox+Dap	Growth
3T3+SP+ Carvacrol	Growth
3T3+SP+ oregano	Growth
3T3+SP+CefU+Dox+ Carvacrol	Growth
3T3+SP+CefU+Dox+ Oregano	Growth
3T3+SP+CefU+Dox+Dap+ Carvacrol	No growth
3T3+SP+CefU+Dox+Dap+ Oregano	No growth

241 [a]After coculture of GFP *B. burgdorferi* with 3T3 fibroblast cells treated with antibiotics and
242 essential oils *ex vivo*, the total content of each well was inoculated into 1 ml of BSK-H medium
243 to determine if any residual bacteria not killed would regrow. Cultures were incubated at 33℃
244 for 21 days and monitored for the presence of viable *B. burgdorferi* with fluorescence
245 microscope. Abbreviations: SP- stationary phase culture of *B. burgdorferi*, 3T3- NIH/3T3
246 murine fibroblast cell line, Dox- doxycycline, CefU- cefuroxime, Dap- daptomycin.

247

248 **Discussion**

249

250 In this study, we used stationary phase *B. burgdorferi*- fibroblast coculture model ex vivo to
251 evaluate whether fibroblasts would protect *B. burgdorferi* from the action of some drugs and
252 whether combinations of drugs and essential oils could eradicate *B. burgdorferi*. The reason why
253 we chose this model are as follows. Firstly, in tuberculosis treatment, persister drug
254 pyrazinamide is more active against stationary phase cells and persisters than against log phase
255 growing cells and could shorten the therapy (32), which justifies the use of stationary phase *B.*
256 *burgdorferi* enriched in persisters as a persister model. Secondly, *B. burgdorferi* is transmitted

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257 firstly into the skin by ticks, where it spreads locally and establishes infection (33, 34).
258 Fibroblasts reside in the skin and interact with *B. burgdorferi*. Thirdly, fibroblasts have been
259 shown to protect *B. burgdorferi* from the action of antibiotics commonly used for its eradication,
260 especially the most powerful Lyme antibiotic ceftriaxone (5). This implies that fibroblast
261 interaction with *B. burgdorferi* may allow persisters to develop that are not killed easily by the
262 current Lyme antibiotics.

263 In this study, we were interested to know whether fibroblasts could also protect *B. burgdorferi*
264 from the action of the powerful persister drug combination CefU, Dox and Dap, which was
265 previously shown to completely eradicate *B. burgdorferi* persisters not killed by the current
266 Lyme antibiotics in vitro (20). Surprisingly, the presence of the fibroblast cells indeed protected
267 the *B. burgdorferi,* from eradication by the persister drug combination CefU, Dox and Dap as
268 shown by regrowth in subculture study. Nevertheless, we did find that CefU, Dox and Dap
269 combination was better against *B. burgdorferi* than each single antibiotics in the fibroblast cell
270 persistence model, since none of them could completely kill *B. burgdorferi* as they were able to
271 regrow and were still visible after 21-day subculture. We then tested 4 active essential oils
272 carvacrol, cinnamon bark, oregano and clove bud which previously were shown to be highly
273 active against *B. burgdorferi* (27) in the *B. burgdorferi*-fibroblast cell coculture model. We found
274 that fibroblasts protected *B. burgdorferi* from eradication by the triple drug combination or
275 carvacrol or oregano at 0.1% as either alone was unable to completely clear the bacteria.
276 Interestingly, we found that when the triple drug combination
277 doxycycline+cefuroxime+daptomycin was combined with essential oils carvacrol or oregano at
278 0.1%, all *B. burgdorferi* cells were completely eradicated such that the cells did not grow back in
279 21-day subculture. This indicates the potential value of essential oils when used in combination
280 with antibiotics. Further studies are needed to confirm this in animal studies.

281 It has been previously demonstrated that *B. burgdorferi* penetrated endothelial cells inside and
282 between these cells (35), or penetrated cultured human umbilical vein endothelial cells (36), but
283 the viability of the intracellularly located spirochetes was not assessed. In our study, we were
284 mainly focused on evaluating the effect of drug combination and essential oils on eradicating *B.*
285 *burgdorferi* in the fibroblast persistence model, and whether the GFP *B. burgdorferi* is located
286 inside or on the surface of the fibroblast cells is less clear. Future studies are required to precisely
287 define the locale of the protective effect of fibroblasts for *B. burgdorferi* as well as whether other
288 drugs with different modes of action and different ability to penetrate cells would eliminate the
289 spirochetes even in the presence of fibroblast cells. One possibility that may explain why
290 fibroblasts protected *B. burgdorferi* from the drug combination is that association of *B.*
291 *burgdorferi* with fibroblast cells allows the bacteria to change the metabolic status and develop
292 more persisters. Alternatively, association of *B. burgdorferi* with fibroblast allows the bacteria to
293 attach to the fibroblast and facilitate biofilm formation such that even the powerful triple drug
294 combination is still unable to eradicate the bacteria. It is also possible that *B. burgdorferi* could
295 penetrate cells and survive in fibroblasts as shown by previous studies (36) and the intracellular
296 survival would provide protection against antibiotics, since many antibiotics are much less
297 concentrated in the cells than in extracellular spaces. In contrast, essential oils carvacrol and
298 oregano due to their lipophilicity may have a better cell membrane association and penetration as
299 well as intracellular distribution, which may explain the more effective killing and eradication of
300 *B. burgdorferi* by essential oils carvacrol or oregano combined with the triple drug combination

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301 doxycycline, cefuroxime, daptomycin even in the presence of the fibroblast cells. Further studies
302 are needed to elucidate the mechanisms involved.

303 In summary, the results in this study showed host fibroblast cells could protect *B. burgdorferi*
304 from the killing by drug combination doxycycline, cefuroxime and daptomycin, but addition of
305 essential oil carvacrol or oregano at 0.1% to this drug combination could completely eradicate *B.*
306 *burgdorferi* with no regrowth. Future studies will be carried out to assess their safety and
307 efficacy against *B. burgdorferi* infection in animal models.

308

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313

314 **Conflicts of Interest**

315 The authors declare no conflict of interest.

316

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